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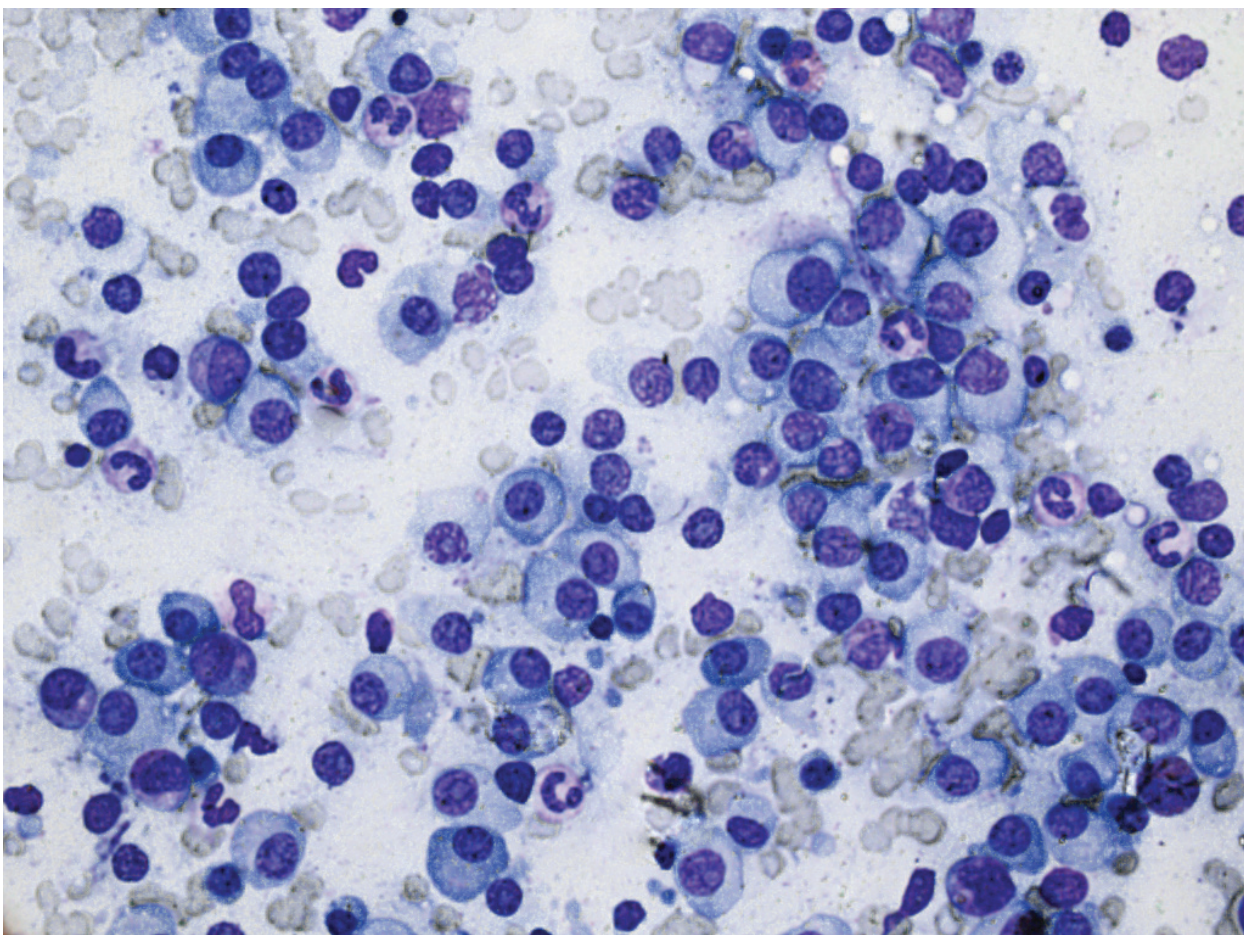
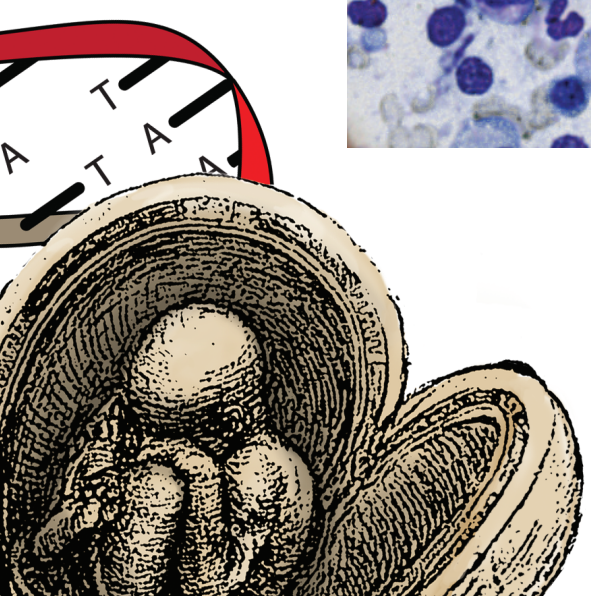
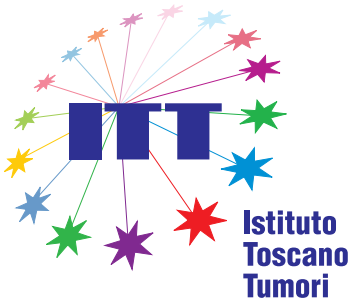
Istituto Toscano Tumori

Istituto Toscano Tumori
SCIENTIFIC REPORT 2005-2009







Istituto Toscano Tumori

SCIENTIFIC REPORT 2005-2009

ITT SCIENTIFIC REPORT 2005-2009

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The Istituto Toscano Tumori has been established in 2003 by the Tuscany Legislative Assembly through the foresight and thanks to the leadership of Enrico Rossi, Health Secretary in Tuscany from 2000 to 2010, now Governor of the Tuscany Region.

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CONTENTS

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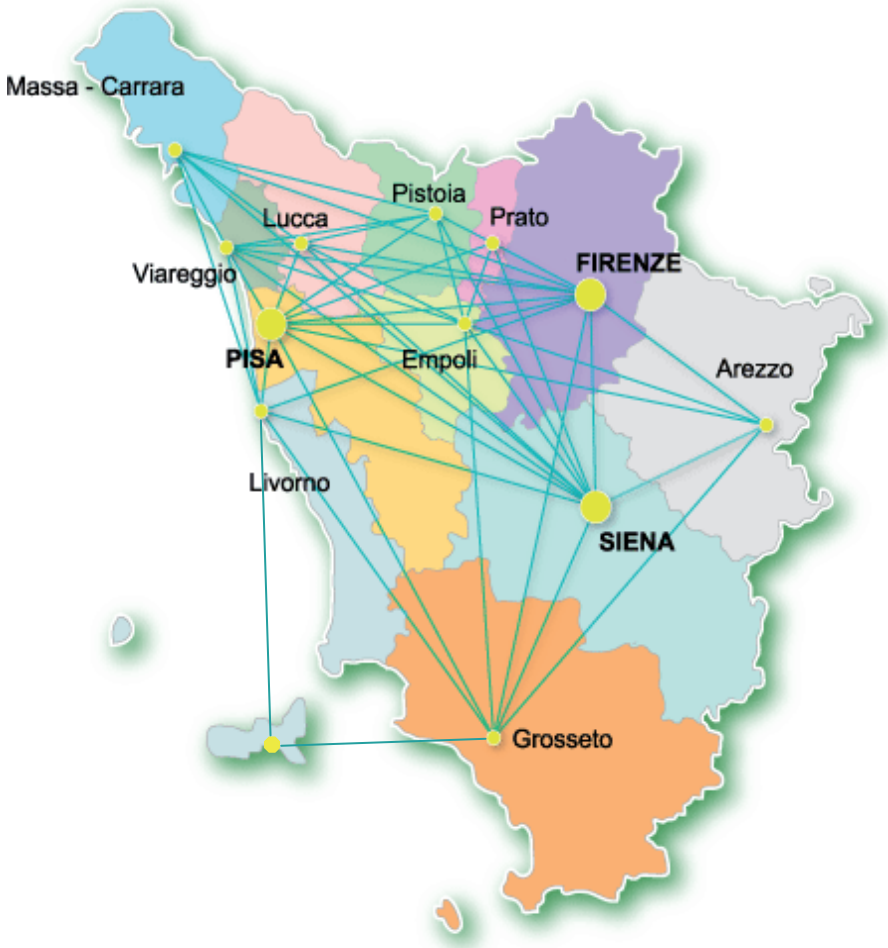


TO UNDERSTAND, TO PREVENT
AND TO TREAT CANCER
AT BEST FOR ALL

ISTITUTO TOSCANO TUMORI

The *Istituto Toscano Tumori* (ITT) was established through a Regional Law in 2003 and started to operate in 2005: ITT strives to operate with a keen, pervasive and continuing awareness of its mission: **to understand, to prevent, and to treat cancer at best for all.**

This means that we must devote our efforts primarily to the care of patients, to screening programmes, and to high quality research. Here we can only outline some of the steps that have been taken, and the main activities we are pursuing in the Institute (see also our site www.ittumori.it).

The *Istituto per lo Studio e la Prevenzione Oncologica* (ISPO, formerly CSPO) has been at the forefront of cancer prevention for over 40 years. Thanks to ISPO, population-wide screening programmes for cervical cancer, for breast cancer and for colon cancer have been well established in Tuscany, and have achieved a high rate of compliance, among the highest in Italy. The standards of these programmes and of the research studies associated with them have earned for ISPO a national and international reputation. ITT is fortunate to have ISPO as one of its founding and major components since its inception.

Epidemiology. Accurate and up-to-date information on the incidence of and mortality from tumors is essential not only for cancer research but also for the rational organization of health services. A high standard *Tumor Registry* has covered the two provinces of Firenze and Prato for about 30 years. In 2006 ITT has been instrumental in extending coverage to all 10 provinces of Tuscany.

Cancer Care. The clinical network of ITT consists of 16 Medical Oncology Units, 10 Radiotherapy Units, 16 Surgical Units, 3 Hematology Units, and other Units dealing with specific types of cancer. In addition, a major Oncology Unit is active at the Meyer Pediatric Teaching Hospital in Firenze. Details of many of these Units are given in the main body of this report. The philosophy of ITT is that the backbone for the management of patients must consist of Disease Management Teams (*Gruppi Oncologici Multidisciplinari*, or GOM); these are accessed through entry points (*Accoglienza*) that operate in each Hospital of the Region.

Clinical Recommendations. Since its inception, ITT has set up a study group for each of the major types of cancer. Each group has worked out management protocols for breast cancer, lung cancer, prostate cancer, colon cancer, and gynaecological cancer. Of course the protocols conform to international standards, and they have been formally adopted in July 2005. By the end of 2005 ITT has promoted a clinical epidemiology project with the specific aim to monitor the rate of compliance to the approved protocols. In May 2007 clinical recommendations for melanoma were also adopted. In addition, a study group on inherited cancer has published a preliminary report, and this group is currently establishing standards for genetic counseling and genetic tests for cancer-prone families throughout the Region.

Clinical Research. Although naturally research activities are more developed in the three University-affiliated Teaching Hospitals in Firenze, Siena and Pisa, the majority of the other Clinical Units are also engaged in research – clinical and translational – as well as patient care (see the main body of this report). At the interface between the two, it is important to offer patients participation in clinical trials when appropriate. At the moment there are over 200 active clinical trials in the Region. In order to coordinate clinical research activities ITT has set up a Clinical Trials Coordinating Center (*Centro di Coordinamento Sperimentazioni Cliniche*, *CCSC*), and has appointed as director **Dr Luca BONI**, who has a distinguished record of previous experience in clinical epidemiology and biostatistics. The CCSC is formally part of the CRL (see below).

ITT Projects. Basic cancer research is carried out in many laboratories, in University preclinical and clinical Departments, in laboratories of the National Research Council (CNR), and elsewhere. Many of these research activities pre-date the inception of ITT, and ITT does not claim credit for their existence: but it has welcomed their participation to ITT retreats, to collaborative projects, as well as their contribution to this report, which aims to give a reasonably comprehensive portrait of current cancer research in Tuscany. It was felt from the outset that one ought to give high priority to encouraging cancer research throughout the Region, and to this end ITT has decided to double up as a granting agency, and has put out calls for Project Grant applications, by research Units within Tuscany (duration up to 3 years), on all aspects of cancer research, including but not limited to:

- Molecular basis of tumours and somatic mutations.
- Environmental and hereditary factors that cause or influence the development of cancer.
- Novel approaches in tumour prevention.
- Innovation in cancer diagnosis and treatment.
- New approaches to supportive and palliative therapy.
- New approaches to communication with patients and with the public.

The only restriction is that, even though collaborations are encouraged, the funds must be spent within Tuscany. All can apply except members of the ITT CRL (see below). The Projects are subjected to international Peer Review, with a database of over 500 referees. The final decision is taken at a full meeting of the International Scientific Advisory Board (ISAB), based on a composite assessment of Originality, Scientific value, Relevance to current research on cancer/oncology, Preliminary results, Feasibility, and Scientific track record of the Principal Investigator and of external collaborators, appropriateness of the scientific environment, and coherence between the research proposal and the level of funding requested. In the first call (2008) we had 85 applications and 25 were funded; in the second call (2009) we had 109 applications and 33 were funded. The annual budget is approximately M€ 1.8.

Core Research Laboratory (CRL). As stated in the previous section, ITT has capitalized substantially on pre-existing laboratories in order to build up its Research network. At the same time, it was deemed essential to develop some new research initiatives by establishing a small central laboratory, the CRL. The Regional Goverment has indicated that this should be located in Firenze, and it has given approval for an L-shaped building on the Careggi campus of the Medical School, near an existing research building called 'il Cubo'. Construction work has started in May 2009 and it is due for completion in November 2011. In order to activate the CRL as soon as possible, there was no alternative but to 'borrow space'. Thus far we have been able to activate 4 Units.

Genetics and Gene Transfer in Oncology
Dr Rosario NOTARO (2007)

Molecular Mechanisms of Oncogenesis
Dr Silvo CONTICELLO (2007)

Signal Transduction
Dr Mario CHIARIELLO (2008: Siena)

Tumor Cell Biology
Dr Barbara STECCA (2009)

In keeping with the network structure of ITT, CRL already has one of its Units in Siena, and a new Unit will be activated in Pisa as soon as possible.

Core Facilities. In order to support the research activities of ITT there is of course a need for infrastructure: some of this is provided by Universities and Hospitals. ITT intends to strengthen this infrastructure for all its sites. For the moment we have established (a) an animal facility mainly for animal models of human

tumors (see L.I.Ge.M.A. page 407); (b) a Clinical Trials Coordinating Center (see page 545) for planning and running clinical trials.

Training activities. Currently there are active training programmes in Oncology and Hematology at the three Teaching Hospitals in Firenze, Pisa and Siena. Trainees are posted or rotate through some, but not all of the non-Teaching Hospitals associated with ITT. We have had several meetings aiming to integrate training programmes: for this purpose ITT has sponsored some Trainees, thus increasing their number. ITT has also set up a scheme for brief research and training stages in other institutions, in Italy or abroad, open to ITT staff, post-doctoral Fellows and clinical Trainees.

Staff rounds/Journal Club sessions. Given the network nature of ITT, it is important that joint discussions are held at regular intervals. Apart from numerous meetings taking place all over the Region and announced on our site, we have a weekly session (Tuesdays from 2:30 to 3:30 pm) in which all Units are joined in a video conference, for which we have 18 video stations. Each Unit presents in turn clinical cases, or reports on recent journal articles, recent research meetings, stages held abroad, or current clinical trials.

The ITT Foundation. In 2009 the Tuscany Region has established the *Fondazione dell'Istituto Toscano Tumori*. This Foundation has the mission to support institutional activities of ITT; in the aim to promote and support, with the help of citizens, the fight against cancer through research, prevention, diagnosis and all forms of therapy. The founding members have been the three Teaching Hospitals of Florence (Careggi), Pisa and Siena; the Meyer Pediatric Teaching Hospital; and all 12 of the local Health Authorities in Tuscany. The Foundation will have the status of a Charity, facilitating fund-raising for the benefit of ITT. The first meeting of the Board of Directors has taken place on February 5, 2010. Dr Giuseppina Cabras was appointed as Executive Secretary.

The building site of the Core Reserarch Laboratory of ITT, near the "Cubo" building at Careggi. Photograph taken on May 4, 2010

ITT INTERNATIONAL SCIENTIFIC ADVISORY BOARD (ISAB)

All the research and related activities of ITT are supervised by a Board that meets at least once a year, on the occasion of the scientific retreat of ITT, usually at the beginning of July. In addition, the Board is responsible for the award of funds for ITT Research Projects and is consulted on such matters as seem appropriate. Currently the Board consists of:

Sydney Brenner (Chairman)
Nobel Prize in Medicine 2002

Dino Amadori
Director of the Istituto Oncologico Romagnolo (IOR), Forlì; Oncologist and Investigator of international repute

Andrea Ardizzoni
Director of Medical Oncology Unit, Azienda Ospedaliero Universitaria, Parma; Coordinator of Lung Cancer Group of the European Organization for Research and Treatment of Cancer (EORTC)

Paolo Bruzzi
Istituto Nazionale per la Ricerca sul Cancro (IST), Genova; Clinical Epidemiologist of international repute

Giannino Del Sal
Director of Molecular Oncology Unit, University of Trieste; a leader in Molecular Oncology, member of the European Molecular Biology Organization (EMBO)

Jean-Claude Horiot
Institut Multidisciplinaire d'Oncologie, Clinique de Genolier; a leader in the field of Radiotherapy, past President of the European Society for Therapeutic Radiology and Oncology (ESTRO)

Pier Paolo Pandolfi
Director of Cancer Genetics Program, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston; word leader in cancer research

Robert Pinedo
Cancer Center Amsterdam; Oncologist and Investigator of international repute

Paolo Vineis
Professor of Environmental Epidemiology, Imperial College, London; leader in Epidemiology and Environmental Cancerogenesis; one of the founders of the EPIC project (European Prospective Investigation into Cancer and Nutrition)


Annual Scientific Conference

ITT STEERING COMMITTEE

ITT has a Steering Committee (Ufficio di Direzione) that acts as advisory body to the Operative Director and Scientific Director. This Steering Committee includes representatives of the main geographical areas within the Tuscany Region, a representative of the Istituto per lo Studio e la Prevenzione Oncologica, a representative of General Practitioners and an Officer of the Tuscany Region Administration. Currently the Steering Committee consists of:

Gianni Amunni
Operative Director

Lucio Luzzatto
Scientific Director

Luigi Biancalani
Representative of General Practitioners

Maurizio Cantore
Representative, "Area Vasta Nord Ovest"

Luigi Cataliotti
Representative, "Area Vasta Centro"

Luca Cionini
Coordinator, "Polo Oncologico Area Vasta Nord Ovest"

Sergio Crispino
Coordinator, "Polo Oncologico Area Vasta Sud Est"

Carolina Cuzzoni
Medical Director, Istituto per lo Studio e la Prevenzione Oncologica

Valerio Del Ministro
Officer of Tuscany Region Administration

Luisa Fioretto
Coordinator, "Polo Oncologico Area Vasta Centro"

Michele Maio
Representative, "Area Vasta Sud Est"

FOREWORD

Gianni Amunni

ITT Operative Director

The *Istituto Toscano Tumori* (ITT) is a network to which all Oncology Units of the Region belong. ITT is characterized by a number of strategic objectives in the areas of diagnosis, treatment and research, including the following:

- merging into one system of governance of structures operating in oncology, ranging from cancer prevention to research, from basic diagnosis to highly specialized clinical investigation;
- optimization of a network capable of capturing the demand of the population in terms of cancer services and to organize and manage synergistically a variety of different institutions, including those devoted to innovation;
- geographic diffusion of entry points to ITT throughout the Region through which integrated management can be provided at all levels of clinical complexity;
- ensuring uniformity of clinical services through the adoption of shared management protocols adhering to strict criteria of quality, appropriateness, timeliness, efficiency.

Each one of the above topics is strengthened by consistent enforcing of one of the fundamental principles of the ITT: namely, to promote scientific research, which is regarded not as an option, but as an integral and indispensable part of the quality of care, without any institutional or geographic hierarchies.

Out of these objectives have emerged significant choices regarding the distribution of resources. This in turn has resulted in a detailed program, which includes the following:

- setting up a *Core Research Laboratory* in Florence comprising five Units, supplemented by and integrated with two satellite laboratories, one in Pisa and another in Siena;
- setting up a project grant program open to all ITT Units;
- activation of a central facility for the coordination of clinical research, particularly in order to promote clinical trials, which are not necessarily industry sponsored; and to provide statistical expertise to local projects;
- setting up a facility for animal models of cancer;
- support for research projects assessed as strategic for ITT by funding training programs in external institutions in Italy or abroad;
- preparing and coordinating grant applications to national and international agencies.

The above points are part of a regional context which is rich in individual and group experiences, operating in basic, translational and clinical oncology research.

This Report contains a summary of some of the ongoing research and future projects of many but not all the research groups which come under the aegis of the ITT. The material in this book can be used for various purposes:

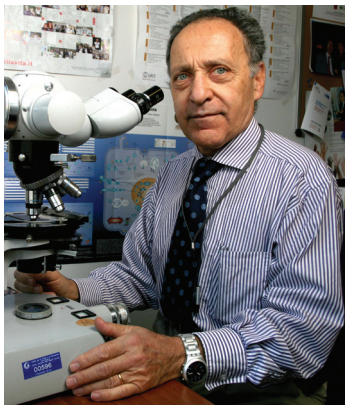
- to illustrate the state of the art of oncological research in Tuscany;
- to provide knowledge of the sectors where it is possible to create synergy between researchers to have similar and complementary research objectives;

- to provide a basis for discussion of the ideas circulating in these various fields so that they can be critiqued as well as encouraged;
- to identify new research ideas and areas requiring study so they can be promoted and funded;
- to offer the International Scientific Advisory Board (ISAB) a panorama of the research activities of the ITT for purposes of evaluation and future development.

We are deeply convinced that this collection of research summaries will help the process of integration and collaboration between clinics and researchers, which is the basis of an advanced and competitive health system.

Along these lines, the ITT intends to work toward the following goals:

- diffusion of patient participation in clinical research as one of the ways to improve the quality of care;
- promotion of rapid transfer of innovations in care as a result of the collaboration between research and care structures;
- use of a system of regional case lists as an instrument to reinforce and speed up strategic clinical research;
- reciprocal coordination between the world of research and that of care with the aim of sharing needs and future prospects.



INTRODUCTION

Lucio Luzzatto

ITT Scientific Director

The study of cancer today is a vast research field in its own right, that has evolved from previously established disciplines, including pathology, genetics, biochemistry, cell biology. Although any attempt to identify historical milestones is risky, I dare mentioning three. (a) Around 1845 Rudolf Virchow stated the notion that the regulatory mechanisms controlling normal tissues are defied by cancer, because it is made up of seriously deranged cells. This suggested that the primary problem does not come from outside: rather, it is internal to the body, indeed, to certain cells within the body. (b) In 1913 Theodor Boveri surmised that cancer originated from an abnormality in the chromosomes: although at the time this was hardly more than intuition, it had a spectacular confirmation nearly half a century later, when Nowell and Hungerford[1] discovered the Philadelphia chromosome, a cytogenetic abnormality specifically associated with chronic myeloid leukemia. (c) In 1989 Michael Bishop and Harold Varmus received a Nobel prize[2,3] for the discovery of what became known as oncogenes, *i.e.* mutant genes arising from normal cellular genes through somatic mutations, capable of transforming normal cells into cancer cells. Thus, cancer is now recognized as a genetic disease of somatic cells[4,5].

Side by side to these developments, the advances in cancer management over the past generation have been impressive, through a combination of imaging techniques and sophisticated histopathology that afford earlier and more accurate diagnosis and characterization of tumors; advances in surgical techniques, including less invasive and robotized methodologies; advances in radiation technology that improve tumor targeting and reduce toxicity of radiotherapy; an ever increasing range of agents for pharmacotherapy of tumors; as well as the increasing adherence of specialized multi-disciplinary management teams to evidence-based detailed guidelines for individual tumor types: the latter has been an early landmark of the strategy of the *Istituto Toscano Tumori* (ITT).

Based on these foundations, during the first decade of this millennium cancer research has been explosive: and perhaps its most distinctive feature has been a more productive relationship between basic cancer research and clinical oncology. Previously, progress in these two areas has tended to take place, as just mentioned, side by side: even if basic scientists have always hoped that their efforts might help to cure cancer, this was often regarded merely as a more or less probable extra bonus. More recently, research projects are frequently developed aiming to a specific clinical goal; and increasingly clinical oncology is posing questions that basic research has the means to attack. The bedside to bench approach is at least as important as the bench to bedside approach; and a full integration of basic research, translational research and clinical research is on top of the agenda of ITT. With this in mind we list here some of the major questions that contemporary cancer research is tackling and we intend to pursue [6].

1. *What makes a normal cell become a cancer cell?*
2. *What changes can override growth control, mitotic checkpoints, and danger signals that ought to activate apoptosis?*
3. *What endows a cancer cell with the potential for metastasis?*
4. *What inherited genes predispose to cancer?*

5. *What is the quantitative impact of environmental factors, including diet, and can we effectively influence lifestyles in the aim to decrease the number of avoidable cancers?*
6. *What is the role of genomic instability*
7. *What is the role of epigenetic changes?*

And, most important of all,

8. *How will this massive body of new knowledge help us to help cancer patients?*

Today we have the means to answer these questions, and significant progress has been made already.

Molecular basis of oncogenic transformation

The most direct approach to answering the first of the above questions is to identify those genes that are mutated in cancer. Until recently this task has been managed essentially in three ways. (a) Cytogenetic analysis has been highly successful in the discovery of fusion genes and of tumor suppressor genes[7,8]. (b) Positional cloning has identified, in families with increased incidence of cancer, genes with germ-line mutations; and the same genes may be somatically mutated in sporadic cancer[5]. (c) In some cases genes thought to be involved in oncogenesis on grounds of their function, *i.e.* candidate genes, such as *RAS*, have been directly tested for mutations[9].

Recently a new approach has been explored, which can be called a blanket approach: sequencing thousands of genes in a set of tumors[10,11]. This approach combines the power of high throughput sequencing technology with contemporary bio-informatics. In a landmark paper on 10 samples of colon cancer and 10 samples of breast cancer some six hundred genes with somatic mutations were identified in one or more individual tumors. Algorithms were introduced aiming to distinguish causative, or 'driver' mutations from 'passenger' mutations[12], and one cannot be sure that the classification is accurate in every case. Although this approach can be dubbed as based on *force brute*, it has among others one great advantage: it is totally unbiased. Thus, not only it makes it possible to detect point mutations that would escape even high resolution cytogenetic or comparative genome hybridization (CGH) methodologies[8]; equally important, it has detected genes that had not been and may never have been spotted as good candidates.

The seminal work by Sjoblom et al[10] was followed by a flurry of papers with even greater coverage of the genome, or the exome or the transcriptome in melanoma[13,14], breast cancer[15], renal cancer[16], lung cancer[17]. As for the nature of oncogenic mutations, perhaps the most ground-breaking discovery has been that fusion genes are present not just in prostate cancer (in which *TMPRSS2* fusion genes were known already[18]; but also in lung cancer (although the significance of *EML4-ALK*[19-21] is still under debate[22,23]), and probably in all major epithelial cancers[24]. Paired comparison of germ-line genome and cancer genome promises to be a powerful tool for the ultimate characterization of the genetic events that produce any type of cancer; indeed any individual cancer[21,25-27], and also to follow the evolution of its sub-clones[28]. Of course, this is not to say that cleverly targeted approaches will no longer play a role: for instance, identifying genes important in carcinogenesis by comparative oncogenomics[29], or by systematic analysis of the sites of insertion of retroviruses that are followed by the development of tumors[30].

Somatic mutations in tumors are often in genes involved in growth control, cell cycle checkpoints, and apoptosis

In first approximation, we can expect that control mechanisms and checkpoints are disrupted in cancer precisely because genes involved in these functions are mutated. This is no longer a mere hypothesis: we have numerous real-life illustrative examples (see Table 1). Dr Falaschi has investigated for many years the structure and mode of operation of the DNA replication origins: he and his group have outlined

functional interactions of the c-MYC oncoprotein with lamin B2; and very recent work in the same lab, within ITT, has revealed that the homeotic protein HOXC13 is a component of human DNA replication complexes[31] (see Figure 1). What makes this finding specially interesting is that gene fusion of *HOXC13* with *NUP98* (encoding a nucleolar protein) was already known to be pathogenetically involved in acute myeloid leukaemia[32].

Another mechanism has emerged as an important barrier to the development of tumors: it has been named oncogenes-induced senescence and it is often referred to simply as cell senescence[33]: once again p53 is pivotal to this process. Indeed, a targeted loss of function screen has identified *BRD7* as a crucial ancillary to this process. When p53 is mutated oncogenesis can be prevented provided *BRD7* is intact: but if it too is inactivated tumor formation becomes inevitable[34]. On the other hand, Skp2 inactivation may trigger cell senescence in p53 or Pten deficient cells[35]. The role of suppressor genes such as Pten can be finely modulated in subtle ways, including micro RNAs, which have been investigated

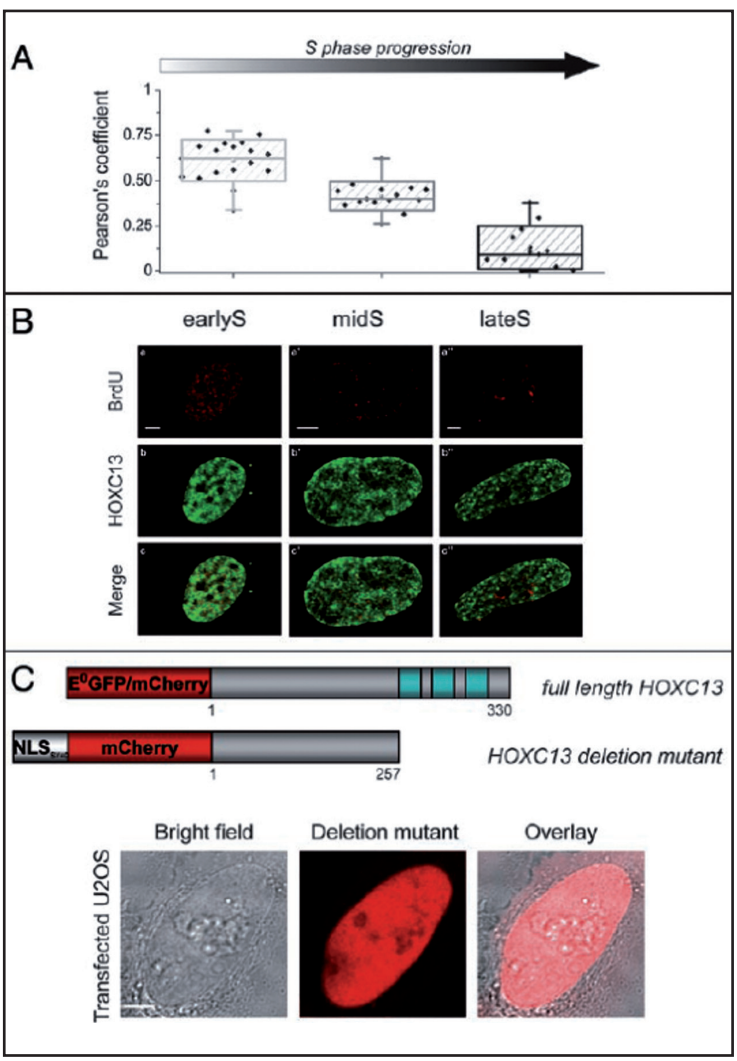


Figure 1 - *HOXC13 co-localizes with DNA replication foci and its homeodomain is crucial for restricting co-localization to early S phase*. Pulsed-BrdUrd immunofluorescence was performed in U2OS cells transfected with E0GFP-HOXC13. Depending on the BrdUrd foci nuclear distribution, cells were classified to be in early, mid, late S phases.12 Co-localization of HOXC13 with replication foci was calculated for each phase by deconvolution microscopy. (A) Measurement of the Pearson's co-localization coefficient. The distributions of obtained p values from individual early, mid, late S phase cells are plotted: the 5–95 percentile distribution is reported as a vertical line, the mean p as a sphere enclosed in a box, whose vertical length represents the SD. p values of the three S phase populations were found to be statistically different, as reported in the Methods section. (B) Significant z-projections of U2OS cells displaying early (a), mid (a'), late (a") S BrdUrd patterns and expressing E0GFPHOXC13 (b and b"). The merge of the two fluorescence channels (c and c") reveals a marked co-localization of HOXC13 with early S, but not with mid and late S BrdUrd foci. (C) Top: two schematic pictures of the fluorolabelled full length HOXC13 and of the deletion mutant, which is devoid of the homeodomain (residues 258–330). Bottom: the deletion mutant expressed in U2OS cells displayed a homogeneous nuclear staining. Thus, the nuclear pattern created by the presence of the homeodomain is crucial to restrict the co-localization with replication foci to early S phase. Scale bar: 5 μm. From Ref. 31

Table 1 - Examples of genes that have undergone somatic mutation in cancer

Gene symbol	*Functional classification*	*Main 'pathway'*	*Tumor(s) in which somatic mutations found*	*Germ-line mutations known*	*Clinical implications*	*Comments*
p53	Regulation of transcription	p53 (genome patrol etc)	All types	YES (Li-Fraumeni syndrome)	Genotype-phenotype correlations exist	Also involved in apoptosis
APC	Signal transduction	Catenin-cadherin	Colon; others	YES (intestinal polyposis)	Pre-natal counselling possible	Sometimes inactivated by promoter methylation
ATP8B1	Membrane transport		Breast	YES (familial cholestasis)		Found by unbiased sequencing[10]
BCL2	Control of apoptosis	Mitochondrial	B cell lymphoma			Oncogenic when translocated to c-*MYC*
BRAF	Signal trasduction	RAS-ERK	Melanoma; colon; thyroid	YES (cardio-facio-cutaneous sindrome)	Correlates with clinical course	Usually not mutated when *RAS* mutated (suggesting different pathways)
EGFR	Signal transduction	Growth factor receptor	Lung; others	YES (one lung cancer prone family	Some mutations are good targets for gefitinib	
GLI1	Regulation of transcription	Hedgehog	Breast			Knock-out may cause gut malformations
KRAS	Signal transduction	RAS-ERK	Colon; pancreas; others		Mutations influence therapeutic options	Commonest somatically mutated oncogene
NPM	Nucleolar protein	Ribosome; centrosome	Acute myeloid leukemia		Commonest mutation in AML that do not have a fusion gene	
PKHD1	Cell adhesion & motility		Colon	YES (polycystic kidney)		Found by unbiased sequencing[10]
PTEN	Cell cycle control	PI3-AKT	Glioblastoma; prostate; others	YES (Cowden and hamartoma syndromes)	Expression may be target of small molecules	One of the most commonly mutated genes in cancer
RET	Signal transduction	RAS-ERK	Thyroid; colon	YES (MEN2; Hirschprung)	TyrKin inhibitors currently being tested	Rare example on inherited oncogene
VHL	Response to Hypoxia	Control of HIF1-α	Renal (clear cell)	YES (von Hippel-Lindau syndrome)		Unique germ-line mutation causes inherited erythrocytosis

Genes are arranged in alphabetical order, except for p53.

for years in Dr Rainaldi's lab[36] within ITT. Indeed, Dr Poliseno in Dr Pandolfi's lab has discovered that certain miRs (encoded within an intron of the *MCM7* gene) targeting Pten do significantly affect the development of prostate tumors in mice[37].

As for apoptosis, new pathways keep emerging that may have considerable clinical relevance. For instance, the Shc family adapter p66Shc, by uncoupling the B cell receptor from the Erk and Akt-dependent survival pathways, favours B-cell apoptosis to the extent that work within ITT has found p66Shc expression to correlate with the clinical course and clinical outcome of chronic lymphocytic leukemia[38] (see Figure 2).

Mutations in distinct genes may endow tumor cells with the extra ability to generate metastasis

If until recently cancer was for many an unpronounceable word, now the taboo has shifted to the word metastasis, because all too often it marks a turning point boding ill for the final outcome in the clinical history of a patient. The very notion of metastasis has been forever a divide between oncology and hematology. Indeed, leukemia has been called a liquid tumor because it literally pervades the entire body: the disease is systemic from the start, and the very concept of metastasis is meaningless in the case of leukemia. Lymphoma as such is solid: but there is so much movement of lymphoid cells between a lymph node and lymph, and also between a lymph node and blood, that lymphoma as well must be regarded always as a systemic disease, even when it appears to involve only one or very few nodes. By contrast, in the case of a solid tumor, such as a carcinoma, metastasis is neither automatic nor a trivial event: the best evidence for this is that complete removal of a primary tumor is often curative even when no additional treatment is carried out at all. The relative weights of seed *versus* soil in the generation of metastasis have been debated for over a century[39]; but this debate is probably more relevant to where, rather than to whether metastasis takes place. In this respect, the prediction that one component of tumor growth may be self-seeding[40] was recently verified[41].

A crucial issue, then, is what makes a cell competent for metastasis[6,42]. There must be several components. (i) *Invasion* and *mobility*. Most lymphoma cells are naturally mobile, and there is no basal

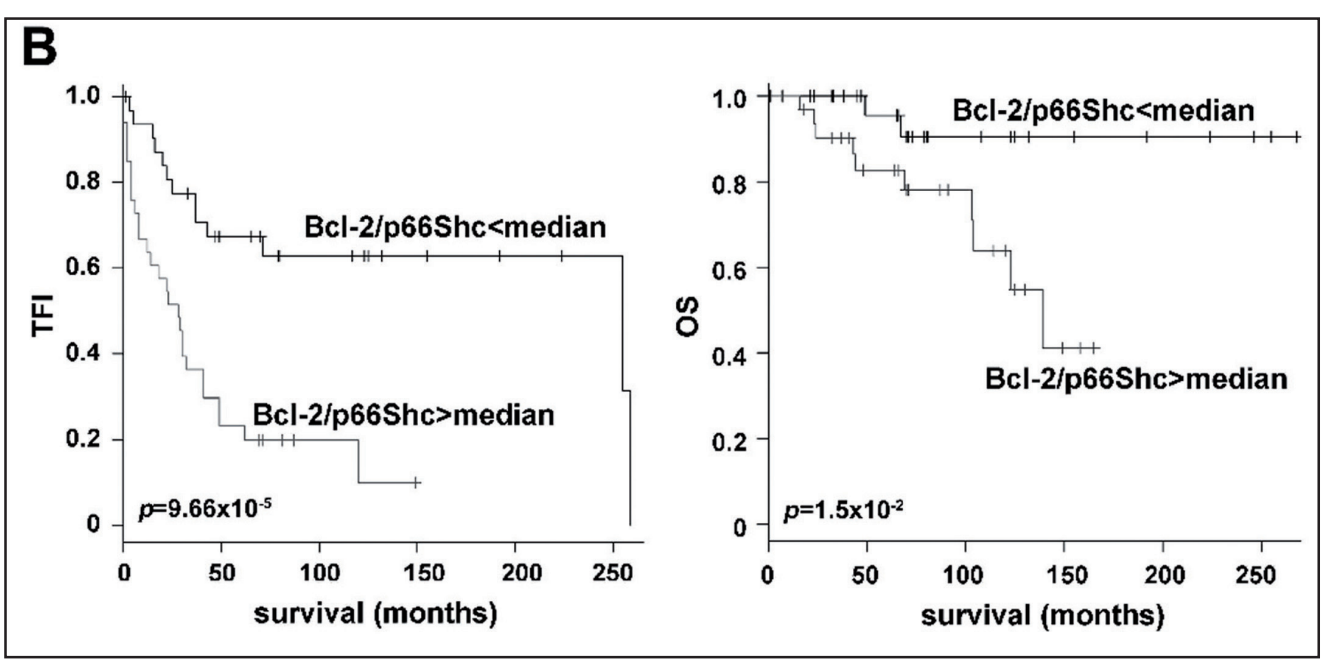


Figure 2 - *p66Shc levels correlate with clinical corse of CLL*. Treatment-free interval (TFI: *left hand panel*) and Overall survival (OS: *right hand panel*) in CLL patients, as a function of p66Shc mRNA, normalized to the level of bcl-2 mRNA. From Ref. 38

membrane to trespass: but carcinoma cells must acquire these properties, presumably through one or more mutations. Invasion and mobility may be all that is needed for a carcinoma cell to reach regional lymph nodes. Work within ITT has shown how, in the case of melanoma cells, invasion and motility depend on cell-autonomous properties, but may be influenced by environmental conditions[43]. (ii) *Trans-endothelial migration*. In most cases this is a pre-requisite for a tumor to succeed in the hematogenous spread that gives rise to the most dreaded complication: distant metastasis. For this to occur, trans-endothelial migration must take place twice: first at the site of the primary tumor, and then at the site of arrival (*extravasation*). In most cases it seems likely that tumor cells are in transit in the peripheral blood in the form of small clusters, rather then as single cells: this would explain how colon cancer and other abdominal tumors give metastasis in the liver, through the portal vein system; and how tumors from any part of body give metastasis to the lungs, through the systemic venous circulation. However, to explain metastasis to other sites we must either invoke a septal defect in the heart[44], or we must assume that single tumor cells (CTCs) do circulate and can evade entrapment in the lungs. (iii) *Establishing a colony*. Everybody who has experience with mammalian cell cultures *in vitro* knows that for a colony to grow from a single cell is not trivial: most cells, including tumor cells, have a low cloning efficiency. Today the concept of tumor stem cell (TSC) is well established[45]: and we can speculate that finding circulating tumor cells (CTC) does not automatically mean that we will find metastasis, precisely because the majority of CTCs are not TSCs. However, it stands to reason that the greater the number of CTCs, the greater the probability that some of them are TSCs: therefore finding, quantitating and characterizing of CTCs[46] may be of great significance not only in predicting metastasis but also in making adjustments to treatment protocols. Work within ITT has shown, for instance, that the HER2 status of breast cancer cells, currently an accepted criterion in the management algorithm, is not always the same in the primary tumor *versus* CTCs[47] (see Figure 3). The relationship between TSCc, CTCs and cells competent for metastasis is complex: certainly the three phrases are not synonymous, and the very notion of TCS must be critically reviewed in operational terms[48]; however, very interestingly, an epigenetic pattern reminiscent of normal stem cells is seen in cancer cells[49].

Thus, not every tumor has metastatic potential; and even within a tumor that has metastatic potential not all cells share that potential. These notions are not new; but a remarkable recent achievement has been tracking down the identity of some of those genes that are responsible for this extra property of cancer cells. In the case of human breast cancer metastasizing to the lung, for instance, these include the transcription factor *EREG*, the metalloproteinases *MMP1*, *MMP2*, and the cyclooxygenase *COX2*[42]. It is not yet clear whether these genes are over-expressed in metastatic cancer because they are mutated, or because of a mutation in another gene: but it is clear that the main determinants of metastasis are in the cancer cell[50,51], and that even the organ-specificity of metastasis depends largely on the features of

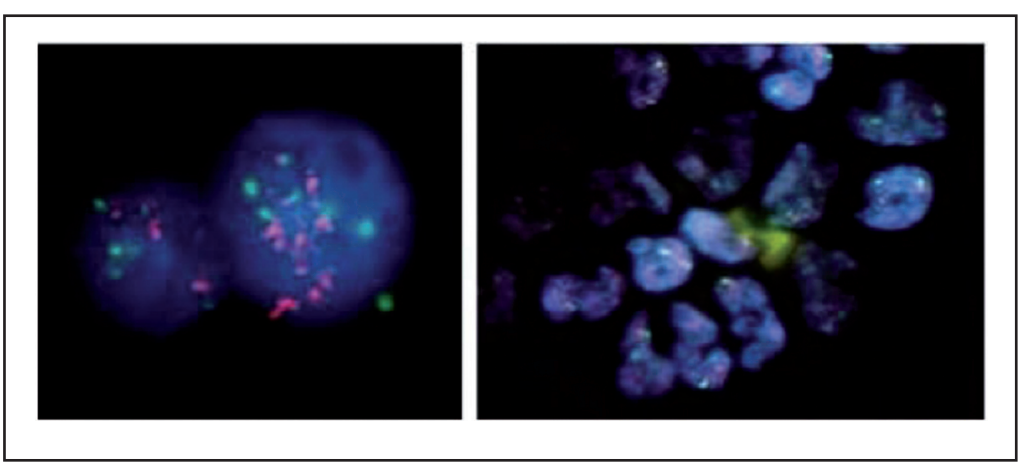


Figure 3 - *Morphology of circulating tumor cells (CTCs) isolated from the peripheral blood of patients with breast cancer.* Left panel: CTC with HER2 amplification; Right panel: CTC without HER2 amplification. From Ref. 47

the cancer cell involved[52,53] (see Figure 4). Interestingly, like in oncogenesis itself, also in the production of metastasis it is not just oncogenes that are involved: tumor-suppressor genes such as p53 and p63 can play a crucial role[54,55] as well.

High penetrance genes and low penetrance genes can predispose to cancer

The so-called hereditary cancer syndromes identify genes that, when mutated, carry a high risk of developing cancer. The list now comprises about 30 such genes[5]: some are highly specific for a certain type of cancer[56], whereas others predispose to several types of cancer. The penetrance of at least some mutations must be high: otherwise these genes would not have been discovered. However, not all mutations have the same penetrance: and this can give us insight in how they work. The most extensive data are available for inherited mutations of p53, largely thanks to the publicly available IARC database[57]. A detailed genotype-phenotype analysis[58] has shown not only that different mutations are associated with different disease severity, in terms of number of tumors and age of onset; but also that clinical expression correlates with the spectrum of target genes for which each specific mutation alters the transcriptional activity of p53 (see Figure 5). For families in which mutations of these genes are known or suspected, it is imperative that Cancer Genetics Clinics with the appropriate expertise are available within ITT[59,60], in order that appropriate counselling can be offered according to guidelines that need periodic revision[61].

A different issue is that of low penetrance cancer susceptibility genes[62-68], the existence of which has been predicted for a long time, especially because for several types of cancer family history is a risk factor[69]. Unlike high penetrance genes, low penetrance genes cannot be identified, almost by definition,

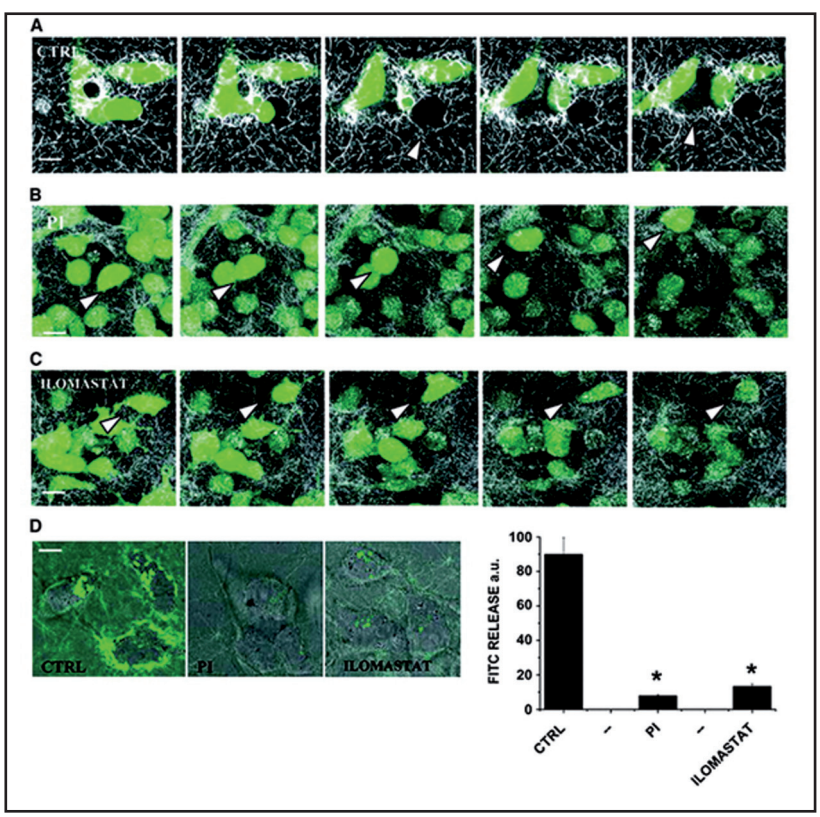


Figure 4 - *Expression of the tyrosine kinase EphA2 enables migration of melanoma cells in a collagen lattice*. A, morphology of F10-M3 melanoma cells migrating through a three-dimensional collagen I lattice. Arrowheads mark areas of degradation of collagen. Bar, 6 µm. B, as in A, except that a protease inhibitor cocktail (PI) was added to the lattice before polymerization as well as to the medium. Arrowheads indicate round-shape squeezing movement of one cell across collagen I fibers. Bar, 20 µm. C, as in B, except that the broad-range MMP inhibitor ilomastat was added to the lattice before polymerization as well as to the medium. Arrowheads indicate round-shape squeezing movement of one cell across collagen I fibers. Bar, 20 µm. D, migration-associated lysis of collagen produced by F10-M3 melanoma cells was quantified by measuring FITC release after 40 h of migration in the presence or absence of above-mentioned inhibitors (see also bar diagram on the right). Bar, 5 µm. From Ref. 43

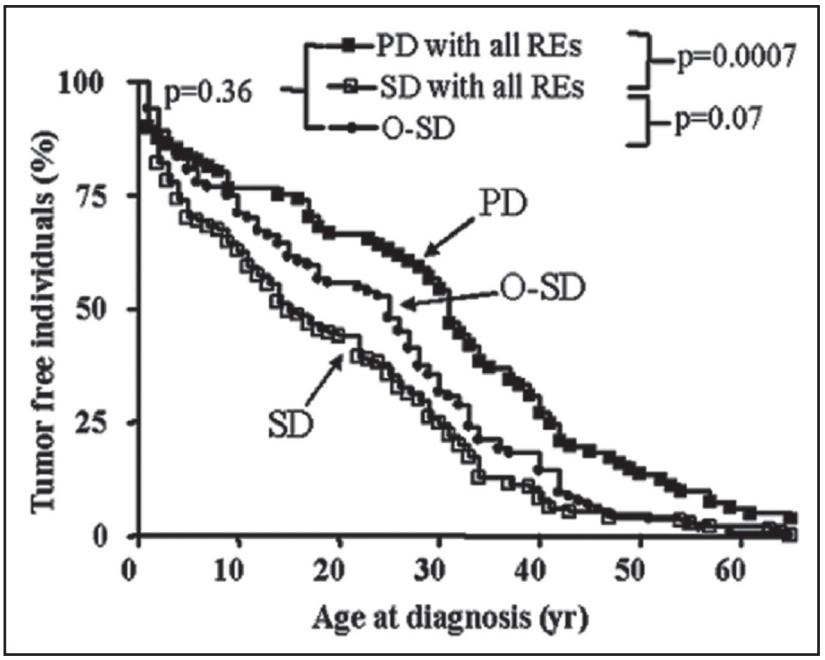


Figure 5 - *Genotype-phenotype correlation in inherited mutations of p53*. Transcriptional functionality of different p53 mutant proteins was plotted against age at which heterozygotes for inherited mutations of *p53* (from families with Li-fraumeni or related syndromes) were still tumor-free. PD = partial transcriptional deficiency; SD = severe transcriptionalo deficiency; O-SD: obligate transcriptional deficiency (due to null mutations). The percentage of tumor-free individuals is plotted as a function of age up to age 65 (Kaplan-Meier method). The P values shown were obtained from of a within-cluster re-sampling analysis. For patients with multiple tumors, only the first tumor was considered. From Ref. 58

through linkage analysis: therefore finding such genes has been difficult by traditional approaches, such as twin studies[70] or kinship analysis[71]: however, some have turned up[56] by searching among candidates[72,73] or by serendipity[74,75]. It was predictable that low penetrance genes would be amenable to being tracked by genome wide association studies (GWAS): increasingly so as we now have several millions SNPs available. In fact, although the long-awaited '*BCRA3*' gene has not yet been identified by this approach either[76], the harvest has been generally rich[77-81]. For all of these genes the relative risk of cancer, compared to the general population, is estimated to be 1.3 or less[82]: therefore the practical implications in terms of counseling are problematic or questionable[83]. However, at the population level the overall cancer burden for which these genes account may be quite substantial, since some high susceptibility alleles may be present at a high frequency, which of course may vary from one population to another[84]. In addition, these genes may be of great interest in terms of their mechanism of action[85]: for instance, in some cases it is precisely low penetrance genes that may condition the effect of exogenous carcinogens[86]; or, in the case of the 15q25 locus known to be linked to the risk of lung cancer, this effect may be mediated through an allele that increases the tendency to nicotine addiction[87].

What is the quantitative impact of environmental factors, including diet, and can we effectively influence lifestyles in the aim to decrease the number of avoidable cancers?

The discovery of the role of cigarette smoking in causing lung cancer and other cancers has become a textbook example of the heuristic and practical value of clinical epidemiology. It has taken some thirty years, approximately one generation, for that discovery to produce a significant decrease in the incidence of lung cancer in men in many populations: thus, it has become also a textbook example of how an epidemiological discovery can translate into successful preventive medicine[88]. However, we must admit that the results have been only partial (and not yet appreciable in women): to bring about changes in behavior appears to be even more difficult than doing epidemiological research. Nevertheless, it has been natural to try and extend the notion that cancers can be avoided by behavioral modification to lifestyles other than smoking. To obtain the necessary background evidence has not been easy. Concerning diet, and also other components of life-style, considerable credit must be given

to the monumental EPIC study – in which ISPO has been present since EPIC's inception – which has already paid dividends[89-91], and is likely to continue to do so for many years. From a sort of meta-analysis[92] several lifestyle components, in addition to smoking, have emerged as being significantly associated with an increased risk of cancer: alcohol use, low fruit and vegetable intake, overweight, obesity, and sexually transmitted HPV. The relative and absolute weights of these items of course vary in different parts of the world, and they are markedly influenced by economic factors. Stomach cancer is a high priority area for ITT, because there is an excess of cases, particularly in certain areas of Tuscany: studies are needed to evaluate the role of specific factors (diet, lifestyle, environmental exposures, infections, individual susceptibility) in developing gastric cancer in specific areas of the Tuscany, and significant progress has been made already[93-95].

At the moment, cancer epidemiology faces several new challenges: suffice it to mention two. On one hand, cancer incidence and cancer mortality are ominous as well as inconvenient endpoints for any project: therefore there is a great need for surrogate end-points, but these must be rigorously validated[96]. On the other hand, how far will it be possible to convert important information on the impact of lifestyles into effective preventive measures? A problematic example regards breast cancer. We know that early pregnancies, a high number of pregnancies, prolonged breast-feeding, avoiding the use of contraceptive pills will all decrease the risk of breast cancer: but who will advocate for women in industrialized societies to do all of these things?

The role of epigenetics

There has been lively controversy (see for instance[97]) about the human epigenome project[98], and also on the role of epigenetics in the pathogenesis of cancer[99,100]. If the term epigenetics is used to cover anything that is 'over' (= επι), or downstream of genes, then it will include all aspects of gene expression at every level, and the term will become too loose to serve any useful purpose. However, according to a current definition, essentially in keeping with Waddington's original notion[101] – *an epigenetic trait is a stably inherited phenotype resulting from changes in a chromosome without alterations in the DNA sequence*[102]. Changes that can be inherited by somatic cells (see Figure 6) are crucial for the

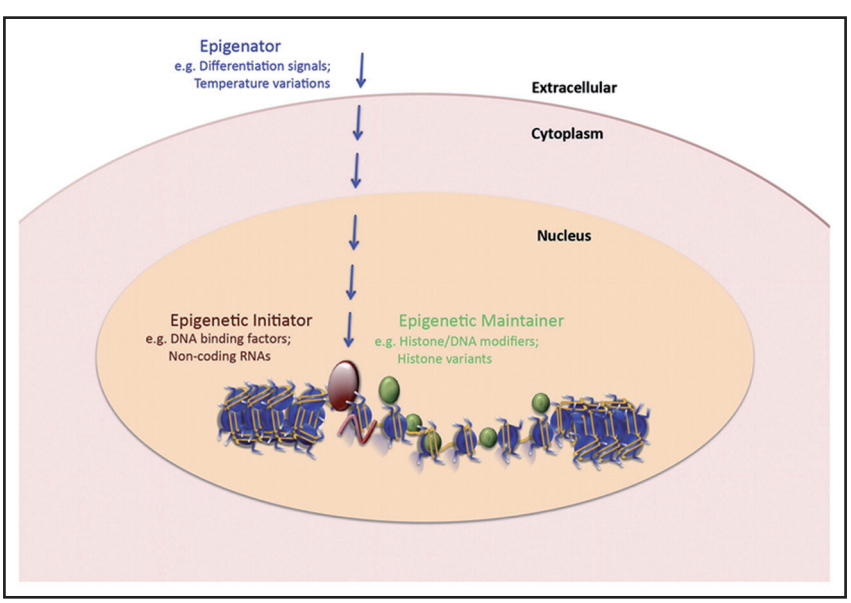


Figure 6 - *The epigenetic pathway.* Three categories of signals are proposed to operate in the establishment of a stably heritable epigenetic state. An extracellular signal referred to as the "Epigenator" (shown in blue) originates from the environment and can trigger the start of the epigenetic pathway. The "Epigenetic Initiator" (shown in red) receives the signal from the "Epigenator" and is capable of determining the precise chromatin location and/or DNA environment for the establishment of the epigenetic pathway. The "Epigenetic Maintainer" (shown in green) functions to sustain the chromatin environment in the initial and succeeding generations. Persistence of the chromatin milieu may require cooperation between the Initiator and the Maintainer. Examples for each category are shown below each heading. Chromatin is depicted in blue. From Ref. 102

development of a tumor, and it appears that in some cases such changes can be epigenetic rather than genetic[103]. Methylation of specific sites can silence a gene; and once a site is methylated, in most cases its methylated state will be faithfully replicated along with the DNA itself. Thus, it is clear that at the somatic cell level epigenetic phenomena have the potential to mimic closely somatic mutations: and since the latter produce cancer, it is highly relevant to ask to what extent epigenetic changes can act *in lieu* of somatic mutations: if a tumor suppressor gene is inactivated through methylation, the consequence may be essentially the same as if it were inactivated through mutation (if demethylation did occur in some cells, they would be probably at a selective disadvantage, and therefore not relevant). It is much less certain that chromatin changes, such as those associated with the acetylation status of histones, can be equally perpetuated with successive cell divisions[104,105]. All in all, it does not seem likely that in any particular tumor the successive steps required for oncogenesis can be all epigenetic: for instance, there is no way that epigenetics can surrogate for genetic events such as fusion genes or gain of function mutations, which are known to be pathogenic in many cases.

In experimental systems it has been shown that by switching on a transgene encoding the *de novo* DNA methylating enzyme Dnmt3b tumors are produced in mice[106]; however, we don't know how often this is relevant to human tumors. Work within ITT has shown that cancer testis antigen (CTA) expression can be affected by epigenetic changes in melanoma[107]: this could affect drastically the efficacy of CTA-based immunotherapy. Of course one of the most crucial outstanding questions is what signals are the initiators[102] of specific epigenetic changes in chromatin. Recent work within ITT has revealed that *MYC* and *PIM1* play a critical role in this process[108], which has presumably evolved for the control of normal develoment and differentiation. However, since both *MYC* and *PIM1* are proto-oncogenes, there is evidence that their role in epigenetic coding has been hijacked by neoplastic cells, so that it is also relevant to the mechanism of oncogenesis[109].

Genetic instability in tumors and the intrinsic mutation rate

Based on cytogenetic analysis and on microsatellite analysis there is evidence of genomic instability in certain types of cancer. However, when abnormalities are found by these methodologies in *advanced* cancers, the simplest explanation is that, once the cancer cell population is massive, all sorts of mutations can take place by chance, including mutations that compromise in one way or another the stability of the genome. Therefore the real issue is not whether there is genomic instability in cancer, but whether this is an early event or a late event: only in the former case should we say that genomic instability plays a role in causing cancer, rather than being an epiphenomenon. On this too there has been lively debate[110,111]: but there is strong evidence that, whereas rare somatic mutations occur in every normal person, an *increased* rate of mutation is not a requirement for cancer to develop[112].

In collaboration with Alessandro Vindigni in Trieste, work within ITT has shown that two of the five RecQ helicases (several mutations in whose genes cause predisposition to cancer) interact specifically with lamin B2 at DNA replication origins, but at different stages in the cell cycle: RecQ4 joins the origin in late G1-G1/S and RecQ1 at the onset of S, while both leave the origin after replication initiation[113] (see Figure 7). Depletion of either helicase reduces initiation frequency and cell proliferation, indicating that these enzymes play distinct important roles in replication regulation; while at the same time they are essential for the fidelity of DNA replication. Indeed, the most compelling evidence that genomic instability predisposes to cancer is provided by inherited conditions in which DNA repair or related functions are defective, and in which there is a markedly increased risk of cancer[5,114,115]: these include *xeroderma pigmentosum*, Fanconi anemia, Bloom syndrome, Nijmegen breakage syndrome, ataxia telangectasia, hereditary non-polyposis colon cancer associated with microsatellite instability, and others.

Thus, on one hand genomic instability is not necessary for the onset of cancer; on the other hand, various forms of inherited genomic instability do predispose to cancer. As for the majority of 'sporadic' cancers (*i.e.*, those without a significant excess in the family history), there has been no evidence

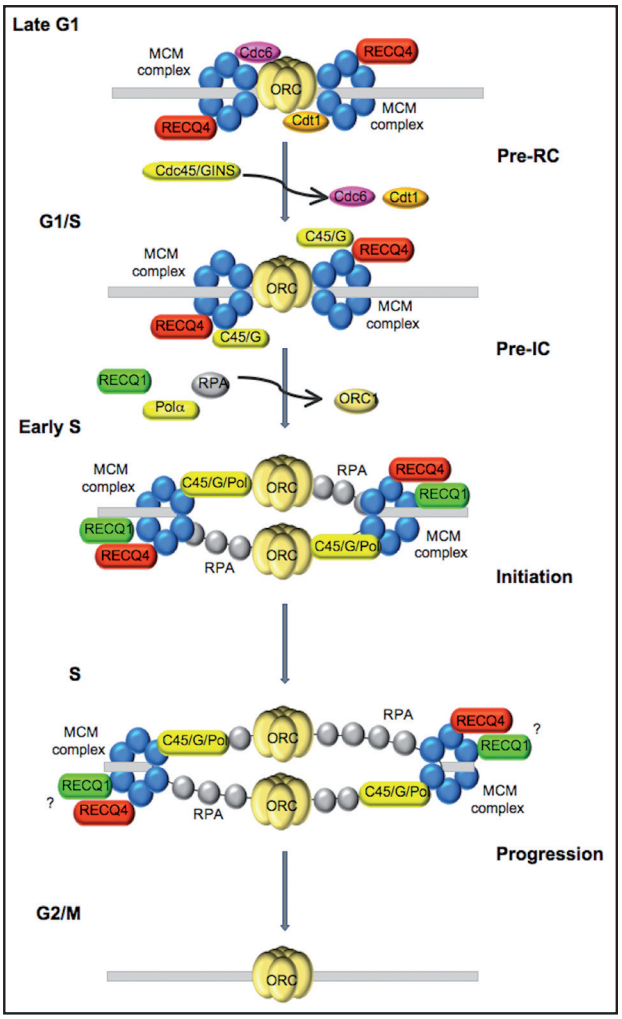


Figure 7 - *Model of cell cycle-dependent loading of RECQ and RECQ proteins onto DNA replication origins*. RECQ4 is recruited to origins in late G1 as part of pre-RC assembly. At the G1/S transition, CDC6 release signals pre-initiation complex (pre-IC) formation. RECQ1, as well as additional RECQ4, is recruited in early S phase, after the release of ORC1. Both RECQ1 and RECQ4 are no longer detected on the lamin B2 origin by mid-S phase, when either or both may be associated with active replisomes. This cell cycle phase-dependent loading and the subsequent loss of RECQ1 and RECQ4 for origins of replication suggest specific roles for each protein in replication initiation and, potentially, other specific aspects of DNA replication, such as fork progression. From Ref. 113

hitherto for abnormal genomic instability. Therefore we have started exploring the normal range in the distribution of the rate of somatic mutation in the population (see Figure 8[116]), as measured through a novel methodology which has been extensively validated[117]: we are embarking on several studies aiming to estimate to what extent this is a determinant of the risk of cancer.

Preclinical models of cancer

The recurrent finding of somatic mutations in a certain gene in a certain type or in several types of cancer is highly suggestive of driver mutations. The ideal complementary evidence (which can be regarded as a version of the famous Koch's postulate transferred from microbiology into oncology) is that, by engineering such mutations into the mouse genome we produce cancer[118]. By using transgenic and knock-out technology it is probably possible to obtain an animal model for practically any human tumor[119,120]; and the model will be probably more faithful, the more the genetic lesions reflect known molecular lesions of the human tumor one is trying to model[121-123]. This approach has already contributed tremendously to understanding the role of oncogenes and tumor suppressor genes: but mice with 'human' tumors will turn out to be also valuable for studying the pathophysiology of clinically relevant tumor-host interactions; and, as ideal surrogate patients, they will be probably invaluable in working out therapeutic protocols[124].

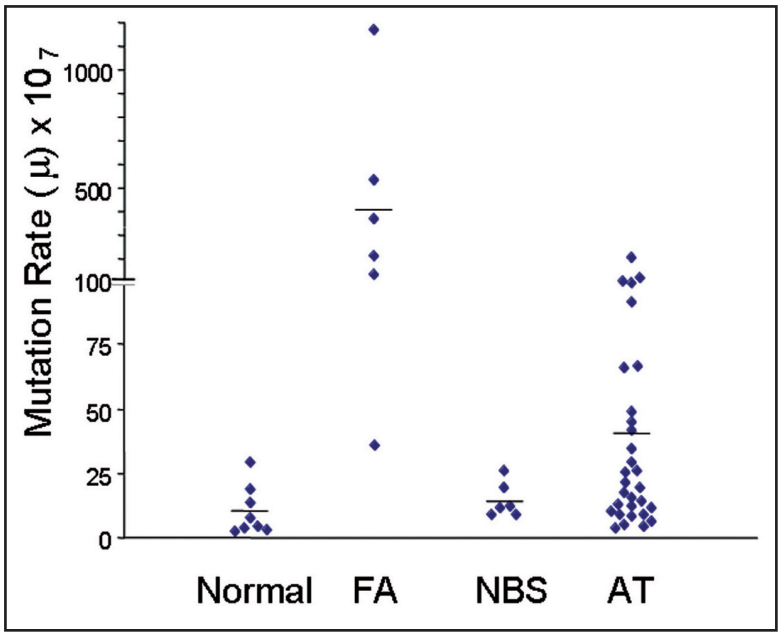


Figure 8 - *The mutation rate is increased in conditions associated with increased susceptibility to cancer.* The data were obtained by using the X-linked *PIG-A* gene as sentinel gene (see Ref. 106) in lymphoblastoid cell lines from normal controls (Normal), from patients with Fanconi anemia (FA), from patients with the Neijmegen breakage syndrone (NBS), and from patients with ataxia-telangectasia (ATM). From Ref. 116

How will this massive body of new knowledge help us to help cancer patients?

In the area of early diagnosis of cancer (which is commonly called secondary prevention) the hottest news in my own view has to do with the prostate. Whereas testing for serum levels of Prostate Specific Antigen (PSA) is widespread (and has also become a major business), it had never been accepted by the experts in clinical epidemiology as an evidence-based medical act. Two recently published papers[125,126], of which S Ciatto and M Zappa of ISPO are co-authors (see Figure 9), within ITT, have changed matters in a substantial way, by showing for the first time that PSA screening can save lives. The relative risk of death from prostate cancer was about 0.8 in the screening group compared to the control group. This work has just been awarded the Trial of the Year prize by the International Society for

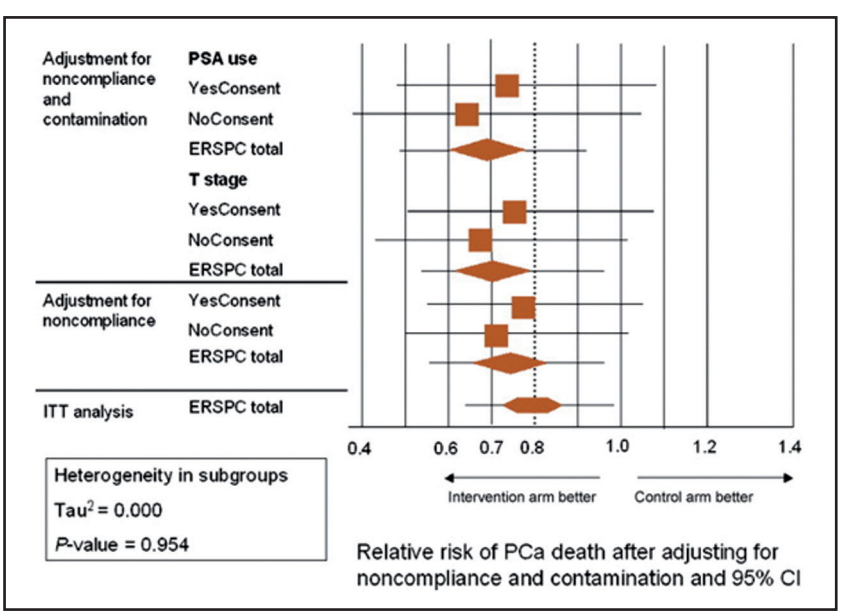


Figure 9 - *PSA screening decreases significantly the risk of dying from prostate cancer.* From this Forest plot of the unadjusted and adjusted relative risks of prostate cancer mortality it is clear that the result is robust. From Ref. 126

Clinical Trials and Project ImpACT, precisely because it was regarded as of high scientific caliber and likely to have a high public health impact. This major innovative result does not come free of charge: it is estimated that for every life saved there are 1410 screened men without prostate cancer (which does not seem a problem), and 48 'over-diagnosed' men, which must be regarded as a problem. Therefore how to translate this new finding into intelligent public health policy, in the face of screening habits that already prevailing, will be quite a challenge.

Precise and detailed characterization of tumors is also important. For instance, it has long been known that Ig chain usage is non-random in chronic lymphatic leukemia (CLL)[127,128], and that the extent of somatically acquired mutations is important[129]: indeed, ITT is sponsoring a Region-wide study of CLL patients based on the expertise of F Lauria's Unit in Siena[130]; and recent work by that group has shown that CLL clones may have signatures of IgH molecules suggesting prior engagement by antigens (see Figure 10) from common pathogens[131]: if these are identified it might even help to prevent CLL. In AML, detailed characterization of leukemic blasts at a very early stage in treatment may help to optimize the treatment itself[132] (see Figure 11).

The mainstay of the treatment of solid tumors, which are the majority of tumors, still consists of surgery and radiotherapy: therefore, all advances in these areas must be highly regarded. In surgery, the sentinel node approach for breast cancer and for melanoma is evidence-based[133-136]; and it is developing for other tumors as well[137]. The same applies to minimally invasive surgery in an increasing number of situations[138-141]. In radiotherapy the sophistication of focusing techniques and treatment modalities, including intensity modulation, has improved the ratio of tumor cell killing to tissue damage by one or two orders of magnitude[142-146]; and at the same time, the importance of contemporary approaches to limiting side-effects such as vomiting should not be belittled[147]. The refinement of imaging methods (see Figure 12) for early diagnosis of primary cancer and of metastasis is spectacular[148-150]: so much so, that one major new issue has become over-diagnosis[151,152].

Among innovative approaches to cancer treatment two comprise a large proportion of scientific literature in the basic research and in the preclinical domains: immunology[153,154] and gene transfer[155,156]. The former is certainly not new, as early attempts to 'vaccinate' people against Hodgkin's lymphoma were carried out before World War II[157]. Since that time, enormous research efforts have been invested in the aim to overcome several problems in cancer immunology[158]: amongst which the most fundamental is that the body is tolerant

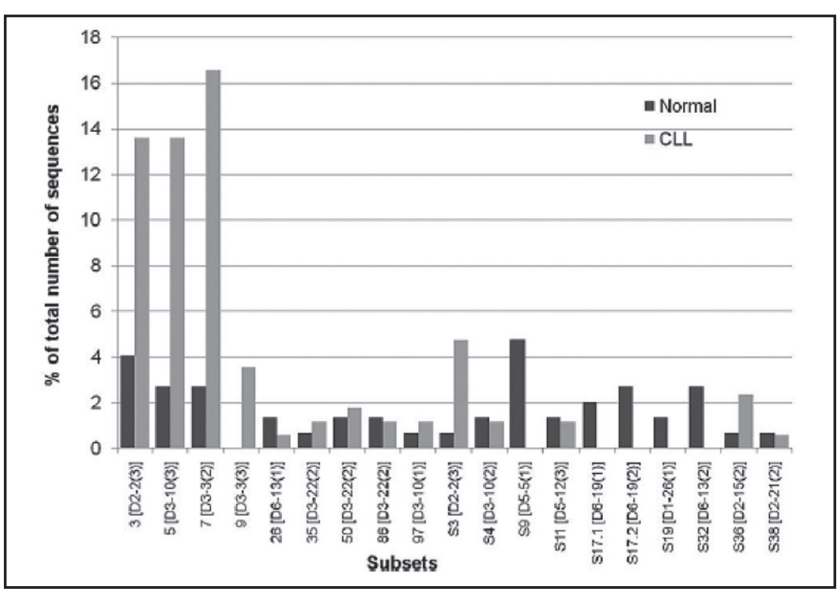


Figure 10 - *Certain stereotypic sequences of the IgH J region are much more common in CLL than in normal B cells*. Sequences were assigned to known subsets or to new subsets (prefix S). For each subset, code, IGHD gene, and reading frame are indicated. From Ref. 131

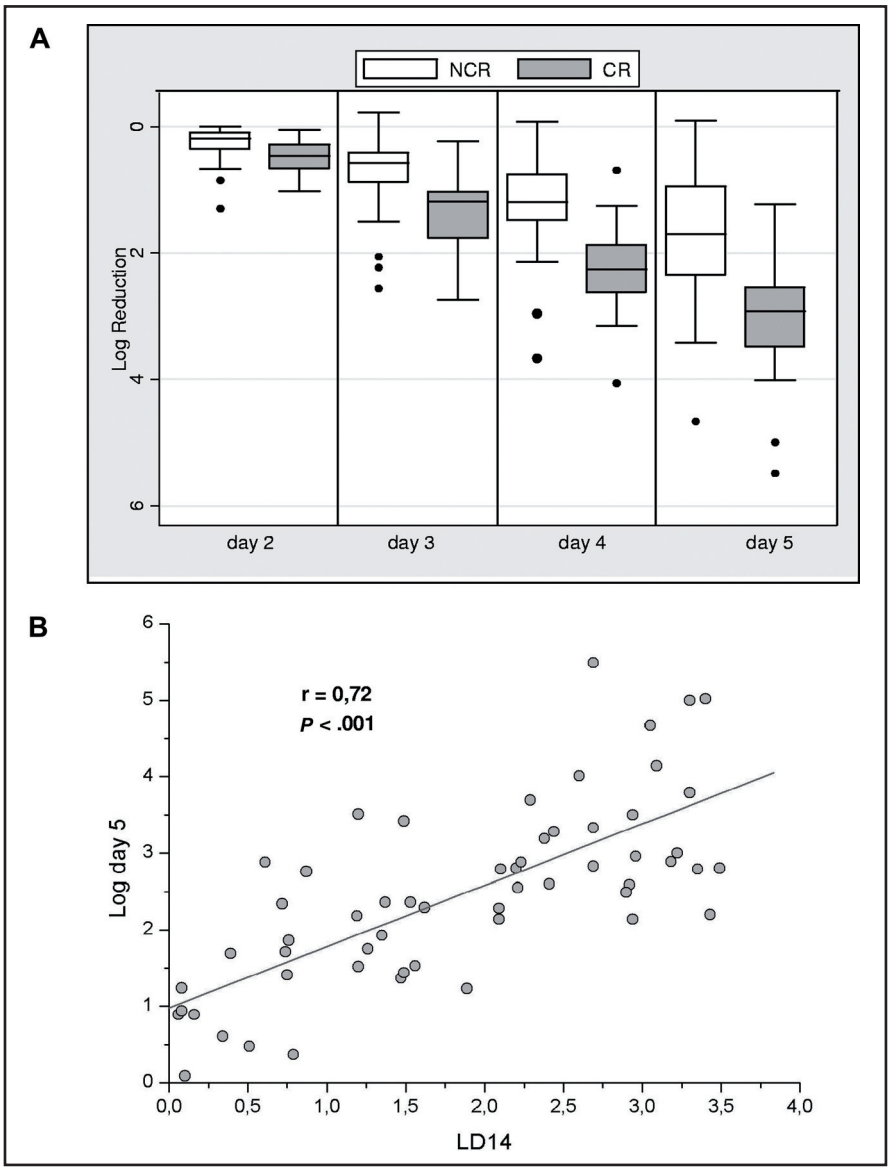


Figure 11 - *Clearance of blasts from peripheral blood after one day of treatment predicts bone marrow response 3 weeks later.* (A) Peripheral blood blast (PBB) clearance resolves responders (CR) from nonresponder (NCR) patients. Log reduction is the ratio between baseline and daily absolute leukemic cells with aberrant immunophenotype (LAIP)-positive blast counts are converted to a logarithmic scale. The ranges of log reduction show minimal overlap between the 2 groups. Horizontal bars are medians, boxes are 25th percentiles, and whiskers are 75th percentiles. Dots are outliers. (B) Bone marrow blast clearance correlates with PBB clearance. A linear statistically significant correlation is found as from day 2. From Ref. 132

to most tumors that we know. Of course the main reason for this is that cancer cells are self-cells: when they are not, as in the extraordinary case of the canine transmissible venereal tumor (CTVT), the cancer cells are eventually rejected, as long as the host is immuno-competent[159]. However, in every tumor there are non-self molecules: namely, those that are modified as a direct or indirect result of somatic mutations. The most distinctive products of somatically mutated genes are fusion proteins: peptides straddling the fusion point could be legitimately immunogenic. In fact, within ITT, administration of BCR-ABL fusion peptides have been shown to decrease significantly minimal residual disease in patients with chronic myeloid leukemia over and above what can be achieved by conventional therapy[160]. Also, within ITT, attempts to modulate the immune system in a variety of ways are being subjected to the demanding test of clinical efficacy, with some promising results involving thymosin $\alpha 1$[161] and ipilimumab[162].

As for gene transfer, one must admit that attempts to restore by this technique the activity of missing or non-functional tumor suppressor genes, such as *p53*, could not be really expected to produce substantial clinical benefit[163] (two phase III clinical trials are still ongoing in advanced head and neck cancer). In fact, even assuming that the gene transferred can effectively correct the tumor cell phenotype, unless the efficiency

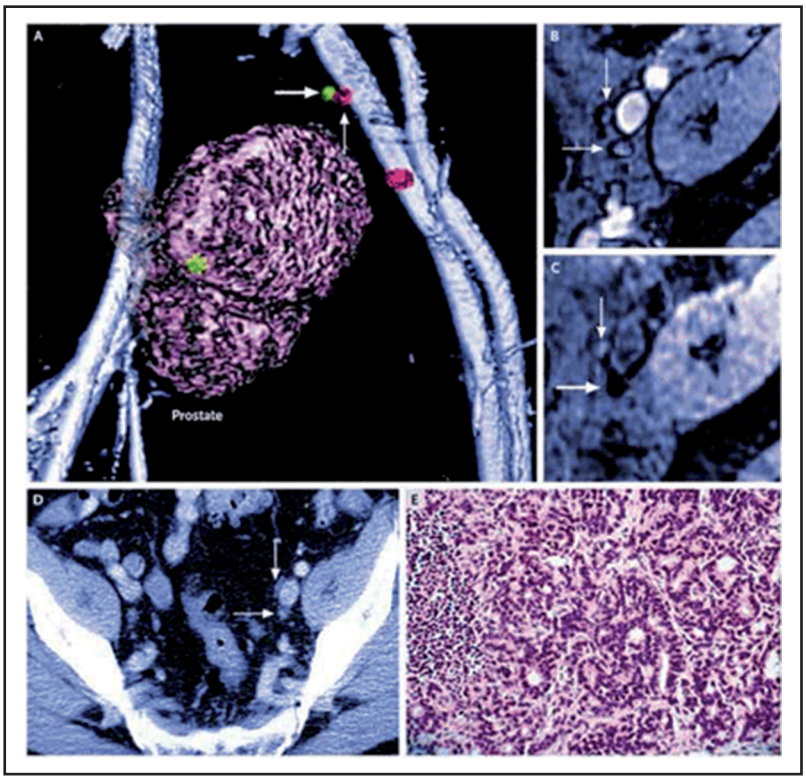


Figure 12 - *Three-Dimensional Reconstruction of prostate and Pelvic Lymph Nodes*. Panel A shows the prostate, iliac vessels, and metastatic (red) and nonmetastatic (green) lymph nodes, to assist in the planning of surgery and radiotherapy. There is a malignant node (thick arrow) immediately adjacent to the normal node (thin arrow) posteromedial to the iliac vessels. Panel B: From conventional MRI the signal intensity appears the same in the two nodes (arrows). Panel C: MRI with lymphotropic superparamagnetic nanoparticles shows that the signal in the normal node is decreased (thick arrow) but that it is high in the metastatic node (thin arrow). Panel D, abdominal CT fails to differentiate between the two lymph nodes (arrows). Panel E, histopathological examination of the malignant lymph node reveals sheaths of carcinoma cells (hematoxylin and eosin, x200). From Ref. 150, with permission (Copyrigth © 2003 Massachussets Medical Society. All rights reserved)

of transfer and expression is 100%, uncorrected cells will retain such a growth advantage over the residual tumor cells as to quickly overtake whatever temporary respite might have been obtained by the transfer of normal *p53*. Nevertheless, research in these areas remains vigorous, as many approaches are being explored: possibly the most promising being those based on lateral thinking. A natural suggestion is that, if immunological and gene transfer approaches to cancer treatment have not yet blossomed separately, they might still bear fruit in combination. Indeed, the obstacles to breaking through tolerance have been largely that (i) tumor cells are poor antigen presenters; (ii) they often have decreased expression of MHC molecules; and (iii) they lack co-stimulatory molecules: any or all of these shortcoming are amenable to correction by gene transfer[164-166].

Overall, the limelight today is on new drugs. Probably the most popular buzzword is 'biological drugs'; but from a conceptual point of view the highest premium should be clearly on the most finely targeted drugs. Trastuzumab is attractive not because it is biological, but because it targets a molecule that is important for the growth of breast cancer cells: and that is why, in spite of its cardiotoxicity[167] it has proven successful[168,169]. Imatinib is not biological at all; but it has been even more successful, in the management of chronic myeloid leukemia[170,171], because it targets the very molecule that is encoded by the fusion gene that causes the disease. Other tyrosine kinase inhibitors are proving useful for difficult problems in oncology, such as advanced renal cancer[172]. Among 'conventional' drugs, taxol is certainly biological (it has been so difficult to produce by organic synthesis that it is still extracted from trees), whereas cyclophosphamide is not: they are both effective, and they are both toxic, because of their limited specificity. Biological drugs are certainly not free of toxicity: suffice it to mention the experience that is currently accumulating with respect to bevacizumab[173]. At the same time, work within ITT has shown how important molecular characterics of tumors may be in terms of the response to biological agents such as bevacizumab[174] or cetuximab[175] (see Figures 13-15).

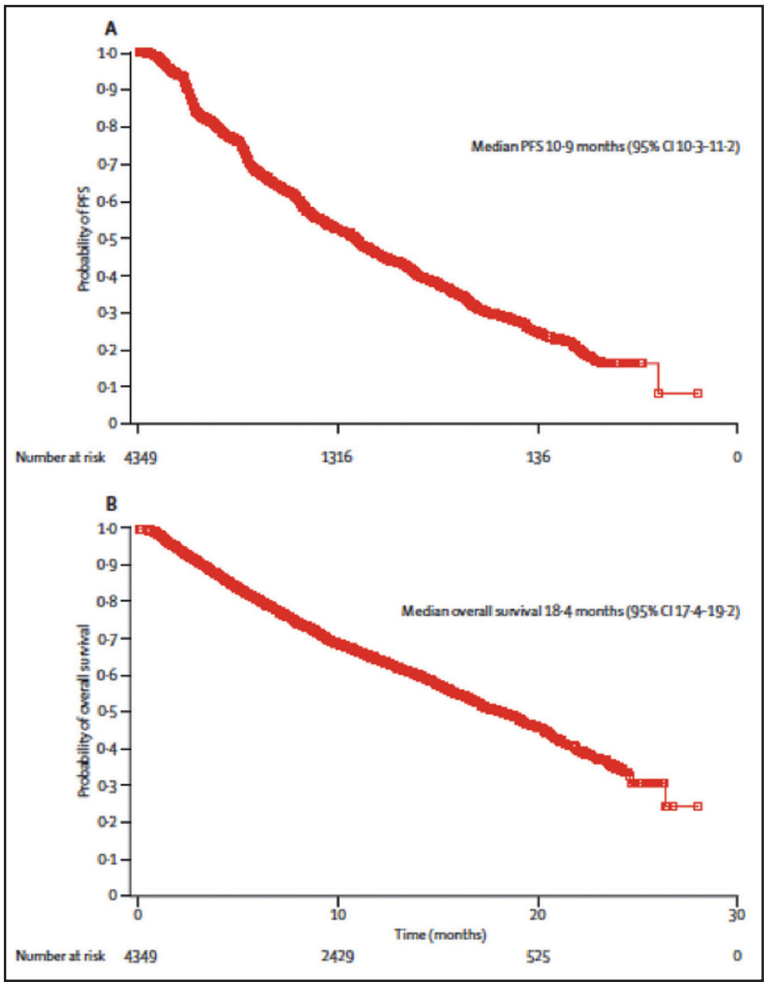


Figure 13 - *Efficacy of sunitinib in the management of patients with metastatic renal-cell carcinoma regarded as trial-ineligible*. From Ref. 172

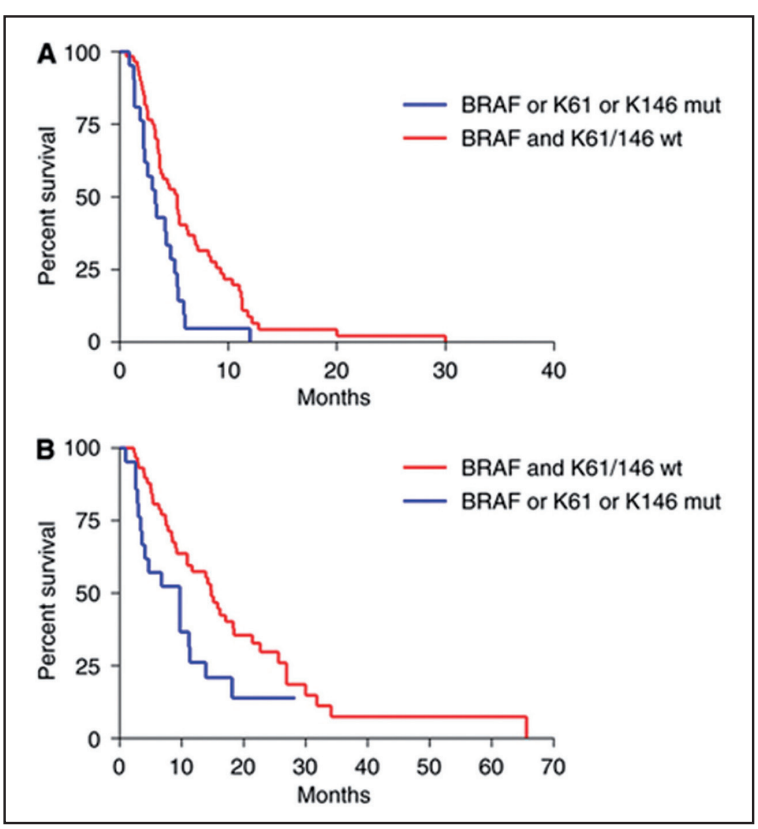


Figure 14 - *Beneficial effect of cetuximab in the treatment of colon cancer is a function of molecular lesions present in the tumor.* Different survival curves are a function of *BRAF* and K-*RAS* mutations. From Ref. 174

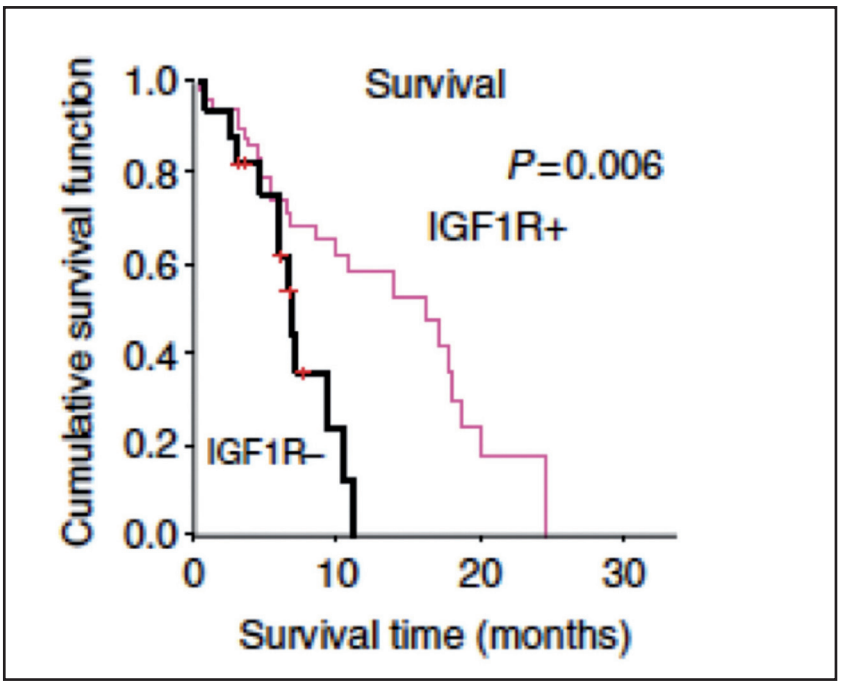


Figure 15 - *Expression of the Insulin Growth Factor 1 Receptor (IGF1R) influences significantly the response to cetuximab of patients with colon cancer.* From Ref. 175

Thus, the real advance is clearly a much greater specificity of the target: for instance, a growth factor receptor *versus* tubulin; tyrosine kinase *versus* DNA; and so on. Specificity, of course, has to do with the target molecule as well as the cells expressing it; and specificity is the major factor in limiting toxicity. The link between finding the genes that are mutated in cancer and finding the cognate drugs is direct: it defines, perhaps as well as anything, what we should mean by translational research. Thus, it becomes clear that the perfect target is not just a gene that in a certain type of tumor is over-expressed or mutated, but the mutation itself. In this respect, the best current example is probably gefitinib, for the subset of bronchogenic adenocarcinomas that have certain mutations of the EGF receptor[176-178]. In this respect, ITT was probably the first cancer institution in Italy that activated a centralized facility for testing for these mutations in biopsy samples from the entire Region[179]. We have also participated in a leading position to the international trial that has led to the licensing of a new drug, the monoclonal antibody eculizumab, for the control of hemolysis in the severe non-neoplastic blood disorder paroxysmal nocturnal hemoglobinuria (PNH)[180].

With this paradigm in mind, there are two important considerations. (a) If in terms of specificity, and therefore minimal toxicity, each individual mutation is a perfect target, an obvious problem is the multitude of individual mutations that can be found in tumors, even within the same gene. Customized therapy is another current buzz-phrase: but producing a new drug for each patient is certainly not the dream of the drug industry. From this point of view, priority targets would be the rare situations where the same mutation obstinately recurs: *e.g.* the V600E mutation in *BRAF* in melanoma[181], or the V617F mutation in *JAK2* in myeloproliferative disorders[182]. (b) The issue of acquired drug-resistance: which is, of course, not a new problem. It is not likely that a cell can become resistant to cyclophosphamide or resistant to cisplatin: but the development of drug resistance has been well characterized, for instance, in the case of methotrexate (MTX), where the target gene, dihydrofolate reductase, can undergo mutations[183] that bind MTX less well. We can surmise that, the more the target is narrow, the easier it will be for a drug to select resistant mutant cells[184] – just as antibiotics do with bacteria. On this topic we have already learnt a lot from the widespread use of imatinib[185]: and for optimal long-term results one will need probably to use appropriate combinations of highly targeted drugs and less targeted drugs, including conventional chemotherapeutic agents.

Finally, it should be mentioned that, if immunotherapy of cancer is mostly still at the experimental stage rather than routine clinical applications, there are at least two examples of vaccination procedures that are already preventing cancer. The first example has not been publicized in this way: but vaccination against the hepatitis B virus, by preventing chronic hepatitis, has certainly avoided a significant number of liver cancers arising in the context of cirrhosis. The second example is current: vaccination against four strains of human papilloma virus has been shown recently to be effective in the prevention of cancer of the cervix[186]. The vaccine is now commercially available, and it is being adopted as a public health measure in many countries: paradoxically, it will be used more in developed countries in which the incidence of this type of cancer has already declined. It is now a real challenge for the international community to make this vaccine available, at a reasonable cost, in those countries in which cancer of the cervix is still rampant[187], and which therefore need the vaccine most.

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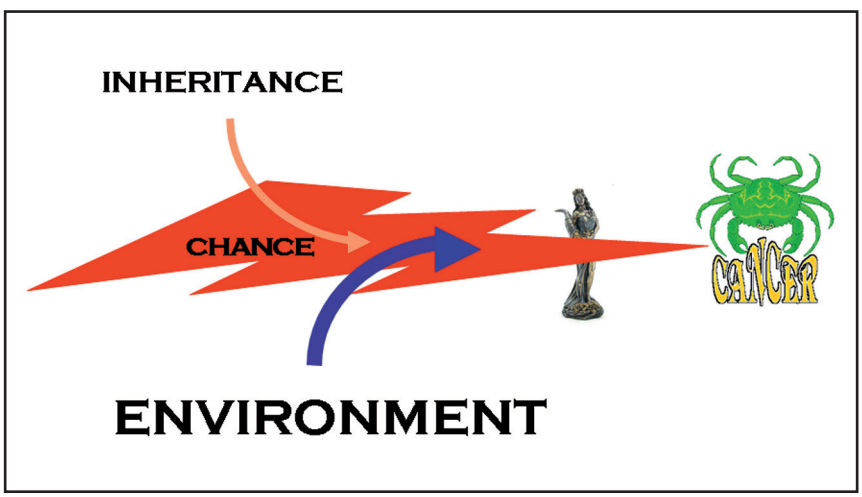

The main factors in the origin of cancer. Heredity (H) and environment (E) are well known to be important. However, since somatic mutations are stochastis or random events, the major role of chance has been probably not uncommonly under-estimated; and even H and E act essentially through an accelerated pace of somatic mutations, which by definition are random events. Across the chance thunderbolt arrow is a figurine of the Roman goddess Fortune.

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Introduction

Since 1994, the Unit has been working on loco-regional chemotherapies using an angiographic approach via intra-arterial administration. Pancreatic cancer, biliary-tree tumors and liver metastases are the main pathologies treated and we receive more than 120 cases every year from all over Italy.

Since January 2006, we have been using external hyperthermia in the treatment of patients with cancers confined to a specific organ or region, usually associated with systemic chemotherapy.
In patients with pancreatic cancer or biliary tract tumors, we combine external hyperthermia with intra-arterial chemotherapy.
We are also evaluating the impact of external hyperthermia in patients with liver metastases from colorectal cancer and hepatocellular carcinoma after the failure of all other systemic approaches.
The daily activity in our Institution is characterized by particular attention paid to the "humanization" aspects of the structures.

Main Research Themes

1. Management of pancreatic cancer

Main achievements:

- One of Italian Oncological Unit with the highest number of patients treated for pancreatic cancer over the last three years (Figure 1).
- Multivariate analysis of our ten-year experience (211 patients) and identification of prognostic factors.
- Scientific evaluation of single nucleotide polymorphisms like prognostic factor.
- Publication of our experience in an adjuvant setting.
- Scientific Basis for the definition of pancreatic cancer clinical guide-line (Alleanza Contro il Cancro, www.alleanzacontroilcancro.it): intra-arterial chemotherapy is suggested in adjuvant setting and for metastatic disease.

Current work: Multi-modality treatment of locally advanced diseases: intra-arterially and sistemic chemotherapy, radiotherapy and thermoablation with radio-frequency.

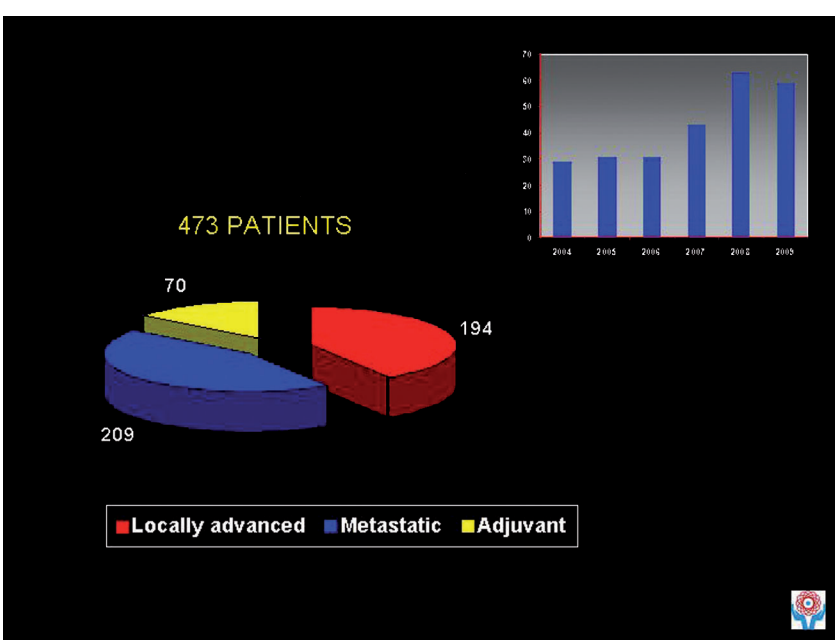

Figure 1 - Number of patients with pancreatic cancer treated with loco-regional chemotherapy, 1993-2009

2. Management of biliary duct cancer

Main achievements: One of Italian Oncological Unit with the highest number of patients treated with combined intra-arterial and systemic chemotherapy.

Current work: Scientific evaluation of single nucleotide polymorphisms like prognostic factor (2).

3. Music donors

More than 100 events inside the oncological ward, with the most famous Italian and European musicians. A psychological evaluating group is studying this phenomen.

Clinical Trials

1. Breast cancer

Description	Year	Sponsor	Number of patients recruited to date
GIM4: study of treatment duration with letrozole as an adjuvant therapy in post-menopausal women with breast cancer; long-term *versus* short-term treatment	2006	Oncotech	18
GIM5: adjuvant therapy with letrozole after tamoxifen. Study of the correlation between the CYP19 gene and the efficacy of letrozole in post-menopausal patients with breast cancer	2006	Oncotech	2
Spontaneous study: study of the genetic factors that control individual sensitivity to chemotherapy and radiotherapy in oncological patients	2006		32
THOR: a randomized, comparative, phase III study between ongoing administration *versus* discontinued administration of trastuzumab in association with second-line chemotherapy after progression of the disease following first-line chemotherapy combined with trastuzumab for patients suffering from metastatic cancer with hyper-expression of HER2	2006	Roche	2
Prediction of docetaxel toxicity and efficacy in patients with metastatic breast cancer using pharmacogenetic profiling	2006	Azienda Ospedaliero Universitaria di Parma	5
GIM2: a randomized phase III study of EC followed by paclitaxel *versus* FEC followed by paclitaxel, all administered either every three weeks or every two weeks, supported by pegfilgrastim, for node-positive breast cancer patients	2004	Oncotech	23

2. Colon-rectal cancer

Description	Year	Sponsor	Number of patients recruited to date
Acupressure use to manage chemotherapy-induced nausea and vomiting: blind randomized study	2009	University of Florence	1
TOSCA: randomized study to evaluate the FOLFOX4 regimen duration (three *versus* six months) ± bevacizumab in adjuvant therapy in patients with stage II, high risk/III colon cancer	2008	Istituto di Ricerche Farmacologiche "Mario Negri"	20
STAR: 5FU *versus* 5FU+oxaliplatin in combination with pelvic radiotherapy as preoperative treatment for resectable locally advanced rectal cancer; a randomized phase III study	2004	Sanofi-Aventis	39

3. Biliary tract cancer

Description	Year	Sponsor	Number of patients recruited to date
Efficacy and safety of RAD001 (everolimus) in patients affected by biliary tract cancer progressing after prior chemotherapy: a phase II Italian Trials in Medical Oncology (ITMO) study	2009		4

4. Pancreatic cancer

Description	Year	Sponsor	Number of patients recruited to date
Maintenance therapy with sunitinib in metastatic pancreatic cancer: phase II randomized study	2008	Istituto "San Raffaele"	2
Prospective, observational study of pancreatic-duodenal endocrine tumors	2005	Associazione Italiana per lo Studio del Pancreas	1

5. Lung cancer

Description	Year	Sponsor	Number of patients recruited to date
Phase II study of first-line use of injectable alternating with oral vinorelbine in combination with cisplatin, followed by consolidation therapy with oral vinorelbine in locally inoperable advanced or metastatic NSCLC	2005	Pierre Fabre Pharma	1

Description	Year	Sponsor	Number of patients recruited to date
Assessment of the efficacy of vinorelbine in replacement of platin and with the addition of isofosfamide to a two-drug chemotherapeutic regime in patients with non-small-cell lung cancer (NSCLC) at an advanced or metastatic stage: a randomized, prospective, factorial, multicentric, clinical phase III study. FAST Protocol	2002	Association Biomedical Technology Assessment (BETA)	5

6. Stomach cancer

Description	Year	Sponsor	Number of patients recruited to date
Randomized, open, multicentric phase III study of adjuvant chemotherapy for a radically operated adenocarcinoma of the stomach and gastro-esophageal junction: comparison between sequential treatment (CPT-11+5-FU/LV ® TXT + CDDP) and a 5-FU/LV regime	2004	Intergruppo Nazionale Adiuvante Gastrico and Istituto di Ricerche Farmacologiche "Mario Negri"	25

7. Hepatocellular carcinoma

Description	Year	Sponsor	Number of patients recruited to date
GIDEON: global investigation of therapeutic decisions in hepatocellular carcinoma and its treatment with sorafenib	2009	Bayer Healthcare	0

8. Head-neck cancer

Description	Year	Sponsor	Number of patients recruited to date
Spontaneous study: study of the genetic factors of susceptibility to cancer of the head-neck area	2006		80

Main Collaborations

With other Italian and Foreign Institutions/Organizations

» Hepato Pancreatic Biliary Unit, University of Verona
» Department of Radiotherapy, Centro di Riferimento Oncologico (CRO), Aviano (Pordenone)

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Introduction

The Unit started working in the area of brachytherapy in 2005. The main clinical research interest of the Unit is the treatment of breast and prostate cancers with partial irradiation with an interstitial High Dose Rate (HDR) brachytherapy. In the first part of the research, a feasibility study of interstitial HDR treatment was performed in order to evaluate the possibility to export this technique to other hospitals.

Sixty-nine point two percent of the implantations were performed in the Ospedale “Campo di Marte” (Lucca), while the others were done in the Ospedale “Versilia” (Lido di Camaiore).
The prostate Brachytherapy (BT) team began its activity in 2004. At present, we have treated 231 patients for low-intermediate risk prostate cancer, reporting an overall disease-free percentage of 89% among patients with at least 12 months of follow-up.
This activity of external radiation therapy with a hypofractionated regimen started in December 2006. The aim of the study is to evaluate the results of the hypofractionated regimen in the treatment of prostate gland neoplasia with external 3D-beam irradiation. The first evaluation is in terms of the acute and late effects of the treatment and in a later period, with an adequate follow-up time, the survival rate.

Main Research Themes

1. Partial breast irradiation with interstitial HDR BT: experimental study phase II

Main achievement: Combining lumpectomy and axillary dissection with whole breast irradiation has resulted in a safe and effective alternative to mastectomy, with low toxicity and only 3% of breast recurrence. More recent partial breast irradiation has been aimed at decreasing local toxicity and overall time of treatment.

The Accelerated Partial Breast Irradiation (APBI) approaches described are: interstitial BT LDR and HDR (low and high dose rate), endocavitary BT using a balloon catheter (MammoSite) and single-fraction IntraOperative Radiation Therapy (IORT) using 6-9 MeV electron energy with a linear accelerator or 50 kV orthovoltage radiation.

From January 2005 until today, 330 patients received an implant during surgery and were treated using a Microselectron-HDR unit. Chemotherapy was used in 15.9% of the patients, hormonal therapy in 76.8%. After the operative implant, CT slices were acquired and sent to a TPS Plato v 3.3.4 (Nucletron) to simulate treatment. The dose was calculated by the Paris Dose System (PDS): the reference dose was taken as 85% of the mean basal dose (that is, the mean value of the local minimum doses between each set of three adjacent source lines within the source pattern).

Catheters were implanted in a double- or triple-plane in 98.4% of the patients and in a single- or quadruple-plane in 1.6% of the patients.

Current work: Evaluation of the feasibility of the operative multicatheter implant, cosmetic results, acute and late toxicity, local control and overall survival. Our data demonstrate that the interstitial intraoperative BT implant is a feasible method with good tolerance and cosmetics results. Within a three-years follow-up, only 1.3% of local relapses was observed. Three patients have shown a local relapse outside the tumor bed and in three patients we observed metastases.

Future work: Evaluation of the local control rate and overall survival with a follow-up of five years.

2. Prostate BT permanent implant

a) Pre- and post-planning dosimetry analysis of interstitial BT with iodine-125 permanent implant in the treatment of prostate cancer

Main achievement: Permanent seed implantation is often used in the management of localized prostate cancer as a alternative to radical prostatectomy and transcutaneal radiotherapy (3D-conformational and IMRT). Post-implant Computed Tomography (CT)-based dosimetry is the most common method to

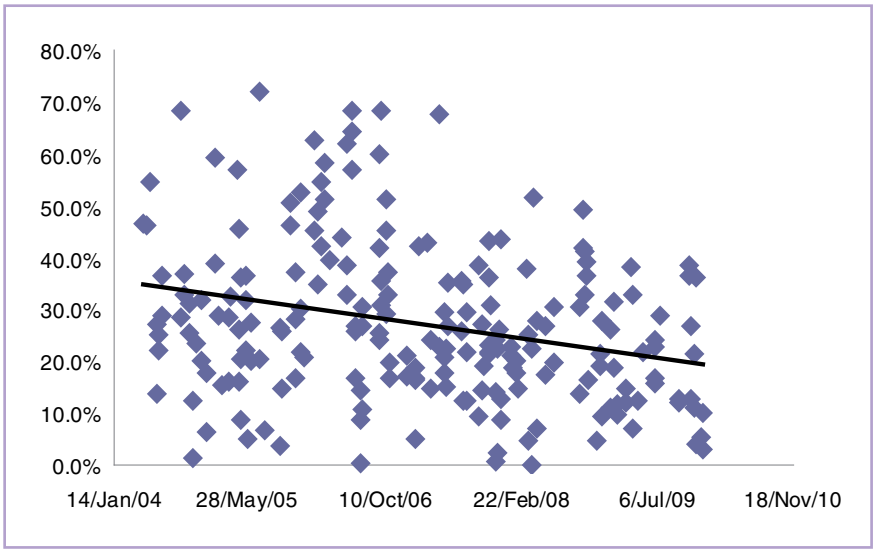

Figure 1 - Differences in percentage between pre- and post-implant D90

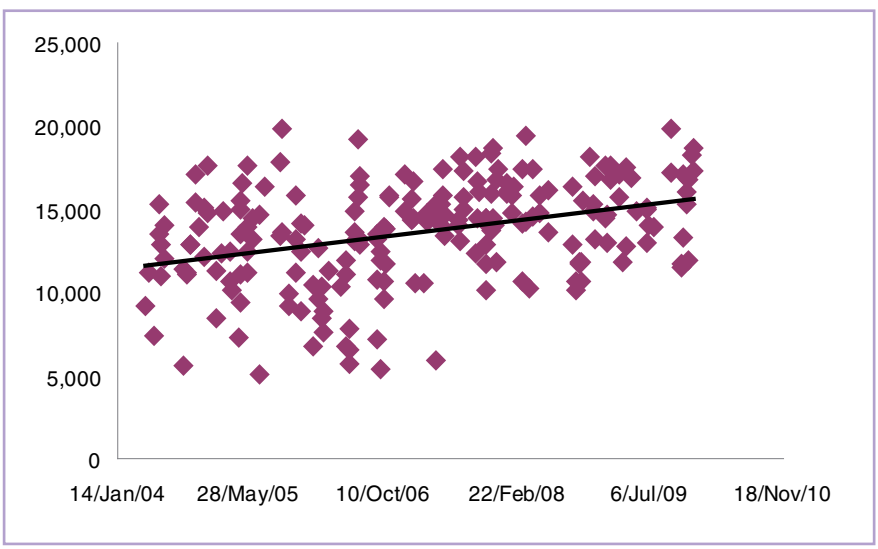

Figure 2 - D90 post-(cGy)

assess the quality of permanent prostate BT, the actual dose delivered to the prostate and the normal surrounding structures.

Two hundred nine patients with an intracapsular tumor, were treated with transperineal ^{125}I pre-planned BT (Rapid Strand, Amersham Health). ^{125}I seeds (model 6711-NIST 99) with an activity ranging from 0.383 to 0.450 mCi/source were used. A post-implant assessment was performed one month after implant, taking 5 mm abutting CT slices. We compared the D90, V100, V150 and V90 values and the volumes of the prostate from both planned and post-implant dosimetry for all patients, in order to improve the implantation technique.

Current work: We analyzed our data for pre-planned dosimetry. The mean values for D90, V100, V90 and V150 values for the entire set of patients were respectively: 186 Gy, 99%, 99.7% and 70.7%. For post-implantation, these values become respectively: 136 Gy, 86%, 89.6% and 59%. During this time, however, we changed technique in order to improve dosimetric results, realizing that our implants were lacking at the base. The main change in technique is due to the kind of images used to guide the

implant in terms of depth of release. Indeed, by passing from fluoroscopy to echography, we have made the first significant improvement in terms of quality, passing from an average value of D90 of 122 Gy to 147 Gy. Another improvement can be found in the introduction of real-time dosimetry, which offers the possibility to correct for mistakes in release of the seeds. This has led to slight improvement in the mean value, but to significant improvement in the standard deviation of D90 (from 30 Gy to 25 Gy, without no D90 value under 100 Gy).

Future work: In spite of this good mean value of D90, we still have patients with value of D90 that are not optimal (around 100-110 Gy), which corresponds to a lack in the coverage of the base. This problem has been deeply analyzed and we think that it can be solved in two different and concurrent ways. On one hand, improving the quality of the echographic images in order to better define the base, and on the other hand, arranging to have the seed at the top of the needle instead of having a piece of surgical thread. Moreover, if both the quality of the image and the echo-visibility of the seeds are better, it would be possible to take full advantage of the real-time dosimetric re-calculation and to correct a wrong implant immediately in the operating room.

b) Definition of PSA kinetic and research on an early prognostic parameter

Main achievement: We analyzed the PSA percentage curve in two groups of patients, n = 24 patients with proven biochemical and clinical relapse after BT (group B) and n = 50 patients still disease-free, chosen on the basis of the longer follow-up (mean 51.6 months, range 48-60 months) as the control group (group A). None of the patients considered had taken antiandrogens before treatment.

Considering PSA percentages at six months of follow-up, we observed strong difference between the two groups. A PSA decrement > 70% of the pre-treatment value is present in 47:50 patients in group A (disease-free) and only in 2:24 patients in group B (relapse), with a sensitivity of 91.67%, a positive predictive value of 88%, a specificity of 94% and a negative predictive value of 95.9%.

Despite the fact that broader studies are needed to verify our data, we think that PSA percentage could became a useful prognostic tool in follow-up management of patients after BT for low-intermediate risk prostate cancer.

Current work: It is evident that patient clinical outcome is correlated to the quality of the implant, expressed by means of the dosimetric parameters, such as D90 and V100, representing the coverage of the prostate by the prescription dose. In addition, in some cases, the overall D90 (that is, the dose covering 90% of the entire prostate gland) is affected by the presence of unintentional cold areas due to imprecise implantation. Despite this dosimetric failure, patients showed a good outcome. Using sector analysis, we can define what are the critical areas where the procedure could be repeated.

Future work: PSA percentage decrease, seems to correlate well with short-medium term outcomes with high sensitivity (91.67%) and specificity (94%). With the aim of getting further confirmation of our data, we are planning to realize a prospective study, selecting patients on the basis of PSA percentage decrement, and overseeing them along their postoperative course.

c) Hypofractionated external beam radiation therapy in the treatment of prostate neoplasia. A phase II study

Main achievement: Our Department of Radiation Oncology has carried out a phase II experimental study on the hypofractionated regimen with conformal 3D radiation therapy in the treatment of the prostate gland tumor.

Current work: The protocol used in our center for the hypofractionated treatments has been established by analyzing the results of retrospective studies and other prospective studies. From April 2007 to October 2008, we planned the external beam radiotherapy treatments of 40 patients.

All patients were classified as high, intermediate and low risk. For each of them, IIEF5 (Table 1), IPSS (Table 2) and, eventually, hormonotherapy were evaluated (Table 3).

Table 1 - IIEF-5 patients classification

IIEF-5	0-25	26-40	41-75	Na
Exclusive	12	3	-	6
Recurrence	6	-	-	3
Adjuvant	8	-	-	2

Na = not available.

Table 2 - IPSS patients classification

IPSS	0-7 (light)	8-19 (moderate)	20-35 (severe)	Na
Exclusive	7	9	2	3
Recurrence	3	4	-	2
Adjuvant	4	3	1	2

Na = not available.

Table 3 - Hormonotherapy

	Neo-adjuvant	Neo-adjuvant + concomitant	Adjuvant
Exclusive	5	6	-
Recurrence	1	1	1
Adjuvant	-	-	-

We used an immobilization system (Combifix), a fixed bladder and rectum volume (emptying the rectum with an enema and oral hydration with 500cc water half an hour before CT simulation and each treatment sessions).

For exclusive treatment, the beginning volume of treatment was composed of the prostate gland and seminal vesicles, while the boost was planned on only the prostate gland:

- for low-risk patients, the planned total dose was 44 Gy (4.4 Gy/fr, 2 fr/week; 30.8 Gy + 13.2 Gy boost), corresponding to 70.4 Gy with conventional fractionation;
- for intermediate-high risk patients, the total dose was 46.9 Gy (4.69 Gy/fr, 2 fr/week; 32.8 Gy + 14.1 Gy boost), corresponding to 78 Gy with conventional fractionation.

For adjuvant treatment, the total dose to the prostate bed was 40 Gy (4 Gy/fr, 2 fr/week), corresponding to 60 Gy with conventional fractionation.

For local clinical or histological recurrence, the total dose to the prostate bed was, respectively, 46.9 Gy/fr (4.69 Gy/fr, 2 fr/week) and 44 Gy (4.4 Gy/fr, 2 fr/week).

Before each treatment, the patient set-up was verified by comparing DRR images to portal images (at 0° and 90°) using the software PrimeView 3i (Siemens).

Follow-up results at one year:

- for exclusive treatment, six patients were NED; one patient had persistent local disease and one had distant disease;
- for local clinical or histological recurrence, five patients were NED and two had distant disease;

- for adjuvant treatment, only one patients had distant disease;
- seventeen patients did not attended the follow-up at one year, so it was not possible to say anything regarding their outcome;
- nobody developed G3 rectal and bladder toxicities (RTOG scale).

Conclusions: The hypofractionated regimen could be a potential treatment technique to improve the therapeutic index, taking into account that the alpha/beta ratio for the prostate tumor is 1.5-2 Gy, and for rectal late toxicity, it is 3-5 Gy.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
Hypofractionated external beam radiation therapy in the treatment of prostate neoplasia. A phase II study	2007	None	40
Definition of PSA kinetics and research of an early prognostics parameter	2006	None	209
Partial breast irradiation with interstitial HDR BT experimental study phase II	2004	None	330
Pre- and post-planning dosimetry analysis of interstitial BT with iodine-125 permanent implant in the treatment of prostate cancer	2004	None	209

Main Collaboration

With Units within ITT

» Breast Surgery Unit, Azienda USL 12 Viareggio

Pistoia Area

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Introduction

The Unit was established in 2005.
Our research activity consists mainly in clinical trials.
Over the last three years, we have concentrated on gastrointestinal, urological, breast and lung cancer and myeloma.

Main Collaborations

With Units within ITT
» Medical Oncology, Azienda USL 5 Pisa
» Department of Radiotherapy, Azienda Ospedaliero Universitaria Careggi (AOU Careggi), Firenze
» Department of Oncology, Azienda Ospedaliero Universitaria Pisana (AOU Pisana)

With other Italian and Foreign Institutions/Organizations
» Istituto di Ricerche Farmacologiche "Mario Negri", Milano
» Gruppo Oncologico del Nord Ovest (GONO)
» Gruppo Oncologico Chirurgico Cooperativo Italiano (GOCCI)

Publications

1. Roila F, Ballatori E, Labianca R, et al: *Off-label prescription of antineoplastic drugs: an Italian prospective, observational, multicenter survey*. Tumori 2009; 95: 647-51.
2. Ciolli S, Leoni F, Casini C, Breschi C, Santini V, Bosi A: *The addition of liposomal doxorubicin to bortezomib, thalidomide and dexamethasone significantly improves clinical outcome of advanced multiple myeloma*. Br J Haematol 2008 Jun; 141(6): 814-9.

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Valentina Panella, Psychologist
Daniela Magni, Nurse
Carlo Marata, Radiology Technician
Roberta Marini, Radiology Technician
Silvia Nastasi, Radiology Technician
Lara Mori, Radiology Technician

Introduction

In 1993, in Pistoia, the Radiotherapy Unit started its oncological activity as an integral part of the "Area Vasta" of Florence. Since June 2006, a new linear accelerator, Varian Clinac DHX, has been available for the newest radiation technology. We can acquire 3D volume imaging using a CT simulator, the Philips Brilliant Big Bore, and by the use of a very sophisticated Treatment Planning System software, Pinnacle, we are able to provide very targeted radiation therapies.

Our main purpose has been to optimize Intensity Modulated Radiotherapy (IMRT) in a selected series of patients with prostate or head and neck cancer. We intend to continue our experience with prostate cancer brachytherapy using ^{125}I seeds, and metabolic radiotherapy in the palliative treatment of metastatic prostate and breast carcinoma.
Our research activity consists mainly of clinical trials.
Over the last three years, we have concentrated on:

- breast cancer;
- prostate cancer;
- fractionation of the dose.

Main Research Themes

1. Dose hypofractionation in postoperative breast cancer treatment

Main achievement: Experience with 275 cGy x 5/week.

Current work: Up-grading our experience.

2. Dose hypofractionation in prostate cancer radiotherapy

Current work: Preparing a protocol with the Radiotherapy Unit in the "Area Vasta" of Florence.

Future work: Use this protocol in intracapsular cancer.

3. Brachytherapy of prostatic cancer

Main achievement: Experience with more than 120 patients.

Future work: Set up a new technique with ^{125}I seeds.

4. Preoperative radiochemotherapy in rectum cancer

Main achievement: Use of capecitabine and Oxaliplatinum in association with radiotherapy.

Current work: Up-grading our experience.

5. Metabolic radiotherapy

Main achievement: Use of strontium^{89} in patients with bone metastases in prostate cancer.

Current work: Use of samarium in patients with bone metastases in breast cancer.

Future work: Use metabolic radiotherapy in association with chemotherapy.

6. IntraOperative Radiation Therapy (IORT)

In the next months, an intraoperative accelerator unit will be made available to our Radiotherapy Unit.

Main Collaborations

With Units within ITT

» Radiotherapy Units of "Area Vasta" of Florence
» Radiotherapy Units of Pisa, Arezzo, Siena and Lucca

Publications

1. Strigari L, Orlandini LC, Andriani I, et al: *A mathematical approach for evaluating the influence of dose heterogeneity on Tcp for prostate cancer brachytherapy treatment*. Phys Med Biol 2008; 53: 5045-59.
2. Livi L, Stefanacci M, Scoccianti S, et al: *Adjuvant hypofractionated radiation therapy for breast cancer after conserving surgery*. Clin Oncol 2007; 19: 120-4.
3. Magrini SM, Bertoni F, Vavassori V, et al: *Practice patterns for prostate cancer in nine central and Northern Italy Radiation Oncology centers: a survey including 1759 patients treated during two decades (1980-1998)*. Int J Radiat Oncol Biol Phys 2002; 52 1310-9.
4. Aikaterini D, Colamussi P, Giganti M, et al: *A multicentre observational study of radionuclide therapy in patients with painful bone metastases of prostate cancer*. Eur J Nucl Med 2001; 788-98.

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Chiara Biagioni, Data Manager
Silvia Cappadona, Data Manager
Donata Cavaciocchi, Data Manager
Francesca Del Monte, Data Manager
Simone Anderini, Research Nurse
Alessandra Baldi, Research Nurse
Elizabeth Florea, Scientific Secreriat

Introduction

The Clinical Research Unit of the Medical Oncology Department in Prato was formally created in the year 2003 thanks to the generous support of the "Sandro Pitigliani" Association, and to the interaction between this association and the public health care provider in Prato. The Italian Association for Cancer Research (AIRC) has also contributed to the creation and development of the Unit by supporting research projects and fellowships. The aim is to structure this new Research Unit in a way that dedicated personnel from both the Medical Oncology and Pathology Departments can work together. Research activities are oriented toward the identification of new drugs with a targeted mechanism of action and to the discovery of molecular markers and signatures predicting activity and toxicity of anti-cancer agents.

Clinical Trials

1. Breast cancer

Description	Year	Sponsor	Number of patients recruited to date
HER-2 and Topoisomerase II α as markers predicting the efficacy of anthracycline-based adjuvant chemotherapy in node-positive breast cancer patients. A meta-analysis of four phase III clinical trials with centralized evaluation of biological markers	2009	AUSL 4 Prato	65
A phase III trial evaluating the role of ovarian function suppression and the role of Exemestane as adjuvant therapies for premenopausal women with endocrine responsive breast cancer, and the suppression of ovarian function trial (SOFT), a phase III trial evaluating the role of exemestane plus a GnRH analogue as adjuvant therapy for premenopausal women with endocrine responsive breast cancer. Tamoxifen and Exemestane Trial (TEXT)	Until 2008	International Breast Cancer Study Group (IBCSG)	29
Metabolic profiles of early breast cancer patients for diagnostic and prognostic purposes	2007-2009	AUSL 4 Prato	64
A pilot study testing the feasibility of two different regimens incorporating dose-dense docetaxel and capecitabine-oral vinorelbine in the adjuvant treatment of early breast cancer patients	from 2006-ongoing	AUSL 4 Prato	52
CTC Study: HER-1 and HER-2 inhibition in advanced breast cancer patients with HER-2 non-amplified primary tumors and HER-2-positive circulating tumor cells. A phase II study	2005-2008	AUSL 4 Prato	66
ATHENA Study (ML19391): a study of Avastin (bevacizumab) plus taxane-based therapy in patients with locally recurrent or metastatic breast cancer	2007-2008	Roche	14

Description	Year	Sponsor	Number of patients recruited to date
Urine metabolic profiles and serum BNP level changes as early markers of cardiac toxicity in early breast cancer patients receiving an anthracycline-based adjuvant therapy. A pilot study	2005-2008	AUSL 4 Prato	72
NMR-based spectroscopy analysis: evaluation of urine metabolites as markers predicting response to therapy in a population of advanced breast cancer patients	2005-2008	AUSL 4 Prato	53
Study A6181077: a randomized, phase II study of SU011248 *versus* standard-of-care for patients with previously treated, advanced, triple receptor negative (ER, PgR, and HER-2) breast cancer	2005-2008	Pfizer	8
CONFIRM: a randomized, double-blind, parallel-group, multicenter, phase III study comparing the efficacy and tolerability of fulvestrant (Faslodex™) 500 mg with fulvestrant (Faslodex™) 250 mg in post-menopausal women with estrogen receptor-positive advanced breast cancer progressing or relapsing after previous endocrine therapy	2005-2007	AstraZeneca	6
A randomized, multicenter, double-blind, placebo-controlled, two-arm, phase III study of oral GW572016 in combination with paclitaxel in subjects previously untreated for advanced or metastatic breast cancer	Until 2005	GlaxoSmithKline	4
Chemotherapy Adjuvant Study for women at advanced Age (CASA) Trial: a phase III trial evaluating the role of adjuvant pegylated liposomal doxorubicin (PLD, Caelyx®, Doxil®) in women (age 66 years or older) with endocrine non-responsive breast cancer who are not suitable candidates for a “standard chemotherapy regimen”		IBCSG	0
p-53 as a predictive marker in early breast cancer patients receiving chemotherapy in a phase III trial		AUSL 4 Prato	0

2. Non-small cell lung cancer

Description	Year	Sponsor	Number of patients recruited to date
TORCH Study: an international randomized phase III study of first-line erlotinib followed by second-line cisplatin + gemcitabine *versus* first-line cisplatin + gemcitabine followed by second-line erlotinib in advanced non-small cell lung cancer	2007-2010	Roche	6

Description	Year	Sponsor	Number of patients recruited to date
Study A8501001: an international, randomized, open-label, phase III trial of paclitaxel/carboplatin + PF3512676 *versus* paclitaxel/carboplatin alone as first-line treatment of patients with advanced non-small cell lung cancer	2007-2008	Pfizer	0
CALC-1 Study: a randomized phase II study of cetuximab in combination with gemcitabine or gemcitabine followed by cetuximab in advanced non-small cell lung cancer patients who are not candidates for platinum-based therapy	2005-2006	Istituto “Pascale”	2
Multicentric randomized study comparing gefitinib *versus* platinum-based chemotherapy in EGFR FISH-positive NSCLC patients (RANGE)		Istituto “Humanitas”	0

3. Colorectal cancer

Description	Year	Sponsor	Number of patients recruited to date
Open-label, multicenter, phase II study of first-line, bi-weekly irinotecan, oxaliplatin and infusional 5 fu/LV (folfoxiri) in combination with bevacizumab in patients with metastatic colorectal cancer	2007-2009	AUSL 6 Livorno	12
Randomized phase II trial testing the efficacy of three bevacizumab-containing, first-line regimens for metastatic colorectal cancer	2005-2007	Roche	15

4. Gastric cancer

Description	Year	Sponsor	Number of patients recruited to date
Open-label, randomized, multicenter, phase III study of adjuvant chemotherapy in radically resected adenocarcinoma of the stomach or gastroesophageal junction: comparison of a sequential treatment (CPT-11+5-FU/LV → TXT+CDDP) *versus* a 5-FU/LV regimen	2005-2008	Istituto di Ricerche Farmacologiche “Mario Negri”	24

5. Onco-Hematology

Description	Year	Sponsor	Number of patients recruited to date
Schering-Plough Research Institute Study P02978: a pivotal randomized study of lonafarnib *versus* placebo in the treatment of subjects with MyeloDysplastic Syndrome (MDS) or Chronic MyeloMonocytic Leukemia (CMML) who are platelet transfusion-dependent with or without anemia	2006-2007	Shering-Plough	1

6. Elderly

Description	Year	Sponsor	Number of patients recruited to date
ONCOGER 1 Study: the effect of chemotherapy on the functional reserve of elderly cancer patients. A coordinated multidisciplinary evaluation	2005-2006	AUSL 4 Prato	28

Main Collaborations

With Units within ITT

» Azienda USL 6 Livorno
» Azienda Ospedaliero Universitaria Pisana (AOU Pisana)
» Other ITT Units including the Faculty of Medicine and the School of Medical Oncology, University of Florence, and Center for Magnetic Resonance (CERM), Scientific Pole, University of Florence, Sesto Fiorentino (Firenze)

With other Italian and Foreign Institutions/Organizations

» Institute Bordet, Brussels (Belgium)
» International Breast Cancer Study Group (IBCSG)
» Early Breast Cancer Trialists Collaborative Group (EBCTCG)
» Breast International Group (BIG)
» Yale University (USA)

Publications

1. Magné N, Castadot P, Chargari C, Di Leo A, Philippson C, Van Houtte P: *Special focus on cardiac toxicity of different sequences of adjuvant doxorubicin/docetaxel/CMF regimens combined with radiotherapy in breast cancer patients*. Radiother Oncol 2009; 90:116-21.
2. Oakman C, Bessi S, Zafarana E, Galardi F, Biganzoli L, Di Leo A: *Recent advances in systemic therapy. New diagnostics and biological predictors of outcome in early breast cancer*. Breast Cancer Res 2009; 11: 205.
3. Finn RS, Press MF, Dering J, et al: *Estrogen receptor, progesterone receptor, human Epidermal Growth Factor Receptor 2 (HER2), and epidermal growth factor receptor expression and benefit from lapatinib in a randomized trial of paclitaxel with lapatinib or placebo as first-line treatment in HER2-negative or unknown metastatic breast cancer*. J Clin Oncol 2009; 27: 3908-15.
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11. Biganzoli L, Licitra S, Moretti E, Pestrin M, Zafarana E, Di Leo A: *Taxanes in the elderly: can we gain as much and be less toxic?* Crit Rev Oncol Hematol 2009; 70: 262-71.
12. Francis P, Crown J, Di Leo A, et al: *Adjuvant chemotherapy with sequential or concurrent anthracycline and docetaxel: Breast International Group 02-98 randomized trial*. J Natl Cancer Inst 2008; 100: 121-33.
13. Larsimont D, Durbecq V, Awada A, Di Leo A: *HER2 and topoisomerase II alpha: useful clinical markers in breast cancer*. Bull Cancer 2008; 95: 344-51.
14. Galmarini CM, Treilleux I, Cardoso F, et al: *Class III beta-tubulin isotype predicts response in advanced breast cancer patients randomly treated either with single-agent doxorubicin or docetaxel*. Clin Cancer Res 2008; 14: 4511-6.
15. Di Leo A, Moretti E: *Anthracyclines: the first generation of cytotoxic targeted agents? A possible dream*. J Clin Oncol 2008; 26: 5011-3.
16. Guarneri V, Frassoldati A, Bruzzi P, et al: *Multicentric, randomized phase III trial of two different adjuvant chemotherapy regimens plus three versus twelve months of trastuzumab in patients with HER2- positive breast cancer (Short-HER Trial; NCT00629278)*. Clin Breast Cancer 2008; 8: 453-6.
17. Di Leo A, Gomez HL, Aziz Z, et al: *Phase III, double-blind, randomized study comparing lapatinib plus paclitaxel with placebo plus paclitaxel as first-line treatment for metastatic breast cancer*. J Clin Oncol 2008; 26: 5544-52.
18. Di Leo A, Biganzoli L, Claudino W, Licitra S, Pestrin M, Larsimont D: *Topoisomerase II alpha as a marker predicting anthracyclines' activity in early breast cancer patients: ready for the primetime?* Eur J Cancer 2008; 44: 2791-8.
19. Press MF, Finn RS, Cameron D, et al: *HER-2 gene amplification, HER-2 and epidermal growth factor receptor mRNA and protein expression, and lapatinib efficacy in women with metastatic breast cancer*. Clin Cancer Res 2008; 14: 7861-70.

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Introduction

The Medical Oncology Unit of the AUSL 5 Pisa (Valdera and Alta Val di Cecina) was founded on 23 October 2008. The Unit, which cares for an area of over 150,000 residents, is active in two hospitals: “Felice Lotti” in Pontedera (Pisa) and “Santa Maria Maddalena” in Volterra (Pisa). The Unit operates through two Day Hospitals (DHs) facilities which provide a total of 6 beds and 16 seats for the administration of antineoplastic drugs. Both DHs contain rooms dedicated to the preparation of anticancer drugs, for hosting and for outpatient visits. Daily activities are carried out based on first and follow-up visits,

chemotherapy treatments and consultations with other hospital divisions. In the last year, a total of 566 first visits, more than 2,900 chemotherapy treatments and over 4,300 follow-up visits were performed. In addition to the daily clinical care provided to cancer patients, an activity of clinical research has been developed. At present, we are conducting a series of non-profit clinical trials in different tumor types. In particular, an active collaboration with the Division of Pharmacology and Pathology of the University of Pisa on observational studies has been developed.

Main Research Themes

1. Pilot study of the correlation between polymorphism of pro-/anti-angiogenic genetic factors and outcome in metastatic breast cancer patients (MBCP) treated with a combination including bevacizumab and taxanes

- Identify genetic polymorphisms of pro- and anti-angiogenic mediators, involved in neovascularization, associated with response to treatment in terms of activity and efficacy in MBCP.
- Collection of blood and histological samples of tumor tissue.
- A prospective study based on the results.

2. The role of molecular determinants in predicting the response to trastuzumab in patients diagnosed with metastatic breast cancer (MBC) with overexpression of HER-2

- Analyze the state of molecular determinants predictive of the response to trastuzumab (p95, HER2, loss of PTEN, overexpression of IGF-R1, PI3K/Akt mutation, polymorphism of FcγRIIIa) on a large series of MBC patients treated with trastuzumab, and correlate the results with the clinical outcome.
- Collection of blood and histological samples of tumor tissue.
- A prospective study based on the results.

Main Collaborations

With Units within ITT

» General Surgery Unit, Ospedale “Felice Lotti”, AUSL 5 Pisa
» Medical Oncology Unit, Azienda Ospedaliero Universitaria Pisana (AOU Pisana)
» Department of Pathology, AOU Pisana
» Section of Senology Surgery, Ospedale “Santa Chiara”, AOU Pisana
» Department of Internal Medicine, University of Pisa

GENERAL SURGERY

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Dario Pietrasanta, Surgeon
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Introduction

The Unit provides assessment, diagnosis, surgery and multidisciplinary management for abdominal pathologies, mainly cancer conditions. New patients are usually seen within a week and access to surgery is also available in a short time frame at the state of the art. Cancer cases are discussed by surgeons, radiologists, pathologists, radiation and medical oncologists. Particular attention is directed to minimally invasive approach. We take special care to multimodal treatment of hepatic metastasis and approach of elderly patients. The Unit is also dedicated to endocrine surgery. Collaborative treatment plans for individual patients are developed and recommended.

Main Research Themes

1. Thyroid cancer

a) *Markers of cell proliferation, apoptosis, and angiogenesis in thyroid adenomas: a comparative immunohistochemical and genetic investigation of functioning and non-functioning nodules*. The objective of this study was to perform *i*) an immunohistochemical investigation of cell proliferation, apoptosis, angiogenesis and malignancy markers in 15 functioning and 15 non-functioning thyroid adenomas, and in normal adjacent tissue, and *ii*) a genetic analysis of the Thyroid-Stimulating Hormone receptor (TSH-r), Gsalpha, and RAS mutations in the same group of adenomas, in order to describe their expression within tissues and to correlate them with hormonal functioning. Thirty patients who underwent surgery for a solitary thyroid nodule were included in the study. Adenomas and normal adjacent tissues were evaluated by immunohistochemistry using the following antibodies: MIB-1 for proliferative activity, bcl-2 and mutant p53 for apoptosis control, Vascular Endothelial Growth Factor-A (VEGF-A) for angiogenic activity, and galectin-3 as a marker for malignancy. To calculate microvascular density, "hot spots" were selected and defined by cells positive for CD34 staining. Genetic analysis for TSH-r, Gsalpha, and H-, K-, and N-RAS mutations was performed on adenoma specimens.

Our results indicated that a proportion of both functioning and non-functioning adenomas showed immunohistochemical phenotypes similar to normal adjacent tissue. No differences were found between functioning and non-functioning thyroid adenomas with regard to the expression of markers associated to angiogenesis (VEGF-A, microvascular density) and apoptosis control (mutant p53, bcl-2). All adenomas resulted negative for galectin-3 immunostaining. MIB-1 was the only marker showing a substantial difference in expression between the two groups of adenomas. TSH-r mutations were found in 12 out of 15 functioning adenomas, whereas the absence of Gsalpha and H-, K-, and N-RAS mutations was demonstrated in all adenomas.

b) *Clinical management of suspicious thyroid nodule: the role of surgeon-performed ultrasonography in the prediction of malignancy*. Thyroid nodules are extremely common and should be considered a diagnostic challenge in clinical practice. Approximately 4% to 8% of adults have palpable thyroid nodules and up to 40% have nodules visible on Ultrasonography (US). Despite Fine Needle Aspiration Cytology (FNAC), precise identification of malignancy remains difficult. Although several US characteristics have been studied as potential predictors of malignancy, there is also an overlap in the nodule's appearance. The purpose of our study was to analyze whether surgeon-controlled high resolution US with a color Doppler study could recognize malignancy in the thyroid nodule and compare it to FNAC results. A total of 122 patients with 178 solid thyroid nodules underwent high resolution US, color Doppler analysis and FNAC between 2004 and 2006, and were retrospectively analyzed. Imaging and ultrasound-guided FNAC was performed by the same operators (endocrine surgeons). Based on the US results, 78% of the nodules were considered benign and 22% suspicious or doubtful for malignancy. Based on the FNAC results, 73% were benign, 15% suspicious or suggestive of a neoplasm, 5% positive for papillary carcinoma and 7% were atypical. All thyroid nodules considered benign based on the US had benign FNAC results. Out of the suspicious nodules, 36% were really carcinoma, with no difference between the two methods.

2. Abdominal cancer

a) *Minimally invasive approach for focal hepatic lesions*. Radio-frequency thermal ablation is a safe and effective local treatment for hepatic focal lesions during laparoscopic surgery. Current indications are: patients with non-surgical lesions or refusing surgery with hepatocarcinoma or hepatic metastases from colorectal carcinoma or endocrine tumors. In lesions smaller than 3 cm in diameter, a single insertion of the electrode needle in the center of the lesion is enough to destroy the entire lesion with a treatment session. In tumors exceeding 3 cm, multiple needle insertions are required to cover

the entire volume. The precise role of laparoscopy in resection of neoplastic hepatic lesions remains controversial and, perhaps, is indicated only in few patients with malignant liver disease. The same oncological rules should be applied as in open surgery; by using laparoscopic ultrasound, a 1 cm-free surgical margin should be routinely obtained. Tumor selection in our series concerned small, superficial or peripheral lesions, located in the left lateral segment of the liver or in the anterior segments of the right liver including the anterior part of the IV, V and VI segments.

b) *Laparoscopic approach to pancreatic insulinoma: from enucleation to distal pancreatectomy*. Thanks to technical advancements and endoscopic surgical skills, the laparoscopic approach topancreatic tumors is being increasingly performed. Minimally invasive approaches for resection is useful to treat benign cystic tumors, chronic pancreatitis and neuroendocrine tumors. Pancreatic insulinoma, typically single, benign and small is the most suitable for laparoscopic resection. Although neuroendocrine tumors of the pancreas are often managed traditionally, these rare neoplasms may be amenable to laparoscopic surgical resection. Laparoscopic Sonography (LUS) examination of the pancreas must be performed carefully and is essential to determine the most suitable surgical procedure, either a laparoscopic distal pancreatectomy or enucleation, according to location of the tumor, its depth in the pancreatic parenchyma and its proximity to portomesenteric vessels and main pancreatic ducts. Hospital stay and time to recover may be shortened by using laparoscopic pancreatic resection. The most common complication is a formation of a peripancreatic collection followed by a pancreatic fistula. Rates of pancreatic leaks reported in laparoscopic surgery (8-33%) are slightly higher than those for the open pancreatectomy series (5-23%). However, in most cases fluid collections and pancreatic leaks were resolved non-operatively with percutaneous drainage, parenteral nutrition and therapy with octreotide.

c) *Treatment with 5-fluorouracil/folinic acid, oxaliplatin and irinotecan enables surgical resection of metastases in patients with initially unresectable metastatic Colorectal Cancer (CRC)*. The prognosis of unresectable metastatic CRC might be improved if a radical surgical resection of the metastases could be performed after a response to chemotherapy. We treated 74 patients with unresectable metastatic CRC (not selected for a neoadjuvant approach) with irinotecan, oxaliplatin, and 5-fuorouracil/leucovorin (FOLFOXIRI and simplified FOLFOXIRI). Because of the high activity of these regimens (response rate, 72%), a secondary curative operation could be performed in 19 patients (26%).

The FOLFOXIRI regimens we studied have significant anti-tumor activity and permit radical surgical resection of metastases in patients with initially unresectable metastatic CRC not selected for a neoadjuvant approach, and also those with extrahepatic disease. The median survival of patients with resected disease is promising.

d) *CRC in the elderly*. CRC is the third most common cancer for both sexes: elderly patients are often viewed as high-risk surgical candidates with high rates of emergency presentations and perioperative mortality. The aim of our study was to examine the characteristics and perioperative morbidity and mortality rate of elderly patients submitted to CRC surgery.

We retrospectively studied 248 patients who underwent surgery for CRC at our institution between July 2003 and December 2005. Risk factors included sex, age, cancer localization, Duke's classification, blood transfusion, preoperative POSSUM score and mode of presentation. Primary outcome was perioperative mortality.

The study consisted of 143 males and 105 females; 134 patients (54%) were over 75 years old. In the two older groups, cancer was situated more commonly in the proximal colon than in the youngest age group ($p = 0.001$). Of the 25 resections performed as emergency treatment, 20 were in the over 75s ($p < 0.001$) group. In elective procedure, perioperative mortality scores were 3.1% in the over 75s *versus* 0% in the under 75s, meanwhile in emergency, rates of 24% *versus* 0% (P = NS) were registered. In Cox multivariate regression analysis, age and mode of presentation reached statistical significance.

Old age itself is not an independent negative prognostic factor for CRC surgery.

e) *Long-term outcome of initially unresectable metastatic CRC patients treated with 5-Fluorouracil/ Leucovorin, Oxaliplatin and Irinotecan (FOLFOXIRI) followed by radical surgery of metastases*. The GONO-FOLFOXIRI regimen improved the rate of R0 secondary resection of metastases in initially unresectable metastatic CRC. The objective of this study was to evaluate the long-term outcome of resected patients and the impact of FOLFOXIRI on perioperative morbidities, mortality, and chemotherapy induced hepatotoxicity. Overall, 196 patients with initially unresectable metastatic CRC were treated with FOLFOXIRI in two phase II and one phase III trials. This regimen was associated with an elevated response rate (70.4%) and 37 patients (19%) could undergo a secondary R0 surgery on metastases. This study was registered with the Australian New Zealand Clinical Trials Registry Database at http://www.anzctr.org.au/Statistics.aspx, ID number ACTRN12608000615381.
The main characteristics of the 37 radically resected patients were: median age 64 years (45-73), Eastern Cooperative Oncology Group Performance Status (ECOG) PS > or = 1 in 30%, synchronous metastases in 65%, multiple sites of disease in 22% and metastases confined to the liver in 68%. Preoperative FOLFOXIRI was administered for a median of 5.5 months. There was no perioperative mortality and all morbidities (27% of patients) resolved without sequelae. After a median follow-up of 67 months, 5-year and 8-year survival are 42% and 33%, respectively. At five years, 29% of patients are free of disease. The analysis of treatment-induced liver injury showed neither G3 vascular toxicity nor G4 steatosis, and steatohepatitis in only 5% of patients.

Main Collaborations

With Units with ITT
- Medical Oncology Unit, Azienda Ospedaliero Universitaria Pisana (AOU Pisana)
- Hematology Unit, AOU Pisana
- Department of Oncology, Transplantation and New Technologies, University of Pisa

With other Italian and Foreign Institutions/Organizations
- Hopital Paul Brousse, Hepato-biliary Centre, Université de Paris

Publications

1. Masi G, Loupakis F, Pollina L, et al: *Long-term outcome of initially unresectable metastatic colorectal cancer patients treated with 5-fluorouracil/leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) followed by radical surgery of metastases*. Ann Surg 2009; 249: 420-5.
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4. Masi G, Cupini S, Marcucci L, et al: *Treatment with 5-fluorouracil/folinic acid, oxaliplatin, and irinotecan enables surgical resection of metastases in patients with initially unresectable metastatic colorectal cancer*. Ann Surg Oncol 2006; 13: 58-65.

MEDICAL ONCOLOGY

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Introduction

The Medical Oncology 2 Unit is part of the "Polo Oncologico Area Vasta Nord Ovest" that was instituted in 2009. Its activity is focused on patient care, research and educational activities for the treatment of solid neoplasms. The team is composed of nine medical oncologists, one gynecology-oncologist, ten post graduate students, three fellow physicians, three data managers and several fellows.
The Polo Oncologico is located in Ospedale "Santa Chiara" and it is arranged on three floors. The ground floor has a welcome service called Focal Point, managed by the Istituto Toscano Tumori (ITT), and the multidisciplinary outpatient offices of the Centro Oncologico di Riferimento Dipartimentale (CORD), where the Gruppo Oncologico Multidisciplinare (GOM) meets. This is where first and follow-up visits are performed. Visit timetables are organized on the basis of the primary cancer diagnosis (gastrointestinal colorectal, gastrointestinal non-colorectal, genitourinary, breast, lung, rare tumors). On the second and third floors can be found the out-patient and in-patient facilities. The research activity of the Unit consists mainly in clinical trials and translational research. Many independent or endorsed clinical trials are already ongoing or under evaluation.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
GONO-TRIBE: phase III randomized study of FOLFOXIRI combination chemotherapy and bevacizumab *versus* FOLFIRI combination chemotherapy and bevacizumab as first-line treatment in patients with unresectable metastatic Colorectal Cancer (CRC) (approved by the EC 30/04/2009, obtained administrative authorization)			
GONO-BEBYP: phase III randomized study of second-line combination chemotherapy with bevacizumab *versus* without bevacizumab in patients with metastatic CRC who have received first-line chemotherapy with bevacizumab (submitted EC)			
ProVeTTA: prospective evaluation of -1498 C/T VEGF polymorphism in the prediction of benefit from first-line folfiri + bevacizumab in metastatic CRC patients (submitted EC)			
ISTRICE-BEACH: phase II trial of induction FOLFOXIRI + cetuximab followed by maintenance with bevacizumab and 5-FU as first-line treatment for KRAS and BRAF wild-type metastatic CRC (submitted EC)			
A randomized, double-blind, placebo-controlled phase III study of regorafenib + BSC *versus* placebo + BSC in patients with metastatic CRC who have progressed after standard therapy. Protocol BAY 73-4506/14387 (submitted EC)			

Description	Year	Sponsor	Number of patients recruited to date
TOSCA: a randomized trial investigating the role of FOLFOX4 or XELOX (three *versus* six months) regimen duration and bevacizumab as adjuvant therapy for patients with stage II/III colon cancer (submitted EC)			
PROMET-3: a phase II study on metronomic chemotherapy with oral vinorelbine and desametazone in patients with advanced stage hormone-resistant prostate cancer: a pharmacokinetic and pharmacodynamic evaluation. ASL609LIOM01 EudraCT 2009-015116-17 (submitted EC)			
Janus trial: a phase II translational study investigating the biological effects of zoledronic acid as neoadjuvant therapy on invasive prostate cancer (submitted EC)			
A randomised, multicenter, phase II, parallel-group trial of vandetanib monotherapy or vandetanib in combination with gemcitabine *versus* gemcitabine + vandetanib matching placebo in subjects with advanced biliary tract cancer (gallbladder cancer, cancer of the extrahepatic bile duct, intrahepatic cholangiocarcinoma and ampullary carcinoma). Protocol AstraZeneca D4200L00007 (approved by the EC 03/12/2009, pending administrative authorization)			
A randomized, open-label, multicenter phase II study to compare AUY922 with docetaxel or irinotecan in adult patients with advanced gastric cancer, who have progressed after one line of chemotherapy. Protocol CAUY922A2202 (approved by the EC 03/12/2009, obtained administrative authorization)			
Multicenter, open, randomized phase III study comparing bevacizumab and cisplatin-etoposide in combination *versus* cisplatin-etoposide alone as first-line treatment of Small Cell Lung Cancer (SCLC). Extended Disease (ED). Protocol FARM6PMFJM (approved by the EC 26/11/2009, obtained administrative authorization)			
Randomized, double-blind, study of controls *versus* placebo conducted with neratinib (HKI-272) after trastuzumab in women affected with early stage breast cancer characterized by overexpression/amplification of HER-2/neu. Protocol 3144A2-3004-WW (submitted EC)			
Randomized, stage III, open, two-arm study with neratinib in association with paclitaxel *versus* trastuzumab in association with paclitaxel as first-line treatment of locally reoccurring or metastatic ERRBB-2-positive breast cancer. Protocol 3144A2-3005-WW (submitted EC)			

Main Collaborations

With Units within ITT

» All the Medical Oncology Units within ITT

With other Italian and Foreign Institutions/Organizations

» Several Italian Medical Oncology Units
» Department of Biochemistry and Molecular Biology "G. Fornaini", University of Urbino
» Medical Oncology Division, University of Southern California, Norris Comprehensive Cancer Center, Los Angeles, California (USA)

Publications

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4. Vasile E, Masi G, Fornaro L, et al: *A multicenter phase II study of the combination of oxaliplatin, irinotecan and capecitabine in the first-line treatment of metastatic colorectal cancer.* Br J Cancer 2009 Jun; 100(11): 1720-4.
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12. Graziano F, Ruzzo A, Loupakis F, et al: *Liver-only metastatic colorectal cancer patients and thymidylate synthase polymorphisms for predicting response to 5-fluorouracil-based chemotherapy.* Br J Cancer 2008 Sep; 99(5): 716-21.
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21. Ruzzo A, Graziano F, Loupakis F, et al: *Pharmacogenetic profiling in patients with advanced colorectal cancer treated with first-line FOLFOX4 chemotherapy.* J Clin Oncol 2007 Apr; 25(10): 1247-54.
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HEMATOLOGY

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Introduction

The Hematology Unit is a Division of the Department of Oncology, Transplants and New Advances in Medicine, afferent also to the University of Pisa.
The Division is organized into three specific and interacting sections: *a*) the "out-patients" section, dedicated to diagnosis of hematological diseases, decision and performance of planned treatments when feasible in a day-hospital regimen, and where patients enrolled into clinical trials are strictly followed and monitored; *b*) the coagulation section; *c*) the "in-patients" section, including the intensive care and bone marrow transplantation units.
Morphology, cytofluorimetry, molecular biology, and cell culture and manipulation are covered by the laboratories included in the Division.
Pisa Hematology collaborates with several national and international groups and is presently involved in more than 40 clinical trials, most of them representing multicentric and phase II-III innovative protocols. Among them, some appear very relevant, such as the DNA anti-idiotype vaccination project, the induction of gamma delta lymphocyte reactivity against plasma cells in melanoma (auto)transplanted patients and some molecularly targeted treatment protocols.
The use of radio-labeled monoclonal antibodies in lymphoma therapy is an interesting area developed in collaboration with the Nuclear Medicine Division.
Finally, the molecular laboratory is the referent laboratory for national trials on follicular lymphoma and is involved in a molecular network dedicated to the standardization of methods for detection of the Minimal Residual Disease (MRD) in chronic myeloid leukemia and lymphomas.

Main Research Themes

1. Minimal Residual Disease

Main achievement: Demonstration of the MRD role in different hematological diseases (acute myeloid leukemias, lymphomas, multiple myeloma).

Current work: Role of MRD in patients receiving allogeneic bone marrow transplantation, with attention to different conditioning regimens.

Future work: Setting of new, advanced molecular and immunophenotyping methods to improve MRD detection, also in the context of two national networks (Labnet for chronic myeloid leukemia and IIL-MRDnet for lymphomas).

2. Multidrug resistance

Main achievement: Demonstration of the clinical role of MDR in acute leukemia and mantle cell lymphoma.

Current work: Study of gene polymorphisms.

Future work: Clinical application of the MDR concept (treatments would be planned or modified according to these results).

3. Polymorphism detection

Main achievement: Demonstration of the clinical role of polymorphisms in multiple myeloma patients.

Current work: Study of further gene polymorphisms in myeloma and lymphomas.

Future work: Use detected polymorphisms to plan, from diagnosis, the most appropriate treatment.

4. Mesodermal stem cells

Main achievement: Identification and purification of a previously unknown precursor cell population.

Current work: Identification of selective intracellular pathways.

Future work: Clinical application in regenerative medicine.

5. Anti-idiotype DNA vaccine

Main achievement: Demonstration of effectiveness of these vaccines in animal models; approval of the protocol by ISS.

Current work: Recruitment of patients affected by follicular lymphoma into a phase I study.

Future work: Expanding to follow protocol phases.

6. Post-transplant immunological disease control

Main achievement: Demonstration of the gamma/delta T lymphocyte role in graft *versus* myeloma phenomenon.

Current work: Patient recruitment for MRD control in a clinical study on *in vivo* expansion of gamma delta T-cells in multiple myeloma.

Future work: Elaboration of clinical data from the above-mentioned trial; study of the effects on graft-*versus*-host disease of histone acetylase inhibitors in a murine model.

7. Infectious disease in hematological patients

Main achievement: Evaluation of the incidence of PML in rituximab-treated patients.

Current work: Identification of new risk factors.

Future work: Setting new effective and more specifically targeted therapies.

Main Collaborations

With Units within ITT

» Hematological Net, "Area Vasta Nord Ovest"
» Hematology Unit, Azienda Ospedaliero Universitaria Careggi, Firenze
» Hematology Unit, Azienda Ospedaliero Universitaria Senese

With other Italian and Foreign Organizations/Institutions

» Gruppo Italiano Studio Linfomi (GISL), Modena
» Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA), Roma
» Intergruppo Italiano Linfomi
» European Group for Blood and Marrow Transplantation (EBMT), London (UK)

Publications

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MOLECULAR PATHOLOGY

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Introduction

The Laboratory, established in the 1980s, focused on the biomolecular aspects of cancer. The fact that it is inside a Division of Pathology makes the use of human material for research projects easier to obtain. The Lab introduced Molecular Pathology in Italy, being one of the first Molecular Pathology Labs in Europe. At the same time, the Lab was the first Italian group leading in the field of hereditary breast tumors.
Present research activity is focused on the molecular aspects of human cancer, in particular, the model of breast carcinoma.
Special attention is given to the development of new molecular tools for the characterization of tumors in terms of diagnosis, prognosis and therapy.

Main Research Themes

1. Is human breast cancer caused by a virus? Study on the etiological role, pathogenetic mechanisms and spreading of the Human Mammary Tumor Virus (HMTV) infection. Development of new therapeutic and vaccine approaches

The project has the main objective to study and understand the relationship between HMTV and human Breast Cancer (BC), with particular interest in defining the causal action, pathogenetic mechanisms inducing carcinogenesis, infection spreading and possible development of vaccines and new therapeutic strategies. It is not easy to establish in a conclusive way a viral etiology for a specific cancer. Regarding HMTV, several evidentiary criteria have been met, but the proof of its infective and transforming potential or its presence before the development of the cancer is still lacking. HMTV sequences are present in 33% of invasive breast carcinoma and absent in normal human breast tissues, in blood donors from healthy volunteers and in other neoplastic tissues. We were able to detect, for the first time, at high frequency (79.6%) the presence of HMTV sequences in the early stages of BC progression (pre-invasive lesions, ductal carcinoma *in situ*) confirming a possible etiological role of HMTV virus in the onset of the disease. The ongoing study will focus on: *a*) confirming the presence of the HMTV virus in pre- and neoplastic lesions; *b*) defining the possible pathogenetic mechanism of carcinogenesis induction; *c*) conducting molecular and epidemiological studies in order to analyze the spreading of the virus in the infected population, in individuals carrying tumors and in well defined epidemiology clusters (relatives, close communities) or in other situations that would provide further clues about possible HMTV transmission mechanisms; *d*) defining therapeutic strategies to control the replication of the virus; *e*) designing therapeutic and prophylactic vaccine strategies; *f*) developing an animal model which will confirm the role of HMTV or an equal one in the carcinogenesis of BC, and which will allow validation of the vaccine and therapeutic approaches described in points *d*) and *e*) (a).

2. Molecular markers in cytological diagnosis of thyroid nodules

We have recently demonstrated the usefulness of the molecular approach to the preoperative cytological evaluation of thyroid tumors. In particular, the detection of the BRAF V600E mutation was revealed as relevant in diagnosis of papillary carcinoma. The status of the BRAF gene is actually studied in thyroid microcarcinomas, with the aim of better defining prognosis.

At the same time, a 6-gene model (SYNGR2, LSM7, KIT, Hs.296031, c21orf4, Hs.145049) is under evaluation for differentiating benign from malignant thyroid tumors. This model was defined by Chiara Mazzanti using an array-based gene expression approach. Finally, the role of hTERT in thyroid neoplasms is under investigation (b).

3. Analytical and clinical validation of biomarkers for non-invasive early diagnosis of bladder cancer

Although up to 75-80% of new cases of bladder cancer present as non-invasive (Ta), superficially invasive (T1) or carcinoma *in situ* (Tis) disease, the remaining 20-25% present as muscle-invasive (T2-4) or more advanced disease with a poor prognosis. Furthermore, although approximately 20% of Ta and T1 tumors are cured, after initial removal 60-70% recur at least once in five years, increasing up to 90% within 15 years and 10-20% progress to muscle-invasive cancer (≥ T2). Cystoscopy and urinary cytological analysis are currently the standard modalities to monitor recurrence and progression. Cystoscopy is an efficient method, however, it is invasive, causes patient discomfort, may be associated with a risk of urethral and bladder neck stricture, and might not detect flat tumors or carcinoma *in situ*. Urinary cytology is non-invasive and accurate in diagnosing high grade lesions with a sensitivity up to 95% and specificity close to 100%, but it has a low sensitivity in detecting grade I lesions. Results of recent studies on the efficacy of cystoscopy with biopsy suggest that tumors are missed in 10-40% of patients who have undergone transurethral resection because the sensitivity of these techniques is often too low during the early course of disease. Thus, the development of highly reliable non-invasive tools for bladder cancer diagnosis would facilitate early detection of tumors and help to define the role of molecular markers in prognostic evaluation of patients with bladder cancer at the time of initial diagnosis. Urine represents an easy substrate on which morphological and molecular characteristics can be analyzed. In addition to the classic cytological approach, the study of genetic alterations and the identification of proteins specifically secreted in bladder tumors today represent relevant tools. Indeed, particular mutations and polymorphisms on genes like p53, K-Ras and Fibroblast Growth Factor Receptor 3 (FGFR3) are often present in urothelial carcinoma cells, the most common type of bladder cancer. The aim of this project is the identification and validation of molecular markers present in the urine sediment of individuals at risk for bladder cancer and of patients affected by bladder cancer for early detection of the disease and/or recurrence. The study is performed on individuals at risk for bladder cancer (workers exposed to chemical agents) and on patients affected by bladder cancer, with the goal of early detection of the disease and of its recurrence, respectively. In this project, we want to study different patterns of cancer-related genes in order to identify tumor markers that can be indicative or predictive of the prognosis (grade and/or progression and/or recurrence) in bladder cancer. FISH analysis is performed on urothelial cells for evaluation of the status of chromosomes 3, 7, 9 and 17, which are more frequently involved in this neoplastic process. DNA analysis is performed to investigate the status of p53 and FGFR3 genes and to evaluate Microsatellite Instability (MSI). TRAP assay will be used for telomerase activity. RNA analysis focuses on the expression of NM23, CK18, CK19, CK20, CD44, hTERT and survivin genes. We expect to detect different combinations of chromosomal alterations and/or genetic mutations that can be associated with the aggressiveness of the tumor. On the other hand, we want to analyze the fraction of cancer cells in voided urine in order to determine marker combinations that can have diagnostic power for progression and recurrence, avoiding the use of diagnostically invasive methods (c).

4. Role of molecular determinants in predicting resistance to trastuzumab in patients diagnosed with overexpressing Human Epidermal growth factor 2 (HER2) in Metastatic Breast Cancer (MBC)

BC is the second highest cause of death worldwide. In Italy, there are approximately 40,000 new cases per year and 11,000 deaths, representing one of the major causes of morbidity and mortality cancer in females. In Tuscany, it is estimated that in 2010 there will be approximately 4,100 new diagnoses of BC and 850 deaths. Many preclinical and clinical trials were performed to investigate and establish effective treatments. The HER2 receptor is expressed in 20-30% of BC cases and it has been chosen to be an effective therapeutic target. Trastuzumab (herceptin), a monoclonal antibody, was approved by the FDA as an anti-HER2 therapy. The mechanism by which trastuzumab exerts its antitumor activity is not fully

understood. It has been suggested that trastuzumab induces Antibody-Dependent Cellular Cytotoxicity (ADCC), inhibits HER2 extracellular domain cleavage or inhibits PI3K/ AKT survival signaling, either by down-regulating HER2 signaling or by increasing PTEN membrane localization and phosphatase activity, leading to a decline in PI3K/AKT pathway activation and inhibition of proliferation. In addition, activation of HER-related receptors, such as the Insulin-like Growth Factor 1 receptor (IGF-R1), has been suggested in preclinical studies to increase PI3K/AKT signaling, thereby limiting trastuzumab efficacy. In spite of this, it is still largely unclear why almost half of BC patients overexpressing HER2 are initially not responsive to trastuzumab-based therapy, even when combined with chemotherapy, or eventually become resistant to trastuzumab during treatment. An understanding of the resistance mechanisms would stimulate the development of rational drug combinations to circumvent resistance and allow better selection of patients likely to respond. This study aims to analyze some molecular determinants predictive of resistance to trastuzumab – p95HER2, Loss Of Heterozygosity (LOH) and mutations in PTEN, overexpression of IGF-R1, PI3K/Akt and HER2 mutations; loss of HER2 amplification following trastuzumab therapy, polymorphism of FcγRIIIa – on a large series of MBC patients, over 200, treated with trastuzumab and correlate the results obtained with the clinical outcome of these patients. The gold standard will be the development of a clinical assay permitting accurate diagnosis of the implicated resistance mechanism in each subset of patients that may help design more efficient treatment protocols. Moreover, E.A. Mittendorf et al demonstrated that one third of patients resistant to trastuzumab no longer had amplification of the HER2 gene. These non-amplified HER2 patients had a significantly worse recurrence-free survival than those with tumors that retained HER2 amplification. The second step of the project is to evaluate the status of all the above-mentioned molecular determinants directly on Circulating Tumor Cells (CTCs) to detect possible change in tumor phenotype. Accumulating reports show that the detection of CTCs in body fluids has considerable potential to improve the clinical management of patients with MBC (d).

5. Genetic role of pro- and anti-angiogenic factors to predict response to treatment with bevacizumab in MBC patients

MBC is an incurable disease, with a median survival of 18-24 months depending on biological aggressiveness, site or extent of the disease. Palliative treatment of symptoms, better life quality and an increase in progression-free survival remain the main objectives of medical therapy. In the case of patients with MBC that expresses hormone receptors or in patients who are not candidates for chemotherapy due to advanced age, hormone therapy is the first therapeutic choice. For patients with metastatic hormone resistance who are hormone receptor-negative, or for patients with metastatic disease at a visceral localization and rapid development, chemotherapy is the treatment of choice. Based on the results of several studies, to date, first-line chemotherapy in patients diagnosed with MBC generally involves the use of anthracycline and a taxane, in combination. Clinical and experimental evidence show that the process of neoangiogenesis plays an important role in tumor progression and metastatization. Vascular Endothelial Growth Factor (VEGF) is one of the most important factors involved in neoangiogenesis because it stimulates proliferation and migration of endothelial cells, inhibits endothelial apoptosis and increases vasodilatation. In the last decade, new inhibitors of angiogenesis have been developed to selectively hit endothelial cells and block tumoral angiogenesis. Recently, several studies introduced bevacizumab, a monoclonal antibody against VEGF-A isoform, as a new drug in the treatment of MBC patients. A phase III trial study showed that the combination of bevacizumab + paclitaxel in MBC patients caused a statistically significant increase in progression-free survival (median PFS 11.8 *versus* 5.9 months, p = 0.001) and increased the objective response rate (36.9% *versus* 21.2%, p = 0.001) rather than treatment with paclitaxel alone. On the basis of these data, the combination of bevacizumab + paclitaxel nowadays represents the first treatment for HER2 negative MBC patients. Overexpression of HER2 or its amplification are found in about 15-25% of breast carcinomas and are associated to a more aggressive tumor behavior. New studies have been published about the significant association between Single Nucleotide Polymorphisms (SNPs) in genes

involved in angiogenesis and the risk of developing a breast carcinoma. In fact, patients treated with paclitaxel + bevacizumab showed a correlation between VEGF SNPs and therapy efficacy or toxicity; in particular VEGF-2578AA and -1154AA are predictive of better overall survival, while VEGF-634CC and -1498TT are correlated with minor incidence of level 3-4 hypertension, according to the NCI scale, if compared to the other genotypes. Feedback mechanisms induced by bevacizumab therapy are not linked only to VEGF expression, but probably other factors influencing angiogenesis are involved, like Thrombospondin-1 (TPS-1). Our objectives are the identification of SNPs in pro- and anti-angiogenic genes, involved in neovascularization correlated with response to bevacizumab treatment in 100 MBC patients. We expect, with our results, to identify genetic markers predictive of response to bevacizumab treatment in MBC patients, so that subgroups of patients who would react positively to this treatment can be selected. In this way, it would be possible to optimize the use of these drugs with important clinical and economic results (e).

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Research Grants

Year	Funding Agency	Amount
2008	Ministero della Salute, Progetto Integrato Oncologia	€ 45,000
2008	Ministero della Salute, Progetto Integrato Oncologia	€ 420,000
2007	European Union	€ 40,000

Main Collaborations

With other Italian and Foreign Institutions/Organizations

» Istituto Nazionale Tumori (INT), Milano
» Istituto Europeo di Oncologia (IEO), Milano
» Istituto Oncologico Veneto (IOV), Padova
» IstitutoTumori "Giovanni Paolo II", Bari
» Medical Genetics Unit, University of Bologna
» Laboratory of Invasion and Metastasis, University of Liege (Belgium)
» Department of Pathology, University of Leiden (The Netherlands)
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Publications

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ENDOCRINOLOGY AND METABOLIC DISEASES

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Introduction

The Department of Endocrinology of Pisa is a WHO Collaborating Center for the Diagnosis and Treatment of thyroid cancer and other Thyroid diseases. More than 8000 patients with thyroid cancer, treated from 1969 to 2010, are currently and actively followed and their clinical data have been recorded in a computerized database. About 400 new cases of thyroid cancer are diagnosed per year at our Department, where a group of researchers and medical doctors are fully dedicated to the oncological patients and their management, both at a diagnostic and therapeutic level.

The Department of Endocrinology of Pisa has a tissue bank of snap-frozen thyroid tissues of more than 1,500 samples and a blood bank of about 2,500 Papillary Thyroid Cancer (PTC) and 1,000 Medullary Thyroid Cancer (MTC) samples for DNA extraction.

The Department research laboratory is well equipped and the personnel involved in the project have a great deal of experience in molecular and cellular biology applied to research on thyroid carcinoma. It has also been involved in several projects dealing with radiation-induced thyroid cancer following the Chernobyl nuclear accident. For this reason, some irradiated papillary thyroid carcinoma tissues are also available at the Department's tissue bank. Several clinical trials for the treatment of thyroid cancer patients with new targeted drugs are ongoing.

Main Research Themes

1. Thyroid carcinoma (clinical research)

- *Analysis of clinical and pathological features of 4187 differentiated thyroid carcinoma*. In the last decades, a significant increase in the prevalence of Differentiated Thyroid Cancer (DTC) has been observed worldwide. According to several studies, the increase has been due mainly to small PTC (a). The aim of our study was to evaluate the changing features of DTC referred to our institution between 1969 and 2004. To this purpose, clinical and pathological features and prognostic factors were analyzed in 4187 DTC patients, subdivided into two groups: Group 1 (n = 1215) and Group 2 (n = 2972) diagnosed before and after 1990, respectively.
  We concluded that DTC patients diagnosed after 1990 have smaller tumors with a less advanced stage and a better prognosis. Despite these differences, both advanced stage and older age still represent the most important poor prognostic factors for survival. Epidemiological studies on the risk factors for recurrences of DTC are ongoing.
- *Follow-up of low-risk DTC patients who underwent radioiodine ablation of postsurgical thyroid remnants after either recombinant human thyrotropin or thyroid hormone withdrawal*. In the last 10 years, a new important tool has been introduced in the management of DTC: the recombinant human TSH (rhTSH) (b). It allows the stimulation of both normal and neoplastic follicular cells without the need to withdraw l-thyroxine substitutive therapy, which determines the increase in endogenous TSH. Many studies have demonstrated that rhTSH has the same stimulation power than endogenous TSH. We previously demonstrated comparable thyroid remnant ablation rates in postoperative low-risk thyroid cancer patients prepared for administration of 3.7GBq ^{131}I (100 mCi) after rhTSH during thyroxine (L-T4) therapy *versus* withholding L-T4 (euthyroid *versus* hypothyroid groups). After a median of four years, low-risk thyroid cancer patients prepared for postoperative remnant ablation either with rhTSH or after L-T4 withdrawal were confirmed to have had their thyroid remnants ablated and to have comparable rates of tumor recurrence and persistence.
  At present, we are evaluating the possibility of reducing the dose of ^{131}I up to 30 mCi, which is more safe than 100 mCi in terms of side effects. Different protocols of rhTSH administration are currently under investigation.

- *Second Primary Cancer (SPC) risk after radioiodine treatment for DTC*. Controversial studies have been published on the risk of developing a SPC in patients with DTC treated with radioiodine (^{131}I). The aims of this study were to evaluate: *a*) the association of a SPC and DTC, *b*) the risk to develop a SPC in DTC patients compared to the general population and, *c*) the correlation of SPC with ^{131}I or other epidemiological parameters.
  To this purpose, we retrospectively studied epidemiological, clinical and pathological features of 530 consecutive DTC patients. We demonstrated that SPC is present in about 11% of DTC patients. The risk to develop SPC is higher in patients treated with ^{131}I, without any relationship with the cumulative dose, and in older patients as well. However, our data show that patients with DTC, both ^{131}I-treated and untreated, have an increased generic risk to develop any SPC compared to the general population.
- *Clinical trials for the treatment of thyroid cancer with tyrosine kinase inhibitors.* In the last years, the Department of Endocrinology has taken a main role in a series of international clinical trials with new targeted therapies for the treatment of patients with advanced thyroid cancer not responding to conventional therapies (c). We used motesanib (AMG706), vandetanib (ZD6474), axitinib (AG-013736), XL-184, E7080 and sorafenib (BAY 43-9006) for the treatment of either MTC or dedifferentiated PTC. A total of 84 patients were enrolled and 35 are still under therapy. The drugs share the property to inhibit the VEGF-receptors plus other TK receptors, such as RET, Kit and BRAF. In this regard, it is worth noting that these oncogenes are the ones most frequently involved in the pathogenesis of both PTC and MTC. Patients were treated for more than one year. The results show that vandetanib is very active in patients affected with MTC and sorafenib in thyroid cancer of follicular origin, but characterized by radioiodine resistance. We did not obtain any completely cured cases, but in about 70% of the cases a durable (more than six months), partial remission was observed.
  Since about 40% of the more aggressive thyroid tumors are characterized by the presence of BRAF mutations, BAY-43-9006 has been studied *in vitro* and *in vivo* in thyroid tumor models. A phase II study showed partial response (26%) and clinical benefit (68%) in patients affected with aggressive thyroid tumors, results are shown in Figure 1.
  Ours and other groups' results induced the pharmaceutical company to start an official phase III protocol in which we are taking active part. The results of the new protocol will be available in a couple of years.

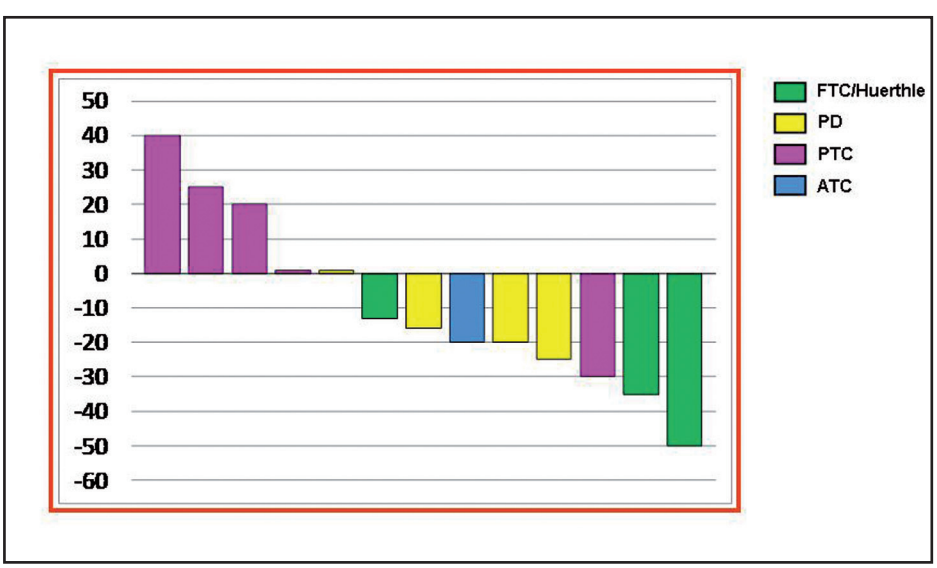

Figure 1 - Results of 13 patients with advanced thyroid cancer treated with Sorafenib for at least three-six months. FTC: Follicular Thyroid Cancer. PD: Poorly Differentiated thyroid cancer. PTC: Papillary Thyroid Cancer. ATC: Anaplastic Thyroid Cancer

2. Multiple Endocrine Neoplasia type 2 (MEN2) syndromes (clinical research)

- *Reassessment of clinical features of patients with MEN2 syndromes*. MEN2 is classified into three subtypes: MEN2A (65%), familial MTC (FMTC) (15%) and MEN2B (20%). All three subtypes carry a high risk (100%) of developing MTC; MEN2A and MEN2B carry an intermediate risk (45-50%) for Pheochromocytoma (PHEO); MEN2A also carries a risk (30%) for Hyperparathyroidism (HPTH) and for Cutaneous Lichen Amyloidosis (CLA) (10%). In MEN2B, a high risk of developing mucosal neurinomas (100%), habitus marphanoid (80%) and megacolon (60%) is also present. In our series, we observed some differences with respect to the literature data. For this reason, we decided to analyze the prevalence of the three different types of MEN and the clinically developed neoplasias.
  Results show: *a*) a significantly higher prevalence of FMTC with respect to the prevalence reported in the literature, thus a different percentage of MEN2 syndromes is now configured; *b*) a lower percentage of HPTH than expected in MEN2A, in accordance with the literature data, and a greater penetrance of HPTH when PHEO was also present. A multicentric study including all the centers participating in the ItaMEN network is ongoing: we will collect all the clinical information on the 250 Italian families recruited in this network.
- *Genotyping an Italian series of MEN 2: results of the ItaMEN network.* MEN 2 is a genetic disease characterized by MTC, associated (MEN 2A and 2B) or not (FMTC) to other endocrine neoplasia due to germline RET gene mutations (d). Over the years, several different RET mutations have been described, but their prevalence is not well defined due to the small size of the series and only a few European studies collecting data from several centers have been published. We have collected data on germline RET mutations in 220 Italian families with hereditary MTC followed in 19 different Italian centers. Our aim was to realize a large series of MEN2 families to analyze the distribution and frequency of RET mutations and to compare this prevalence to what is reported in previous series of European families of affected individuals.
  Overall, 39 different germline RET mutations were identified. The comparison of the prevalence of RET germline mutations in the present series with those found in the series published by other European Study Groups showed a statistically significant difference ($p < 0.0001$). In particular, in the Italian series there was a significantly higher prevalence of Val804Met ($p < 0.0001$) and Ser891Ala ($p = 0.0004$) mutations, which were relatively rare in the European series. When we compared the percentage of RET mutations affecting cysteine and non-cysteine codons, we found a statistically significant difference among other groups with a higher prevalence of mutations affecting non-cysteine codons in our series ($p < 0.0001$). This analysis showed a statistically significant different pattern of germline RET mutations in Italian MEN 2 affected families with respect to other European series. A European network of MEN 2 families will be the objective of this field of research in the next years: we will organize a network through the already active European Thyroid Cancer Research Network in which this group plays a leading role.
- *Screening of RET proto-oncogene mutations in MTC, diagnostic and prognostic value*. We performed RET genetic screening in 807 subjects: 485 with an apparently sporadic MTC, 33 with clinical evidence of MEN 2 and 289 relatives. All subjects were also clinically investigated. Forty-four gene carriers underwent total thyroidectomy after clinical and biochemical examination. One hundred MTC tissue samples were also analyzed for RET somatic mutations. The correlation between the presence/absence of a somatic RET mutation, the clinical/pathological features and outcome of MTC patients has been evaluated.
  We found a germline RET mutation in 39:485 (8%) MTC patients presenting as sporadic cases and in 32:33 patients with clinical evidence of hereditary MTC. A somatic RET mutation, mostly M918T, was found in 43% of sporadic MTC; furthermore, the presence of a somatic RET mutation correlates with a worse outcome in MTC patients.
  Data derived from our series confirm that RET genetic screening is the most powerful tool for the identification of all forms of MEN 2 and especially for FMTC, which are clinically frequently misdiagnosed as non-hereditable, sporadic cases.

According to the observation that the RET somatic mutation is more frequently associated with larger tumors, in the near future RET genetic analysis will be performed in MTC tumoral nodules smaller than 1 cm to determine if the RET somatic mutation is an early event in thyroid tumorigenesis

3. Analysis of gene alterations in PTC, diagnostic and prognostic value (basic research)

Putative oncogenes in thyroid carcinoma have been widely studied and some of them, such as BRAF and RET, are of particular importance from a pathogenetic point of view (e). Their role in clinical practice in the performance of presurgical diagnosis is still being debated. The problem of presurgical diagnosis is of particular interest in follicular neoplasms, which can be benign in 80% and malignant in 20% of cases and, at the present, only histology can indicate the conclusion. We have developed a molecular study to find biological markers whose gene expression profile could differ enough between benign and malignant thyroid nodules that it could be used for a better definition of follicular neoplasms. By real-time PCR we analyzed the mRNA expression of 11 genes [6 thyroid differentiation genes (TTF1, PAX-8, TPO, TSH-R, NIS and Tg) and 5 genes involved in thyroid tumorigenesis (PPARg, Gal-3, EGF-R, MET and OnfFN)] in a total of 174 human thyroid tissues (87 tumor samples and 87 corresponding normal tissue) obtained from 72 patients operated on for PTC and from 15 patients operated on for Follicular Adenomas (FA).

We found that all thyroid differentiation genes and PPARg were significantly less expressed in PTC than in the corresponding normal tissue, while onfFN, MET and Gal-3 were significantly more expressed in PTC than in normal tissues. No difference was observed in the level of expression of EGF-R mRNA in PTC and their corresponding normal tissue. A completely different pattern of gene expression was found in FA. In particular, a statistical difference between mRNA expression levels in tumoral and normal tissue was observed only for PPARg, similar to PTC. All the other genes were expressed at no different level in FA compared to their corresponding normal tissue. Our data show that almost all selected genes are differentially expressed in PTC, with respect to normal tissue. Using the ROC Curve and the logistic regression analysis, we identified a highly specific (accuracy 0.947) theoretical predictive model of gene expression able to predict the relative risk of a sample to be malignant.

At present, we are trying to develop a mathematical algorithm to be applied to the presurgical diagnosis of follicular neoplasms. The analysis of the level of expression of these genes in fine needle aspirates might represent a helpful and innovative method for the presurgical definition of follicular neoplasms.

4. RET oncogene (basic research)

- *Functional studies of new RET mutations.* The aim of this study was the functional study of the biological features of six never described and six already known RET mutations.
  We have obtained fibroblast cell lines stably transfected with RET mutated at Ala883Thr and Met848Thr codons, which are new mutations, and at Cys634Arg, Leu790Phe and Val804Met codons, which are already known mutations. We then compared the ability of the above described cell lines to form foci and grow in soft agar with the non-transfected 3T3 fibroblasts. The analysis showed that all cell lines formed a comparable number of foci, except for Ala883Thr cells, which did not have this ability, and that only Cys634Arg and Ala883Thr cells were able to grow in soft agar. The growth of all cell lines, except for Val804Met cells, was inhibited by the treatment with the tyrosine kinase inhibitor PP1, confirming data on PP1 resistance of this mutation.
  Recently, we have obtained cell lines stably transfected with the new RET mutations, Val648Ile, Ser904Phe, Met918Val and Thr338Ile, and with the already known RET mutations Met918Thr, Gly691Ser and Tyr791Phe. Currently we are performing the focus formation assay, soft agar growth assay and growth curves with and without PP1 on these cell lines.
  We are planning to perform an *in vivo* analysis of the transforming abilities of all these RET mutations by xenografting nude mice with each of the above-mentioned RET mutations in one

leg, and non-transfected 3T3 in the another leg, as a control. Data on the variable transforming activities of RET mutations will be compared.

- *Other possible mechanisms of activation of the RET oncogene*. We analyzed the RET gene copy number alterations in a large series of hereditary and sporadic MTCs in order to identify possible alternative mechanisms of RET activation. To this end, we studied RET mutations and RET gene copy number alterations in 66 MTCs (12 hereditary and 54 sporadic).
  A RET germline mutation was found in 12:12 (100%) and 23:54 (42.6%) hereditary and sporadic MTCs, respectively. Fluoresce-in-situ-hybridization analysis revealed RET gene copy number alterations in 17:66 (25.8%) MTCs (Figure 2). A significantly higher prevalence of RET gene copy number alterations, mainly chromosome 10 aneuploidy, was observed in sporadic MTCs with mutated RET compared to non-mutated MTCs ($P < 0.003$). No difference was observed when somatic RET mutations were considered. In hereditary MTC, the prevalence of RET gene copy number alterations was lower than in sporadic cases and was exclusively represented by RET gene amplification. No cases of chromosome 10 monosomy or RET gene loss of heterozygosity were found.
  These findings suggest that different roles might be played by RET gene copy number alterations; in sporadic cases, chromosome 10 aneuploidy might favor the occurrence of RET gene point mutations, while in hereditary cases RET gene amplification might enhance the transforming activity of the germline RET mutation. Despite these findings, the sporadic MTCs without RET mutations remain orphans of genetic events responsible for their tumoral transformation, and other studies are ongoing to verify other mechanisms that could be involved in the activation of the RET oncogene, such as the overexpression of the mRNA or mutations in regulatory regions of the gene, such as its promoter.

5. Treatment with anti-tumoral drugs (ciglitazone, Retinoic Acid – RA, azacytidine, tyrosine kinase inhibitors, cyclooxygenase inhibitors, deacetylase inhibitors) of thyroid carcinoma cell lines (basic research)

Since not all metastatic thyroid cancer patients can enter in clinical trials with tyrosine kinase drugs or can be allergic or not tolerant to these drugs, in the last five years, several drugs have been tested *in vitro* in our laboratory to verify their ability to interfere with the cell growth or to revert the ability to take up iodine. Here are described the main results we obtained with some of these anti-tumoral drugs.

- *In vitro effects of treatment with a Cyclooxygenase (COX-2) inhibitor on a MTC cell line (TT)*. Multi Drug Resistance 1 gene (MDR-1) codes for a multidrug transporter located on the cell plasma membrane; this protein enables tumoral cells to resist to chemotherapy by carrying chemotherapeutic

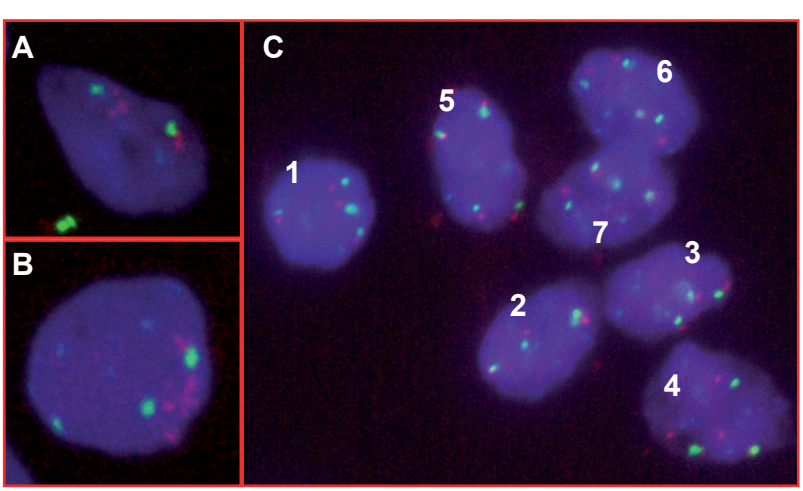

Figure 2 - RET gene (red dots) and chromosome 10 (green dots). FISH analysis of MTC. A) Diploid normal cell (two green and two red dots). B) Diploid cell: chromosoma 10 (two green dots) with RET gene amplification (many red dots). C) Mixture of triploid cells (1-4) and tetraploid cells (5-7)

drugs outside the cells. MDR-1 mRNA expression is positively regulated by Cyclooxygenase-2 (Cox-2).

MTC resistance to conventional chemotherapy is probably due, at least in part, to MDR-1 overexpression. The aim of this research has been the analysis of the effects of celecoxib, a COX-2 inhibitor, on TT cells cell growth, proliferation, necrosis and apoptosis and on COX-2 and MDR-1 mRNA expression. The associations of celecoxib with chemotherapeutic drugs, doxorubicin and vinorelbine, were also analyzed.

We observed that celecoxib was not able to significantly decrease viable cell number but it induced some apoptosis. Celecoxib was able to reduce both COX-2 and MDR-1 mRNA expressions in TT cells, and this inhibition was progressively reversed to approximate initial levels by the suspension of the treatment. We also demonstrated that elecoxib was able to increase the retention of drugs inside cells, and to conversely decrease the drug efflux in cell medium.

The association of celecoxib with vinorelbine reduced the number of viable cells, but did not affect the sensitivity to doxorubicin. The reduction in viable cell number induced by celecoxib combined with vinorelbine was mainly due to apoptosis.

This study provided evidence that we could improve the treatment with chemotherapeutic agents in metastatic MTC patients, who cannot be treated with tyrosine kinase inhibitors and need to be treated with conventional chemotherapy. An *in vivo* clinical trial should be performed to better demonstrate such effectiveness of celecoxib.

- *In vitro effects of treatment with RA, azacytidine (5-Aza-Cdr) singularly and in combination on follicular, anaplastic and MTC cell lines*. Conventional chemotherapy and radiotherapy are ineffective for the treatment of advanced thyroid tumors like poorly differentiated PTC, Anaplastic (ATC) and MTC. In the attempt to evaluate the possibility of using either RA, which is a re-differentiating agent, azacytidin (5-Aza-CdR, which is a de-methylating agent, or the combination of the two drugs in the treatment of thyroid cancer refractory to conventional therapy, we studied the effect of RA, 5-Aza-CdR singularly and in combination on three human thyroid cancer cell lines (WRO from FTC, FRO from ATC and TT from MTC) on cell growth, proliferation, apoptosis, iodide uptake and gene expression.

  We observed that RA inhibited cell growth in only the FTC and WRO cell lines. In basal conditions, WRO, FRO and TT cells expressed only some thyroid differentiation genes and we found that RA treatment was unable to reinduce the expression of the differentiation genes. In particular, no recovery of NIS mRNA expression was seen, and as expected none of the cell lines was able to take up radioiodine. As WRO growth was found to be sensitive to RA treatment, we investigated the mRNA expression of its receptors RAR α, β and γ in basal and RA-treated cell lines. We found that both RAR α and RAR γ mRNA were expressed in all cell lines, while RAR β was expressed only in WRO and that its expression was significantly increased during RA treatment. We showed that the RARβ2 promoter was not methylated in the three analyzed cell lines. Nevertheless, the treatment of cell lines with 5-Aza-CdR was able to inhibit cell growth in all three cell lines, by means of apoptosis and inhibition of DNA synthesis. Interestingly, the treatment with 5-Aza-CdR was able to induce an increase in RARβ mRNA levels in FRO cell lines and the expression of NIS mRNA in TT cells. Unfortunately, the ^{125}I uptake test did not show any active iodine concentration.

  WRO and FRO cells did not show any redifferentiation when treated with 5-Aza-CdR, but the combined treatment inhibited cell growth in all cell lines more strongly with respect to the single drugs. The growth in soft agar was reduced by 5-Aza-CdR alone in all cell lines, by RA alone only in WRO cells and by the combination of the two drugs more strongly in all cell lines. No data could be generated with the TT cell line, which was unable to grow in soft agar after two months of culture. The growth inhibition obtained with the combined treatment was mainly due to the apoptosis and inhibition of DNA cells. The combined treatment determined an increase in RARβ mRNA in all cell lines and in NIS mRNA in FRO and TT cells, but we did not observed any significant redifferentiation of cells. The ^{125}I uptake test was negative, thus clearly demonstrating that the ability to take up

iodine was not regained by any of the analyzed cell lines. Immunofluorescence suggested that the induced NIS protein was not functional because it did not reach the right localization on the plasma membrane.

These studies have been performed in parallel with a clinical *in vivo* study in which metastatic patients affected with tumors which were no longer able to take up iodine were treated with RA for three months and then retested for the ability to take up iodine. The *in vivo* study demonstrated the ability to regain the iodine uptake in only 40% of patients. Furthermore, the ^{131}I treatment of these patients did not obtain any clinical advantage because the level of iodine uptake was very low and very transient. These studies have been concluded at the moment.

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Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
Protocol XL184-301: an international, randomized, double-blinded, phase III, efficacy study of XL184 *versus* placebo in subjects with unresectable, locally advanced, or metastatic MTC	2009	Exelixis	9
Protocol E7080-G00-201: a phase II multicentric open label single arm trial to evaluate the safety and efficacy of oral E7080 in medullary and ^{131}I refractory, unresectable DTCs, stratified by histology	2009	Eisai	3
A multicenter, open-label, randomized, phase II/III study to evaluate the safety and efficacy of combretastatin A-4 phosphate in combination with paclitaxel and carboplatin in comparison with paclitaxel an carboplatin against anaplastic thyroid carcinoma. Protocol No. OXC4T4-302	2008	Oxygene	12

Description	Year	Sponsor	Number of patients recruited to date
Study D4200C00058: an international, phase II, randomized, double-blinded, placebo-controlled, multicenter study to assess the efficacy of ZD6474 (ZACTIMA™) *versus* placebo in subjects with unresectable locally advanced or metastatic MTC	2007	AstraZeneca	24
Study D4200C00068: a phase II, open-label study to assess the efficacy and tolerability of ZD6474 (ZACTIMA™) 100mg monotherapy in subjects with locally advanced or metastatic hereditary MTC	2007	AstraZeneca	4
Study AG-0137736: a phase II study of the anti-angiogenesis agent AG-013736 patients with metastatic or unresectable locally-advanced thyroid cancer refractory to, or not suitable candidates for ^{131}I treatment	2007	Pfizer	3
AMG 706 20050130: an open label treatment extension study of AMG 706	2006	Amgen	6
AMG 706 20040273: a phase II, open label study of AMG 706 to treat subjects with locally advanced or metastatic thyroid cancer	2005	Amgen	12
Clinical study for follow-up of thyroid cancer patients who received thyroid remnant ablation, THYR01605	2004	Genzyme	13

Research Grants

Year	Funding Agency	Amount
2007-2009	Ministero della Salute, Regione Campania	€ 62,750
2007-2009	Istituto Toscano Tumori	€ 225,000
2007-2009	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 70,000/ year
2007-2008	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR)	€ 84,286
2007-2008	MIUR	€ 52,857
2006	AIRC	€ 20,000
2005-2007	AIRC	€ 75,000/ year
2005-2006	MIUR	€ 71,500
2005-2006	AIRC	€ 40,000/ year
2005	AIRC	€ 20,000

Main Collaborations

With Units within ITT

» Department of Surgery, Section of Pathology, AOU Pisana
» Department of Internal Medicine, Section of Pharmacology and Chemotherapy, AOU Pisana
» Department of Oncology, Transplantation and New Medical Technology. AOU Pisana
» Department of Internal Medicine, Azienda Ospedaliero Universitaria Careggi, Firenze
» Department of Internal Medicine, Endocrinology and Metabolism, Section of Endocrinology, Azienda Ospedaliero Universitaria Senese

With other Italian and Foreign Institutions/Organizations

» Department of Internal Medicine, University of Perugia
» Department of Cellular and Molecular Biology and Pathology, University of Naples
» Institute of Endocrine Sciences, University of Milan
» Section of Nuclear Medicine, Institute Gustave Roussy, Villejuif (France)
» Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire, Université Libre de Bruxelles (Belgium)
» Human Cancer Studies Group, Swansea Clinical School, University of Wales (UK)
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» Memorial Sloan-Kettering Cancer Center, Division of Endocrinology, New York (USA)
» Johns Hopkins University, Division of Endocrinology, Baltimore, Maryland (USA)
» University of Pittsburgh, Division of Pathology, Pittsburgh, Pennsylvania (USA)
» University of Texas, M.D. Anderson Cancer Center, Division of Internal Medicine Houston, Texas (USA)
» National Cancer Institution (NCI), National Institute of Health (NIH), Bethesda, Maryland (USA)

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NUCLEAR MEDICINE

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Introduction

In addition to delivering the routine applications of diagnostic and therapeutic nuclear medicine within the Azienda Ospedaliero Universitaria Pisana (AOU Pisana), the Regional Center of Nuclear Medicine is the

site of the Post Graduate Specialty School in Nuclear Medicine of the University of Pisa Medical School, presently with 12 full-time Residents. The center also serves as a training institution for the International Atomic Energy Agency (IAEA, Wien), and carries out close collaborations with the Institute of Clinical Physiology of the Consiglio Nazionale delle Ricerche (IFC-CNR, where both the small-animal scanner YAP-(S)PET and the cyclotron for radiopharmaceutical production are located), with the National Institute of Nuclear Physics and with both preclinical and clinical institutes within the Universities of Pisa and Florence. Furthermore, the center has coordinated inter-university government-funded research projects focused on oncology, neurology, and infectious/inflammatory disease (some of which are still ongoing). In particular, studies in the oncological field are focused on breast cancer diagnosis and tumor treatment with new tumor-seeking radiopharmaceuticals. MDR mechanisms of drug-resistance and apoptosis have been the core of preclinical and translational studies aiming at the development of new radiopharmaceuticals. Our studies also concern the development of a new form of receptor-mediated radiometabolic therapy, based on the use of a Lutetium-177-labeled somatostatin analog. Moreover, a number of preclinical evaluation studies, still ongoing, have been performed in a mouse model of breast cancer. The center is also involved in the clinical development of new radiopharmaceuticals for radioimmunotherapy (RIT). Currently, we are running two phase I trials with stromal-antigen-specific small immunoproteins (SIP format) radiolabeled with iodine-131. Clinical trials evaluating the efficacy of combined chemo-radiotherapy regimens, specifically a bone seeking agent (^{153}Sm-EDTMP) and docetaxel in hormone-refractory metastatic prostate cancer, and RIT + EBRT + chemotherapy in advanced lung cancer patients are also ongoing. Furthermore, since current nuclear medicine techniques are part of any new pharmacological clinical trial (such as, *e.g.*, bone scintigraphy or [^{18}F]FDG-PET/TC), the center is involved in a number of clinical developmental protocols, working in a GCP compliance modality.

Main Research Themes

1. ^{99m}Tc-Annexin V

Study of response to chemotherapy with taxol in a murine model of spontaneous breast cancer. Animals were treated with a single dose of chemotherapy and sacrificed after 1, 3, 6 and 24 hours after treatment to assess the biodistribution of ^{99m}Tc-Annexin V and other parameters related to apoptosis.

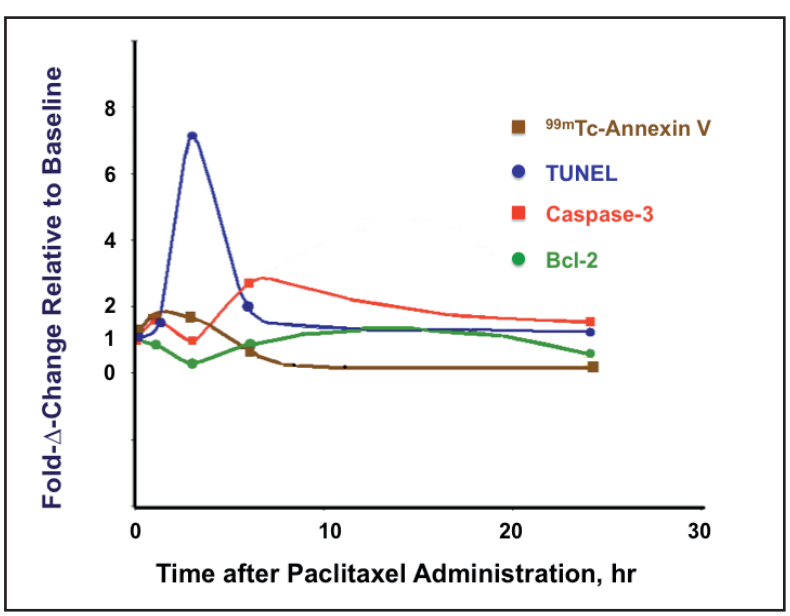

Figure1

2. ^{111}In-Biotin

- ^{111}In-Biotin can be used as an infection indicator.
- *In vitro* assays are performed on different bacteria to demonstrate the radiopharmaceutical uptake.

3. *In vitro/in vivo* characterization of new SST DOTA analogs

- The aim of this project is to evaluate new SST-analogs recently prepared through a ring-closing metathesis of the on-resin linear octapeptide carrying two allyl glycines in the place of the corresponding cysteine residues. These molecules keep the same sequence as octreotide, the standard SST-analog in clinical practice, but do not contain the disulphide bridge that may be cleaved by endogenous reducing agents. The project is focused on the evaluation of the new analogs after labeling with radiometals (^{111}In/^{90}Y/^{177}Lu), as far as binding, internalization and cellular retention are concerned on cell lines expressing SSTRs, and the biodistribution profiles of these analogs.
- Evaluation of ^{111}In/^{177}Lu DOTA-SST analogs on cell-lines expressing SSTR2 and biodistribution of the same radiopeptides in nude mice with xenografted tumors.
- Evaluation of the apoptotic pathway and cytotoxic activity induced by treatment with SST analogs.

Reference

Lazzeri E, Erba P, Perri M, Doria R, Tascini C, Mariani G: *Clinical impact of SPECT/CT with In-111 biotin on the management of patients with suspected spine infection*. Clin Nucl Med 2010; 35: 12-7.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
A phase I/II dose finding and efficacy study of the tumor targeting human ^{131}I-L19 monoclonal antibody in patients with cancer	2008	Philogen	23
A phase I/II dose finding and efficacy study of the tumor targeting human ^{131}I-F16 monoclonal antibody in patients with cancer	2009	Philogen	12
Multicenter study for lymphoma staging by PET-CT with contrast agent	2009	AOU Pisana	1
Multicenter study for lung cancer staging and restaging by 18F-FDG PET-CT, with and without contrast agent	2009	AOU Pisana	2
Myocardial dissynchrony and sympathetic activity by cardiac gated – SPECT in patients with heart failure treated with cardiac resynchronization	2009	AOU Pisana	15
Multicenter, randomized, phase III clinical study comparing 153Sm-EDTMP and docetaxel + prednison *versus* docetaxel + prednisone in patients with hormone-refractory prostate cancer, Taxane naïve	2008	AOU Pisana	6

Research Grants

Year	Funding Agency	Amount
2008	Fondazione Cassa di Risparmio di Lucca	€ 25,000
2006	Fondazione Cassa di Risparmio di Pisa	€ 50,000
2005	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) – PRIN	€ 124,000
2003	MIUR – PRIN	€ 185,000

Main Collaborations

With Units within ITT
- » Medical Oncology Unit, AOU Pisana
- » Hematology Unit, AOU Pisana
- » Department of Radiotherapy, AOU Pisana
- » Breast Surgery Unit, AOU Pisana
- » Medical Oncology Unit, Azienda USL 1 Massa e Carrara
- » Breast Surgery Unit, Azienda USL 12 Viareggio

With other Italian and Foreign Institutions/Organizations
- » Istituto Europeo di Oncologia (IEO), Milano
- » Istituto Clinico "Humanitas", Milano
- » Institute of Clinical Physiology, CNR, Pisa
- » Istituto Nazionale di Fisica Nucleare, Pisa
- » University Medical Center, Groningen (The Netherlands)
- » Memorial Sloan-Kettering Cancer Center, New York (USA)
- » Dana Farber Cancer Center, Boston, Massachusetts (USA)

Publications

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8. Ricci S, Boni G, Pastina I et al: *Clinical benefit of bone-targeted radiometabolic therapy with (153)Sm-EDTMP combined with chemotherapy in patients with metastatic hormone-refractory prostate cancer.* Eur J Nuclear Med Mol Imaging 2007; 34: 1023-30.
9. Manassero F, Erba P, Mariani G, Giannarini G, Mogorovich A, Selli C: *Metastatic prostate cancer after orchiectomy, radiotherapy and testosterone replacement in a patient with bilateral seminoma.* J Androl 2007; 28: 10-3.
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14. Ambrogi MC, Dini P, Boni G, et al: *A strategy for thoracoscopic resection of small pulmonary nodules.* Surgical Endoscopy-Ultrasound and Interventional Techniques 2005; 19: 1644-7.
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GENETIC ONCOLOGY

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Introduction

This Unit began its activity in the Division of Pathological Anatomy and Molecular and Ultrastructural Diagnosis – Department of Oncology, University of Pisa, director Prof. Generoso Bevilacqua, in 1989. At this time, the main research interest of this Unit is the genetic basis of hereditary cancer, particularly

breast and ovarian cancers. Specifically, we are concerned with the identification of new breast cancer and susceptibility genes, the molecular basis of BRCA1 and 2-driven carcinogenesis, the definition of BRCA1 and 2 mutational spectrum in the Italian population and identification of the genetic modifiers of BRCA1 and 2 gene penetrances. The Unit participates to the main Italian networks on hereditary cancer, such as InTEF, and it is also involved in European collaborative research groups, such as the European Breast Cancer Linkage Consortium, funded as the European Concerted Actions by BIOMED I and II European Community, and it is now part of the CIMBA (Consortium of Investigator of Modifiers of BRCA).

Main Research Themes

1. Assessment of biological effects of BRCA1 and BRCA2 unknown pathological variants by developing *in vitro* functional assays and multimodal approaches

Main achievements:

- Multimodal assessment of protein functional deficiency supported the pathogenicity of BRCA1 p.V1688del (3).
- A yeast recombination assay was proposed as useful to characterize human BRCA1 missense variants of unknown pathological significance (8).
- Characterization of gene expression profiles of yeast cells expressing BRCA1 missense variants (5).

Current work: Recombination based-assays to characterize BRCA1 missense variants in Hela human cells.

Future work: Assessment of BRCA2 missense variants by using functional assays in yeast *S. cerevisiae* and in human breast cancer cell lines.

2. RNA-based analysis of BRCA1 and BRCA2 variants localized in canonical and non-canonical splice-sites

Main achievements: RNA-based analysis of BRCA1 and BRCA2 gene alterations (17). Two mutations of the BRCA2 gene at the exon and splicing site in a woman who underwent oncogenetic counseling (6).

Current and future work: Analysis of BRCA genes variants in non-canonical splice-sites.

3. Identification of genetic variants in known low penetrance breast cancer susceptibility genes

Main achievements:

- Germline mutations of the BARD1 gene were found in breast and breast/ovarian families negative for BRCA1 and 2 alterations (29).
- A novel germ-line mutation impairing protein stability and function was identified in BRIP1 (FANCJ/BACH1) in 5% of hereditary breast cancer families (9).
- Identification of novel alternatively spliced BRCA1-associated RING domain (BARD1) messenger RNAs in human peripheral blood lymphocytes and in sporadic breast cancer tissues (15).
- The CHK21100delC mutation plays an irrelevant role in breast cancer predisposition in Italy (24).

Current work: Analysis of germline mutations in the PALB2 gene in HBC families lead to the identification of a novel truncating mutation.

Future work: Further mutational screening of candidate genes in high risk breast cancer patients tested negative for BRCA1 and BRCA2 germline mutations.

4. BRCA1 founder mutations

Main achievement: Reconstructing the genealogy of a BRCA1 founder mutation 1499insA by phylogenetic analysis (11).

5. Study of genetic modifiers of cancer risk in BRCA1 and BRCA2 mutation carriers

Most of these studies are in collaboration with an international collaborative group of researchers (CIMBA). The aim of CIMBA is to provide sufficient sample sizes to allow large scale studies, in order to reliably evaluate the effects of genetic modifiers.

Main achievements:

- Evaluation of a candidate breast cancer associated SNP in ERCC4 as a risk modifier in BRCA1 and BRCA2 mutation carriers (1).
- Common variants in LSP1, 2q35 and 8q24 and breast cancer risk for BRCA1 and BRCA2 mutation carriers (4).
- No evidence that GATA3 rs570613 SNP modifies breast cancer risk (7).
- Common breast cancer predisposition alleles are associated with breast cancer risk in BRCA1 and BRCA2 mutation carriers (10).
- Methyl group metabolism gene polymorphisms as modifier of breast cancer risk in Italian BRCA1 and 2 carriers (16).

Research Grants

Year	Funding Agency	Amount
2008-2009	Fondazione Cassa di Risparmio di Pisa	€ 80,000
2007-2010	Ministero della Salute, Progetto Integrato Oncologia	€ 250,000
2005-2007	Associazione Italiana per la Ricerca sul Cancro (AIRC), Toscana	€ 146,000

Main Collaborations

With Units within ITT

» Center of Statistical Genetics, University of Pisa
» Laboratory of Gene and Molecular Therapy, Institute of Clinical Physiology, Consiglio Nazionale delle Ricerche (CNR), Pisa
» Senology Unit, Azienda Ospedaliero Universitaria Pisana (AOU Pisana)
» Radiodiagnostic Unit, AOU Pisana
» Molecular and Nutritional Epidemiology Unit, Istituto per lo Studio e la Prevenzione Oncologica (ISPO), Firenze

With other Italian and Foreign Institutions/Organizations

- Department of Experimental Pathology, Medical Biotechnology, Epidemiology and Infectious Diseases, University of Pisa
- Istituto Nazionale Tumori (INT), Milano
- Istituto Europeo di Oncologia (IEO), Milano
- Istituto Nazionale per la Ricerca sul Cancro (IST), Genova
- Istituto Superiore di Sanità (ISS), Roma
- Screening and Follow-up for Hereditary and Familial Cancer Unit, Department of Molecular and Clinical Endocrinology and Oncology, "Federico II" University, Napoli
- Departments of Cancer Biology and Medical Oncology and Center for Cancer Systems Biology, Dana-Farber Cancer Institute, Boston, Massachusetts (USA)
- CIMBA

Publications

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2. Michelucci A, Di Cristofano C, Lami A, et al: *PIK3CA in breast carcinoma: a mutational analysis of sporadic and hereditary cases.* Diagn Mol Pathol 2009; 18: 200-5.
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12. Becherini F, Castagna M, Iannelli A, et al: *Choroid plexus carcinoma: a new case associated with a novel TP53 germ line mutation.* Neuropathol Appl Neurobiol 2008; 34: 564-8.
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23. Tancredi M, Sensi E, Cipollini G, et al: *Haplotype analysis of BRCA1 gene reveals a new gene rearrangement: characterization of a 19.9 KBP deletion.* Eur J Hum Genet 2004; 12: 775-7.
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25. Sensi E, Tancredi M, Aretini P, et al: *P53 inactivation is a rare event in familial breast tumors negative for BRCA1 and BRCA2 mutations.* Breast Cancer Res Treat 2003; 82: 1-9.
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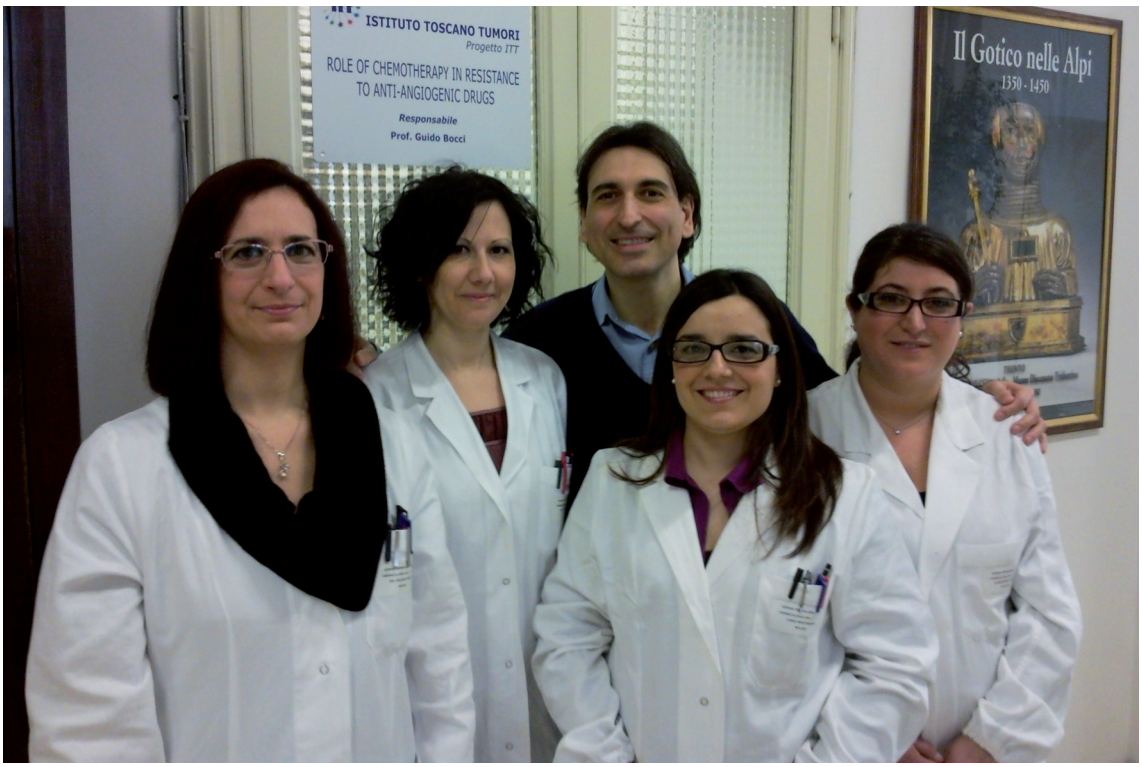
TRANSLATIONAL ONCOLOGY AND ANTIANGIOGENIC THERAPIES



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Introduction

The scientific activity of the Unit started at the beginning of 2004 in the laboratories of the Division of Pharmacology and Chemotherapy of the University of Pisa. The main research activity of the Unit has been focused on antiangiogenic research, including *in vitro* and *in vivo* experimental settings, and

clinical pharmacology of chemotherapeutic drugs, including pharmacokinetic and pharmacodynamic studies in cancer patients. Recently, the Unit has been involved in the development of the metronomic/antiangiogenic chemotherapy concept in both preclinical and clinical trials, in surrogate markers for antiangiogenic therapies such as monoclonal antibodies and tyrosine kinase inhibitors and in the pharmacogenetic of drugs used in the treatment of colorectal cancer. Moreover, the Unit has participated in research on angiogenesis-related pathologies, such as endometriosis and age-related macular degeneration, in order to find novel pharmacological approaches to the treatment of these diseases.

Main Research Themes

1. Preclinical and clinical metronomic chemotherapy

Main achievements: Role of thrombospondin-1 in the mechanism of action of metronomic chemotherapy; determination of cost-effectiveness of metronomic treatments; preclinical and clinical activity of metronomic irinotecan schedules in gastrointestinal tumors; preclinical and clinical efficacy and pharmacodynamic markers of metronomic cyclophosphamide combined with celecoxib in metastatic prostate cancer patients.

Current work: Preclinical and clinical development of new metronomic chemotherapies (*e.g.* oral fluoropyrimidines) in colorectal and prostate cancer and the related surrogate markers for their antiangiogenic and antitumor activities.

Future work: Preclinical activity of low dose ceramide analogs on pancreas cancer models and pharmacokinetics, pharmacodynamics and pharmacogenetics of metronomic chemotherapies.

2. Pharmacodynamics and pharmacogenetics of antiangiogenic therapies

Main achievements: Preclinical identification of ligand plasma levels as surrogate markers for the optimal dose of anti-VEGFR-2 antibodies; identification of possible biomarkers of bevacizumab treatment, such as plasma VEGF, in immunodepleted samples of patients; identification of genetic discordance between tumor and germline VEGF genotype.

Current work: Pharmacokinetic, pharmacodynamic and pharmacogenetic profiles of patients treated with chemotherapy and antiangiogenic therapies (*e.g.* bevacizumab, sorafenib and cetuximab) in phase II and phase III clinical trials; preclinical models of combined schedules of chemotherapeutic drugs and antiangiogenic compounds (*e.g.* irinotecan in combination with axitinib or sunitinib in pancreas and thyroid cancer) and related biomarkers of efficacy and toxicity.

Future work: Development of mathematical and software tools based on pharmacokinetic, pharmacodynamic and pharmacogenetic data to identify candidate patients to successful antiangiogenic therapies.

3. Synthesis and pharmacological activity of novel tyrosine kinase inhibitors

Main achievements: Synthesis and patenting of 15 novel tyrosine kinase inhibitors (RTKIs) for EGFR, VEGFR-2 and RET and their *in vitro* activities in different cancer and endothelial cell lines.

Current work: *In vivo* activity of new RTKIs in thyroid cancer models; combination of new RTKIs with chemotherapeutic drugs (*e.g.* irinotecan); synthesis of further compounds.

Future work: Preclinical development of the most promising compound in other cancer models, such as lung, colorectal and pancreatic carcinomas.

4. Role of chemotherapy in antiangiogenic drug resistance

Main achievements: Identification of VEGF upregulation *in vitro* and *in vivo* as a possible mechanism of the acquired resistance to the antiangiogenic activity of metronomic irinotecan; indeed, protracted low concentrations of oxaliplatin and 5-FU do not significantly affect VEGF secretion in cancer cells; on the contrary, a small but significant inhibition of VEGF secretion is induced by SN-38 (active metabolite of irinotecan), whereas there was a significant increase in VEGF levels in cells exposed to the three-drug combination; moreover, *in vivo*, the simple association of 5-FU and L-OHP to irinotecan determines a resistance to the antitumor and antiangiogenic effect of metronomic irinotecan alone, as shown by the tumor volume and microvessel density measures.

Current work: Setting up of representative preclinical *in vitro* and *in vivo* models that mimic the current standard combination therapies (chemotherapy + antiangiogenic drugs) in gastrointestinal cancers in order to obtain useful data on the modulation of antiangiogenic resistance by chemotherapeutic drugs.

Future work: Investigation in a preclinical model and in clinical settings (phase II and phase III clinical trials) of the role of different pro- and antiangiogenic factors and other related proteins in the resistance of antiangiogenic therapies (metronomic chemotherapy, RTKIs, bevacizumab) and how these proteins could be modulated by the use of different chemotherapeutic drugs.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
5-Fluoro Test: prospective pharmacokinetic study on patients diagnosed with colorectal cancer who are candidates for treatment with fluoropyrimidine	2007	University of Pisa	190
Degene: pilot study on the association of VEGF gene polymorphism with clinical efficacy and tolerability in patients affected with wet-type age-related macular degeneration (neovascular)	2007	University of Pisa	43

Research Grants

Year	Funding Agency	Amount
2009	Fondazione Cassa di Risparmio di Lucca	€ 30,000
2007	Ministero dell'Istruzione, dell'Università e della Ricerca – PRIN (two year grant)	€ 35,000
2005	Fondazione Cassa di Risparmio di Lucca	€ 25,000
2004	Associazione Italiana per la Ricerca sul Cancro (AIRC) (three year grant)	€ 105,000

Main Collaborations

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» Medical Oncology Unit 2, Azienda Ospedaliero Universitaria Pisana (AOU Pisana)
» Medical Oncology Unit, Azienda USL 5 Pisa
» Division of Pathological Anatomy, Department of Surgery, University of Pisa

With other Italian and Foreign Institutions/Organizations

» Laboratory of Clinical Experimental Oncology, Istituto Tumori "Giovanni Paolo II", Bari
» Department of Pharmaceutical Sciences, University of Pisa
» Sunnybrook Health Sciences Centre, University of Toronto (Canada)

Publications

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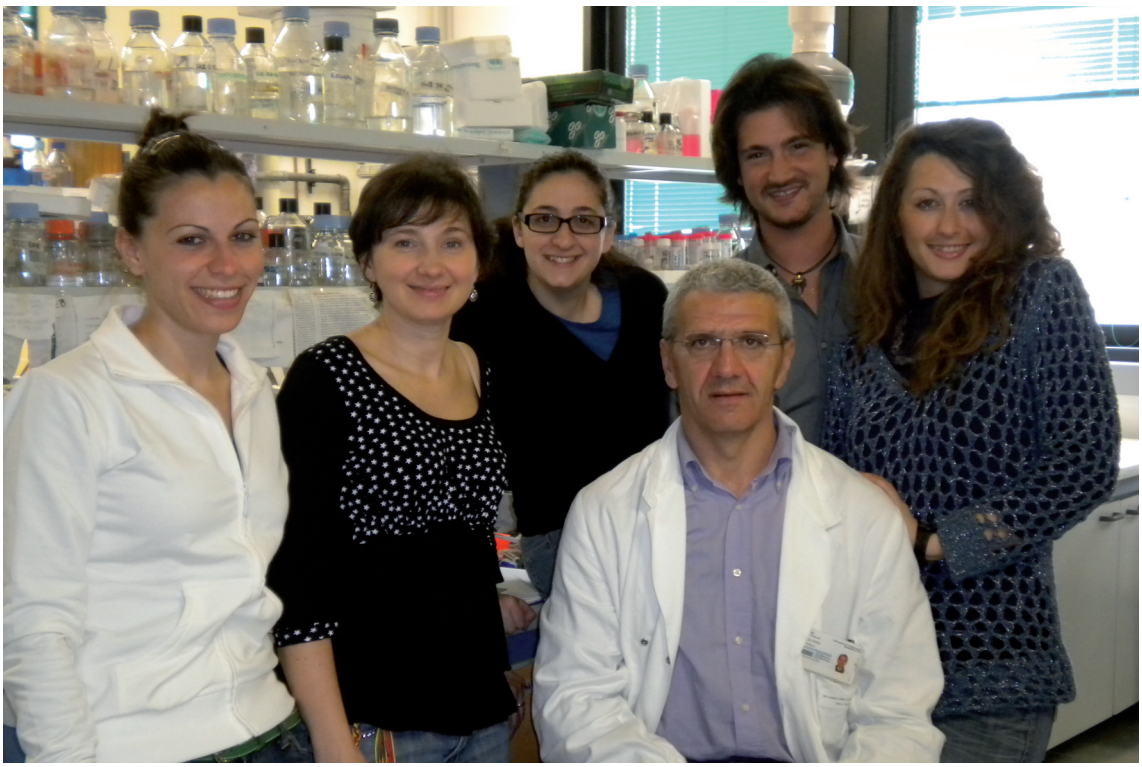
MOLECULAR GENETICS



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Introduction

Our group has been working on yeast genetics and recombination for many years. We have a great collection of yeast strains carrying mutations in the main pathways of homologous and non-homologous recombinations. Therefore, we are developing new tools to investigate the molecular basis of BRCA1/2 carcinogenesis by expressing these proteins in yeast strains carrying those mutation. Our group had

achieved an excellent experience on how to express BRCA1 mutant proteins in yeast and characterize the phenotype.
We are currently using the standard techniques and equipment to characterize the phenotype of the yeast strain carrying the mutations. We currently use site specific mutagenesis, PCR-mediated gene targeting, immunoprecipitation and immunofluorescence. We are also taking advantage of this excellent organism to encapsidate the adeno-associated virus, which represents a very promising tool for gene therapy.

Main Research Themes

1. Developing a yeast-based assay to characterize BRCA1 missense variants

Main achievements: Hereditary Breast/Ovarian Cancer (HBOC) is caused by, in 25-40% of cases, germ-line mutations in two major high-penetrance susceptibility genes: BRCA1 and BRCA2. Individuals carrying germ-line mutations in BRCA genes have a high risk of developing breast and/or ovarian cancer with an estimated lifetime risk of 35% to 80%.The tumor suppressor BRCA1 gene encodes a nuclear phosphoprotein whose principal role is in preserving genomic stability in DNA Double Strand Break repair by both Homologous Recombination (HR) and Non-Homologous End-Joining (NHEJ), and also in sister chromatid cohesion. The yeast *S. cerevisiae* is an excellent system to study the genetic control associated to DNA repair and checkpoint functions that are conserved in most eukaryotes (a). Yeast is a good organism model for studying BRCA1 functions since heterologous expression of human wild type BRCA1 inhibits growth, and this phenotype has been exploited to characterize several missense mutations localized within the BRCT domains. We recently reported that the expression of some BRCA1 cancer-related missense variants increased HR in yeast, while the neutral polymorphisms and BRCA1wt did not. We are trying to characterize the BRCA1 mutant and develop a novel functional assay to distinguish mutants from neutral polymorphisms.

Current and future work:

- *Evaluation of the effect of BRCA1 expression (both wild type and missense variants) on the genome stability*. To complete the analysis of BRCA1 effects in yeast genome instability or DNA repair, we will determine if BRCA1 affects the frequency of mutation on chromosome gain (aneuploidy) and on DNA DNB repair by NHEJ.
- *Effect of BRCA1 expression in HR and mismatch repair-defective yeast strains*. To identify which pathway is crucial for BRCA1-induced genome instability. We will express BRCA1 and the variants in yeast strains carrying deletion of the gene involved in HR and mismatch repair strains and measure the recombination and mutation frequency.
- *Evaluation of the novelly identified BRCA1 partners in human breast cancer susceptibility*. As most breast cancer genetic susceptibility still remains unexplained, we would like to take advantage of the "yeast-based" functional approach to identify new potential predisposing genes.

2. The yeast *Saccharomyces cerevisiae* as a cellular environment to study Adeno-Associated Virus (AAV) replication, integration and encapsidation

Main achievements: AAV vectors are among the most promising tools for human gene therapy. Ideally, a genetic disease can be treated by the expression of the functional gene or the correction of the mutated gene. As AAV transduces a variety of tissues and cells, these vectors are under investigation for treatment of a large number of diseases (b). Unfortunately, fundamental processes of AAV biology, such as replication, integration and genome dynamics are not completely understood. The characterization

of the mechanisms playing a role in AAV maintenance, AAV integration and transgene expression is essential in order to understand the potential of AAV in gene therapy. The yeast *S. cerevisiae* has been proven to be a very useful tool for the study of viruses. Therefore, the overall objective of this project is to study DNA replication and integration in yeast in order to use this model system for AAV vector production.

The objectives we want to achieve are a further characterization of AAV genome replication, understanding the role of Rep proteins in AAV DNA replication and integration, and succeed in eventual AAV encapsidation for vector production in yeast. A more profound knowledge of AAV biology will provide essential background to understand and evaluate the potentially of AAV vectors for therapeutic application.

Current and future work: To increase the knowledge of AAV biology in order to better understand the potential of AAV in terms of application in gene therapy, we are using the yeast *Saccharomyces cerevisiae* as genetic system.

- *Role of cis and trans elements for AAV DNA replication*. The current AAV DNA replication model is subdivided into several steps, which include ITR-primed polymerization, nicking of the ITR, DNA double strand displacement and formation of a single-stranded DNA).
  Our results showed that AAV DNA is indeed able to autonomously replicate in yeast. Moreover, a single-stranded DNA molecule has been identified only in those clones derived from yeast expressing the Rep68 protein. To further characterize the molecular mechanism of AAV replication in yeast, we will confirm the presence of single-stranded DNA in a greater number of clones; we will then investigate the role of ITR and other parts of the AAV genome, such as the Rep sequence and the promoters. It has been reported that helicase activity and DNA nicking activity of the Rep proteins are required for AAV replication in human cells We will therefore determine the effect of the expression of Rep proteins on AAV DNA replication in yeast.
- *Role of host proteins for AAV DNA replication*. The AAV has a very simple genome and does not carry any sequence encoding proteins directly involved in DNA replication, such as DNA polymerases or ligase. Several viruses, such as Adenovirus, Herpes virus, Vaccinia virus and Human Papilloma virus, can provide the helper activity required for AAV growth. The protein, Rad52, has been proposed to have a role in AAV genome processing by binding the single-stranded and the protein Mre11 associates with the incoming AAV genome, inhibiting AAV genome processing. To better understand the role of Rad52 and other DNA binding proteins, and to identify novel factors involved in AAV replication, we will then take advantage of yeast genetics. We will investigate the role of the recombination proteins XRS2, RAD50, MRE11, RAD52, Ku70 and Ku80, and DNA replication protein POL3 in a set of yeast strains isogenic to wild type RSY12 where the AAV DNA replicates efficiently.
- *Cell factors favoring AAV integration*. To date, very little is known about the molecular events of AAV infection in whole organisms. Recently, in an analysis of human tissues from children, AAV was found both as an episome and a random integrant. No site–specific integration has been found (c). As already mentioned, AAV can mediate gene targeting with a remarkably high efficiency, therefore AAV vectors are very promising tools for both scientific and therapeutic purposes (d,e).
  One of the most important limitations in the application of AAV to gene therapy is that AAV vectors, just like other viral vectors, can integrate randomly (d). This may potentially mutate functional genes that are essential for the cell. In yeast, we preliminarily showed that, when no homology is present between the AAV vector and chromosomal DNA, AAV DNA integrates as a single copy in basically all clones tested, whereas when a linear non–homologous DNA fragment without any AAV sequence is inserted in yeast, multiple integrations are detected.
  These intriguing results prompt us to investigate the molecular basis and the genetic requirement of this event. We will carry out experiments to test the effect of homologous and non-homologous DNA recombination genes and DNA replication genes on AAV integration when no homology between

the AAV vector and chromosomal DNA exists. As already reported in several non-homologous integration experiments, we will sequence the junctions where AAV is integrated to elucidate the molecular events and whether this integration event is mediated by micro-homology.

- *AAV protein expression and assembly in yeast*. Another aspect poorly understood in AAV biology is the regulation of the expression of Rep proteins, capsid proteins and their assembly. Indeed, their expression is thought to be a rate–limiting step in the production of rAAV. Rep gene expression appears to be critical for all steps of the AAV life cycle, including Cap expression It has been suggested that AAV–2 assembly occurs in two steps involving first the formation of empty capsids followed by the introduction of the newly replicated capsids into the preformed ones. The capsid consists of three structural proteins, VP1, VP2 and VP3, in a ratio of 1:1:10, which are expressed from the same open reading frame by using alternative splicing and an atypical start codon. In order to generate the three proteins, we used a 2.6 kb transcript from the p40 promoter spliced into two 2.3 kb mRNAs using the same splice donor site but different splice acceptor sites.

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Research Grants

Year	Funding Agency	Amount
2009-2011	Telethon	€ 60,500/year
2008-2009	Fondazione Cassa di Risparmio Pisa	€ 10,000/year
2003-2005	Associazione Italiana per la Ricerca sul Cancro (AIRC), Toscana	€ 15,000/year

Main Collaborations

With Units within ITT
» Genetic Oncology Unit, University of Pisa
» Department of Biochemistry, University of Pisa

With other Italian and Foreign Institutions/Organisations
» Scuola Normale Superiore, Pisa

Publications

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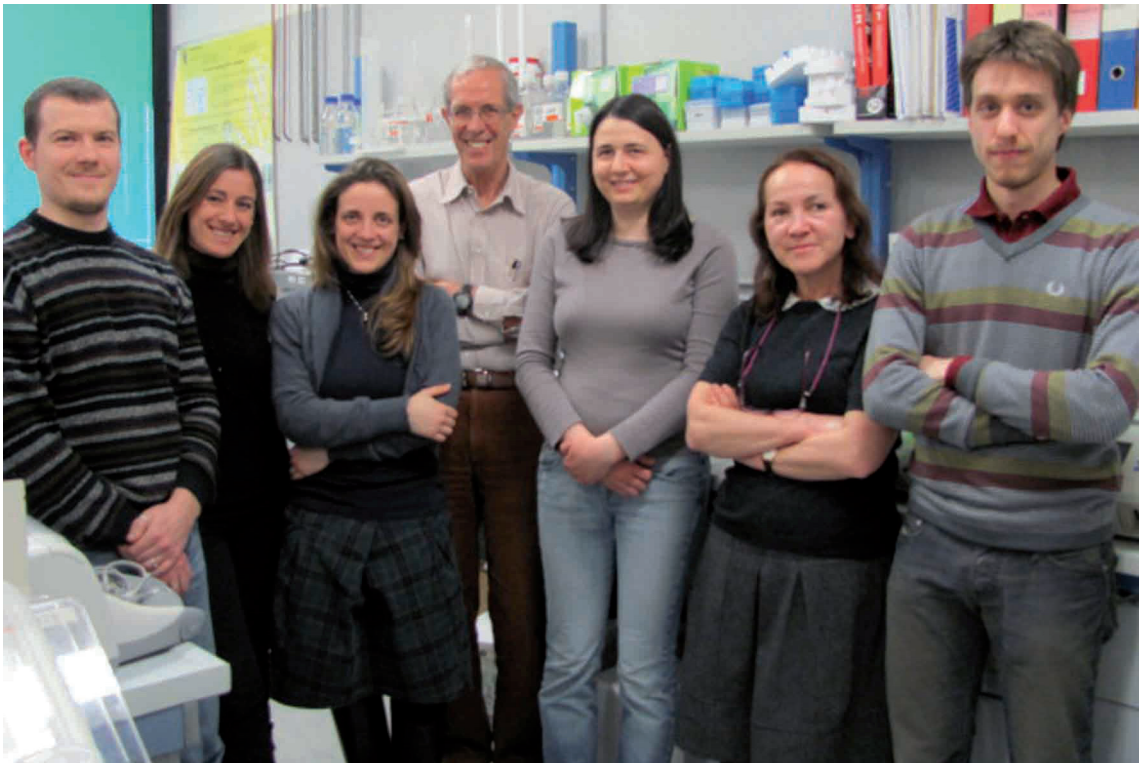
MOLECULAR BIOLOGY



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Introduction

The Laboratory of Molecular Biology of the Scuola Normale Superiore (LMB) is housed in the Institute of Clinical Physiology of the Consiglio Nazionale delle Ricerche (CNR) within the Institute's Laboratory of Gene and Molecular Therapy, with which it maintains a close scientific collaboration. Participating in the programs pertaining to oncology in the LMB are the Director, a senior researcher, a post-doctoral fellow, five graduate

students, one undergraduate student and one technician. The Laboratory is endowed with all the equipment and infrastructures needed for conducting research in cell biology, molecular biology and biochemistry.

Main Research Themes

1. DNA replication regulation, DNA topology and chromatin organization

Main achievements: DNA topology is known to play a key role in the activation of replication origins. In recent years, the interactions of topoisomerase I and II with the lamin B2 origin were investigated by the PI's lab (a). Both enzymes interact with different, precise sites – on either side of the start sites of bidirectional synthesis, on the templates of the leading strands, within the area covered by the replicative complexes – each at different moments of the cell cycle. Abolition of topoisomerase I activity at the G1/S transition inhibits the start of synthesis; both enzymes interact with the replication complex protein ORC2 on the origin at different moment of the cell cycle (a). The specific interactions of the two topoisomerases with the origin can be reproduced *in vitro*, either by the pure enzyme (topoisomerase I, hinting at a possible role of this enzyme in origin specification) or in the context of a multi-protein complex assembled on the origin (topoisomerase II) (a). Thus, modulation of the DNA topology in the origin area plays an important role in origin function. Furthermore, topoisomerase II binds *in vivo* to one more site, 170 bp removed to the left of the replicative complex area (a). This binding remains constant throughout the cell cycle and could correspond to a scaffold attachment region, considering that about 50% of the scaffold mass is given by topoisomerase II and the likelihood that a function of the scaffold is to isolate the chromosomal loops (each possibly corresponding to a replicon) from the structural and topological variations occurring in neighboring ones.

Local DNA topology and local chromatin organization are closely correlated. Possible function-related modifications of this structure could be reflected in a modification of the local DNA topology and affect the replicative complex transactions and the origin function. In the PI's lab, these correlated aspects of origin structure have recently been addressed in two ways. Firstly, the topological status of the origin area along its functional cycle was probed by UV-photofootprinting, a procedure based on the principle that the pyrimidine dimer formation induced by UV irradiation is blocked or enhanced by changes in DNA flexibility caused by the binding of proteins. The photofootprinting pattern of DNA extracted from cells synchronized in M, mid G1 and early S undergoes significant cell cycle-related variations, and topological alterations are detectable particularly in the region of the start sites, where RC proteins are bound. This is analogous to the described changes in DNA topology caused by the binding of different proteins to the ARS1 region of *S. cerevisiae*. Treatment with the histone deacetylase inhibitor trichostatin A (TSA) significantly modifies the pattern observed around the start sites. This disturbance reduces the ability of the origin to function, as well as to bind the replicative complex protein CDC6 and the newly discovered (see research theme 2) CDC6 partner, HOXC13.

Current work: More recently, a study of nucleosome distribution and composition around the RC area has been initiated based on the immuno-precipitation with specific anti-histone antibodies of mono-nucleosomes followed by the probing of the precipitates for the abundance of 12 DNA fragments of ≈ 160 bp each, spanning the origin area. Preliminary data demonstrate a high concentration of micrococcal nuclease hypersensitive sites in the RC area, in agreement with the contention of the presence of an "open" structure therein.

Future work: We intend to explore the chromatin organization and topology of the origin area along the cell cycle by the technique described above, utilizing antibodies *versus* specific modified histones, with the aim of obtaining a complete time-resolved picture of the position and chemical structure of

the nucleosomes around the area. The dynamics of these properties will be related with the dynamics of interaction with the replicative complexes of the two topoisomerases; also, the possibility that topoisomerase II residue bound 170 bp to the left of the replicative complex may correspond to a scaffold attachment site will be explored.

2. The lamin B2 origin and homeotic proteins

Main achievements: A search for human proteins binding specifically to the lamin B2 origin, performed with a one-hybrid screen, brought to the identification of only three homeotic proteins, HOXA13, HOXC10 and HOXC13 (b). HOXC10 and HOXC13 have specific affinities *in vitro* for the lamin B2 origin, in the area covered by the replicative complexes. HOXC10 and HOXC13 are present only in proliferating cells. The HOXC10 protein is a target of the proteasome pathway and disappears in mitosis, whereas mutations that make it resistant to ubiquitinylation disturb the cell cycle and cells accumulate in metaphase. Fluorescence microscopy analysis demonstrated that HOXC13 has an exclusively nuclear localization, co-localizes with replication foci thanks to its homeo-domain, and binds the lamin B2 (and two other early replicating origins, close to the *TOP1* and *MCM4* genes) in asynchronous cells (c). Thus, it is conceivable that HOX complexes may contribute to the identification of origins and assembly of replicative complexes on them, in association with other proteins and specific chromatin and topological configurations, and that homeotic transcription factors may offer tools for a cross-talk between the developmental processes and the regulation of genome duplication (d).

Current work: Subsequent studies (in collaboration with the lab of Prof. Fabio Beltram in Pisa) by FRAP (fluorescence recovery after photobleaching) and ChIP (chromatin immuno-precipitation), show that HOXC13 is very stably bound to chromatin, that it joins the lamin B2 origin in G1, binds it within the RC, reaches a maximum at the G1/S transition, leaves it during S and is absent in M and G0. Also HOXC10 binds the origin *in vivo*. HOXC13 interacts *in vitro* with ORC1, ORC2 and CDC6. FLIM (fluorescence lifetime imaging microscopy) analysis shows *in vivo* interaction of HOXC13 with ORC2 and CDC6 exclusively in G1. The interaction of HOXC13 (probably together with other homeoproteins) with replicative complexes is of general nature and not restricted to one particular origin.

Future work: We intend to study the composition of the homeo-protein complex specifically interacting with the origin, assessing whether the other homeoproteins demonstrated to bind it [HOXC10, see above and (b,d)], and other homeotic co-factors like the Meis1 protein, are present therein; investigate whether the homeocomplex bound to the lamin B2 origin has always the same composition or some components are interchangeable in different alleles or cells and in different origins capable to bind HOXC13; explore the possible involvement of homeoproteins in replication origins firing in different moments of the S phase.

3. The lamin B2 origin and oncogenic proteins

Main achievements: Chromosome translocations fusing the 5' portion of the *NUP98* gene with the *HOXC13* (and several other homeotic) gene(s), cause leukemia; also, translocations of the same portion of the same gene with the genes for topoisomerase I or II cause leukemia (d). The functional interaction of a well known oncoprotein, the product of the *c-MYC* proto-oncogene, with the lamin B2 origin, was demonstrated. Recently, in the PI's lab the presence of the product of the *c-Fos* proto-oncogene within the replicative complex of the lamin B2 origin and its specific affinity for the origin *in vitro* have been discovered. Furthermore, in the same lab, the presence of the HMGA1 protein in the replicative complex has also been demonstrated; this protein is involved in many DNA transactions and, although not specifically identified as oncoprotein, is systematically overexpressed in tumor cells; this protein has several AT-hooks (like the *S pombe* ORC4), binds AT-rich areas in the minor groove and specifically binds the origin *in vivo* and *in vitro*, within the pre-replicative complex area. Finally, in collaboration with the group of Dr. Alessandro Vindigni in Trieste we have demonstrated that two of the five RecQ helicases (several mutations in whose genes cause genome

instability and predisposition to cancer) interact specifically with the lamin B2 (and two other) origin(s): RecQ4 joins the origin in late G1-G1/S and RecQ1 at the onset of S, while both leave the origin after replication initiation. Depletion of either helicase reduces initiation frequency and cell proliferation, indicating that these enzymes play distinct important roles in replication regulation (e). Thus it appears that established and recently discovered members of the replicative complexes, when quantitatively or qualitatively altered, may disrupt the fine regulation of genome duplication and hence of cell proliferation.

Current work: We are currently studying the precise sites, moments of interaction with the origin and functional roles of the HMGA1, MYC and FOS proteins; we are also studying, by chemical cross-linking and biophysical (FRET-FLIM) methods, the interactions of these proteins with the other members of the replicative complexes; we are studying the interactions of the RecQ1 and RecQ4 helicases with replication proteins, in particular as concerns RecQ4 and the pre-initiation complex and as concerns RecQ1 and the growing fork proteins, to assess the possibility of specific roles of these two enzymes in initiation and fork advancement, respectively (e).

Future work: We intend to search for new members of the replicative complexes by *in vitro* studies, taking advantage of our ability to reproduce, with nuclear extracts, a large sequence-specific protein-DNA complex on the lamin B2 origin, resembling the pre-replicative complex (a), purifying the involved proteins and identifying them by immunologic and proteomic analysis. We also intend to study the structural deformations of the origin sequence interacting with replicative complex proteins by the use of advanced nanotechnology-based techniques: atomic force microscopy, optical tweezers and small-angle X-ray scattering (in collaboration with Prof. Giacinto Scoles of the ELETTRA synchrotron in Trieste), and, in collaboration with Prof. Beltram of the NEST laboratories of the Scuola Normale, nanoplasmonic resonance studies on origin fragments derivatized with gold nano-particles.

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Research Grants

Year	Funding Agency	Amount
2007-2009	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 150,000
2007-2009	Istituto Toscano Tumori	€ 180,000
2007-2008	Fondazione Monte dei Paschi di Siena	€ 100,000

Main Collaborations

With Units within ITT

» Laboratory of Gene and Molecular Therapy, Institute of Clinical Physiology, CNR, Pisa

With other Italian and Foreign Institutions/Organizations

» NEST Laboratories, Scuola Normale Superiore, Pisa
» Institute of Molecular Genetics, CNR, Pavia
» Genome Stability Group, International Centre for Genetic Engineering and Biotechnology, Trieste
» ELETTRA Synchrotron, Trieste

Publications

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GENE AND MOLECULAR THERAPY

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Introduction

The “RNA Interference” Research Unit was established in the Laboratory of Gene and Molecular Therapy (LTGM) of the Clinical Institute of Physiology in 2002. Since 2004, the main research focus has been microRNAs, which are trans-acting factors that regulate the expression of target mRNAs at a post-transcriptional level, using the RNA interference enzymatic machinery. miRNAs constitute almost 1% of the predicted human genes and each of them is believed to modulate, either alone or in combination with other miRNAs, the expression of an average of six different genes.

Main Research Theme

miRNA and gene expression networks in Mouse Embryo Fibroblasts (MEF) and lymphoma cells

Main achievements: Cellular senescence represents a stress response whereby cells experience irreversible cell cycle arrest. In MEF senescence can be induced by cellular stresses, such as the persistent mitogenic stimulation during propagation in culture, the overexpression or underexpression of single oncogenes, DNA damaging drugs. We demonstrated that in MEF, at early passages, miR-20a is able to induce cellular senescence. The direct downregulation of LRF, with the consequent induction of p19ARF, is a key mediator of the process, but cooperation with other pathways, represented by E2F1 down-regulation and p16 upregulation, appears to contribute. The finding that miR-20a is able to induce premature senescence is, in our opinion, interesting as it may have potential clinical relevance as an anti-tumorigenic drug.

Moreover, we also showed that LRF can affect MEF proliferation, senescence and apoptosis by controlling not only the p19ARF/p53 pathway, but also miRNA modulation. By comparing miRNA expression profiles of MEF transfected with scrambled or LRF-specific short interfering RNAs, we identified two intragenic miRNAs, miR-28 and miR-505, modulated by LRF. Using transactivation assay, we showed that LRF inhibits the promoter of the *Lpp* gene, which hosts miR-28, suggesting direct control of LRF on miR-28 expression, while direct interaction of LRF with the miR-505 host gene promoter could not be assessed. Both miR-28 and miR-505 are predicted to target the Alternative Splicing Factor/Splicing Factor2 (ASF/SF2). In vertebrates, loss or inactivation of ASF/SF2 may result in genomic instability and apoptosis. We showed that in MEF miR-28 and miR-505 modulate ASF/SF2 expression by directly binding ASF/SF2 3’UTR and that LRF downregulation results in an ASF/SF2 decrease. We found that the miR-28/miR-505/ASF/SF2 axis is coordinately modulated during MEF passages in culture. Moreover, alteration of each of the members of the axis affects MEF proliferation, as well as the number of senescent and apoptotic cells. Given the importance of alternative splicing in cell proliferation and cancer progression, the miRNA-mediated cooperation between the two proto-oncogenes, LRF and ASF/SF2, has many interesting implications.

We also demonstrated that miR-290 in MEF is causatively implicated in both culture-induced as well as NCZ-induced senescence, thus expanding the physiological role of this important miRNA previously reported as an embryonic stem cell-specific miRNA. It is interesting to note that miR-290 appears to be upregulated in connection with LRF downregulation, raising the interesting possibility that LRF, directly or indirectly, controls this miRNA. Moreover, miR-290 upregulation is always accompanied by the upregulation of the *Ink4A* locus (especially *p16*). In this regard, it will be interesting to establish whether there is a direct link between miR-290 and the *Ink4A* locus via inhibition of repressor/s.

All these results indicate that microRNAs and genes are integrated in the senescence regulatory network.

Current work: LRF and BCL6 are both oncogenic POK proteins aberrantly overexpressed in human lymphomas: little is known about the mechanism of this co-expression. We are currently involved in studying the role of microRNA (interference) in the LRF/BCL6 network of the Dohh2 lymphoma cell line. At present, we have data showing that the transient downregulation of BCL6 elicits a series of regulatory miRNAs/transcription factor circuitries which cause an adverse effect in the antiproliferative response. Interestingly, we have found that BCL6 silencing results in c-myc downregulation, which is counteracted by E2F1 upregulation, thus establishing a "futile" circuit. It is possible that Dohh2 lymphoma cells utilize this circuit in order to counterbalance the stop signals and continue cell proliferation. In order to avoid these counteractive effects and direct Dohh2 cells towards apoptosis, we propose to target, at the same time, components of the circuit which have opposing effects. For this purpose a Dohh2 cell line stably overexpressing miR-145, which negatively controls c-myc, will be transfected with either an E2F1-specific si-RNA or miR-20a, which negatively controls E2F1, in order to destroy the feedback loop, c-myc-miR-20a-E2F1.

Finally, we are starting a project aimed to replace miRNAs in tumor cells All types of cancer are characterized by abnormal miRNA expression profiles and on the basis of their over/underexpression they have been classified as onco/Tumor Suppressor (TS) miRNAs. Recent advances in this field have highlighted the role of TSmiRNAs as a new exciting frontier in the field of cancer therapy. The assumption upon which this project is based is that miRNAs which are homogeneously highly expressed in most normal tissues and underexpressed in most tumors could have broad antitumorigenic properties so that their restoration could inhibit tumor growth (replacement strategy). The aim of this project is to test the therapeutic properties of these miRNAs, using a replacement strategy first *in vitro* (tumor cell lines) and then *in vivo* (xenografts in nude mice).

Research Grants

Year	Funding Agency	Amount
2008-2010	Istituto Superiore di Sanità (ISS)	€ 160,000
2007-2010	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 150,000

Main Collaborations

With Units within ITT

» Department of Experimental Pathology and Oncology, Azienda Ospedaliero Universitaria Careggi, Firenze
» Department of Biology, Azienda Ospedaliero Universitaria Pisana (AOU Pisana)
» Department of Human Development and Applied Biology, AOU Pisana

With other Italian and Foreign Institutions/Organizations

» Scuola Normale Superiore, Pisa
» Scuola Superiore di Studi Universitari e di Perfezionamento "Sant'Anna", Pisa
» Department of Clinical, Morphological and Technological Sciences, University of Trieste
» Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York (USA)

Publications

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2. Decembrini S, Bressan D, Vignali R, et al: *MicroRNAs couple cell fate and developmental timing in retina*. Proc Natl Acad Sci USA 2009; 106: 21179-84.
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4. Turco SD, Basta G, Lazzerini G, et al: *Effect of the administration of n-3 polyunsaturated fatty acids on circulating levels of microparticles in patients with a previous myocardial infarction*. Haematologica 2008; 93: 892-9.
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Livorno Area

MEDICAL ONCOLOGY

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Manolo D'Arcangelo, Trainer in Medical Oncology
Gabriele Minuti, Trainer in Medical Oncology
Alessio Petronici, Nurse
Antonella Dal Canto, Nurse

Introduction

The Oncology Research Unit of "Ospedale Civile" in Livorno has started its activity recently and is formed by a young group of researchers. Some changes in the Unit's direction took place, bringing

about new lines of research. The Unit is now actively involved in clinical and translational trials on the three major cancer diseases (lung, colorectal and breast cancer). As a consequence of the high incidence of thoracic cancer in the geographical area of Livorno, the greatest effort of the Unit will be focused on this set, *i.e.* lung cancer and pleural mesothelioma. With respect to lung cancer, both clinical and translational projects will be conducted as an "inheritance" of the most important trials of the principal investigator. Regardless, the research line will double for all cancer types, including both clinical and translational aspects.

Main Research Themes

1. Lung Unit

Main achievements: The main achievements of the Lung Unit is to ensure new treatment options for lung cancer and pleural mesothelioma patients, offering complete clinical assistance.

Current work: Current research is directed toward evaluating standard chemotherapy in specific subsets of patients with advanced lung cancer, such as elderly patients and second-line treatment. Moreover, our effort is directed toward investigating tyrosine kinase inhibition with drugs acting on VEGF-R and EGFR pathways. With regard to pleural mesothelioma, we are working toward establish a specific route for multimodal adjuvant treatment, including not just the surgeon, but also the oncologist and radiotherapist, in a coordinated fashion.

Future work: In the future, our efforts will be directed along two principal paths. Firstly, basic research to evaluate biomolecular mechanisms of response and resistance to the new target therapies. Secondly, we will consider new trials testing innovative drugs inhibiting specific molecular pathways, such as MET, IGFR-1 and others.

2. Breast Unit

Main achievements: Research has been directed toward both the adjuvant and metastatic sets, with special regard to new treatment options and the possibility of choosing what treatment is best for a patient on the basis of tumor molecular characteristics.

Current work: At the moment, the Unit is working on a trial evaluating the efficacy of a new drug in patients with specific biological features.

Future work: In the near future, we are contemplating having strong collaborations with other ITT Units principally involved in breast cancer research.

3. Colorectal Unit

Main achievements: With respect to Colorectal Cancer (CRC), the Unit is working on ongoing clinical trials as a continuation of our past direction.

Current work: Currently, we are participating in trials testing triplet chemotherapy in association with anti-VEGF monoclonal antibodies and the use of bevacizumab after disease progression in the metastatic set.

Future work: Our efforts will be directed toward the biological aspects of CRC, especially those regarding the EGFR pathway and resistance to anti-EGFR agents. It would be of interest to our Unit to test new drugs, especially those with molecular targets, both in the adjuvant and advanced sets.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
A phase III, multicenter, placebo-controlled trial of sorafenib (BAY-43-9006) in patients with relapsed or refractory advanced, predominantly non-squamous, NSCLC after two or three previous treatment regimens. BAY43-9006/13266	2009	Bayer	1
Multicentric, open-label trial of expanded access of RAD001 in patients with metastatic renal cancer in progressive disease after therapy with vascular endothelial growth factor tyrosine-kinase receptor inhibitors or intolerance to this therapy – CRAD00122410	2009	Novartis	4
VANGOGH Study: vandetanib + gemcitabine or placebo + gemcitabine or vandetanib monotherapy in patients with advanced (unresectable or metastatic) biliary tract cancer	2009	AstraZeneca	3
A phase II, single-arm trial of BIBW 2992 (Tovok™) in EGFR FISH-positive NSCLC patients	2008	Boehringer Ingelheim	3
TRIBE study: a phase III, randomised trial of FOLFOXIRI + bevacizumab *versus* FOLFIRI + bevacizumab as first-line treatment for metastatic colorectal cancer	2008	Gruppo Oncologico del Nord Ovest (GONO)	26
An open-label, multicenter, randomized, phase III study of second-line chemotherapy with or without bevacizumab in metastatic colorectal cancer patients who have received first-line chemotherapy + bevacizumab. BEBYP-ASL607LIOM03	2008	GONO	21
Phase II, multicenter trial evaluating efficacy and safety parameters of treatment with daily doses of lapatinib in patients with advanced breast cancer with HER2-negative primary tumor and HER2 or EGFR-positive circulating cancer cells	2008	GlaxoSmithKline	7
PROMET-3: phase II clinical trial of metronomic chemotherapy with oral vinorelbine and dexamethasone in patients with advanced hormone-refractory prostate cancer: pharmacodynamic and pharmacogenomic evaluation	2008	Independent	38
GLIMESOR Study: sorafenib in combination with metronomic therapy with temozolomide in patients with glioblastoma multiforme after failure of first-line chemotherapy: phase II clinical study with pharmacodynamic, pharmacokinetic and pharmacogenomic evaluation	2008	Independent	23

Description	Year	Sponsor	Number of patients recruited to date
Phase II, multicenter trial of sequential chemotherapy with cisplatin/gemcitabine followed by docetaxel in elderly patients with advanced Non-Small Cell Lung Cancer (NSCLC)	2007	Independent	24
Phase II, randomized, double-blind, two-arm, parallel study of vandetanib (ZACTIMA™, ZD6474) + gemcitabine (Gemzar®) or gemcitabine + placebo as first-line treatment of advanced (stage IIIb or IV) NSCLC elderly patients. D4200L00012	2007	AstraZeneca	0
Study MK-0646: a phase II/III study of MK-0646 treatment in combination with cetuximab and irinotecan for patients with metastatic colorectal cancer	2007	Merck	1
Randomized, phase II study of pemetrexed *versus* pemetrexed and carboplatin as second-line chemotherapy in advanced NSCLC. Protocol GOIRC 02/2006. EudraCT number: 2006-004009-24	2006	Gruppo Oncologico Italiano di Ricerca Clinica (GOIRC)	4
PROMET-2: docetaxel and prednisone in combination with metronomic therapy with cyclophosphamide and celecoxib in hormone-refractory prostate cancer patients. Phase II clinical trial with pharmacodynamic and pharmacogenomic evaluation	2006	Independent	24

Main Collaborations

With Units within ITT
- » Radiotherapy Unity, Azienda USL 6 Livorno
- » Department of Pathology, University of Pisa
- » Division of Pneumology, Ospedale “Cisanello”, Pisa
- » Other ITT Oncology Units

With other Italian and Foreign Institutions/Organizations
- » Maugeri Foundation, Pavia
- » Monteluce Polyclinic, Perugia
- » Istituto Superiore Sanità (ISS), Roma
- » Colorado Cancer Center (USA)
- » Harvard University (USA)
- » Basilea University (Switzerland)

Publications

1. Metro G, Cappuzzo F: *Emerging drugs for small-cell lung cancer*. Expert Opin Emerg Drugs 2009; 14: 591-606.
2. Varella-Garcia M, Diebold J, Eberhard DA, et al: *EGFR fluorescence in situ hybridisation assay: guidelines for application to non-small-cell lung cancer*. J Clin Pathol 2009; 62: 970-7.
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5. Hirsch FR, Varella-Garcia M, Cappuzzo F: *Predictive value of EGFR and HER2 overexpression in advanced non-small-cell lung cancer*. Oncogene 2009; 28 Suppl 1: S32-7.
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9. Cappuzzo F, Jänne PA, Skokan M, et al: *MET increased gene copy number and primary resistance to gefitinib therapy in non-small-cell lung cancer patients*. Ann Oncol 2009; 20: 298-304.
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29. Metro G, Finocchiaro G, Cappuzzo F: *Anti-cancer therapy with EGFR inhibitors: factors of prognostic and predictive significance*. Ann Oncol 2006; 17 Suppl 2: ii42-45.
30. Cappuzzo F, Toschi L, Tallini G, et al: *Insulin-like growth factor receptor 1 (IGFR-1) is significantly associated with longer survival in non-small-cell lung cancer patients treated with gefitinib*. Ann Oncol 2006; 17: 1120-7.

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Introduction

The Division of Radiation Oncology of Livorno has a long tradition. Activity began in the 1930s under the guidance of Dr. Guido Zanotti, utilizing radium. After the Second World War, the Department was headed by Prof. Alberto Anzilotti, who began to treat patients with a Roentgen Unit, and who was then succeeded by Dr. Ario Crinelli in 1966 and Dr. De Marzi and Dr. Guido Atzeni in the years 1978-2002. In the 1970s, a Cobalt Unit was acquired and in the 1990s, linear accelerators were made available with a treatment planning system.

Now the Division consists of a Treatment Unit (two Linacs, one simulator, one plesiotherapy, one 3D TPS, the availability of a CT for 3D simulations, and a mould room) and a Recovery Unit with 10 beds. We are waiting for a new Linac for high-tech Radiotherapy (RT): IGRT, IMRT, volumetric IMRT (Rapid-Arc).

First and follow-up visits are managed in specific areas of the ITT “CORD/Accoglienza” Units.

Our activity is managed in close collaboration with the Health Physics Unit, particularly for dosimetric aspects and the implementation of the new TPS, in close collaboration with Medical Oncology and Surgery. We are also connected with the Oncology Department.

In 2008, we treated 855 patients using RT, distributed as follows:

- primary RT 200 patients;
- preoperative RT 6;
- postoperative RT 337;
- palliative RT 312.

We are also connected with the Gruppo Oncologico Multidisciplinare (GOM), dealing with the breast, lung, CNS and urology and gynecology.

Our past interest focused on accelerated hyperfractionated RT (with or without chemotherapy) in head and neck cancer, conducting some self-governed studies, and others in collaboration with Florence University RT and INT Milan (Oropharynx Cancer). We also took part in the STAR Study together with Pisa University RT.

We studied the role of local intra-arterial chemotherapy in malignant glioma in the 1990s.

Chemo-RT in larynx and hypopharynx cancer for organ preservation in collaboration with the Head and Neck Cancer Group of the AIRO (the Italian Association of Radiation Oncology)

Our present interest is concomitant chemo-RT in stage III lung cancer as a radical approach and threefold therapy in mesothelioma in collaboration with Medical Oncology and Thorax Surgery of Livorno.

Siena Area

MEDICAL ONCOLOGY

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Introduction

The clinical activity of the Oncology Department of AUSL 7 Siena is aimed to the treatment of cancer patients mainly focusing on breast, colorectal and lung cancer.
The activity is carried out in three locations: Medical Oncology of Campostaggia Hospital – Valdelsa, Medical Oncology of Nottola Hospital – Valdichiana and Medical Oncology of Amiata Hospital.
Within the Department, the activity of cancer home care with the provision of palliative care is also carried out in the four areas of the AUSL 7: Siena, Valdelsa, Valdichiana and Amiata.
The Department also collaborates with other local, regional and national hospitals.
Clinical research activities are conducted within the two hospitals. Several sponsored and spontaneous trials are currently being conducted.

Clinical Trials

1. Breast cancer

Description	Year	Sponsor	Number of patients recruited to date
EGF106708-ALTTO: a randomized, multicenter, open-label, phase III study of adjuvant lapatinib, trastuzumab, their sequence and their combination in patients with HER2/ErbB2-positive primary breast cancer	2008	GlaxoSmithKline	4
MO 19391 ATHENA: open label study of bevacizumab + taxanes, monotherapy or in combination, for first-line treatment of patients with locally recurrent or metastatic breast cancer	2007	Roche	8
TOP TRIAL: a randomized phase III clinical trial of trastuzumab optimization in patients with locally advanced and/or metastatic breast cancer overexpressing HER2 after first-line chemotherapy plus trastuzumab	2007	Istituto di Ricerche Farmacologiche “Mario Negri”	0

2. Colorectal cancer

Description	Year	Sponsor	Number of patients recruited to date
BEBYP: an open-label, multicenter, randomized, phase III study of second line chemotherapy with or without bevacizumab in metastatic colorectal cancer patients who have received first-line chemotherapy plus bevacizumab	2008	Gruppo Oncologico del Nord Ovest (GONO)	1
TOSCA: a randomized trial investigating the role of FOLFOX4 regimen duration (three *versus* six months) and bevacizumab as adjuvant therapy for patients with stage II/III colon cancer	2007	Istituto di Ricerche Farmacologiche “Mario Negri”	4

Description	Year	Sponsor	Number of patients recruited to date
ML 18522: phase II study of the combination of bevacizumab + capecitabine with preoperative standard radiotherapy in patients with locally advanced rectal cancer	2005	Roche	3

3. Lung cancer

Description	Year	Sponsor	Number of patients recruited to date
TAILOR: optimization of erlotinib for the treatment of patients with advanced Non-Small Cell Lung Cancer (NSCLC): an Italian randomized trial	2008	Istituto di Ricerche Farmacologiche "Mario Negri"	3
Phase II of GOIRC: gene expression as predictive markers of outcome in NSCLC patients (stage IIIB with pleural effusion and stage IV) treated with chemotherapy		Gruppo Oncologico Italiano di Ricerca Clinica (GOIRC)	Activation phase

4. Pancreatic cancer

Description	Year	Sponsor	Number of patients recruited to date
LIPOGEM PII 1L PANCR: multicenter phase II/ III clinical study of lipoplatin + gemcitabine as first-line treatment in inoperable, locally advanced or metastatic pancreatic cancer		Regulon Inc.	Activation phase

5. Observational study

Description	Year	Sponsor	Number of patients recruited to date
SOFISEP: a randomized clinical multicenter trial for the evaluation of the "lock" efficacy of CVC with physiological solution *versus* heparin solution	2009	Polo Oncologico di Biella	20
MASTER ONCOLOGY: multicenter, case-control on the epidemiology and risk factors for thromboembolic events in cancer patients and on the influence of VTE on patient outcome	2008	Sanofi-Aventis	9
OHERA: the observational study of cardiac events in patients with Her2-positive early breast cancer treated with trastuzumab. Phase IV study	2008	Roche	3
REEF: relief and efficacy on oral mucositis using Gelclair		Collegio Italiano dei Primari Oncologici Medici Ospedalieri (CIPOMO)	Activation phase

Description	Year	Sponsor	Number of patients recruited to date
RITMA4: a prospective observational study of breast cancer in the T4 stage, inflammatory and non-inflammatory. Role of the CYP19 Ex11+410G>T and other genetic polymorphisms in response to exemestane as first-line treatment in patients with metastatic breast carcinoma		Associazione Italiana di Oncologia Medica (AIOM), CIPOMO and Lega Italiana per la Lotta contro i Tumori (LILT)	0
P6: acupressure in the control of nausea and vomiting induced by chemotherapy		Dipartimento Sanità Pubblica di Firenze, ASP Catanzaro and Associazione Italiana Infermieri di Oncologia	Activation phase

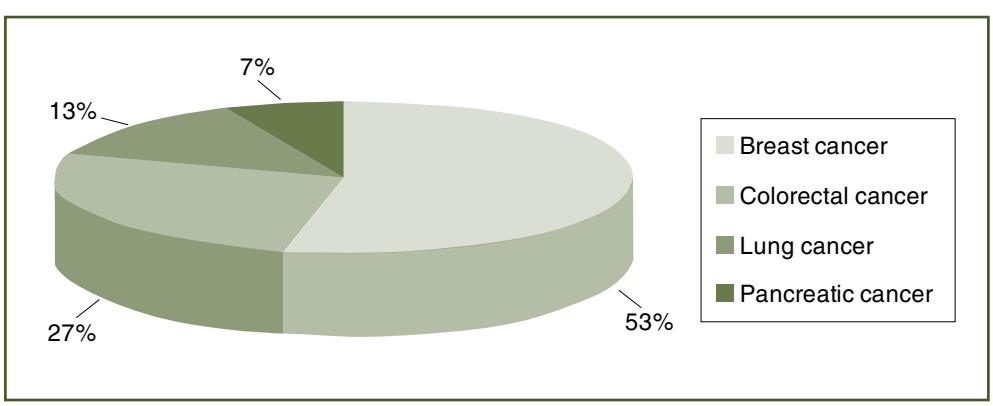

Figure 1 - Percentage of clinical trials for different diseases

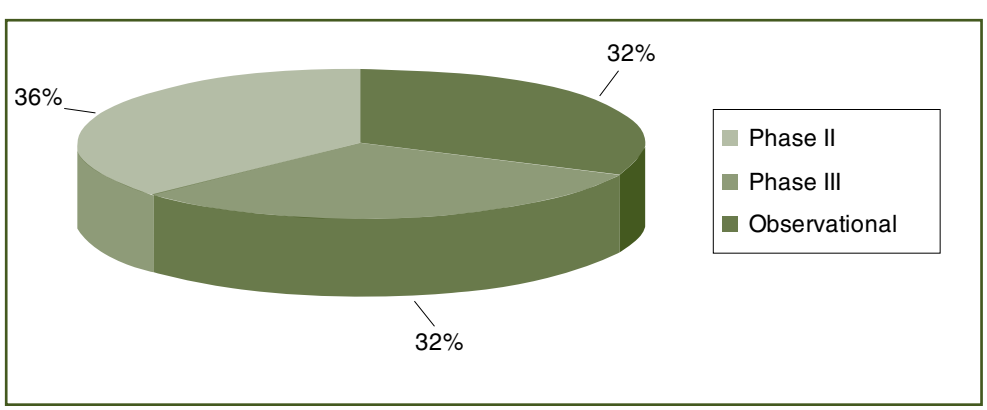

Figure 2 - Percentage of trials for clinical phase

Main Collaborations

With Units within ITT
» Department of Radiotherapy, Azienda Ospedaliero Universitaria Senese
» Medical Oncology Unit, Azienda USL 4 Prato
» Department of Radiotherapy, Azienda USL 2 Lucca
» Breast Surgery Unit, Azienda Ospedaliero Universitaria Careggi, Firenze

With other Italian and Foreign Institutions/Organizations
» Istituto Nazionale Tumori (INT), Milano
» Centro Riferimento Oncologico (CRO), Aviano (Pordenone)
» Istituto Europeo di Oncologia (IEO), Milano
» Istituto di Ricerche Farmacologiche "Mario Negri", Milano
» Memorial Sloan-Kettering, Cancer Center, New York (USA)

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MEDICAL ONCOLOGY

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Introduction

Our Medical Oncology Unit intends to provide the patient with a diagnostic-therapeutic pathway and medical treatment based on the most modern principles of experimental and clinical oncology.

Our activity is aimed at the diagnosis and treatment of the most common oncological pathologies. The operative Unit is an integral part of the regional center for the diagnosis and treatment of bone metastases attending to patients affected by primary and secondary bone tumors, and is an integral part of the Center of Oncopharmacological Research (CROF) of the University of Siena, which studies Translational Oncology and Immunotherapy. In the Department, several prospective studies, independent or sponsored in accordance with Good Clinical Practice requirements, on the pharmacological and/or immunological treatment of gastroenteric, urogenital, lung and breast cancers are currently ongoing. These studies focus on clinical testing of new drug associations and therapeutic strategies, with the goal to improve patient survival and quality of life.

Main Research Themes

1. Pathogenesis and therapy of bone metastases.
2. Chemotherapy in the treatment of solid tumors.
3. Active specific immunotherapy for solid tumors.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
Master Oncology: multicenter, case-control, observational study, on the epidemiology and risk factors for thromboembolic events in cancer patients, and on the influence of VTE on patient outcome	2009	Sanofi-Aventis	4
PETACC 8: adjuvant therapy with FOLFOX4 *versus* FOLFOX4 + cetuximab for completely resected stage III colon cancers	2008	None	10
H3E-IT-JMHK: open-label safety study of ALIMTA (pemetrexed) single agent as alternative chemotherapy for previously treated patients with locally advanced or metastatic Non-Small Cell Lung Cancer (NSCLC)	2005	Eli Lilly	6

Main Collaboration

With other Italian and Foreign Institutions/Organizations

» Mayo Clinic, Rochester, Minnesota (USA)

Publications

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MEDICAL ONCOLOGY AND IMMUNOTHERAPY

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Introduction

The Division of Medical Oncology and Immunotherapy was established as a new Unit of Medical Oncology at AOU Senese in 2004. Major clinical goals were to provide patients with the newest and most promising experimental approaches to treat cancer, as well as to make available to them the most recent, effective and validated therapeutic options. Clinical and preclinical research programs have been developed in cooperation with other Units at AOU Senese, additional ITT Units of Medical Oncology, as well as major national and international cancer institutions. Innovative therapeutic approaches have been investigated in several areas (*e.g.* melanoma, mesothelioma, prostate, kidney, lung, pancreatic and gastrointestinal carcinomas), mostly focusing on novel immunotherapeutic agents utilized alone or in combination with cytotoxic/cytostatic agents within clinical trials approved by the local Ethical Committee (Figure 1). In accordance with the tradition of the professionals that set-up the Unit in 2004, clinical research is tightly connected with preclinical research programs to comprehensively develop a true translational approach to cancer patients. The synergy between clinical and research activities is also demonstrated by high impact publications, and by the financial support provided to the Unit by several national and international granting agencies on a competitive basis. An additional indication of the effectiveness of the comprehensive program implemented in the initial years of the Division's activity is provided by its attractiveness to cancer patients referred to the Unit by major oncological institutions nationwide (Figure 2).

Main Research Themes

1. Epigenetics

- *Diagnostic, prognostic and therapeutic implications of aberrant DNA methylation in solid tumors*. Alterations in genomic DNA methylation patterns profoundly influence cancer biology, thus representing attractive targets for the definition of new molecular markers of prognosis and for novel therapeutic strategies. Along these lines, the research activity of the Unit has greatly contributed to identifying epigenetic alterations as a major mechanism impairing immunogenicity and immune recognition of cancer cells, and to define relevant immunobiological activities of DNA Hypomethylating Agents (DHA) (a). The development of this line of research is currently focused on: *a*) the investigation of genome-wide DNA methylation patterns in neoplastic cells to identify

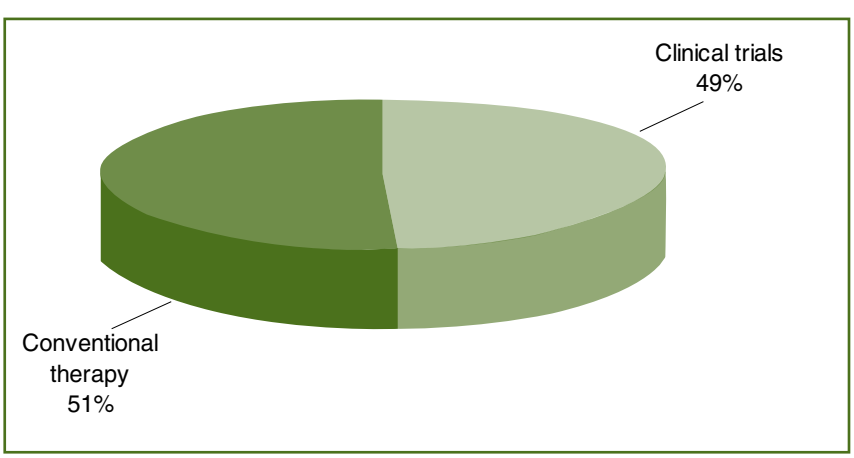

Figure 1 - Type of treatment, 2009

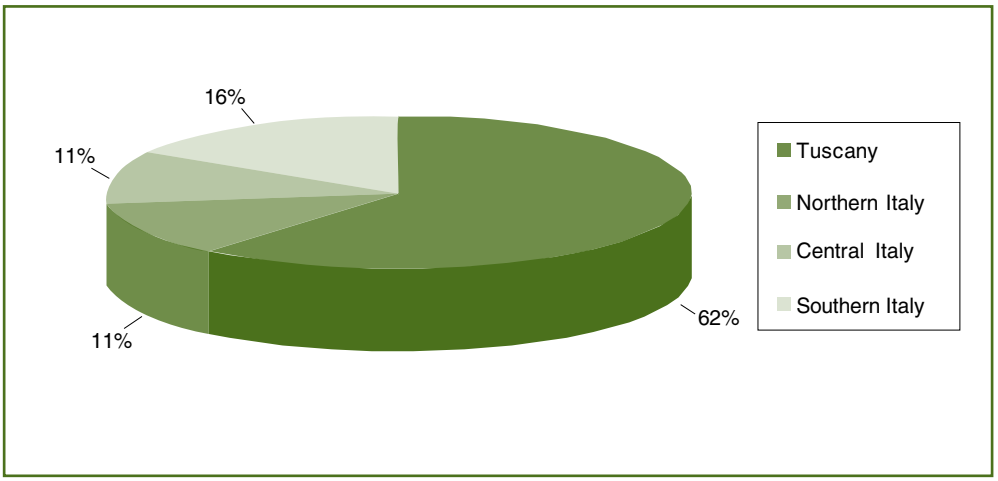

Figure 2 - Geographical origin of treated patients, 2009

potential diagnostic, prognostic and predictive markers of disease in cancer patients, which could help to set up novel clinical and instrumental follow-up procedures, as well as personalize therapeutic approaches; *b*) the analysis of the *in vitro* and *in vivo* immunomodulatory activities of new DHA on cancer cells and their therapeutic potential when utilized alone or in combination with immunotherapeutic approaches.

2. Therapeutic antibodies

- *Immunochemical, functional and clinical characterization of therapeutic monoclonal Antibodies (mAb)*. MAb are currently utilized as pleiotropic therapeutic agents in cancer. The Unit has: *a*) generated an anti-CD105 mAb and characterized its diagnostic and potential clinical activity as an anti-angiogenic agent (b); *b*) contributed to defining the clinical activity of different immunomodulatory mAbs.
  Current and future objectives of our research are to evaluate therapeutic mAbs, available and/or currently under development, for *a*) their binding activity to their target(s); *b*) their functional effects on neoplastic and normal cells.
- *Immunomonitoring of cancer patients treated with therapeutic mAb*. Within the implementation of clinical trials with immunomodulatory mAbs in cancer patients with solid tumors, the Unit has built up a large biobank including sera, plasma, Peripheral Blood Mononuclear Cells (PBMCs) and tumor tissues from enrolled patients, for which detailed follow-up data are available. Our research is currently focused on investigating the "immune efficacy" of immunomodulatory antibodies in cancer patients, by simultaneously characterizing phenotypic, humoral and cellular immune changes induced by the treatment, as well as their correlation with clinical outcome (2). The results will provide the scientific background for improving the clinical use of these therapeutic agents.

3. Communication network

- *Favoring research on cancer biotherapy through the promotion and establishment of national and international networks*. The Unit has promoted the foundation of the Italian Network for Tumor Biotherapy (NIBIT), an association of Italian Institutes, leaders in cancer immunotherapy. Currently, seven annual scientific meetings of the Network have been held (c). Different itinerating courses organized by NIBIT members have been held and are being planned, and an informative booklet on "The use of therapeutic vaccines in cancer" has been prepared and distributed by the Fondazione Federico Calabresi, a non-profit association, nationwide. The website www.nibit.org was created to

access the different activities of the Network. The main goals and objectives of the NIBIT are: *a*) to promote and foster a stronger scientific and operative interaction among professionals belonging to Academia, the Biotech/Pharmaceutical Industry and Regulatory Bodies involved in the biotherapy of cancer; *b*) to develop innovative, multicenter clinical studies on cancer bio-immunotherapy at a national level; *c*) to set-up initiatives to inform patients about the potentials and limitations of cancer bio-immunotherapy and ongoing clinical trials. Within its institutional goals, the NIBIT has designed the clinical trial "A phase II study of the combination of ipilimumab and fotemustine in patients with unresectable locally advanced or metastatic malignant melanoma" (NIBIT-MI), for which it will act as Sponsor, and in which eight Italian Cancer Institutions will be involved.

4. Targeting of cancer stem cells

- Neoplastic populations with stem cell potential, Melanoma Stem Cells (MSCs), have been identified in human cutaneous melanoma (5), and represent the most desirable target for therapeutic intervention. We have demonstrated that MSC express the epigenetically-regulated Cancer Testis Antigens (CTA), thus being potentially recognizable by CTA-specific immune cells. Our present and future activity is focused on extending this observation to additional models of MSC and to study their potential eradication through CTA-based immunotherapeutic interventions.

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Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
A second-line, phase II clinical study with a fully humanized anti-CTLA-4 monoclonal antibody as monotherapy in patients with unresectable malignant mesothelioma. MESOT-TREM-2008	2009	Istituto Nazionale per la Ricerca sul Cancro (IST)	9
Randomized phase III study of tasisulam administered as an intravenous infusion on day 1 of a 28-day cycle *versus* paclitaxel as second-line. treatment in patients with metastatic melanoma. H8K-MC-JZAO	2009	Eli Lilly	-

Description	Year	Sponsor	Number of patients recruited to date
BRIM 3: a randomized, open-label, controlled, multicenter, phase III study in previously untreated patients with unresectable stage IIIC or stage iv melanoma, with V600E BRAF mutation receiving RO5185426 or DTIC. NO25026	2009	Roche	2
PREDICT: an open, single arm trial to assess the activity of recMAGE-A3 + AS15 antigen-specific cancer immunotherapeutic in melanoma patients with unresectable MAGE-A3 positive stage III or stage IV M1a disease (phase II)	2009	GlaxoSmithKline (GSK)	2
An open-label, multicenter, phase III trial of ABI-007 *versus* dacarbazine in previously untreated patients with metastatic malignant melanoma.	2009	Abraxis BioScience	-
A randomized, double-blind, phase III trial comparing ipilimumab *versus* placebo following radiotherapy in subjects with castration resistant prostate cancer that have received prior treatment with docetaxel. CA184043	2009	BristolMyers Squibb (BMS)	5
Randomized, phase III study comparing the combinations of FOLFOXIRI + bevacizumab and FOLFIRI + bevacizumab as first-line treatment of metastatic colorectal cancer	2009	TRIBE, Gruppo Oncologico del Nord Ovest (GONO), IST	-
Dose-defining study and evaluation of tumor-specific monoclonal L19IL2 antibody-cytokine fusion protein activity in combination with dacarbazine in patients with pancreatic tumors. PH-L19IL2DTIC-03/07	2009	Philogen	-
ONCSFN2: randomized, translational, phase III trial: optimization of the FOLFIRI regime in combination with bevacizumab for treatment of Advanced Colorectal Cancer (ACC) based on the genetic polymorphism (pharmacogenetic evaluation of irinotecan) and retrospective analysis of circulating biomarkers	2008	Agenzia Italiana del Farmaco (AIFA)	15
BEBYP: multicenter, randomized, open, phase III study on second-line chemotherapy with or without bevacizumab in patients with metastatic colorectal cancer that have already received first-line chemotherapy treatment in association with bevacizumab	2008	GONO, Investigator Sponsored Trial (IST)	-
Adjuvant immunotherapy with anti-CTLA-4 monoclonal antibody (ipilimumab) *versus* placebo after complete resection of high-risk stage III melanoma: a randomized, double-blind phase III trial. CA 184029/EORTC18071	2008	European Organization for Research and Treatment of Cancer (EORTC)	8

Description	Year	Sponsor	Number of patients recruited to date
A double-blind randomized placebo-controlled phase III study to assess the efficacy of recMAGE-3+AS15 ASCI as adjuvant therapy in patients with MAGE-A3-positive resected stage III melanoma	2008	Derma-GSK	8
A phase III clinical trial to evaluate the safety and efficacy of treatment with 2 mg dose intralesional Allovectin-7® compared to dacarbazine in subjects with recurrent metastatic melanoma. LX01-315-00	2008	VICAL Inc	11
Pilot study with cisplatin at a fixed dose in combination with esomeprazole (dose-ranging) as life-saving treatment in pre-treated patients with advanced/metastatic melanoma	2008	Istituto Nazionale Tumori (INT), ISS, IST	17

Research Grants

Year	Sponsor	Amount
2009	Regione Toscana	€ 280,000
2009	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 110,000
2008-2010	Istituto Toscano Tumori	€ 199,000
2008	Fondazione Buzzi Unicem	€ 40,000
2008	Alleanza Contro il Cancro (ACC)	€ 70,000
2008	ACC	€ 60,000
2008	ACC, Programma Integrato Oncologia (in collaboration with AIFA)	€ 40,000
2008	ACC	€ 25,000

Main Collaborations

With other Italian and Foreign Institutions/Organizations

» Hematology Division, Ospedale "A. Cardarelli", Napoli
» Laboratory of Immunology, Istituto "Regina Elena", Roma
» Immunotherapy of Human Cancer Unit, INT, Milano
» Department of Biotechnology, University of Udine
» Department of Endocrinology and Clinical Oncology, "Federico II" University, Napoli
» MolMed, Istituto Scientifico "San Raffaele", Milano
» Department of Nuclear Medicine, University of Helsinki (Finland)
» Skin Cancer Unit, University Hospital Mannheim (Germany)
» Universitatsklinikum Benjamin Franklin, Berlin (Germany)
» Department of Medicine, University of Halle (Germany)
» Department of Immunology, Rosewell Park Cancer Institute, Buffalo, New York (USA)

» College of Science and Technology, Temple University, Philadelphia (USA)
» The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania (USA)
» Surgery Branch, National Institutes of Health (NCI), Bethesda, Maryland (USA)
» Medical Oncology, The Jefferson University, Philadelphia, Pennsylvania (USA)
» Thomas Jefferson University, Kimmel Cancer Center, Philadelphia, Pennsylvania (USA)

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HEMATOLOGY AND TRANSPLANTS

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Introduction

The Hematology and Transplant Operative Unit is a clinical team dedicated to the diagnosis and treatment of neoplastic and non-neoplastic blood diseases. It includes an in-patient ward, an intensive-care ward for stem cell transplants, a day-hospital ward for out-patient clinics and a diagnostic laboratory. The laboratory collects samples for the diagnosis of hematological diseases from our wards and from patients referred from the Siena University Hospital and from Southern Tuscany. Laboratory activities include those related to stem cell transplantation procedures as well as basic and clinical research activities. For specific research projects also supported by ITT, it performs monitoring of immune responses in vaccination trials for Chronic Myeloid Leukemia (CML) from Italian Centers, and immunogenetic analyses in Chronic Lymphoid Leukemias (CLLs) for all Tuscan Centers referring to the University Hospitals of Florence, Pisa and Siena.

Main Research Themes

1. Chronic Lymphatic Leukemia

a) Immunophenotypic and molecular profiling for the prognosis of chronic B-cell leukemias

Main achievement: Full immunophenotypic and molecular profiling (FISH and immunogenetics) of the CLL clone according to the current clinical standards and guidelines (a).

Current work: Further characterization of established prognosticators and identification of new prognostic parameters of outcome in CLL (IGH gene status and subsets, Angiopoietin-2, p53 defects, etc.); whole genomic profiling of CLL cells; conventional cytogenetics and Fluorescent *In Situ* Hybridization (FISH) of chromosomal aberrations (b); immunophenotypic profiling of CLL with prognostic markers including CD38, ZAP-70 and CD49d.

Future work: Building a multicenter algorithm for the selection of parameters that predict time from diagnosis to progression requiring treatment, resistance to standard treatments and overall survival (multicenter).

b) Molecular profiling of the naïve B-cell repertoire in healthy individuals and in CLL patients

Main achievement: Characterization of the naive B-cell repertoire using IGHV1-69 gene subsets closely related to CLL in healthy subjects.

Current work: Search for CLL-like IGH subsets in normal healthy subjects.

Future work: Immunogenetic profiling of the naïve B-cell clone from which the tumor clone of CLL may derive; identification of genomic aberrations (whole genome profiling on the Affy 6 platform) and definition of the IGH repertoire in the CLL precursor (“monoclonal B-cell lymphocytosis”).

c) Characterization of the interaction of Chronic Lymphatic Leukemia (CLL) B-cells with the microenvironment and of their regulatory activity on T-cells

Main achievement: Inhibition of the proliferation to specific and non-specific immunogenes after incubation of T-cells with CLL B-cells.

Current work: Clinical, immunogenetic and molecular characterization of the most aggressive subset of CLL associated with the dysfunction of p53.

Future work: Identification and characterization of regulatory activities of B-cells of CLL on T-cells.

2. Cytogenic and immunophenotypic study of the plasma cells of plasma cell blood disorders

Main achievement: Referring center for molecular cytogenetic analysis of hematological disorders. Identification of cytogenetic-molecular alterations and immunophenotypic characteristics of plasma cells of patients with monoclonal gammopathy and multiple myeloma and correlations with new therapies (bortezomib lenalidomide, thalidomide).

Current work: Conventional cytogenetic and FISH study of t(4; 14), of the monosomy of chromosome 13 and chromosome 17, of the chromosome ploidy on column-selected plasma cells after incubation with magnetic beads. Clinical, genetic and biological correlations in central nervous system myeloma (GIMEMA trial retrospective).

Future work: Clinical-biological correlations and identification of subsets of patients with different prognostic stratification.

3. Immunogenetic and molecular analyses of Hairy Cell Leukemia (HCL)

Main achievement: Identification of the molecular mechanisms of co-expression of multiple isotypes on the neoplastic cell, an exclusive characteristic of HCL and not of other normal or neoplastic B-cells. Identification of a subset of patients with non-mutated IgHV genes.

Current work: Characterization of distinct clinical subsets on the basis of the status of tumor IGH genes in HCL. Clinical trial ICGHCL2004 (random subcutaneous cladribine 0.5 mg/kg *versus* 0.7 mg/kg). Comparison of immunogenetic, immunophenotypic and immunohistochemical methods for determining minimal residual disease in HCL.

Future work: Research the immunogenetic and molecular mechanisms of lymphomagenesis in HCL. National GIMEMA phase III pilot study “Pentostatin plus ofatumomab in HCL”

4. Vaccine therapy in minimal residual disease in CML and Ph+ Acute Lymphoblastic Leukemia (ALL)

Main achievement: Clinical use of two peptide vaccines developed by our research unit and derived from the *b3a2 or b2a2* fusion point of p210 in CML patients presenting persistent residual minimal disease during therapy with imatinib.

Current work: We are coordinating two multicenter therapeutic peptide vaccine trials, GIMEMA CML0206 to be employed in b3a2/p210 CML patients and CML SI0207 to be employed in b2a2/p210 CML patients. Both studies involve 13 Italian Hematology Units; SI0207 also involves a German Hematology Unit (Leipzig).

Future work: Clinical use in a multicenter study of a p190 derived peptide vaccine (already tested in preclinical models) for controlling minimal residual disease and disease relapse in p190+ lympoblastic leukemia (ALL) patients treated with tyrosine kinase inhibitors and not eligible for allogeneic stem cell transplant.

5. Immunotherapy approach to Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukemia (AML)

Main achievement: Identification within the sequence of the tumor marker WT-1 of peptides suitable for binding with molecules of class II HLA.

Current work: *In vitro* testing of WT1 peptide immunogenicity in AML and MDS patients.

Future work: Phase I/II therapeutic peptide vaccine clinical trial for WT1+ AML patients with or without minimal residual disease after conventional chemotherapy or autologous stem cell transplant.

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Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
Central nervous system and intracranial myeloma. A clinical-biological study	2009	Siena Hematology and Bone Marrow Transplantation Units on behalf of GIMEMA myeloma network	45
Phase I-II international multicenter study of p210-B2A2 derived peptide vaccine in CML patients in complete cytogenetic response with persistent molecular residual disease during imatinib treatment	2008	Siena Hematology and Bone Marrow Transplantation Units	34 out of 49 planned
Phase II multicenter study of p210-B3A2 derived peptide vaccine in CML patients in complete cytogenetic response with persistent molecular residual disease during imatinib treatment	2007	Siena Hematology and Transplant Units and GIMEMA	67 out of 69 planned
Five- *versus* seven-day subcutaneous administration of cladribine in HCL	2005	Hematology and Bone Marrow Transplantation Units on behalf of the Italian Cooperative Group on HCL	157

Research Grants

Year	Funding Agency	Amount
2009	Hairy Cell Leukemia Research Foundation (HCLRF), Research Award, USA	$ 20,000
2009	Fondazione Monte dei Paschi di Siena	€ 25,000
2009	Istituto Toscano Tumori	€ 107,000
2008	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) – PRIN	€ 63,000

Year	Funding Agency	Amount
2007	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 170,000
2006	Ministero della Salute	€ 62,500
2005	HCLRF, Research Award, USA	$ 35,000
2005	MIUR – PRIN	€ 45,000
2004	HCLRF, Research Award, USA	$ 25,000
2004	European Hematology Association	€ 30,000

Main Collaborations

With Units within ITT
- » Hematology and Transplant Unit, Azienda Ospedaliero Universitaria Careggi, Firenze
- » Hematology and Transplant Unit, Azienda Ospedaliero Universitaria Pisana
- » Medical Oncology Unit, Azienda USL 9 Grosseto
- » Medical Oncology Unit, Azienda USL 11 Empoli

With other Italian and Foreign Institutions/Organizations
- » Department of Evolutionary Biology, University of Siena
- » Istituto di Ematologia "Lorenzo e Ariosto Seragnoli", Bologna
- » Hematology and Transplant, "Piemonte Orientale" University, Novara
- » Centro di Riferimento Oncologico (CRO), Aviano (Pordenone)
- » Istituto Oncologico della Svizzera Italiana (IOSI), Bellinzona (Switzerland)
- » Cancer Science Department, University of Southampton (UK)

Publications

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6. Forconi F, Fabbri A, Lenoci M, et al: *Low-dose oral fludarabine plus cyclophosphamide in elderly patients with untreated and relapsed or refractory chronic lymphocytic*. Leukaemia Hematol Oncol 2008; 26: 247-51.
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THORACIC SURGERY

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Introduction

The Unit was established in 1998, although thoracic surgery had been performed in Siena since 1974 by Prof. Adalberto Grossi. The Specialization School in Thoracic Surgery was instituted in 1980. The activity of the Unit has mainly consisted of lung cancer surgery: 2609 patients have been operated on

since 1974 up until today. In 2001 the Unit received authorization to perform lung transplantations (the only center in Tuscany). To date 41 patients have undergone single or double lung transplantation.
The Unit's activity consists mainly in clinical trials and retrospective studies. Over the last three years, we have concentrated on:

- lung cancers;
- lung transplantation.

Main Research Themes

1. Multimodal treatment of locally advanced lung cancer

a) Retrospective clinical study on risk factors for locally advanced lung cancer involving the chest wall and the potential benefit of neo-adjuvant RT and CHT.
b) Randomized clinical study on the role of neo-adjuvant treatment in non-Pancoast T3 lung cancer invading the chest wall.

2. Predictors of postoperative complications after neo-adjuvant therapy for NSCLC

Retrospective clinical study to assess risk factors for postoperative complications after neo-adjuvant treatment for NSCLC: currently collecting patient data.

3. Intrapleural hyperthermic chemo-perfusion with cisplatin after pleurectomy and decortication for malignant pleural mesothelioma

Clinical study to assess survival in two different periods (prior to and following introduction of intrapleural hyperthermic chemo-perfusion) and to report postoperative complications: currently collecting data and adequate follow-up.

Main Collaborations

With Units within ITT

» Medical Oncology Unit, AOU Senese
» Respiratory Diseases Unit, AOU Senese
» Pneumology Unit, AOU Senese

With other Italian and Foreign Institutions/Organizations

» Istituto Europeo di Oncologia (IEO), Milano
» University of Milan

Publications

1. Correale P, Sciandivasci A, Intrivici C, et al: *Chemo-hormone therapy of non-well-differentiated endocrine tumours from different anatomic sites with cisplatinum, etoposide and slow release lanreotide formulation.* Br J Cancer 2006; 96: 1343-7.
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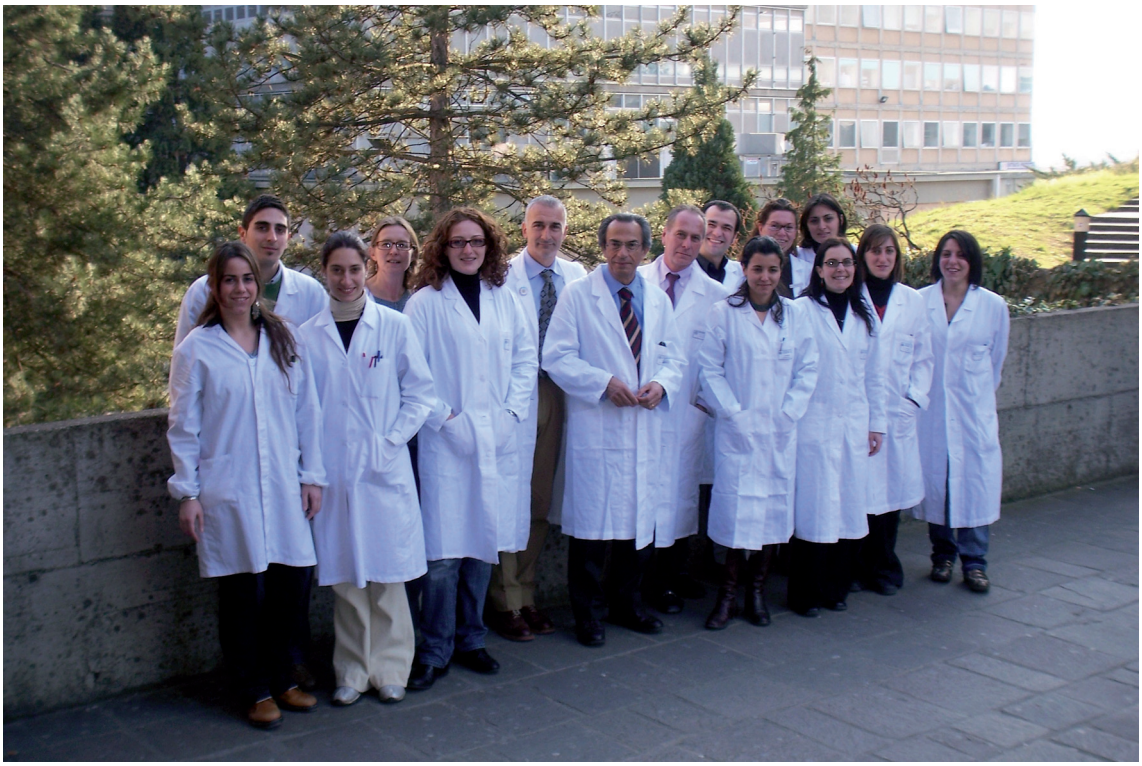
PATHOLOGICAL ANATOMY



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Introduction

The Department of Human Pathology and Oncology of the University of Siena has considerable experience in tumor tissue pathology, ranging from diagnosis and classification to research activities. Mechanisms of regulation of cell proliferation and death in tumors, oncogenes and tumor suppressor genes, immunoglobulin gene mutation and rearrangements, gene and microRNA expression profiles in tumor samples, the role of infectious agents in malignant transformation, such as the assessed role of EBV in the pathogenesis of Burkitt's Lymphoma (BL) for example, are some of the research topics addressed in recent years. Facilities for tissue handling, immunohistochemistry and FISH are available within the Department, as well as fully equipped molecular biology and cell culture (bio-safety level 2) laboratories. Well-trained staff members perform routine diagnostic analyses, as well as research investigations.

Main Research Themes

1. Burkitt's Lymphoma

a) *MicroRNA profiling of BL*. Less than 10% of typical BL cases lack an identifiable *MYC* rearrangement. Additional genetic and epigenetic alterations have been described in different forms of BL besides the *MYC* rearrangement, suggesting that different pathogenetic mechanisms may be involved in the different subtypes of BL. We recently observed that *MYC* overexpression may rely on microRNA dysregulation in BL cases lacking the translocation.
MicroRNAs are a class of small (~22 nt) non-coding RNAs that are able to regulate gene expression at the post-transcriptional level. Several studies have reported their involvement in cancer and their association with fragile sites in the genome. They have also been shown to control cell growth, differentiation and apoptosis, suggesting that these molecules could act as tumor suppressors or oncogenes. Moreover, recent studies have underlined their role in B-cell differentiation. One of our ongoing projects aims at elucidating the microRNA expression profile in BL cases in order to both define the miRNA signature of BL and obtain the complete panel of deregulated miRNAs in BL.

b) *Role of infectious agents in lymphomagenesis*. Thirty-six percent of cancers worldwide are related to infectious diseases, lymphomas representing a big part. Lymphomas may be associated with various pathogens and/or environmental factors which may have a role in malignant transformation and may explain the epidemiological variations and different pathogenic mechanisms. Elucidation of the etiological mechanisms is critical to the understanding of these malignancies and would provide promising alternatives for prevention and treatment of lymphomas. Based on these findings, our ongoing projects aim at unraveling the molecular mechanisms underlying key steps in infection-driven lymphomagenesis. We have collected cases of aggressive B-cell lymphomas, either associated or not with infectious agents (EBV, HIV, HHV8). Gene and microRNA expression profiling of these tumors will be obtained, and the mechanisms through which infectious agent-encoded products may imbalance the host cell's physiological regulation will be thoroughly investigated. These findings will allow the identification of new targets to possibly use in designing novel therapies. In addition, the role of exposure to environmental factors, such as plant extracts commonly used in the traditional medicine of endemic areas, will also be investigated.

2. Analysis of CDH1 mutations in Hereditary Diffuse Gastric Cancer (HDGC)

Aims: germline mutation of the E-cadherin gene (CDH1) accounts for the HDGC syndrome. Fourteen pedigrees with diffuse gastric cancer that fulfilled the International Gastric Cancer Linkage Consortium (IGCLC) criteria were selected and screened for CDH1 germline mutations.

Methods: The entire coding region of the CDH1 gene and all intron-exon boundaries were analyzed by direct sequencing in the 14 families fulfilling the IGCLC criteria. E-cadherin immunohistochemical expression was evaluated in tumors as well as normal formalin-fixed paraffin embedded tissues.

Results: A novel germline missense mutation was found. It was a single C / T substitution in exon 8, resulting in the transition of CCG/CTG (C1118T; Pro373Leu) demonstrated in the proband and her brother. At immunohistochemical analysis, the staining intensity was reduced and considered weakly positive (15%).

Conclusions: The first CDH1 germline mutation of an Italian family is herein reported. The present missense mutation has, to date, never been described so.

3. Analysis of urocortin (UCN) expression in endometrial carcinoma

Urocortin (UCN) is a 40-amino acid neuropeptide sharing 45% sequence homology with Corticotropin-Releasing Factor (CRF). The human endometrium expresses both UCN and CRF, and CRF/UCN receptors type-1 (CRF-R1) and -2 (CRF-R2). CRF-R1 activation inhibits cell growth and proliferation of a tumor cell line derived from the human endometrium, and the UCN signaling pathway has been implicated in tumorigenesis of several tissues. Therefore, we investigated whether UCN mRNA and peptide are expressed by human endometrial adenocarcinoma, and whether their expression changes compared to controls. Samples of well (grade 1; nZ6 endometrioid adenocarcinoma, of whom nZ1 showed squamous differentiation, and nZ1 clear-cell carcinoma) and poorly differentiated (grade 3; nZ3 endometrioid adenocarcinoma) endometrial adenocarcinoma were collected from nine women (age range 61-79 years) enrolled at the time of diagnosis. Healthy endometrium was collected from post-menopausal women (controls; nZ13; age range 64-78 years), who underwent hysterectomy for uterine prolapse. Immunohistochemistry was used to evaluate cellular UCN localization, with the intensity of immunostaining scored on a subjective scale. Quantitative real-time reverse transcriptase (RT)-PCR analysis was used to estimate mRNA expression changes, and restriction analysis was used to confirm PCR product identity. UCN mRNA expression was significantly reduced ($P < 0001$) in endometrial adenocarcinoma than in healthy controls. Immunoreactive UCN was found in luminal and glandular epithelial cells in healthy, but not in neoplastic samples. UCN mRNA and peptide expressions decrease in endometrial adenocarcinoma. These data and the evidence that endometrial cancer expresses UCN receptors and UCN is involved in tumorigenesis of several tissues together suggest a role for UCN in endometrial tumoral cell growth and proliferation.

4. Vascular Endothelial Growth Factor (VEGF) expression in AIDS-related lymphomas

Angiogenic switch marks the beginning of a tumor's strategy to acquire an independent blood supply. In some subtypes of non-Hodgkin's lymphomas, higher local VEGF expression correlates with increased micro-vessel density. However, this local VEGF expression is higher only in tumors with elevated expression of the receptors of the growth factor, suggesting an autocrine growth-promoting feedback loop. Several studies have indicated that VEGF Receptors (VEGFRs) are also targeted by Tat protein from HIV-1-infected cells. Given the similarity of the basic region of Tat to angiogenic factors (bFGF, VEGF), Tat mimics these proteins and binds to their receptors. We evaluated the role of HIV-1 Tat in regulating the level of VEGF expression and micro-vessel density in AIDS-related Diffuse Large B-Cell (DLBCL) and BL. By luciferase assay, we showed that VEGF promoter activity was down-regulated *in vitro* in cells transfected with Tat reduced VEGF protein expression in primary HIV-1-positive BL and DLBCL, compared to the negative cases, supported the findings of promoter down-regulation from the cell lines. Microvascular density assessed by CD34 expression was, however, higher in HIV-1-positive than in HIV-1-negative tumors. These results suggest that Tat has a wider angiogenic role, besides regulation of VEGF expression.

Thus, targeting Tat protein itself and stabilizing transient silencing of VEGF expression or use of monoclonal antibodies against their receptors in AIDS-associated tumors will open a window for future explorable pathways in management of angiogenic phenotypes in AIDS-associated non-Hodgkin's lymphomas.

5. AKT phosphorylation as an indicator of prognosis in gastric cancer

The analysis of phosphorylated Akt (pAkt) expression in gastric carcinomas is another field of investigation. The parameters which are examined include age, sex, site, histotype, stage, survival, mitotic and apoptotic index, expression of cell cycle regulators, such as cyclin D1, cyclin E, Cdk1, p27, and the proliferation index. Our results indicate a statistically significant direct correlation between pAkt and infiltration of the tumor, number of infiltrated lymph nodes and Cdk1 expression. An inverse correlation was instead observed between nuclear pAkt and apoptotic index and between cytoplasmic and nuclear pAkt and survival. No correlation was found with sex, age, tumor site, histotype, mitotic index and cell proliferation.

6. Old and new correlation in the prognosis of breast cancer

Histopathological and immunohistochemical findings on tissue microarrays, Overall Survival (OS), Disease-Free Survival (DFS) and incidence of relapses were recorded and statistically analyzed in 289 breast cancers. A higher R and a shorter DFS were significantly related to large tumors, lymph node invasion, higher tumor grade, absence of Estrogen Receptors (ERs), triple negative tumors and the presence of Lymphovascular Invasion (LVI). Longer OS was observed to be significantly associated with smaller tumor size (T), lymph node negativity, lower tumor grade, absence of LVI, lower Mib-1 expression and with the presence of ER. At multivariate analysis, only T for DFS and lymph node status and triple negativity either for DFS or OS had an independent prognostic value. In the 194 lymph node-negative women, DFS and OS were inversely related to tumor grade, absence of ER, Mib-1 expression in more than 15% of neoplastic cells and, only for DFS, presence of LVI. In the 95 lymph node-positive women, the number of involved nodes was the most discriminating parameter, for either DFS or OS. T, Her-2 status and the presence of LVI were significantly related to DFS. ER negativity was related to higher grade, Progesterone Receptor (PR) negativity, Her-2 negativity, hence to triple negativity, to basal-like type and Mib-1 expression in over 15% of neoplastic cells. Her-2 positivity was related to higher grade, ER positivity and PR positivity. Basal-like type was not an independent prognosticator, while triple negative type has a significant relationship with shorter OS. The Nottingham prognostic index accurately identifies prognostic groupings, and Mib-1 expression and ER signaling are the key biological predictors even in single cases.

7. Epithelial-Mesenchymal Transition (EMT) pathway in thyroid cancer (familial and non-familial forms)

Familial forms are more aggressive than sporadic papillary thyroid carcinoma. We will evaluate genes involved in aggressive forms in both Familial Papillary Thyroid Cancer (FPTC) and sporadic patients. We will investigate the pathways implicated in EMT, and especially TGFbeta family growth factors (activin, inhibin, BMP) and their receptors, and other mechanisms implicated in cell-signaling of neoplastic staminal cells such as Hedgehog-signaling and its relationship with EMT transition.

Methods: genes and gene products (proteins) involved in EMT will be studied by real-time PCR, standard molecular methods, immunohistochemistry and Western blot. We will also use laser capture microdissection, a method which is able to capture from histological slides groups of cells or even isolated ones. By laser microdissection, we can therefore separate the cell populations that are present

in a tissue, in this case, PTC cells from stromal, inflammatory and vascular cells. Thus, we will be able to exam the DNA of neoplastic cells without contamination by non-neoplastic cells.

8. Genetic alterations in Retinoblastoma (RB)

RB is a rare pediatric intraocular tumor (incidence 1:20,000), with a constitutive, hereditary, DNA mutation in about 40% of the patients. Siena represents the national referral center, and we observe about 40 new patients every year. Improvements in conservative therapy are leading to a reduction in surgical procedures and enucleations. In 2009, we had 17 enucleations, 22 in 2008 and 2007, 28 in 2006, and about 25 annually in previous years. The availability of such biological material in fully studied patients (including post-surgery follow-up) has permitted the study of the clinical aspects of this rare tumor, as well as the molecular pathogenesis of malignant transformation. RB represents a well-studied model of carcinogenesis. Such studies have lead to numerous articles. Methods used will include real-time PCR, immunohistochemistry, Western blot and molecular methods.

We will also use laser capture microdissection, a method which is able to capture groups of cells or even isolated ones from histological slides. By laser microdissection, we can therefore separate the cell populations that are present in a tissue, in this case, neoplastic cells from the stromal, inflammatory and vascular cells. Thus, we will be able to exam the DNA of neoplastic cells without contamination by non-neoplastic cells.

9. Studies of Telomerase Activity (TA) in cutaneous melanoma and in melanoma cell lines

a) *Detection of TA and correlation with mitotic and apoptotic indices, Ki-67 and expression of cyclins D1 and A in cutaneous melanoma*. We investigated the TA, Mitotic Index (MI), Apoptotic Index (AI), Ki-67 and nuclear positivity of cyclins D1 and A (Ki-67+N/1,000, cyclin D1+N/1,000, cyclin A+N/1,000) in 42 Primary Cutaneous Melanomas (PCMs). TA was detected in all cases and the results show that it is directly correlated with MI, Ki-67+N/1,000, cyclin D1+N/1,000 and cyclin A+N/1,000 ($p < 0.001$), whereas it was not correlated with AI. When subdividing PCMs into radial (RGPMs) and Vertical Growth Phase Melanomas (VGPMs), the correlation was maintained with MI ($p < 0.005$) and cyclin D1+N/1,000 ($p < 0.005$) only.
Moreover, a high correlation was found between cyclin A+N/1,000 and Ki-67+N/1,000 only ($p < 0.001$) in the RGPM and VGPM groups, thus suggesting that cyclin A is more closely correlated with cell proliferation than cyclin D1. Our results further support the association between TA, tumor cell proliferation and cyclin D1 and A expression in PCM, though it is possible that links between TA and proliferation, and TA and cyclin D1 expression might occur following different pathways.

b) *Quantitative in situ evaluation of telomeres in fluorescence in situ hybridization-processed sections of cutaneous melanocytic lesions and correlation with TA*. In this study, 32 melanocytic lesions were analyzed using FISH, with the aim of investigating possible telomere differences among various benign and malignant lesions and correlating with the TA level.
Results: telomere number per nuclear area was significantly lower in melanomas and metastases than in benign common and Spitz nevi and in control skin (7.24 ± 3.3; 6.11 ± 3 *versus* 14.46 ± 5.6; 16.92 ± 7.8; and 12.59 ± 3.4, respectively; $P < 0.001$). No significant differences were found for the other telomere parameters. In common and Spitz nevi, telomere number was positively correlated with Feret max ($P = 0.046$ and $P < 0.0001$, respectively). TA was significantly higher in melanomas and metastases than in the other groups (70.18 ± 25.2; 105.07 ± 30 *versus* 2.16 ± 2.4; 2.99 ± 2.1; 2 ± 1.2, respectively; $P \leq 0.001$) and was inversely correlated with telomere number per nuclear area in melanomas ($P = 0.0041$). No other significant correlations were found. An extreme shortening of some telomeres probably results in the decrease in telomeric signals and the lower mean number of

detectable telomeres in melanomas and metastases. In melanomas, telomere number per nuclear area is also inversely correlated with TA levels.

c) *Evaluation of MDR1, LRP, MRP and topoisomerase IIalpha gene mRNA transcripts before and after Interferon-alpha (IFN-α), and correlation with the mRNA expression level of the telomerase subunits hTERT and TEP1 in five unselected human melanoma cell lines*. Intrinsic and acquired Multidrug Resistance (MDR) and the activity of the enzyme telomerase have been demonstrated in human melanoma. Direct regulation of the MDR pathways and of telomerase by IFN–α, which is currently used in the therapy of advanced cutaneous melanoma, has also been hypothesized. In this study, we used five melanoma cell lines not selected *in vitro* for drug resistance (Me665/2/21, Me665/2/60, HT-144, SK-MEL-28, and SK-MEL-5), which, in a previous study, had shown different responses to IFN-α in terms of proliferation, apoptosis, TA and mRNA expression for human Telomerase Reverse Transcriptase (hTERT). We investigated the expression of the Multidrug Resistance (MDR1) gene, Multidrug Resistance Protein (MRP), Lung Resistance Protein (LRP), Topoisomerase IIalpha (Topo IIalpha), hTERT and telomerase-associated protein (TEP1), which is shared by telomerase and vault MDR proteins at the mRNA expression level, using Reverse Transcription-Polymerase Chain Reaction (RT-PCR). All cell lines showed an intrinsic expression of hTERT, TEP1 and MDR gene transcripts (only MDR1 mRNA was under detection level in SK-MEL-28 cells). After IFN-α exposure, we observed either no effect, a trend towards a decrease in hTERT, MRP and Topo IIalpha, or an increase in TEP1, MDR1 and LRP mRNA expression in some cell lines. Effects were usually temporary and not always significant. No correlation was found between hTERT and TEP1 mRNA expression, whereas significant positive correlations were found between TEP1 and MDR1 mRNA and between TEP1 and LRP mRNA. IFN-α modulates MDR gene transcripts in human melanoma cell lines differently. A positive correlation between TEP1 and LRP also seems to identify them as common targets of IFN-α effects.

d) *Different effects of IFN-α on melanoma cell lines: a study on hTERT, TA and apoptosis*. Although the antiproliferative and proapoptotic effects of IFN-α are widely recognized, its antitumor mechanisms are not completely known. Recent studies indicate that the derepressed expression of the catalytic subunit of telomerase, hTERT and TA are involved in the process of human carcinogenesis. We studied hTERT mRNA expression, TA and apoptosis in five human melanoma cell lines (Me665/2/21, Me665/2/60, HT-144, SK-Mel-28 and SK-Mel-5) treated with IFN–α. In addition to a variable degree of cell proliferation inhibition in all cell lines tested, we found different responses, ranging from no significant effects in SK-Mel-28 cells to a high degree of apoptosis with no hTERT mRNA expression and TA modification in HT-144 cells, and induction of apoptosis, along with a decrease in hTERT mRNA expression and TA in Me665/2/21 cells. No induction of apoptosis was observed in SK-Mel-5 and Me665/2/60 cells, although an early decrease in hTERT mRNA expression and a minor increase in both hTERT mRNA expression and TA were found, respectively. Our results suggest that the effects of IFN-α on hTERT and TA can result from the induction of apoptosis, but they can also occur through direct modulation of hTERT. We hypothesize that, depending on the cellular context rather than the IFN-α status of the targeted cells, IFN-α can elicit apoptotic cell death. Furthermore, different pathways of apoptosis, not necessarily involving telomerase, can be put into motion.

e) *Evaluation of TA in cutaneous melanocytic proliferations*. In this study, we used a non-isotopic PCR-based Telomeric Repeat Amplification Protocol (TRAP) method to quantify the level of TA in a series of cutaneous melanocytic lesions. Thirty-three benign nevi, 8 dysplastic nevi, 38 malignant melanomas and 4 melanoma metastases were analyzed. The mean relative TA was low in benign nevi (3.5 ± 2.9) and significantly increased in dysplastic nevi (13.1 ± 6.8), malignant melanomas (49.8 ± 29.6) and metastases (121.2 ± 11.2). In addition to the evaluation of TA as a possible diagnostic tool, its increase with tumor progression also suggests a prognostic role in cutaneous melanoma.

10. Studies of prostate in patients subjected to total androgen blockage

a) *Multidisciplinary study* – in vivo *and* in vitro – *of the expression of new proteins in neoplastic and non-neoplastic prostates, with particular reference to variations in the prostate of patients subjected to total androgen blockage*. Combination Endocrine Treatment (CET) is a widely used therapy for Prostate Adenocarcinoma (PA). However, tumor progression and therapy failures are very common in CET-treated patients and, at present, there is no effective therapy for hormone-independent PA. Thus, comprehending the mechanism of androgen action is important for clarifying the role of hormones in PA progression and may also lead to the development of novel anticancer therapies. The relationship between the androgen action and intracellular calcium (Ca2+) in prostatic epithelial cells was established by previous studies that showed that castration-induced regression of prostatic tissues is dependent upon a complex mechanism regulating intracellular Ca2+ levels. Such androgen effects is likely to be mediated, at least in part, through the involvement of Calcium Binding Proteins (CBPs) that, in the gland, buffer intracellular Ca2+ and control the sensitivity to calcium overloads.

Translationally Controlled Tumor Protein (TCTP) is a highly conserved, abundantly expressed protein found in a wide range of organisms of both the animal and plant kingdom. Our group has been the first to identify TCTP as a novel, functioning, highly expressed CBP in the human prostate. More recently, analyzing the expression of TCTP in PA, we observed an inverse correlation between CET-driven histopathological changes and TCTP immunoreactivity, suggesting that TCTP could represent a novel androgen-regulated CBP of the human prostate. On this basis, we propose a research project that aims to: *i*) study the role of TCTP in PA by evaluating the androgen dependence of the protein in prostate cancer and *ii*), determine the relationship between calcium ions, TCTP and apoptosis in PA. The results may better clarify the role of CBPs in PA and PA progression, as well as the mechanism by which androgen controls cell death in prostatic cells.

b) *Research on the evaluation of lymphatics in neoplastic and non-neoplastic prostates in patients subjected to total androgen blockage*. The lymphatic system provides a pathway for metastatic tumor cell migration and growth. In PA, the invasion of peritumoral lymphatic vessels correlates with the Gleason score and the presence of lymph node metastases. This study was designed to fill a gap in the literature on the influence of hormonal withdrawal consequent to CET on lymphatic vascularization in PA. Lymphatic vascularization was assessed by measurement of the Lymphatic Vessel Area (LVA), a parameter that provides important clues about lymphatic vessel function and the ability to favor lymph node metastasis. LVA was measured in 10 adenomectomies and 32 radical prostatectomies performed for PA (16 treated with CET), visualizing lymphatic endothelial cells with a specific monoclonal antibody (D2-40).

In untreated and treated patients, the LVA of interglandular stroma was always lower than that of the periglandular stroma both in non-tumoral and tumoral compartments; LVA inversely correlated with the Gleason grade, and in Gleason grade 5 there were no lymphatic vessels in the interglandular stroma. After CET, LVA in the interglandular stroma of the tumoral compartment of specimens with a conspicuous response to treatment was lower than that of specimens with a low or moderate response. The most remarkable decrease in LVA induced by CET was observed in prostatic intraepithelial neoplasia, where no lymphatic vessels could be detected in the periglandular stroma. These results suggest that hormonal withdrawal, reducing lymphangiogenesis, can improve OS of patients preventing the metastatic diffusion of tumoral cells via lymphatic vessels.

Research Grants

Year	Funding Agency	Amount
2009	Fondazione Monte dei Paschi di Siena (MPS)	€ 50,000
2008	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR)	€ 35,000
2008	Fondazione MPS	€ 50,000
2007	MIUR	€ 21,600
2007	Fondazione MPS	€ 20,000
2006	MIUR	€ 23,200
2006	Fondazione MPS	€ 50,000
2005	MIUR	€ 28,900
2004	MIUR	€ 34,700
2003	MIUR	€ 17,700

Main Collaborations

With Units within ITT

» Department of General Surgery, AOU Senese
» Gynecologic and Obstetric Unit, AOU Senese
» Endocrinology Unit, AOU Senese
» Oculistic Unit, AOU Senese

With other Italian and Foreign Institutions/Organizations

» Department of Hematology and Oncological Science "Lorenzo e Ariosto Seràgnoli", Bologna
» Charité, University of Berlin (Germany)
» Imperial College, London (UK)
» Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid (Spain)

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ENDOCRINOLOGY

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Introduction

This Endocrinology and Metabolism Unit is involved in clinical management and research development of endocrine neoplastic diseases, with particular interest in thyroid cancer, pituitary adenomas and

neuroendocrine tumors. In the clinical setting, the Unit has been provided with all necessary equipment and expertise (including interdisciplinary collaboration) to ensure a complete work-up of the disease from diagnosis to therapy and follow-up. The Unit is involved in national and international research programs that consist of clinical multicentric studies and basic research. The Unit has been involved, as a Coordinating Center, in the definition and publication of European and American Guidelines for Differentiated Thyroid Cancer (DTC). It is actively involved in experimental clinical trials with the tyrosine kinase inhibitor in refractory thyroid cancer.

Main Research Themes

1. Use of rhTSH in well Differentiated Thyroid Cancer (DTC)

a) Our Research Unit participated in several international, randomized controlled studies about the use of recombinant human Thyrotropin (rhTSH) in the follow-up and treatment of differentiated thyroid carcinoma. The research was focused on the role of Thyroglobulin (Tg) measurement as a tumor marker in follow-up, the role of rhTSH in follow-up and in radioiodine treatment of patients with DTC.

Patients with DTC were randomly assigned, after surgery, to receive 1850 MBq or 3700 MBq of ^{131}I after rhTSH. Successful ablation, defined as no visible uptake in diagnostic Whole-Body Scan (WBS) after rhTSH stimulation, was achieved in 88.9% of both groups. Similar rates of ablation were also obtained in patients with lymph node metastases. Dosimetric data showed similar thyroid bed uptake, effective half-life and adsorbed dose in the two groups. Failure to ablate was not correlated with TNM staging, peak TSH levels, thyroid bed uptake and Urinary Iodine Excretion (UIE) at the time of ablation, but was influenced by the absorbed dose of ^{131}I (< or > 300 Gy). Our results demonstrate that therapeutic ^{131}I activities of 1850 MBq are equally effective as 3700 MBq for thyroid ablation in DTC patients prepared with rhTSH, even in the presence of lymph node metastases.

b) We evaluated retrospectively the clinical utility of a second rhTSH-Tg in DTC patients with undetectable basal Tg serum and who had had no evidence of disease on the occasion of their first rhTSH-Tg performed within one year after initial treatment. We concluded that a second rhTSH-Tg was informative in patients in which the first stimulated Tg was detectable but not in those in which the first stimulated Tg was undetectable. Thus, rhTSH-Tg should be repeated only in patients who had a first positive rhTSH-Tg and negative imaging (7).

c) The retrospective study of 201 DTC patients who had received ^{131}I therapy and post-therapy (WBSs) for remnant ablation, after either thyroid hormone withdrawal or recombinant human TSH, was aimed to evaluate the association between UIE and ^{131}I ablation by correlating UIE with the rate of successful ablation (a). There was no significant difference in UIE between ablated or non-ablated patients both in the whole group and the rhTSH or THW groups. Our study indicates that the body iodine content is not an important determinant of thyroid ablation when preparing the patients with either THW or rhTSH.

Recently, several scientific associations, including the American Thyroid Association (ATA) and the European Thyroid Association (ETA) have felt the need to draw guidelines for the management of patients with thyroid nodules and thyroid cancer in order to standardize the follow-up and treatment and to indicate the less invasive and less costly procedures. Our group participated in both the European and American guidelines for differentiated thyroid carcinoma (3) and, more recently, in writing the guidelines for the treatment and follow-up of medullary thyroid carcinoma.

2. Development of a gene expression-based method for the presurgical diagnosis of malignant thyroid disease

The aim of this study was to improve the diagnostic accuracy of fine needle aspiration cytology by searching for proto-oncogene mutations in the cytological material obtained by fine needle aspiration. To this purpose, we studied 174 consecutive patients scheduled for thyroid surgery with cytological indications of suspicious/probable thyroid cancer, indeterminate or inadequate samples or benign nodules. Aspirates were analyzed for the presence of BRAF and RAS point mutations and for RET/Papillary Thyroid Cancer (PTC) rearrangements. The corresponding tissue samples were taken at surgery to validate the molecular analysis of the aspirates. Point mutations were searched for with RT-PCR followed by Denaturing High Performance Chromatography (DHPLC) analysis and direct sequencing. RET/PTC rearrangements were evaluated by RT-PCR followed by Southern Blot. We detected mutations in 67 of the cytology samples (28.5%). BRAF was the most frequently found; RAS mutations were detected in 23 samples and RET/PTC rearrangements in 11 aspirates. The mutation detected in the cytology sample was confirmed in the corresponding tissue samples in 88.2% of the cases. Detection of mutations in the aspirates was associated with thyroid cancer 91.1% of the cases and with follicular adenoma in 8.9% of the cases. BRAF mutation and RET/PTC rearrangements were always associated with cancer, whereas RAS mutations were associated with thyroid cancer in 74% of the cases (b). The combination of molecular analysis and traditional cytology led to improved diagnostic accuracy when compared with the use of either technique alone. Future perspectives are: *a*) to enlarge the number of cases studied; *b*) to refine the technique by defining the amount of cytological material that should be collected in order to maximize the number of informative results and *c*) to explore the possibility to develop a “gene chip” platform that would allow the entire panel of gene mutations to be analyzed at once.

3. Genetic anticipation and alteration of the telomere-telomerase complex in Familial Papillary Thyroid Cancer (FPTC)

PTC is mostly sporadic, but the recurrence of familial cases (FPTC) is well established. Recently, we have demonstrated in a large group of patients affected by FPTC the presence of genetic anticipation, defined as earlier age at onset and/or increased severity of the disease in successive generations (10). Both age at disease manifestation and age at diagnosis were significantly younger in the second generation compared to the first generation and the second generation presented with more aggressive tumors and worst outcome.

In the same families, at a molecular level, we found the presence of germinal alterations in the telomere-telomerase complex. Telomeres are DNA repeated sequences which form chromosome ends. At every replication cycle, telomeres are shortened due to the inability of DNA polymerase to replicate the terminal part of a chromosome (end-replication problem) contributing to cell senescence. Telomerase is the enzyme responsible for the maintenance of telomere length. From a functional point of view, telomerase is a reverse transcriptase enzyme composed of two units: a catalytic unit (hTERT) and a mRNA component (hTR), which serves as a template for hTERT activity. Telomerase is not active in somatic cells. Its reactivation has been described in 90% of neoplastic cells. High telomerase activity and short telomeres have been associated with initiation and progression of some human neoplasia and subjects with telomerase dysfunctions show a higher risk of genetic instability. The tumor-specific activity of telomerase indicates that its activation is responsible for cell immortalization. In thyroid differentiated sporadic cancer, telomerase activity has been reported in 50% of tumoral tissues and the measurement of hTERT activity has been proposed as a marker to differentiate between benign and malignant nodules. Other studies demonstrated that high telomerase activity contributes to anaplastic transformation of differentiated cancers. Patients with FPTC, compared to control groups represented

by sporadic PTC, patients with a nodular goiter, healthy subjects and unaffected siblings of FPTC patients, showed in significantly shorter telomeres the blood, as measured by Q-PCR and by FISH analysis. In addition, hTERT gene amplification was significantly higher in FPTC patients compared to other groups and, in particular, it was significantly greater in the offspring compared to parents. hTERT mRNA expression, as well as telomerase activity, were significantly higher in FPTC patients compared to sporadic PTCs. Relative Telomere Length (RTL), also measured in cancer tissues, was shorter in FPTC patients compared to sporadic PTCs. No mutations in the TERC and hTERT genes were found.

Our study is the first to report on short telomere length, hTERT gene amplification and increased telomerase expression in peripheral white blood cells of patients with familial cancer. Our results suggest that RTL and hTERT activity might play a role in the predisposition of FPTC.

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Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
Efficacy study of XL 184 *versus* placebo in subjects with unresectable, locally advanced or metastatic medullary thyroid cancer	2009	Exelixis	5
Open-label, single arm trial to evaluate the safety and efficacy of oral E7080 in medullary and iodine-131 refractory, unresectable DTCs, stratified by histology	2009	Eisai	2
An international, phase III, randomized, double-blinded, placebo-controlled, multicenter study to assess the efficacy of ZD6474 (ZACTIMATM) *versus* placebo in subjects with unresectable locally advanced or metastatic medullary thyroid cancer	2007	AstraZeneca	6
Study to evaluate the dose, safety and effectiveness of Modified-Release recombinant human Thyroid Stimulating Hormone (MRrhTSH) when used in conjunction with radioiodine for the treatment of multinodular goiter	2007	Genzyme	13

Research Grants

Year	Funding Agency	Amount
2008	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR)	€ 63,000
2007	MIUR	€ 26,000
2007	Istituto Toscano Tumori	€ 45,000
2005	MIUR	€ 50,000
2005	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 82,000
2004	MIUR	€ 33,000
2003	MIUR	€ 43,000

Main Collaborations

With Units within ITT

» Department of Pathology, Radiology, Nuclear Medicine, Surgery, AOU Senese and University of Siena
» Department of Endocrinology and Metabolism, Department of Pathology and Oncology, Azienda Ospedaliero Universitaria Pisana and University of Pisa

With other Italian and Foreign Institutions/Organizations

» Department of Nuclear Medicine and Endocrine Tumor, Institut Gustave Roussy, Villejuif Cedex (France)
» Department of Immunology, University of Illinois, Chicago (USA)

Publications

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MELANOMA

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Introduction

The Siena Melanoma Research Unit was developed more than 30 years ago for the comprehensive management of this malignancy. This includes screening of high risk patients and their families, biopsies of suspected lesions, counseling of patients and their families, as well as the full scope of surgical procedures indicated for management of the various stages of the disease. There has been a long-standing collaboration with dermatologists throughout the Region (Tuscany) and state for the comprehensive care of difficult cases.

The Siena Melanoma Research Unit has been conducting multidisciplinary research into the causes, diagnosis and treatment of melanoma for several decades. As an important diagnostic center in Italy dedicated to melanoma, they have been wholly or partly responsible for a number of important developments in the diagnosis of melanoma.

The nucleus of the Unit is our database. This unique database was initiated in the mid-1980s and has amassed comprehensive clinical details of more than 1,500 melanoma patients. This research resource is used to establish best practices in diagnosis, causes and care of melanoma patients. Moreover, we have in our database more than 1,000 clinical and dermoscopic images of cutaneous malignant melanoma and more than 20,000 clinical and dermoscopic images of other benign and malignant skin lesions. Given that the tumors can all be coupled with a comprehensive patient record, this resource will provide a unique tool for future research.

A recent restructuring of the bio-banking unit will complement the molecular biology research program by providing a more satisfactory infrastructure for extensive bio-specimen banking. This in turn will allow better use to be made of the bio-specimen resources which the Unit is able to procure from its prodigious clinical case load.

Main Research Themes

1. Clinical and instrumental early melanoma diagnosis

Main achievement: With these studies, we demonstrated instruments and new technologies can be extremely useful in the diagnosis of melanoma.

Current work: We are optimizing the techniques of instrumental diagnosis of pigmented and non-pigmented skin lesion cultures of melanocytic cells.

Future work: We would like to evaluate if instruments and new technologies could further improve melanoma diagnosis.

2. Role of telemedicine in the management of geriatric patients with skin cancer

Main achievement: In this project we compared classical face-to-face dermatological examinations in a geriatric ward with the asynchronous store and forward approach of teledermatology, and demonstrated a surprising concordance between diagnosis and therapy.

Current work: We are widening our study to include many geriatric environments (hospital, family physicians, retirement homes, etc).

Future work: We hope to build an assistance model of each specific environment for the elderly so that this age group can avoid problematic and expensive trips to obtain a dermatological consultation.

3. *In vitro* study of the effect of ultraviolet radiation in melanocytic nevi

Main achievement: With these studies, we were able to understand something more about the relationship between keratinocytes and melanocytes (differentiation) and the effects UV has on this relationship.

Current work: We are optimizing the techniques of culturing melanocytic cells.

Future work: We would like to apply these results *in vivo* to evaluate the effects of UV on pigmented skin lesions.

Research Grants

Year	Funding Agency	Amount
2008	Monte dei Paschi di Siena	€ 25,000
2007	Piano di Ateneo per la Ricerca – University of Siena	€ 1,750
2006	Piano di Ateneo per la Ricerca – University of Siena	€ 3,500
2006	Piano di Ateneo per la Ricerca – University of Siena	€ 25,000

Main Collaborations

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» Department of Human Pathology, AOU Senese

With other Italian and Foreign Institutions/Organizations

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Publications

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BRAIN TUMORS

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Introduction

The Research Unit started its activity in the early 2000s when new technology facilities became available at the Radiotherapy Unit, and a new Neurosurgery Unit was started at the Siena University Hospital. A close cooperation aimed at conducting research on High-Grade Gliomas (HGG) began, as a consequence, with the Pathology Unit. The research activity of the Unit is presently dedicated to

several aspects of natural history and to radiation and drug management of brain tumors. However, HGGs are the main field of interest.

Main Research Themes

1. Characterization of biological prognostic factors in HGG

Main achievements: (1,3,5).

Current work: After the identification of several types of gene expression correlated with prognosis in HGG, our present research is aimed to correlate biological prognostic factors to radiation and drug response, on the grounds of the analysis of clinical and pathological material and of experiments on cell cultures (radiobiology research). Particular interest is dedicated to type II programmed cell death (autophagy) and to stem-like tumor cells.

Future work: Translational.

2. Clinical research on radiation and drug management of HGG

Main achievements: (2,4).

Current work: After the demonstration of a trend towards improved prognosis in HGG, drawn through the analysis of the patterns of care and therapeutic results of HGG in a large retrospective, multicenter series, and the results of some experimental treatment protocols, the present research activity is aimed to prospectively test the effectiveness of new therapy modalities.

Future work: Phase II prospective, random clinical trials.

Reference

Scoccianti S, Magrini S, Ricardi U, et al: *Patterns of care and survival in a retrospective analysis of 1059 patients with glioblastoma multiforme treated between 2002 and 2007: a multicenter study by the Central nervous system Study Group of AIRO (Italian Association of Radiation Oncology)*. Neurosurgery 2010; In Press.

Clinical Trial

Description	Year	Sponsor	Number of patients recruited to date
Simultaneous Integrated Boost in Glioblastoma Multiforme (SIB-GBM): a phase II trial	2009		

Research Grants

Year	Funding Agency	Amount
2009	Monte dei Paschi di Siena (MPS)	€ 10,000
2009	Ministero dell'Istruzione, dell'Università e della Ricerca – PRIN 2009	€ 15,000
2008	Schering-Plough	€ 20,000
2008	MPS	€ 20,000
2007	MPS	€ 20,000

Main Collaborations

With Units within ITT

» Oncological Radiotherapy Unit, Azienda Ospedaliero Universitaria Careggi, Firenze
» Oncological Radiotherapy Unit, Azienda USL 4 Prato

With other Italian and Foreign Institutions/Organizations

» Istituto del Radio "O. Alberti", Department of Radiotherapy, University of Brescia
» Department of Radiotherapy, "Piemonte Orientale" University, Novara
» Oncological Radiotherapy Unit, Istituto Oncologico Veneto (IOR), Padova
» Department of Radiotherapy, "Tor Vergata" University, Roma
» Oncological Radiotherapy Unit, University of Turin and Azienda Ospedaliero Universitaria "San Giovanni Battista", Torino
» Department of Genetics and Microbiology, University of Pavia

Publications

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BIOMEDICAL TECHNOLOGIES AND EXPERIMENTAL ONCOLOGY

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Introduction

The activity of this Research Unit is focused on understanding the mechanisms responsible for the disruption of cell cycle regulation in cancer. The main objective of this research is to define the role

of cell cycle regulatory genes in the pathogenesis of several tumor types in order to identify new possible therapeutic strategies. Our research is focused particularly on the Retinoblastoma (RB) family of proteins, which contribute to tumor suppression in different cell types and during different steps of tumorigenesis.

Main Research Themes

1. Small molecules from pRB2/p130 as potential agents for cancer therapy

Over the past decades, cancer research has been mainly aimed at identifying genetic alterations underlying cancer development, in order to design new drugs for molecular therapy. In this regard, the RB family of proteins, consisting of pRB/p105, pRB2/p130 and pRB/p107, has aroused great interest because of its tumor suppressor activity. pRB2/p130 is able to bind the complexes formed by cyclins A and E and cyclin-dependent kinase CDK2, causing the inhibition of their kinase activity, which is necessary for cell cycle progression. All RB proteins consist of two regions, A and B, which are separated by a spacer domain, and the inhibitory activity of pRB2/p130 on CDK2/Cyclin A complex has been attributed to this spacer region.

This project aims at investigating the possible use of small molecules, based on the spacer domain of pRB2/p130, as drugs for molecular cancer therapy. Our previous studies, conducted at the Sbarro Institute for Cancer Research and Molecular Medicine at Temple University in Philadelphia, revealed that the peptide Spa310, derived from the spacer region of pRB2/p130, was able to arrest human lung cancer proliferation in xenotransplanted nude mice (7). These studies will be continued by analyzing the effects of spacer-derived small molecules on cell lines of different tumor types (lung, gastric, breast and ovarian cancers, osteosarcoma, mesothelioma, RB, medulloblastoma and lymphomas). We will evaluate the antiproliferative activity of these molecules using MTS assay, and we will perform cytofluorimetric analyses and caspase assays to assess the induction of apoptosis. We will also analyze the expression of proteins involved in the regulation of apoptosis and the cell cycle using Western blotting and real-time quantitative reverse transcription-PCR. Furthermore, the induction of senescence, another possible cell fate that can be reached as a result of antineoplastic treatments, will be evaluated by senescence–associated beta–galactosidase staining. In order to exclude the potential induction of side effects, these molecules will also be tested on non-neoplastic cells, including mesenchymal stem cells that are necessary to preserve the regenerative ability of the organism.

2. Search for novel susceptibility markers to RB

RB is the most common malignant ocular tumor in childhood. It can affect one eye (unilateral) or both eyes (bilateral) and can occur in both non-hereditary and hereditary forms. The loss of the RB gene has long been recognized as the causative genetic alteration underlying RB but it is increasingly evident that other molecular alterations are required for the tumor to develop.

The aim of this study is to identify new susceptibility markers for RB that might represent new potential preventive and therapeutic targets. The design of new therapeutic strategies is particularly important because, at present, standard treatments are associated with significant toxicities. For this purpose, the expression of cell cycle regulatory genes, other than RB, will be analyzed both in tumor and blood samples by immunohistochemistry and real-time quantitative reverse transcription-PCR. To assess the presence of inheritable alterations, blood samples from relatives of each patient will be also analyzed. We first focused on the p16INK4A tumor suppressor gene because of its possible role in RB pathogenesis and its involvement in the predisposition to familial cancer. We found a significant downregulation of

p16INK4A in about half of the tumor and blood samples from the analyzed RB patients and in most parents of the patients bearing the alteration in p16INK4A expression (a). Moreover, we observed that p16INK4A downregulation seemed to be correlated to hypermethylation of its promoter. These findings suggest that p16INK4A alterations could be novel, inheritable susceptibility markers to RB. The observation that p16INK4A downregulation could occur through an aberrant promoter methylation opens the way for the development of new preventive and therapeutic strategies, based on the use of demethylating agents. Moreover, the finding that p16INK4A promoter methylation seems to be a germline and heritable alteration opens up the new possibility of monitoring high risk families through simple blood sample analyses.

3. miRNA expression profiling of human malignant pleural mesothelioma

Malignant Mesothelioma (MM) is a very aggressive neoplasm that arises from the serosal membranes of the body (pleura, pericardium, peritoneum and tunica vaginalis testis). It is classically subdivided into three main histotypes, named Epithelioid (MME), Sarcomatoid (MMS) and Biphasic (MMB), according to the morphologic differentiation of neoplastic cells. Asbestos exposure has been shown to be the most important risk factor for the development of MM; given the recent ban of asbestos and an onset latency period of 30-40 years from initial exposure, the incidence of this disease is expected to peak over the next two decades in Western countries. The prognosis of MM is very poor, with a median survival of 9-12 months from clinical presentation, even with aggressive multimodal therapy; no curative modality is known for this malignancy.

microRNAs (miRNAs) are a family of small (22-25nts), non-coding RNAs which can downregulate target mRNA translation by binding to their 3' Untranslated Region (UTR). miRNAs are now recognized as one of the major regulatory gene families and their deregulation has been demonstrated to be involved in cancer development (21). So far, very few studies have been published on miRNA alterations in MM.

The aim of this research line is to analyze the miRNA expression profile in pleural mesothelioma tissues and cell lines in order to improve the understanding of cell cycle alterations and biological mechanisms underlying this malignancy and to identify new potential diagnostic/prognostic markers and therapeutic targets. In order to do so, a highly selected casuistry of mesothelioma and non-pathological pleura tissue samples have been analyzed for miRNA expression via hybridization on a comprehensive microarray platform, revealing important miRNA expression differences between normal and neoplastic samples, which we are currently subjecting to validation.

As a future objective, we intend to select the most significant differentially regulated miRNAs and proceed to elucidate their role by characterizing their molecular targets in tissue samples and investigating their biological mechanisms in mesothelial cell line models.

4. New small molecule inhibitors of Src as potential candidates for cancer therapy

Accumulating data show that alterations in the activity of the tyrosine kinase Src play a key role in the development and progression of several human cancers, including breast, colorectal, mesothelial, lung, brain and hematological malignancies. Src has been shown to be an important molecular target in cancer therapy.

This study aims at investigating the effects of new Src inhibitors, which bind the ATP pocket of the Src tyrosine kinase, in a panel of tumors that show high Src kinase activity. Given the central role of Src in regulating several key processes in tumor development, such as proliferation, apoptosis, angiogenesis and invasion, we plan to analyze the effects of Src inhibitors on these processes. We have recently studied the effects of these molecules in medulloblastoma (b). Currently, we are evaluating the antiproliferative activity of these small molecules in mesothelioma and Burkitt's lymphoma cells

using MTS assays, and we are performing cytofluorimetric analyses and caspase assays to assess the induction of apoptosis. We are also analyzing the expression of proteins involved in the regulation of apoptosis and cell cycle by Western blotting and real-time quantitative reverse transcription-PCR. To rule out the potential induction of damages on normal cells, we are analyzing the effects of these molecules on non-neoplastic cells. Furthermore, we propose to study the effects of these inhibitors on tumor invasion, using Matrigel Invasion Assays, and on angiogenesis, using Western blotting analyses of angiogenic factors. Finally, the efficacy of Src inhibitors will be compared to that of the conventional chemotherapeutic agents and their ability to inhibit tumor growth will be also evaluated *in vivo* in mouse models.

5. New pyrazolo-[3,4-*d*]-pyrimidine derivative Src kinase inhibitors may represent a novel antitumoral targeted therapy for medulloblastoma

Medulloblastomas are the most common cerebellar tumors of the central nervous system in childhood. Neuroepithelial tumors arising from the cerebellum, medulloblastomas account for approximately 20% of all intracranial tumors and for 40% of all posterior fossa tumors in children. Therapeutic approaches for medulloblastoma are currently based on a combination of surgery, radiotherapy and chemotherapy. Despite improvements in the overall survival rate following the multimodality treatment, about one third of patients will have a recurrent or progressive disease and, furthermore, current treatments cause neurocognitive sequelae. Therefore, there is a great need for the development of improved therapies that minimize adverse effects. Substantial progress has been made in understanding the molecular features underlying medulloblastoma tumorigenesis and in offering new targets for the development of more effective and specific therapies.

Recently, our research group has conducted preclinical studies aimed at identifying new molecular targets for the treatment of medulloblastoma. One of these therapeutic targets may be represented by telomerase, which is highly activated in medulloblastoma cells and could be required for their continued replication. We have investigated the effects of the anti-telomerase agent Abacavir, an antiviral molecule clinically approved for the treatment of AIDS, on medulloblastoma cells. Our results suggest that Abacavir might be an effective therapeutic strategy for the treatment of this tumor (2).

Currently, our research is focused on the tyrosine kinase Src, another possible target for medulloblastoma therapy. High Src Family Kinase (SFK) activity was identified in pediatric tumors including medulloblastoma, suggesting that Src could have a key role in the development of these tumors. The SFKs are activated in response to cellular signals that promote proliferation, survival, motility and invasiveness, and also angiogenesis and metastasis. Given that Src has been known to be an important molecular target in cancer, highly specific pharmaceutical compounds are currently available and a number of Src inhibitors are being investigated in different tumors. New pyrazolo-[3,4-*d*]-pyrimidine derivative Src kinase inhibitors, binding the ATP pocket of the Src kinase, were recently synthesized and have been demonstrated to have antiproliferative and proapoptotic properties in a broad panel of carcinomas. We have examined the effects of novel Src selective tyrosine inhibitors in human medulloblastoma cells. These pyrimidine derivatives show major inhibitory effects on cell proliferation in medulloblastoma cells, compared with non-neoplastic neuronal cells, and the ability to inhibit tumor growth *in vivo* in a xenograft mouse model of medulloblastoma (b). Our results suggest that pyrimidine derivatives block the cells at the G_2/M phase, through the modulation of cdc2 and CDC25C, and subsequently induce apoptosis via the mitochondria-mediated pathway. Moreover, our findings demonstrate that blocking the Src pathway not only affects cdc2 phosphorylation, as documented, but also its protein levels by post-transcriptional mechanisms (b). We aim to investigate how the pyrimidine derivatives control the cdc2 down-regulation as well as CDC25C(Ser216) phosphorylation. Given that these compounds induce cell cycle arrest and lead to a deregulation of the RB family of proteins, our future aims will be to evaluate the mechanisms by which Src cross-talks with the Rb family protein pathway in medulloblastoma.

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b) Rossi A, Schenone S, Angelucci A, et al: *New pyrazolo-[3,4-d]-pyrimidine derivatives Src kinase inhibitors lead to cell cycle arrest and tumor growth reduction of human medulloblastoma cells*. FASEB J; In press.

Research Grants

Year	Funding Agency	Amount
2008	Fondazione Monte dei Paschi di Siena (MPS)	€ 7,500
2008	Istituto Toscano Tumori	€ 159,000
2008	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) – PRIN	€ 51,600
2005	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 30,000
2004	Fondazione MPS	€ 100,000
2004	AIRC	€ 45,000

Main Collaborations

With other Italian and Foreign Institutions/Organizations

» Centro Ricerche Oncologiche Mercogliano (CROM), Avellino
» "Seconda" University, Napoli
» "La Sapienza" University, Roma
» "Cattolica" University, Roma
» University of Palermo
» University of Sassari
» Human Health Foundation Onlus
» Temple University, Philadelphia, Pennsylvania (USA)

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MOLECULAR BIOLOGY

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Introduction

The Surgical Oncology Unit became an independent ward in 1999. Our surgical activities include treatment of general and oncological pathologies of the gastrointestinal tract, and hepatobiliopancreatic, ovarian and peritoneal cancer. Surgical treatment of stomach cancer, which is of particular concern in the province of Siena, is treated with a Japanese method according to the National Cancer Center of Tokyo Gastric Cancer Section with which there has been an ongoing collaboration since 1995. Our molecular biology research includes genetic screening, identification of circulating biomarkers in cancer

patients and selection of other genetic factors responsible for oncogenesis and especially gastric and r pancreas cancers. Our research center has a large databank of biological material conserved at -80°C. The archive includes tumor and control tissue of gastric origin (about 800 samples), from colon rectal cancer (500) and breast cancer (100). The databank also contains the corresponding genomic DNA and RNA for each sample. Serum from each oncology patient, which has been sampled at various critical phases, is also conserved at -80°C. Due to the interest in hereditary cancer, we also have genomic DNA samples and whole blood from individuals from families affected by hereditary oncological diseases (about 300 families).

Main Research Theme

Molecular studies on gastrointestinal cancer

Main achievements:

a) *Addressing the role of E-cadherin deregulation in Gastric Cancer (GC) progression*. Due to the high incidence of GC in Tuscany, our research group has presented a selection of 14 families meeting the clinical criteria established by the IGCLC in 1999. We performed *CDH1* germline mutation screening in 21 EOGC patients and found two novel constitutional missense sequence variants. The first patient presented a missense 670C>T mutation that affects codon 224 of exon 5 leading to a substitution of the arginine to a cysteine (R224C). In the second patient, the *CDH1* mutation was identified at position -63, located in the 5'UTR region (-63C>A transversion). The R224C mutation was also indentified in a blood donor subject (1/244), suggesting a population frequency of 0.4%. The -63C>A alteration was not found in the control population (0/244). *In vitro* analysis demonstrated that the R224C variant was a non-pathogenic mutation, in fact cells expressing the R224C variant maintain the ability to form compact aggregates as seen in wild-type *CDH1*, different to what is observed for mock cells (Figure 1).

b) *Novel constitutional CDH1 inactivating mechanisms in Hereditary Diffuse GC (HDGC) families*. We screened germline DNA from the 93 mutation-negative probands for large genomic rearrangements using Multiplex Ligation-Dependent Probe Amplification (MLPA) in collaboration with the IPATIMUP of Porto, Portugal. Our Department analyzed three HDGC families coming from Tuscany. Potential

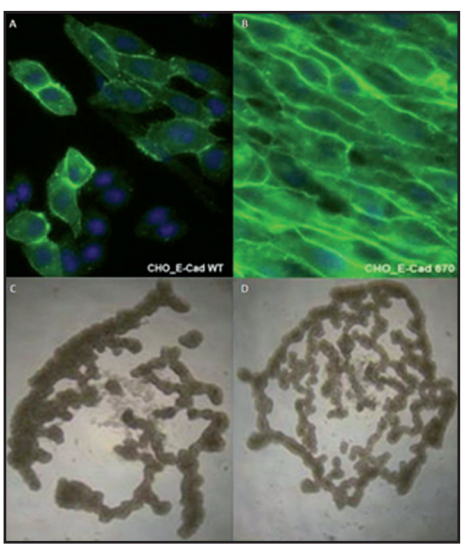

Figure 1 - *In vitro* results of *CDH1* germline mutations 670C>T (details are described in the text)

deletions were validated by RT-PCR and breakpoints cloned using a combination of oligo-CGH-arrays and long-range-PCR. *In silico* analysis of the *CDH1* locus was used to determine a potential mechanism for these rearrangements. Six out of 93 (6.5%) previously described mutation-negative HDGC probands, from low GC incidence populations (UK and North America), carried genomic deletions (UK and North America). Two families carried an identical deletion spanning from 193 to 593 bp, encompassing the full CDH3 sequence and *CDH1* exons 1 and 2. Other deletions affecting exons 1, 2, 15 and/or 16 were identified. The statistically significant over-representation of Alu around breakpoints indicates it as a likely mechanism for these deletions. When all mutations and deletions are considered, the overall frequency of *CDH1* alterations in HDGC is 46% (73/160).

In conclusion, *CDH1* large deletions occur in 4% of HDGC families by mechanisms involving mainly non-allelic homologous recombination in Alu repeat sequences.

c) *Microsatellite instability GC*. Microsatellite analysis was evaluated using five quasimonomorphic mononucleotide repeats named BAT-26, BAT-25, NR-24, NR-21 and NR27.

Seventy-five patients (30%) had at least one first-degree family member affected by GC and 63 patients (25.2%) showed MSI. MSI was significantly more frequent in patients with familial aggregation of GC (38.7% *versus* 19.4%; P = 0.001). A similar frequency of MSI was observed in patients with a positive familial history of GC and in patients with other family members affected by colon cancer (P = 0.96). Conversely, in families with other members affected by lung cancer the frequency of MSI was significantly lower (5.6%; P = 0.007). MSI occurs in GC with familial aggregation. Similar MSI rates have been observed in GC patients with other family members affected by GC or colon cancer. The same does not occur in families with other members affected by lung cancer. A more favorable prognosis was confirmed for the MSI phenotype with univariate (P < .001) and multivariate analysis (P = .051) (Figure 2).

MSI tumors have characteristic clinicopathological features and a better prognosis compared to stable tumors. Considering these five markers, a specific subset of tumors with instability at all five loci and a very good prognosis have been observed.

d) *Single nucleotide polymorphisms and GC risk*. We evaluated the SNP frequencies in three loci at the interferon gamma receptor 1 (*IFNGR1*), at the promoter *CDH1* and promoter *XRCC1* genes.

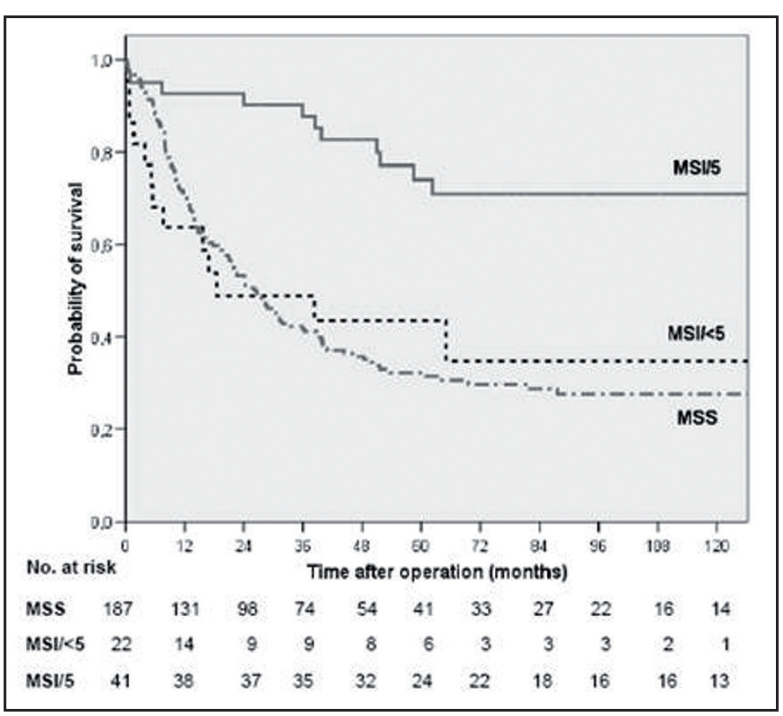

Figure 2 - Long-term prognosis between MSS tumors, tumors with instability at 2-4 markers (MSI/<5) and tumors with instability at all 5 markers (MSI/5)

In collaboration with the IPATIMUP of the University of Porto, we evaluated the frequencies of the *IFNGR1* -56*C/*T SNP and its correlation with increased GC risk. It has been demonstrated that polymorphisms within inflammation-related genes are associated with the risk of GC in people infected with Helicobacter Pylori. Recently, polymorphisms in the gene encoding the *IFNGR1* were found to be associated with increased susceptibility to Helicobacter Pylori infection. We aimed to determine the association between polymorphisms in the *IFNGR1* gene and development of chronic gastritis and GC. In a case-control study including 733 controls, 213 patients with chronic gastritis and 393 patients with GC, the *IFNGR1* -611*G/*A, -56*C/*T, +1004*A/*C and +1400*T/*C polymorphisms were genotyped. A second independent case-control study including 100 controls and 65 patients with GC was used for confirmation of the original results. Our results indicate that the *IFNGR1* -56C/T polymorphism is a relevant host susceptibility factor for GC development. Our data also indicate that this genetic polymorphism is functionally relevant and may be related to the early development of GC.

In the panel of *CDH1* we considered also the putative role of the -160 CA SNP at the promoter region, to assess the effective GC risk development.

The objective of this study was to find out whether C-160A single nucleotide polymorphism of the promoter region of the E-cadherin gene might be a potential genetic marker for identifying individuals at risk for GC. To test this hypothesis, 412 GC patients and 408 controls were analyzed statistically. A PCR-restriction fragment length polymorphism assay was adopted for C-160A single nucleotide polymorphism detection. No statistical differences were found among CC, CA, and AA genotypes and the risk of GC, even stratifying according to age, sex, and area of residence. Similarly, genotype was not associated with intestinal or diffuse histotypes, or with cardia or noncardia carcinomas. In conclusion, the C-160A polymorphism is not associated with GC risk in the Italian population.

Finally, considering that in our pedigree database many families showed multiple cases with diagnosed lung cancer, we analyzed the novel -77T/C SNP of the XRCC1 involved in the lung carcinogenesis. The aim of this is to analyze the -77T/C allelic frequencies in a population composed by 456 primary GC and 507 blood donor controls We found that the -77C/C homozygous genotype is associated significantly with increased risk of gastric cardia carcinoma ($p = 0.023$) with an Odds Ratio (OR) of 1.65 [95% Confidence Interval (CI) 1.14 to 2.4]. In the family history stratification, we report a significant association ($p = 0.043$) between the -77T/C polymorphism and GC cases with familial GC and other family members with lung cancer.

Our results suggest that the XRCC1 -77T/C polymorphism is a relevant host susceptibility factor for gastric cardia cancer development and is associated with familial GC cases also involving an excess of lung cancer.

e) *Determining the type, frequency and timing of E-cadherin deregulation in a large series of clinical samples of GC*. We assessed *CDH1* alterations in 86 diffuse gastric carcinoma and in 160 intestinal gastric carcinoma. We studied the association between alterations in E-cadherin and the clinicopathological features of the patients, cases and family history. Out of the 63 cases, 51 (66%) had promoter hypermethylation, 23 (30%) showed Loss Of Heterozygosity (LOH) and three cases showed somatic mutations. In five cases, LOH and promoter hypermethylation of E-cadherin was detected. We observed a significant association between E-cadherin alterations overall and histological subtype. E-cadherin alterations were more frequently observed in diffuse carcinomas ($p = 0.048$). The hypermethylation of the E-cadherin promoter was significantly associated with the presence of nodal metastatization ($p = 0.024$). We verified a significant association between *CDH1* alterations and depth of invasion, in particular, in the pT2 stage (68.8%). A significant association ($p = .0238$) between hypermethylation and a positive familial history was found for the first time. We determined the survival rate of patients and verified that cases with LOH and E-cadherin mutations showed a worse survival rate, in contrast to cases with hypermethylation or without E-cadherin alterations ($p = 0.048$).

We support previous results showing that E-cadherin alterations are a frequent event in sporadic diffuse gastric carcinoma. We show that epigenetic changes of E-cadherin leading to its decreased protein expression induce an invasive behavior to neoplastic cells. Moreover, we found a new

molecular biomarker of prognosis in GC that can be used to predict the clinical prognosis of the patients (Figure 3).

f) *Determining the association between EGFR and other oncogenic mutations inGC*. Within this project, we aimed to clarify the frequency of activating oncogenic mutations, *EGFR*, *KRAS*, *BRAF*, *PIK3CA,* and *MLK3* (Figure 4), in a large series of 63 MSI GC also studied for *MLH1* promoter methylation status. Mutations were screened in all cases and associations between the molecular data and the clinical pathological features of the patients and tumors, as well as familial aggregation, was investigated. Out of these 63 MSI carcinomas, *MLH1* promoter hypermethylation was detected in 50 (79.4%) cases. We verified *KRAS* mutations in 17.5% (11/63) of all gastric carcinoma. None of the cases showed *BRAF* somatic mutations. The frequency of *PIK3CA* mutations was 14.3% (9/63) in MSI GC. *MLK3* mutations were found in two carcinomas. *EGFR* mutations were screened in all MSI carcinomas, in the hotspot regions of the *EGFR* kinase domain (between exons 18-21) and in the 3' untranslated region of the gene. Within the coding region of *EGFR* no mutations were found. In contrast, 20 (31.7%) of the 63 MSI carcinomas had alterations (deletions and insertions) in the repeat region within the 3' untranslated region of *EGFR*. Interestingly, some of the cases with

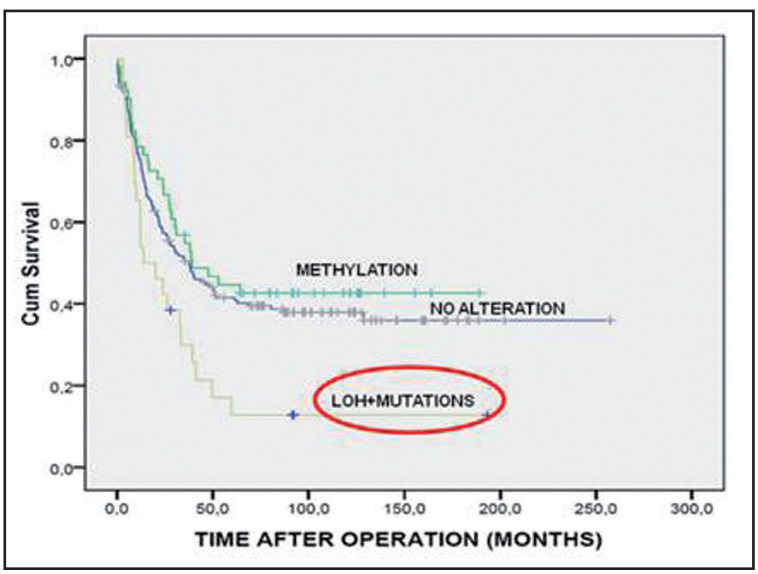

Figure 3 - LOH and E-cadherin mutations showed a worse survival rate, in contrast to cases with hypermethylation or without E-cadherin alterations (p = 0.048)

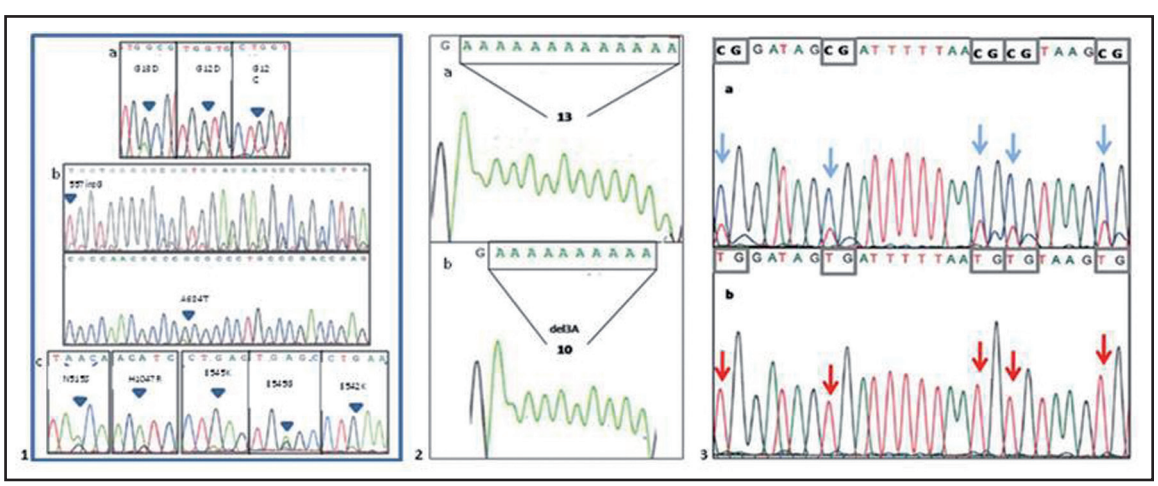

Figure 4 - 1) *KRAS*, *MLK3* and *PIK3CA* mutations. 2) *EGFR* poliA mutations. 3) *MLH1* promoter methylation

large deletions had increased expression of *EGFR*. When comparing cases with EGFR mutations with mutations in other oncogenes (*KRAS*, *PIK3CA*, *MLK3*), we verified that about half of *EGFR* mutations (11/20) occur as single events. In six cases, the *EGFR* mutations occurred concomitantly with *KRAS*, in one case with *PIK3CA*, and in two cases with *KRAS* and *PIK3CA*. These observations are clinically relevant since e they can help to stratify GC cases for anti-*EGFR* therapy.

Future work: In the future, we will complete our activities comparing E-cadherin inactivation results with EGFR expression in our GC series.

To verify the interaction of E-cadherin and the activation status of EGFR, we aim to understand how E-cadherin alterations affect E-cadherin-EGFR interaction and to identify downstream effectors and associated cellular effects. Our colleagues (Suriano and Seruca) verified that in comparison to WT E-cadherin-expressing cells, mutations within the extracellular domain of E-cadherin cause reduced stability of the EGFR/E-cadherin heterodimers. Similarly, weak EGFR/E-cadherin immunocomplexes were obtained for the juxtamembrane mutants. No effect on the interaction between E-cadherin and EGFR was observed for the intracellular cytoplasmic mutants. Ligand binding to a monomeric unit of EGFR activates the cytoplasmic catalytic function, promoting receptor dimerization followed by autophosphorylation on tyrosine residues. Our results show that E-cadherin extracellular and juxtamembrane mutants display increased levels of phosphorylated EGFR upon ligand stimulation, when compared to WT E-cadherin or intracellular cytoplasmic mutants. Furthermore, we demonstrated that these E-cadherin mutant expressing cells show a reversion of the motile phenotype upon EGFR pharmacological inhibition. This is correlated with decreased RhoA activation, supporting the idea that Rho-like proteins are downstream effectors of EGFR activation, and we proposed a model in which upon disruption of EGFR/E-cadherin heterodimers, EGF activation of the receptor takes place, which in turn causes RhoA activation, ultimately leading to increased cell motility. This hypothetical model based on *in vitro* results still needs to be validated in primary samples.

To confirm these results obtained *in vitro*, we should complete the EGFR expression analysis using Fluorescence *In Situ* Hybridization (FISH) analysis and quantitative RT-PCR. The analysis is expected to demonstrate the usefulness of circulating nucleic acid as novel bio-marker in GC.

EGFR and CDH1 immunohistochemistry will be performed on all GC tissues to compare expression with E-cadherin inactivation.

Finally, considering the high incidence of GC with familial aggregation in Tuscany, we will compare CGH array expression between sporadic, familial and hereditary GC, to identify a putative molecular cause for novel biomarkers of GC risk in patients showing family aggregation. In our region, we are also collecting a complete family history of GC in several zones, to amplify our database, offering genetic screening to pedigrees fulfilling the criteria of hereditary forms.

Research Grants

Year	Funding Agency	Amount
2006	Monte dei Paschi di Siena – University of Siena	
2005	Piano Ateneo Ricerca – University of Siena	
2005	Piano Ateneo Ricerca	
2005	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) – PRIN	
2004	Piano Ateneo Ricerca	
2004	MIUR – PRIN	

Main Collaboration

With other Italian and Foreign Institutions/Organizations

» Institute of Molecular Pathology and Immunology, University of Porto (IPATIMUP) (Portugal)

Publications

1. Corso G, Pedrazzani C, Marrelli D, et al: *Correlation of microsatellite instability at multiple loci with long-term survival in advanced gastric carcinoma.* Arch Surg 2009; 144(8): 722-7.
2. Corso G, Pedrazzani C, Marrelli D, Pinto E, Roviello F: *Familial gastric cancer and Li-Fraumeni syndrome.* Eur J Cancer Care 2009 [Epub ahead of print].
3. Corso G, Marrelli D, Pedrazzani C, et al: *Gastric cardia carcinoma is associated with the promoter -77T>C gene polymorphism of X-ray cross-complementing group 1 (XRCC1).* J Gastrointest Surg 2009; 12: 2233-8.
4. Oliveira C, Senz J, Kaurah P, et al: *Germline CDH1 deletions in hereditary diffuse gastric cancer families.* Hum Mol Genet 2009; 9: 1545-55.
5. Corso G, Berardi A, Marrelli D, et al: *CDH1 C-160A promoter polymorphism and gastric cancer risk.* Eur J Cancer Prev 2009; 1: 46-9.
6. Pedrazzani C, Corso G, Velho S, et al: *Evidence of tumor microsatellite instability in gastric cancer with familial aggregation.* Fam Cancer 2009; 3: 215-20.
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MEDICAL GENETICS

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Maria Carmela Epistolato, Research Fellow
Vittoria Di Sciglio, PhD Student
Gabriella Livide, PhD Student

Introduction

The Medical Genetics Unit is involved in both clinical and basic research in human genetics. In particular, research interests focus on the molecular pathogenesis of mental retardation (Rett Syndrome and other forms of mental retardation), hereditary nephropathy (Alport Syndrome) and cancer (Retinoblastoma – RB). The Unit has two ambulatories for genetic counseling and a laboratory equipped with the main items

required for molecular biology activities (cell culture room, five PCR machines, a Real Time quantitative PCR machine, two stations for DHPLC analysis, two machines for automated fluorescent DNA sequencing, a microarray spotter, a microarray hybridization station, a Wash station, a hybridization oven and a microarray scanner). A large bank of DNA, cell lines and tissues from patients with Rett Syndrome, other forms of mental retardation and RB is maintained at the Medical Genetics laboratory. Each patient contributing to the collection has an accurate description of phenotype and genotype. The bank has a dedicated website (http://www.biobank.unisi.it) which is available to the scientific community.

Main Research Theme

Studies on retinoblastoma carcinogenesis

In recent years, we have collected 516 DNA samples from RB patients (176) and healthy relatives (340) through the activity of genetic counseling. We have collected 23 RB tissues from these patients, including two very rare samples also containing areas of retinoma (RN). With regard to inheritance, among the 176 probands, 36 have been classified as familial cases and 130 as sporadic cases (84 unilateral and 46 bilateral). We have performed a *RB1* molecular analysis by DHPLC or direct sequencing, identifying 11 frameshift, 18 nonsense, 11 splice-site and 2 missense mutations. Patients have also been analyzed using MLPA and array CGH to investigate the presence of *RB1* gross rearrangements, and 9 large deletions have been identified. Clinical and molecular information on RB patients, made anonymous through coding, are present in an on-line catalog available at http://www.biobank.unisi.it.

During the last years, our group has successfully used the Agilent platform for array-CGH studies on RB samples. In particular, we used this technique to analyze DNA isolated from retinoma and RB tissues in order to compare the chromosomal aberrations and identify somatic events accompanying malignancy. To accomplish these goals, we used a laser microdissection technique in order to distinguish the areas of RB, retinoma and normal tissues in Formalin-Fixed Paraffin-Embedded (FFPE) samples obtained after enucleation. This protocol creates problems due to the amount of DNA that can be obtained. In the case of a particularly low DNA yield, we used the GenomePlex Whole Genome Amplification (WGA) kit to pre-amplify paraffin-embedded archival specimens before array CGH experiments.

The group investigated, using array-CGH, a series of 18 RB (10 bilateral and 8 unilateral) and 2 RN samples to characterize genomic changes in the two lesions. In RB, a total of 64 rearrangements (47 gains and 17 losses) were detected. In accordance with previous data, recurrent imbalances were found on chromosomes 1, 2, 6, 13 and 16. In addition, three previously undescribed recurrent rearrangements were identified, two on chromosome 9 (9q22.2 and 9q33) and one on chromosome 11 (11q24.3). All these rearrangements indicated interesting candidates for RB progression. Bilateral cases showed a lower number of imbalances (mean, 1) compared to unilateral cases (mean, 7), with statistical significance ($p = 0.002$). The unilateral cases could be divided into low (≤ 4) and high level (≥ 7) chromosomal instability groups, the first group presented younger age at diagnosis (mean, 511 days) compared to the second one (mean, 1,606 days). Array-CGH in RN samples revealed different results. In one RN, ophthalmoscopically diagnosed as a benign lesion, no rearrangements were detected, while adjacent RB displayed seven aberrations. Differently, in the other RN, identified by retrospective histopathological examination, five genomic rearrangements were detected. Among these, three were in common with the adjacent RB (dup5q13.2, dup6p, dup8p23.1), while the remaining two (dup1q32.2 and dup13q31.2) were exclusively detected in RN and did not contain any gene. One rearrangement, dup5p, was RB-specific and included the SKP2 gene, an interesting candidate for tumor progression. The genomic profile therefore uncovered the different molecular nature of the lesions classified as “retinomas” on the basis of their histopathological appearance.

Recent studies in the field of DNA methylation have lead to the awareness that epigenetic changes may represent an alternative or complementary mechanism to mutational events in tumor progression. In particular, methylation in the CpG islands in the promoter regions of a large number of tumor suppressor genes is observed in several human cancers (a). Methylation-specific MLPA has been recently described as a method that allows the simultaneous identification of epigenetic changes at multiple sites (b). Therefore, we decided to use this technique to characterize the role of epigenetic silencing in RB pathogenesis. We analyzed 10 RB samples, comparing the results to those obtained in normal retina (Table 1). Tumor tissues showed frequent hypermethylation of MGMT (7/10, 70%), MSH6 (6/10, 60%), CD44 (5/10, 50%), PAX5 (5/10, 50%) and GATA5 (3/10, 30%). Since these genes are involved in DNA repair (MSH6), cellular differentiation (PAX5 and GATA5), and cell-to-cell communication (CD44), their epigenetic silencing could play an important role in RB initiation and progression. In particular, aberrant methylation of these factors could play a key role in tumor development, especially in bilateral cases, where chromosomal imbalances are less frequently observed.

Table 1 - Summary of tumor samples analyzed for aberrant methylation. %met indicates the gene methylation percentage detected in 10 retinoblastoma samples (#1-10)

Gene	#1 %met	#2 %met	#3 %met	#4 %met	#5 %met	#6 %met	#7 %met	#8 %met	#9 %met	#10 %met	Frequency
MGMT			46%	54%	38%	68%	50%	42%		35%	**7/10**
MSH6	41%			68%	100%	42%	33%			49%	**6/10**
CD44	42%			78%			58%	100%		100%	**5/10**
PAX5A					100%	35%	47%	51%	33%		**5/10**
GATA5				67%	38%		70%				**3/10**
RB1				30%			100%				**2/10**

In order to test the hypothesis that RB phenotypic variability (age of onset, involvement of one/two eyes and therapy necessary) may in part result from the variable function of genes involved in the cell cycle and apoptosis, we investigated the effect of two functional polymorphisms, one in *TP53* (Arg72Pro) and the other in the promoter of *MDM2* (SNP309 T>G), on the age of tumor diagnosis. We tailored specific Pyrosequencing assays for the two SNPs and genotyped 90 RB patients with a characterized *RB1* germline mutation (*i.e.* familial and bilateral cases) belonging to our collection. A descriptive analysis showed an earlier age at diagnosis in patients with bilateral retinoblastoma than in those with unilateral retinoblastoma (median age: 0.57 years *versus* 1.49 years, respectively, $p < 0.001$). Since age of onset is often not exactly known, we considered bilaterality a more robust measure of the variable genetic risk. A multivariate logistic regression model adjusted for age and gender showed the risk of bilateral disease to be higher for splicing and missense mutations than for deletions, duplications, nonsense and frameshift mutations, but not statistically significant (OR = 1.33, 95% CI 0.22-8.22). Regarding MDM2 SNP309, the model revealed a significantly higher risk of bilaterality for the GG genotype than TT (OR = 11.78, 95% CI 2.18-63.65), but was not significant for TG. As for TP53 R72P SNP, the risk of bilaterality is not significant for the PP genotype (Figure 1). Our results suggest, for the first time, that MDM2 and TP53 may be modifiers of RB as well.

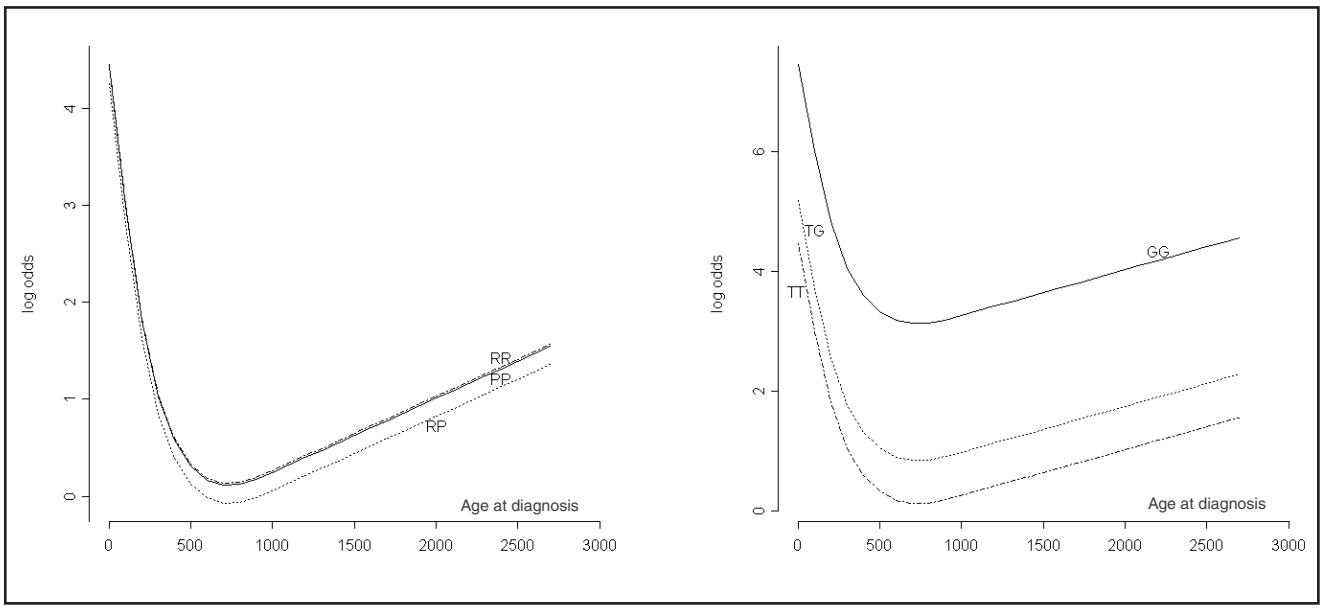

Figure 1 - Graphical representation of multivariate logistic regression model for TP53 (SNP R72P) and MDM2 (SNP309 T>G) polymorphisms adjusted for age and gender. The time is expressed in days from birth. Log odds indicates the risk of bilaterality for each polymorphism

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a) Hegi ME, Diserens AC, Gorlia T, et al: *MGMT gene silencing and benefit from temozolomide in glioblastoma*. N Engl J Med 2005; 352(10): 997-1003.
b) Nygren AO, Ameziane N, Duarte HM, et al: *Methylation-specific MLPA (MS-MLPA): simultaneous detection of CpG methylation and copy number changes of up to 40 sequences*. Nucleic Acids Res 2005; 33(14): 128.

Research Grants

Year	Funding Agency	Amount
2007-2009	Istituto Toscano Tumori	€ 70,000
2007-2008	Ministero dell'Istruzione, dell'Università e della Ricerca – FIRB 2005	€ 113,600
2007-2008	University of Siena – PAR 2006	€ 10,000

Main Collaborations

With Units within ITT

» Department of Human Pathology and Oncology, University of Siena
» Retinoblastoma Referral Center, Department of Ophthalmology, University of Siena
» Department of Pediatrics, University of Siena

With other Italian and Foreign Institutions/Organizations

» Medical Genetics Unit, Azienda Ospedaliero Universitaria “San Luigi”, Orbassano (Torino)
» Institut de Recherches sur le Cancer de Lille (France)

Publications

1. Sampieri K, Amenduni M, Papa FT, et al: *Array comparative genomic hybridization in retinoma and retinoblastoma tissues*. Cancer Sci 2009; 100: 465-71.
2. Sampieri K, Mencarelli MA, Epistolato MC, et al: *Genomic differences between retinoma and retinoblastoma*. Acta Oncol 2008; 47: 1483-92.
3. Caselli R, Speciale C, Pescucci C, et al: *Retinoblastoma and mental retardation microdeletion syndrome: clinical characterization and molecular dissection by array CGH*. J Hum Genet 2007; 52: 535-42.
4. Sampieri K, Hadjistilianou T, Mari F, et al: *Mutational screening of the RB1 gene in Italian patients with retinoblastoma reveals 11 novel mutations*. J Hum Genet 2006; 51: 209-16.

MOLECULAR IMMUNOLOGY

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Orso Maria Lucherini, Biologist, PostDoc Fellow
Nico Giommoni, Biologist, PhD Student
Micol Ferro, Biotechnologist, PhD Student
Giulia Masi, Biologist, PhD Student

Introduction

The Molecular Immunology Unit started its activity at the Department of Evolutionary Biology in 1986 after the Unit head, Prof. Baldari, returned from her post-doctoral training at the European Molecular Biology

Laboratory in Heidelberg (Germany). The research of the Unit has been focused since its inception on the mechanistic dissection of signaling pathways in lymphocytes and innate immune cells and on the identification of signaling dysfunctions in disease, including cancer, autoimmunity, primary immune disorders and bacterial infections. The Unit is now established internationally as one of the leading groups in molecular immunology.

Main Research Theme

Functional characterization of the Shc protein family in lymphocytes

Among the activities of the Unit, the most relevant to cancer research is the study of Shc proteins, which we first identified as key players in TCR signaling. We have been able to associate this family of adaptor proteins with multiple functions in T lymphocytes (Figure 1): p46ShcA and p52ShcA to the transduction of mitogenic stimuli and to the remodeling of actin cytoskeleton and chemotaxis, p66ShcA and ShcC/Rai to oxidative stress-induced apoptosis and to inhibition of mitogenic signaling. We have recently translated these data to the *in vivo* setting of genetically engineered mice lacking expression of either p66Shc or ShcC/Rai, which has led to the discovery of a role for these proteins as attenuators of the immune response and negative regulators of autoimmunity (12). Within the framework of a project funded by ITT, we have discovered that leukemic B cells from B-CLL patients display a defect in p66Shc expression, which we have causally correlated to an imbalance in the expression of pro-apoptotic and anti-apoptotic Bcl-2 family members and which accounts for their impaired apoptosis (a). Importantly, our current evidence, that we are in the process of validating, highlights p66Shc expression as a potential novel prognostic marker. We are, moreover, investigating the mechanisms controlling p66Shc expression and their dysfunction in CLL B cells (Figure 2).

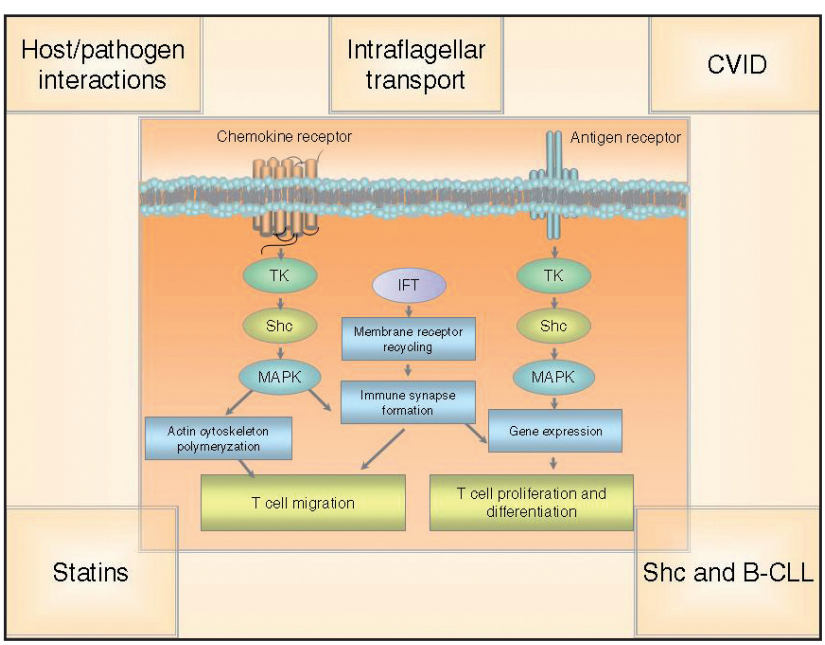

Figure 1 - Shc proteins serve several functions

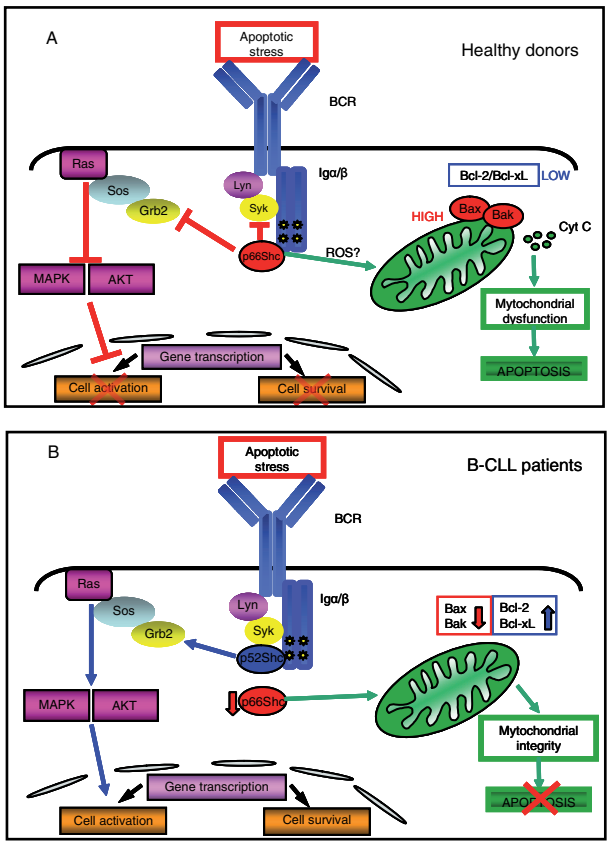

Figure 2 - Model of p66Shc functions in B cells from healthy donors (A) or B-CLL patients (B)

Reference

a) Capitani N, Lucherini OM, Sozzi E, et al: *Impaired expression of p66Shc, a novel regulator of B-cell survival, in chronic lymphocytic leukemia*. Blood 2010 [Epub ahead of print].

Research Grants

Year	Funding Agency	Amount
2009-2010	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) – PRIN	€ 24,000
2008-2010	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 230,000
2006-2008	MIUR – PRIN	€ 39,000
2006	Telethon	€ 70,000
2005-2007	MIUR – FIRB	€ 23,940
2004-2006	MIUR – PRIN	€ 54,000
2004-2006	AIRC	€ 210,000
2003-2006	MIUR – FIRB	€ 138,000

Main Collaborations

With Units within ITT

» Department of Medicine and Immunological Sciences, University of Siena
» Department of Clinical Medicine and Immunological Sciences, University of Siena and Azienda Ospedaliero Universitaria Senese
» Department of Internal Medicine, University of Florence

With other Italian and Foreign Institutions/Organizations

» Novartis Vaccines and Diagnostics Company, Siena
» Istituto Europeo di Oncologia (IEO), Milano
» Department of Biomedical Sciences, University of Padua
» Section of Infection and Immunity, University of Wales, Cardiff (UK)
» Institute for Research in Biomedicine, Bellinzona (Switzerland)
» Pfizer Research, Chesterfield, Missouri (USA)
» University of Massachusetts School of Medicine, Worcester, Massachusetts (USA)
» University of Yale, New Haven, Connecticut (USA)

Publications

1. Finetti F, Savino MT, Baldari CT: *Positive and negative regulation of antigen receptor signaling by the Shc family of protein adapters*. Immunol Rev 2009; 232: 115-34.
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7. Patrussi L, Mariggio S, Paccani SR, et al: *Glycerophosphoinositol-4-phosphate enhances SDF-1alpha-stimulated T-cell chemotaxis through PTK-dependent activation of Vav*. Cell Signal 2007; 19: 2351-60.
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12. Pellegrini M, Pacini S, Baldari CT: *p66SHC: the apoptotic side of Shc proteins*. Apoptosis 2005; 10: 13-8.
13. Patrussi L, Savino MT, Pellegrini M, et al: *Cooperation and selectivity of the two Grb2 binding sites of p52Shc in T-cell antigen receptor signaling to Ras family GTPases and Myc-dependent survival*. Oncogene 2005; 24: 2218-28.

MOLECULAR BIOLOGY

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Francesco Neri, Research Fellow
Caterina Di Clemente, Research Fellow
Anna Krepelova, Research Fellow
Riccardo Serafini, Research Fellow
Beatrice Grandi, Technician

Main Research Theme

Cell response to growth factors and mechanism of cell transformation

The main focus of the Laboratory is the study of cell response to the angiogenic growth factors of the Vascular Endothelial Growth Factor (VEGF) family, which are recognized by VEGFR-1, VEGFR-2 and VEGFR-3 receptors mainly expressed in vascular and lymphatic endothelial cells. We use Human Umbilical Vein Endothelial Cells (HUVEC) and embryonic stem cells for cellular model systems.

Main achievements: The laboratory has identified, cloned, and characterized VEGF-D, a factor that activates angiogenesis and lymphangiogenesis and is involved in tumor metastasis via lymphatic vessels. We characterized the cell signaling originating from the tyrosine kinase receptors of the VEGFR family both in primary human endothelial cells and in embryonal stem cells. We characterized the nuclear response to VEGF and clarified the molecular mechanism of transcriptional activation of *MYC* target genes.

Current work: The main ongoing projects in the Laboratory are focused on the analysis of nuclear response to growth factors of the VEGF family with particular attention focusing on epigenetic modifications. We are studying the molecular mechanisms of the transcriptional activation mediated by chromatin post-translational modifications induced by cell response to growth factors.

Future work: Our Laboratory's main goal is the identification of the molecular effectors of cell transformation and induction of tumor stem cells. We will characterize the *MYC*-target genes involved in cell transformation and induction of the stem cell phenotype.

Research Grants

Year	Funding Agency	Amount
2008-2011	Istituto Toscano Tumori	€ 60,000/year
2008-2011	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 100,000/year

Main Collaborations

With Units within ITT
- » Signal Transduction Unit, University of Siena
- » Pathological Anatomy Unit, Azienda Ospedaliero Universitaria Senese

With other Italian and Foreign Institutions/Organizations
- » University of Leuven (Belgium)
- » Swiss Federal Institute of Technology, Zurich (Switzerland)
- » Université Paris Diderot – Paris 7 (France)
- » University of Texas (USA)

Publications

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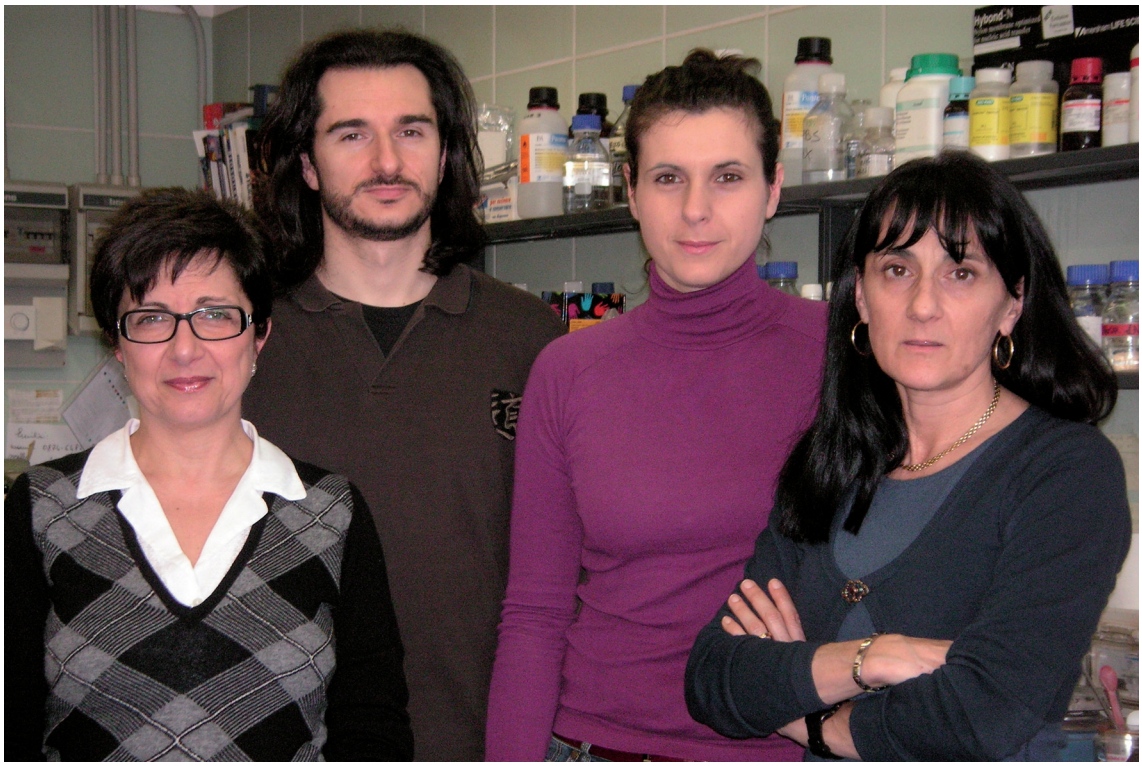
APOPTOTIC CELL DEATH



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Introduction

The primary experimental work of the Unit is basic research in tumor cell biology, focused mainly on the mechanisms of drug-induced apoptotic cell death and on the cross-talk between apoptosis and autophagic response in melanoma cells.

Main Research Themes

Advanced-stage and metastatic melanomas are commonly refractory to the standard therapies, the major contribution to such a resistance and poor prognosis being the low rate of apoptotic cell death. Furthermore, alterations of apoptotic pathways can play a direct role in melanoma progression, from initiation to invasion and metastases. Therefore, the identification of novel pathways involved in apoptosis of sensitive melanoma cells is a challenge to understand melanoma chemoresistance and to reveal possible alterations contributing to melanoma development.

1. Role of calpains in melanoma cell biology

The major focus of our previous research has been to characterize some biochemical mechanisms of cisplatin-induced apoptosis in human melanoma cells (14,6). In the last few years, our research focused on the role played in melanoma biology by calpains, a large family of ubiquitous or tissue-specific cysteine proteases involved in many cellular events, including apoptosis. In cisplatin-treated human melanoma cells we have demonstrated that activation of ubiquitous calpains (1 and 2) play a pro-apoptotic role, and their pharmacological inhibition, down-regulating the cisplatin-induced p53 activation, significantly protects from cell death (3). More recently, in melanoma cells and in melanocytic lesions we have also identified and sequenced two novel splicing variants of muscle-specific calpain 3 (GenBank accession n. EU91850 and EU91851), both endowed with a Nuclear Localization Signal (1). In cisplatin-treated pre-apoptotic cells an increase of both transcription and (auto)proteolytic cleavage of this variants occurs; the latter event, regarded as an activating process, is prevented when also apoptosis is prevented by inhibiting calpains 1/2. Interestingly, among melanocytic lesions, the expression of these novel variants is significantly down-regulated, compared to benign nevi and to early stage lesions, in the most aggressive ones (i.e. in vertical growth phase melanomas and, even more, in metastases), characterized by invasiveness properties, and usually resistant to apoptosis. Along with apoptosis, the molecular mechanisms of autophagy are gaining an increasing interest for tumor cell biology, due to the cross-talk between autophagy and apoptosis, which can interfere with or contribute to drug-induced cell demise.

Our aim is to give further insights on the role played by calpains 1/2 and calpain 3 novel variants in human melanoma cells; in particular, our research will investigate a) mechanisms of calpain proteolytic machinery involved in the interplay between autophagy and apoptosis; b) the role of calpains 1/2 and calpain 3 variants in cell proliferation, motility and apoptosis, in experimental cellular models of calpain overexpression or silencing; c) the functional cross-talk between calpains 1/2 and calpain 3 variants; d) proteolytic targets of calpain 3 variants, with a particular interest to β–catenin levels and its nuclear translocation, in cultured melanoma cells and in human biopsies of melanocytic lesions.

2. Stimuli-sensitive hydrogels as a platform for anticancer drugs

Cisplatin is a commonly used drug in the treatment of a variety of solid tumors, usually administered intravenously. Since about 90% of cisplatin remains bound to plasma proteins in the blood, only a small amount of the drug enters tumor cells, and serious side effects (mainly nephrotoxicity) occur. There is an increasing interest for the design of polymeric hydrogels able to complex cisplatin or other drugs, in order to control the drug release by changing the gel structure in response to environmental stimuli, well-tolerated in vivo and implantable in a solid tumor. Such a new strategy for chemotherapy should allow the delivery of the drug to the local environment of a tumor, with the goal of achieving the effective drug concentration for a sufficient period of time. In collaboration with Prof. Mario Casolaro (Department of Pharmaceutical and Applied Chemistry, University of Siena), who prepared and characterized two novel hydrogels containing α-amino acid residues, we have recently published results showing the

pharmacological efficacy of the cisplatin released from such polymers (2): the platinum species released from hydrogel (cisplatin loading in water) retains its cytotoxic activity towards human melanoma cells, in the same manner shown by the native cisplatin.

Future studies will be aimed to the synthesis of new tunable molecules based on vinyl polymers bearing α-amino acid residues and with a controllable amount of crosslinking agents. These hydrogels, expected to be multiple stimuli-sensitive to control the drug release under different biological conditions, will be complexed with cisplatin or other cytotoxic drugs (as single compounds or as mixtures), and will be tested on cultured tumor cells for their ability to induce cell death, possibly more efficiently than native drugs.

3. Effects of GGT expression on PARP and PARG levels and activities

Oxidant agents are capable to induce post-translational modifications of proteins, often resulting in changes of their functional status; such changes are particularly important in tumor cell biology when key molecules acting in the equilibrium between cell proliferation and cell death are involved. In collaboration with Prof. A. Pompella (University of Pisa) the present research is aimed to evaluate the relevance to these processes of the membrane γ-glutamyltransferase (GGT) activity, whose novel role in cell redox modulation have been characterized by us during recent years (5,10,11,13). Our previous results showed that chemical inhibition of GGT in tumor cells is accompanied by down-regulation of poly(ADP-ribose) polymerase (PARP) activity and onset of apoptosis, suggesting that GGT-mediated pro-oxidant reactions might represent sort of a “life signal” for the cell, possibly through an oxidant-mediated inhibition of caspase activity and consequent up-regulation of PARP.

PARP, whose enzymatic activity is counteracted by poly (ADP-ribose) glycohydrolase (PARG), is activated in the presence of oxidant-induced DNA strand breaks. It is conceivable, therefore, that PARP and PARG activities can be modulated by a GGT-dependent redox modulation; such a correlation will be verified in GGT-overexpressing tumor cells. Since cancer cells endowed (naturally or artificially) with lower levels of PARP are more prone to undergo apoptosis by chemotherapeutics, the relevance of the observed changes will be also assessed in terms of drug sensitivity.

Research Grants

Year	Funding Agency	Amount
2008	Istituto Toscano Tumori	€ 82,000
2008	Fondazione Monte dei Paschi di Siena	€ 20,000
2005	Piano di Ateneo per la Ricerca – University of Siena	€ 28,500
2005	Piano di Ateneo per la Ricerca – University of Siena	€ 15,200
2005	Ministero dell’Istruzione, dell’Università e della Ricerca (MIUR) – PRIN	€ 37,100
2004	MIUR – PRIN	€ 61,000

Main Collaborations

With Units within ITT

» Department of Experimental Pathology and Biomedical Engineering, Section of General Pathology, University of Pisa
» Department of Pharmaceutical and Applied Chemistry, University of Siena

Publications

1. Moretti D, Del Bello B, Cosci E, Biagioli M, Miracco C, Maellaro E: *Novel variants of muscle calpain 3 identified in human melanoma cells: cisplatin-induced changes in vitro and differential expression in melanocytic lesions*. Carcinogenesis 2009; 30(6): 960-7.
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6. Del Bello B, Valentini MA, Comporti M, Maellaro E: *Role of caspase-3 and –7 in Apaf-1 proteolytic cleavage and degradation events during cisplatin-induced apoptosis in melanoma cells*. Exp Cell Res 2004; 293: 302-10.
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10. Pieri L, Dominici S, Del Bello B, Maellaro E, Comporti M, Paolicchi A, et al: *Redox modulation of protein kinase/phosphatase balance in melanoma cells: the role of endogenous and γ-glutamyltransferase-dependent H2O2 production*. Biochim Biophys Acta 2003; 1621: 76-83.
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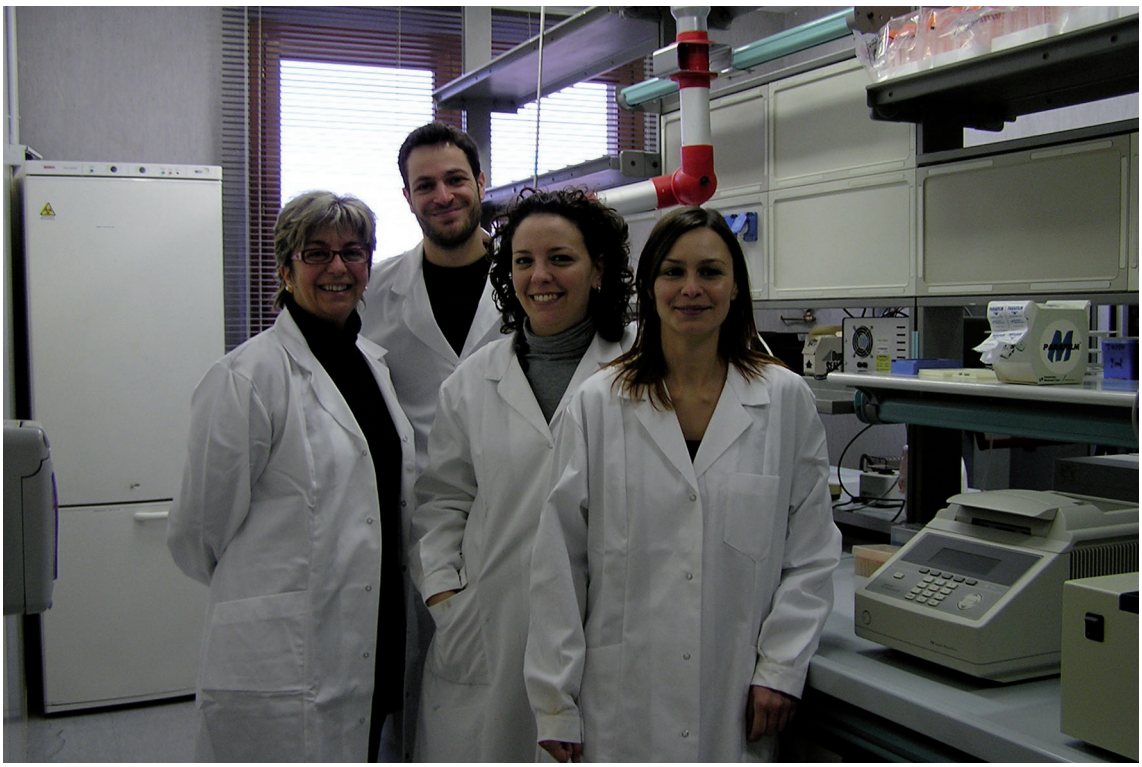
14. Del Bello B, Valentini MA, Zunino F, Comporti M, Maellaro E: *Cleavage of Bcl-2 in oxidant-and cisplatin-induced apoptosis in melanoma cells*. Oncogene 2001; 20: 4591-5.

CLINICAL PHYSIOLOGY



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Johan Carlos Trivino, PhD Student

Introduction

The Siena Unit of the Institute of Clinical Physiology (IFC) – CNR originated in 2003 as a branch of the IFC-CNR of Pisa. Its research activity is in the field of biomedical research and has a central role in basic and technological research in the area of experimental oncology. Its goal is to improve scientific and technological research through interaction with national and local scientific institutions. Since 2005, the IFC-CNR of Siena has become a “Polo Toscano” Unit of the “CNR Medical Research in Italy” (MERIT) network. The IFC-CNR of Siena is located within the Toscana Life Sciences (TLS) laboratories, which are part of “Torre Fiorentina”’s Novartis scientific complex in Siena. The IFC-CNR

Siena Unit has about 160 sq meters of laboratories and 50 sq mtrs of administrative offices. Moreover, it has access to other facilities, such as an animal house facility, a PCL3 laboratory, dedicated rooms for communal analytical equipment, storage of chemicals and radioactive room, as well as meeting rooms, a reception and a recreational area. The staff consists of three Units of CNR personnel employed full-time (two researchers and one administrator) and three CNR grant-funded people.
The activity of the Siena CNR is concentrated on understanding the pathogenetic mechanism of specific tumor histotypes, such as retinoblastoma, osteosarcoma, lung and breast tumors and chemotherapy-sensitive and -resistant tumors, with the aim of identifying tumor-specific molecular markers and develop new therapeutic strategies.

Main Research Theme

Development of new therapeutic and diagnostic strategies

a) Identification of proteins that broaden "gene targeting" in human tumor cells that are sensitive and resistant to chemotherapeutic drugs, and development of new therapeutic strategies
Main achievements:

i) Multi-drug resistant osteosarcoma cell lines have been isolated in our laboratory, starting from a clone of drug-sensitive cells, with the aim to develop an experimental model to study the molecular mechanisms of pharmacoresistance in bone tumors. Molecular markers and the mechanisms of regulation of genes typed to the drug-resistant phenotype were identified. Several *in vitro* experiments of combination therapeutic strategies were conducted (hypomethylation of DNA and chemotherapy) with the aim of evaluating possible new therapeutic protocols to cure drug-resistant osteosarcoma.

ii) *In vitro* experiments on retinoblastoma and Non-Small Lung adenocarcinoma (NSLC) Cell lines have identified some possible mechanisms of gene deregulation. Analyses of promoter methylation (MSP) and gene expression profiles (cDNA microarrays) were conducted on both tumor histotypes. Epigenetic modifications due to hypermethylation of the cytosine on the promoters of some genes are necessary to determine their silencing and can compete together with other genetic alterations to neoplastic transformation. Treatments with appropriate doses of demethylating (5-AZA-2-deoxycytidine) and/or Histone-Deacetylating (HDAC) (Trichostatin A, TSA) agents reactivate the expression of tumor suppressor genes in retinoblastoma and lung adenocarcinoma tumors, suggesting a possible use of this drug in the therapy of these tumors. Through the identification of tumor markers, specific ribozymes targeting mRNAs of genes overexpressed in retinoblastoma and NSLC tumors were synthesized. The objective is to develop new therapeutic protocols based on using hypomethylating agents and ribozymes with the aim to induce tumor mass regression and metastasis inhibition in these two tumor histotypes.

iii) New drug delivery systems have been developed in our laboratories to improve the efficiency and efficacy of therapeutic strategies. Newly engineered human erythrocytes and chemically modified magneto-nanoparticles have been developed to specifically target therapeutic agents at the tumor site by using a static magnetic field.

Current work: Therapeutic strategies based on the use of demethylating agents alone or in combination with chemotherapy drugs or ribozymes are currently being tested *in vitro* in different tumor histotypes sensitive and resistant to chemotherapy drugs. The preliminary results show that the combination of a demethylating agent (5-Aza-dC) with a chemotherapy drug (*i.e.* doxorubicin) or synthetic ribozymes, specifically targeting tumor markers (*i.e.* HSP1, NFKB), increase the

percentage of apoptotic tumor cells. *In vitro* and *in vivo* experiments are in progress to set best conditions for our newly developed drug delivery systems used to improve the therapeutic effects of anticancer compounds.

Future work: With the aim of evaluating the effects of treatments with demethylating/ deacetylating agents alone or in combination with other drugs (chemotherapy or ribozymes) in retinoblastoma and pulmonary adenocarcinoma, *in vivo* experiments will be conducted on three different experimental animal models that develop retinoblastoma and pulmonary adenocarcinoma:

- *Animal models of retinoblastoma*: *i*) female, 6-7 week old CD1 nu/nu mice (nude mice) received an injection in the thigh of approximately 10^6 Y79 or Weri-Rb1 cells; *ii*) transgenic murine models that overexpress the large T antigen of SV40 (SV40-Tag) and demonstrate spontaneous development of hereditary forms of tumors of the retina, *i.e.* similar to the human form of retinoblastoma; *iii*) conditional Ad-Cre Rb -/- transgenic mice that, if induced, develop tumors of the retina.
- *Animal models of pulmonary adenocarcinoma*: mutant LSL-K-Ras G12D murine model that conditionally expresses active K-Ras, after intranasal administration of an adenoviral vector (Ad-Cre), and develop an atypical hyperplasia adenomatosa after two weeks of infection, a papillary adenoma after six weeks, diffuse adenoma at 12 weeks and adenocarcinoma at 16 weeks.

  The therapeutic protocols, based on the use of a demethylating agent alone or in combination with: *i*) chemotherapy drugs; *ii*) new target-specific drugs (ribozymes, synthetic peptides, gene and antibody therapy); *iii*) short-interfering RNAs will be tested in these animal models. New drug delivery systems developed in our laboratories will be used with the objective to improve the efficacy and efficiency of therapeutic protocols.

b) Development of cDNA tumor-specific microarrays for diagnostic use

Main achievements: Various tumor histotypes (renal carcinoma, medullary thyroid carcinoma, malignant mesothelioma, skin melanoma, three different histotypes of non-Hodgkin lymphoma, retinoblastoma, and osteosarcoma) were analyzed using cDNA microarrays (MWG Human Cancer Array) containing 1,853 human genes associated with tumor development and progression. The objective was to evaluate the global expression of the genes that characterize each tumor histotype. Several sets of possible molecular markers linked to the genesis of different tumor histotypes have been identified by using the cDNA microarray analysis.

Current work: We are currently analyzing the data obtained on the tests of cDNA microarrays conducted on different tumor cell lines treated and untreated with demethylating/deacetylating agents.

The analysis of hierarchical clustering, which takes into consideration the quantity of data regarding differential expression of microarrays reported by the various cellular lines, are being analyzed with the aim of identifying the genetic pathways that influence the epigenetic modifications involved in neoplastic transformation of these tumor histotypes.

Future work: A data bank of genes that characterizes each tumor histotype will be constructed with the aim of illustrating cDNA microarrays for diagnostic use. Software that is able to correlate gene profile information with their functional information extrapolated from the data bank (GenBank) will be developed.

Research Grants

Year	Funding Agency	Amount
2008-2011	CNR	€ 84,250
2008-2011	CNR	€ 84,250
2008-2011	CNR	€ 750,000
2003-2008	National Institutes of Health (NIH), Bethesda, Maryland (USA)	$ 1,856,000
2003-2004	Ministero della Salute	€ 30,000
2003-2004	Istituto Superiore Prevenzione e Sicurezza sul Lavoro (ISPESL)	€ 50,000

Main Collaborations

With Units within ITT

» Signal Transduction Unit, ITT Core Research Laboratory (CRL), Siena

With other Italian and Foreign Institutions/Organizations

» Institute of Neurobiology and Molecular Medicine, CNR, Roma
» Institute of Neurosciences, CNR, Pisa
» Fase 1 srl, Cagliari
» Department of Microbiology and Biotechnology, Marshall University, Huntington, West Virginia (USA)

Publications

1. La Sala D, Magnano AR, Estenoz M, Giordano A, Cinti C: *Chromatin remodeling agents for cancer therapy*. Rev Recent Clinical Trial 2008; 3: 192-203.
2. Cinti C, Vindigni C, Zamparelli A, et al: *Activated Akt as an indicator of prognosis in gastric cancer.* Virchows Arch 2008; 453: 449-55.
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4. Tosi GM, Trimarchi C, Macaluso M, et al: *Genetic and epigenetic alterations of RB2/p130 tumor suppressor gene in human sporadic retinoblastoma: implications for pathogenesis and therapeutic approach*. Oncogene 2005; 24: 5827-36.
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9. De Falco G, Bellan C, Lazzi S, et al: *The interaction between HIV-1 Tat and pRb2/p130: a possible mechanism in the pathogenesis of AIDS-related neoplasms*. Oncogene 2003; 22: 6214-19.
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14. Bellan C, De Falco G, Tosi GM, et al: *Missing expression of pRb2/p130 in human retinoblastomas is associated with reduced apoptosis and lesser differentiation*. Inv Ophthal Visual Sci 2002; 43: 3602-8.
15. Leoncini L, Bellan C, Cinti C, Giordano A: *From cancer drug discovery and development: cell cycle inhibitors in cancer therapy*. In Giordano A, Soprano KJ (eds), *Tumor suppressor genes as diagnostic tools*. Totowa (NJ), Humana Press Inc, 2002; Chapter 4, 49-82.
16. Claudio PP, Cinti C, Giordano A: *Application of the primer in situ DNA synthesis (PRINS) technique to titer recombinant virus and evaluation of the efficiency of viral transduction*. Anal Biochem 2001; 29: 96-101.
17. Zamparelli A, Masciullo V, Bovicelli, et al: *Expression of cell-cycle-associated proteins pRb2/p130 and p27kip in vulvar squamous cell carcinomas*. Hum Pathol 2001; 32: 4-9.
18. Maraldi NM, Giordano A, Manzoli L, Falconi M, Pol AD, Cinti C: *Genetic alterations at the nuclear localization signal of the RB2/p130 gene occur in lymphoid tumor but not in osteosarcoma cell lines*. Adv Enzyme Regul 2001; 41: 31-55.

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MEDICAL ONCOLOGY

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Michela Rinaldini, Oncologist
Simona Scali, Oncologist
Alessandra Signorini, Oncologist
Gabriella Rossi, Hematologist
Ori Ishiwa, Oncology Intern
Samanta Capacci, Data Manager
Donatella Nardi, Nurse

Introduction

The Medical Oncology Unit was established in Arezzo in May 1984. It was initially located in the old "Centro Oncologico", built by the Calcit Association close to the "Ospedale Garbasso" in Arezzo, but is now housed in the new "Centro Oncologico", built in 2001 with a contribution from the Calcit Association, close to the new "Ospedale San Donato" in Arezzo.

The activity of the Medical Oncology Unit in the Arezzo area is organized in five different locations, with the main activity in “Ospedale San Donato” in Arezzo, Medical Oncology Services is located in “Ospedale Santa Maria La Gruccia” in Montevarchi, and three Day Hospitals are located in Bibbiena, Sansepolcro and La Fratta.
The Unit, which offers the best available treatment for oncological patients in day hospital and ambulatory regimens, was directed by Dr. Paolo Ghezzi, until the end of 2008. Since June 2009, it has been under the direction of Dr. Sergio Bracarda. Under this new direction, an intensive clinical and translational research activity has been started.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
AXIS Trial: axitinib (ag-013736) as second line therapy for metastatic renal cell cancer. Protocol A4061032	2009	Pfizer	5
An open-label, multicenter, expanded access study of RAD001 in patients with metastatic carcinoma of the kidney who are intolerant of or have progressed despite any available vascular endothelial growth factor receptor tyrosine kinase inhibitor therapy. Protocol CRAD001L2401	2009	Novartis	10
Study VEG108844: a study of pazopanib *versus* sunitinib in the treatment of subjects with locally advanced and/or metastatic renal cell carcinoma. Protocol VEG108844	2009	GlaxoSmithKline	3
READY: a randomized double-blind phase III trial comparing docetaxel combined with dasatinib to docetaxel combined with placebo in castration-resistant prostate cancer. Protocol CA180-227	2009	Bristol-Myers Squibb	1
A phase III, randomized, double-blind, placebo-controlled study of abiraterone acetate (CB7630) + prednisone in asymptomatic or mildly symptomatic patients with metastatic castration resistant-prostate cancer. Protocol COU-AA-302	2009	Cougar Biotechnology Inc.	2
LUX-Lung 3: a randomized, open-label, phase III study of BIBW2992 *versus* chemotherapy as first-line treatment for patients with stage III or IV adenocarcinoma of the lung harboring an EGFR activating mutation. Protocol 1200.32	2009	Boehringer Ingelheim	0
A phase III, randomized trial of FOLFOXIRI + bevacizumab *versus* FOLFIRI + bevacizumab as first-line treatment for metastatic colorectal cancer. Protocol TRIBE-ASL608LIOM04	2008	Gruppo Oncologico del Nord Ovest (GONO)	1

Description	Year	Sponsor	Number of patients recruited to date
A phase II, double-blind, placebo-controlled, multicenter, randomized study of ZD4054 + carboplatin and paclitaxel or placebo + carboplatin and paclitaxel in patients with advanced ovarian cancer sensitive to platinum-based chemotherapy. Protocol D4320C00036	2009	AstraZeneca	0

Publications

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Grosseto Area

MEDICAL ONCOLOGY

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Ilaria Pastina, Oncologist

Introduction

Our Unit was established in 1976 as part of Division of Internal Medicine of Ospedale “Misericordia”, Grosseto.
In 1999, the Division became independent, with its own director and doctors, and was separated from Internal Medicine.

We were able hospitalize patients until June 2007. Since 2008, we have organized five Day Hospitals in Grosseto, Orbetello, Massa Marittima, Casteldelpiano and Pitigliano, with a territory of about 230,000 inhabitants/users.
We treat patients with solid and hematologic tumors and we have several multidisciplinary groups (GOM) to discuss clinical cases with surgeons, radiotherapists, radiologists and pathologists.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
MOTOR: a multicenter, phase II evaluation of torisel as second-line treatment for metastatic RCC patients progressing after cytokine therapy, tyrosine kynase or an angiogenesis inhibitor	2009	GIR – Oncotech	1
SOFISEP: a multicenter, randomized, open clinical trial to evaluate the efficacy of the lock of the completely implantable central venous catheter with physiological solution *versus* eparinate solution	2009	SOFISEP	8
A6181087: a multicenter, randomized, double-blind, controlled phase III trial efficacy and safety study of erlotinib with or without sunitinib in the treatment of advanced/metastatic non-small cell lung cancer	2008	Pfizer	4
A6181094: a phase III study of SU011248 in combination with paclitaxel *versus* bevacizumab with palcitaxel in first-line advanced disease setting in patients with breast cancer	2008	Pfizer	7
COU-AA-301: a phase III, randomized, double-blind, placebo-controlled study of abiraterone acetate + prednisone in patients with metastatic castration-resistant prostate cancer who have failed docetaxel-based chemotherapy	2008	Novella	5
GIM4, Letrozole Adjuvant Therapy Duration (LEAD) study: standard *versus* long treatment. A phase III trial in post-menopausal women with early breast cancer	2005	Oncotech	20
GIM1: a randomized, clinical, phase III study on sequential therapy with Epidoxorubicin and Cyclophosphamide (EC) followed by docetaxel (EC -> D) *versus* 5-Fluorouracil, Epidoxorubicin and Cyclophosphamide (FEC) in combination as an adjuvant treatment in patients with lymph node-negative breast cancer	2004	Oncotech	19
GIM2: randomized, phase III study on EC followed by paclitaxel *versus* FEC followed by paclitaxel, administered every two to three weeks with pegfilgrastim, for patients with previously operated breast cancer (4 BR)	2003	Oncotech	15

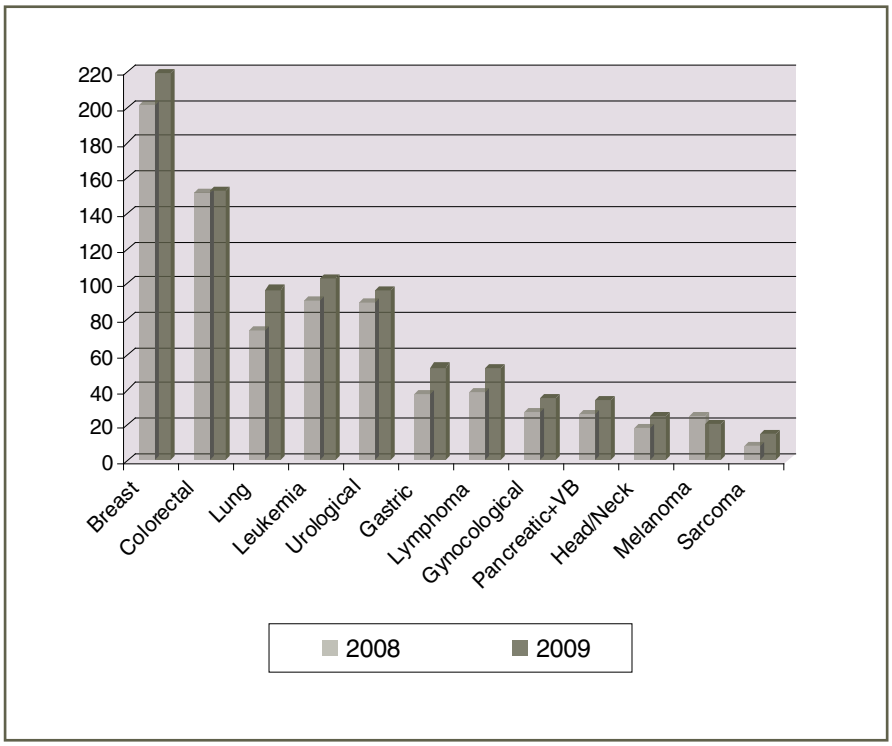

Figure 1 - Types of neoplasia under the care of the Medical Oncology Unit

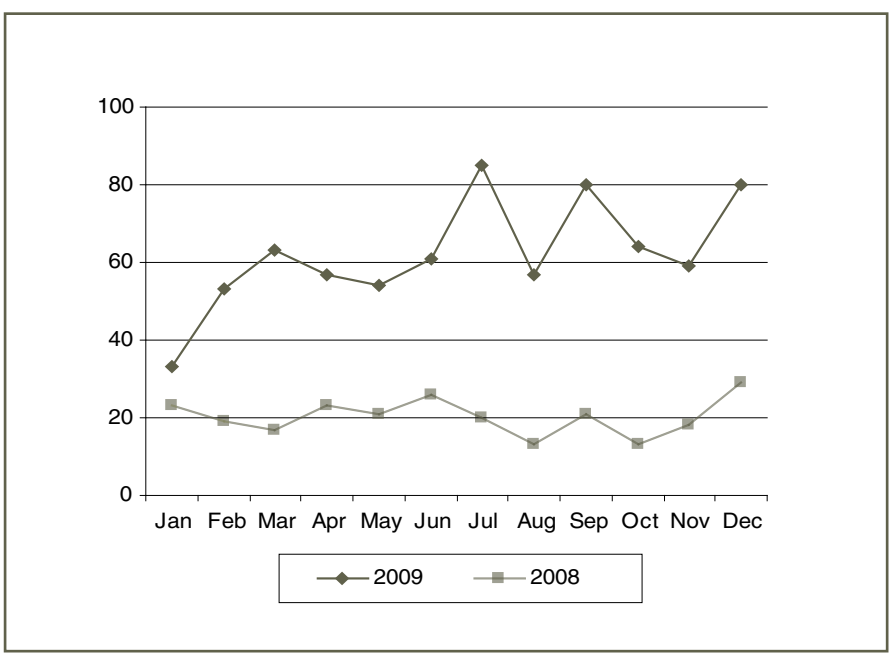

Figure 2 - Comparison of GOM activity in two successive years

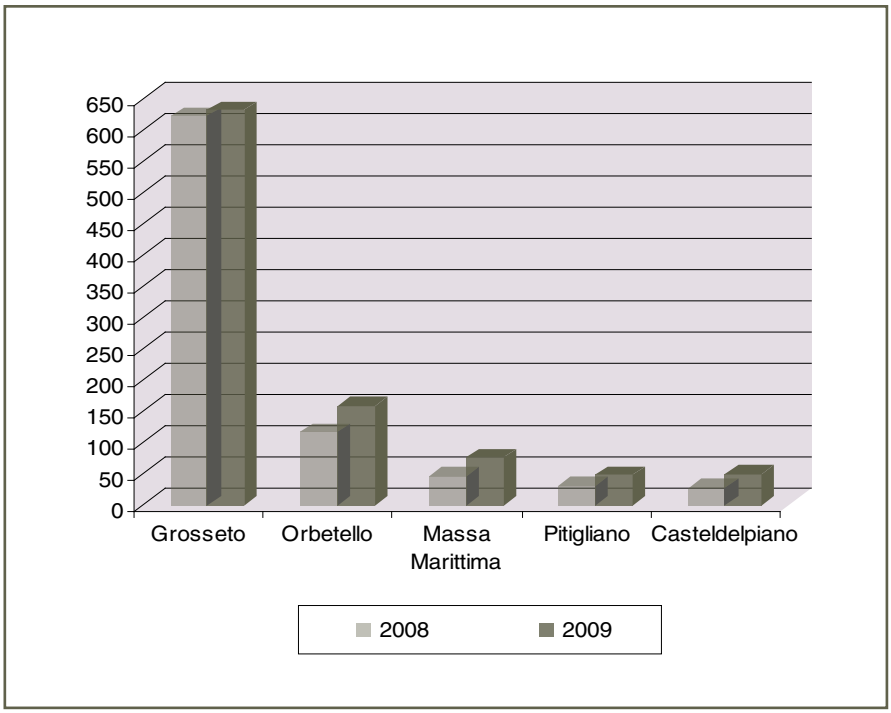

Figure 3 - Patients treated at the Medical Oncology Unit

Main Collaborations

With Units within ITT

» Breast Surgery Unit, Azienda Ospedaliero Universitaria Careggi, Firenze

With other Italian and Foreign Institutions/Organizations

» Istituto Nazionale Tumori (INT), Milano

» Istituto Europeo di Oncologia (IEO), Milano

Firenze Area

MEDICAL ONCOLOGY

Unit Address

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Varesco Martini, Hematologist, Oncologist
Ilaria Meucci, Oncologist
Anna Maria Montanari, Oncologist
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Massimiliano Zisa, Data Manager

Introduction

The Medical Oncology Unit has been working since 1999 as an autonomous unit in Ospedale "Santa Maria Annunziata". Since 2001 the Unit has also included another four Oncological Day Hospitals, located in the Ospedale "Serristori", Ospedale "Santa Maria Nuova", Ospedale "San Giovanni di Dio" and Ospedale "Nuovo del Mugello". In 2007, the Department of Oncology was instituted, which includes Medical Oncology Unit, Radiotherapy Unit, three Palliative Care Units and a Psycho-Oncology Unit. Luisa Fioretto is the Director of the Department of Oncology.
Since 2005 an Oncological Day Service organization has been in operation at Ospedale "Santa Maria Annunziata" and since November 2009 several functional beds have been made available for ordinary oncological hospitalization in this hospital as well. The Unit has 43 available beds in a Day Hospital setting.
The main activities of the Unit are: diagnosis, staging and treatment of the most frequent solid tumors, and hematological neoplasms; in addition, the adoption of lean thinking methodology in the management of clinical pathways. The activities of the Gruppi Oncologici Multidisciplinari (GOM) provides multidisciplinary evaluations and departmental organization leading to continuous care in various settings.
The aim of the activity is to provide better care to cancer patients, offering appropriate diagnostic and treatment procedures, promoting a global health approach for the patient, including psychological, social and familial aspects, by a professional team entirely involved in the process of care.
In 2009, 2,326 first oncological visits were performed, a total of 16,510 oncological visits, 1,089 patients, were managed in a Day Service setting, 1,866 in a Day Hospital setting, mainly for antiblastic therapy.
The Unit also focuses on Clinical Governance, Pharmacoeconomy and Psycho-Oncology.

Main Research Themes

1. Clinical governance

New models of clinical pathway management: OLA project (lean thinking in oncology) and Chronic Care Model in oncology.

Simultaneous care in oncology: an experimental integration of professional skills between palliativist and medical oncologist ("Ponte" project).

Informed consent model in Medical Oncology.

Organizational Model for Caring and Researching in Oncology.

2. Pharmacoeconomy

Cost-effectiveness of PDTA in breast and colorectal cancer: a development of new integrated evaluation instruments (oncologist, pharmacist, administrative working group).

"Drug day": an experimental ongoing program for management, control and waste reduction of high cost drugs.

3. Psycho-oncology

Narrative Medicine.

Quality of Life and expressive-supportive psychotherapy.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
BOLERO-2: a randomized double-blind, placebo-controlled study of everolimus in combination with exemestane in the treatment of post-menopausal women with estrogen receptor-positive locally advanced or metastatic breast cancer who are refractory to letrozole or anastrozole	2009	Novartis	1
CLEOPATRA: a phase III, randomized, double-blind, placebo-controlled clinical trial to evaluate the efficacy and safety of pertuzumab + trastuzumab + docetaxel *versus* lacebo + trastuzumab + docetaxel in previously untreated HER 2-positive metastatic breast cancer	2008	Roche	1
O-HERA: an observational study of cardiac events in patients with HER2-positive early breast cancer treated with herceptin	2008	Roche	13
TRIBE: a phase III randomized trial of FOLFOXIRI + bevacizumab *versus* FOLFIRI + bevacizuman as first-line treatment for metastatic colorectal cancer	2008	Gruppo Oncologico del Nord Ovest (GONO)	5

Main Collaborations

With other Italian and Foreign Institutions/Organizations

» GONO
» Gruppo Oncologico Italiano per la Ricerca (GOIRC)
» Associazione Italiana di Oncologia Medica (AIOM)
» Collegio Italiano dei Primari Oncologi Medici Ospedalieri (CIPOMO)

Publications

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3. Falcone A, Ricci S, Brunetti I, et al: *The triplet combination of infusional 5-Fluorouracil/Leucovorin, Oxaliplatin and Irinotecan (FOLFOXIRI), vs the doublet of infusional 5-Fluorouracil/Leucovorin and Irinotecan (FOLFIRI) as first-line treatment of metastatic colorectal cancer: results of a randomized phase III trial by The Gruppo Oncologico Nord-Ovest (GONO)*. J Clin Oncol 2007; 25: 1670-6.
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6. Angiolini C: *The optimal duration of adjuvant therapy in breast cancer: what kind of evidence?Hormone therapy: what kind of evidence?* Suppl Tumori 2004 Jul-Aug; 3(4): S75-6.
7. Neri B, Cini G, Doni L, et al: *Weekly gemcitabine plus Epirubicin as effective chemotherapy for advanced pancreatic cancer: a multicenter phase II study*. Br J Cancer 2002; 87: 497-501.

RADIOTHERAPY

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Gabriele Salvadori, Technician
Marianna Mastrazzo, Technician
Tamara Priori, Social Operator
Catia Calvellini, Social Operator

Introduction

This Radiotherapy Center began its activity one year ago.
We have at our disposal, two Elekta Synergy linear accelerators, one of which is endowed with a Cone Beam CT for Image-Guided Radio Therapy (IGRT). The varying energy level of the available beams are as follows: photons 6,10,15 MV, and electrons 4,6,9,12,15,18,20 MeV.
We are waiting for a mobile electron accelerator for IntraOperative Radiation Therapy (IORT).
All linear accelerators have a multileaf collimator (MLC), portal imaging detectors and an active breathing control system (ABC). These devices allow for the performance of personalized three-dimensional treatments.
The virtual simulation is performed using CT images, endowed with mobile lasers. The treatment planning system is the 3D Odyssey by PerMedics.
This Radiotherapy Center is able to perform a large variety of treatment types, such as Intensity Modulated Radio Therapy (IMRT).
The main pathologies taken care of in this Center are breast and gynecological cancers, gastrointestinal tumor, urological cancer, lung cancer, head and neck cancer and lymphomas.

Main Research Themes

1. Dosimetric European comparison between EQUAL-ESTRO: "External quality control in Radiotherapy based on postal TLD method" for high energy photons and electron beams.
2. Partial breast irradiation in early breast cancer patients who underwent conservative surgery.
3. IMRT tolerance and treatment in prostate cancer and head and neck cancer.

Main Collaborations

With Units within ITT

» Department of Radiotherapy, Azienda Ospedaliero Universitaria Careggi (AOU Careggi), Firenze
» Breast Unit, AOU Careggi, Firenze
» Oncological and Reconstructive Orthopedics Unit, Centro Traumatologico Ortopedico (CTO), Firenze
» Department of Radiotherapy, Azienda USL 8 Arezzo
» Department of Radiotherapy, Azienda USL 4 Prato

PSYCHO-ONCOLOGY

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Simone Cheli, Researcher

Introduction

Psycho-oncology is defined as the subspecialty of cancer dealing with two psychological dimensions: *a*)the psychological reactions of patients with cancer and their families and the stress on the staff; *b*) the psychological, social, and behavioral factors that contribute as resources for quality of life. The Psycho-oncology Working Group mission focuses on a real action-research, carried out by a team encompassing a professional action researcher, patients and caregivers to improve their situation. We promote integrated research processes, staff and patient training.

Psycho-oncology action interests: clinical intervention, psychotherapy, educational intervention, analysis of quality of life, and staff training.
The Psycho-oncology Unit was put into effect in 2001 as part of the Medical Oncology Unit. At the end of 2009, a Departmental Psycho-oncology Unit was created.

Main Research Themes

1. Project Start 1 / Start 2: evaluation efficacy of support groups

Main achievement: Patients were trained on the specific skills and knowledge pointed out in our psychosocial survey.

Current work: Recruitment of women with metastatic breast cancer for supportive-expressive group therapy; recruitment of patients with colorectal cancer for a supportive-expressive group therapy.

Future work: Start up the supportive-expressive groups therapy and evaluate results.

2. Narrative Medicine Research in oncology

Main achievement: We will carry out a two-year narrative group for cancer patients and oncological staff.

Current work: Recruitment of patients and staff.

Future work: Thematic analysis of cancer metaphors in patients and oncological staff.

3. Staff training and internship: “Ponte” Project

Main achievement: We developed a training project, as a first step, in order to integrate personal and professional skills and perceptions in staff members of all the Departments: Medical Oncology Units, Palliative Units and Radiotherapy Units.

Current work: We are analyzing quantitative and qualitative data, collected during staff training.

Future work: We are outlining a second step project in order to implement experience and measure efficacy of the simultaneous care model.

PALLIATIVE CARE

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Introduction

Since1990, the quality of life of patients in palliative care and the quality of the palliative care offered to them has been our Unit's subject of research. This area is comprehensive of the epidemiology of patients nearing the end of life, with or without palliative care, and the impact of palliative care and hospital access.

This experience has helped us to realize a data base (QUALE) that will very likely become the official database for palliative care for the Regione Toscana.

In 2009-2010, we are introducing the Liverpool Care Pathways in the Medical Department of the ASF.

Main Collaborations

With Units within ITT

» Epidemiology Units of the Istituto per lo Studio e la Prevenzione Oncologica (ISPO), Firenze

With other Italian and Foreign Institutions/Organizations

» Istituto Nazionale per la Ricerca sul Cancro (IST), Genova
» Fondazione Italiana di Leniterapia (FILE)

Publications

1. Borreani C, Brunelli C, Miccinesi G, et al: *Eliciting individual preferences about death: development of the End-of-Life Preferences Interview*. J Pain Symptom Manage 2008; 36: 335-50.
2. Bulli F, Miccinesi G, Biancalani E, et al: *Continuous deep sedation in home palliative care units: case studies in the Florence area in 2000 and in 2003-2004*. Minerva Anestesiol 2007; 73: 291-8.
3. Moro C, Brunelli C, Miccinesi G, et al: *Edmonton symptom assessment scale: Italian validation in two palliative care settings*. Support Care Cancer 2006; 14: 30-7.
4. Miccinesi G, Crocetti E, Morino P, et al: *Palliative home care reduces time spent in hospital wards: a population-based study in the Tuscany Region, Italy*. Cancer Causes Control 2003; 14: 971-7.

MELANOMA

Unit Address

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Vanni Giannotti, MD
Serena Sestini, MD

Introduction

The Unit was established in the Institute of Dermatology in Florence in 1973. The Plastic Surgery Unit moved to Ospedale “Santa Maria Annunziata” in 1997 and was recognized as the Regional Melanoma Referral Center in 2000. Our Unit is currently carrying out clinical and translational research on cutaneous tumors and, particularly, on melanoma. The primary aims of our research projects are: *a*) to develop surgical techniques and instruments; *b*) to study the immuno-biology of dendritic cells within the sentinel node and the melanoma immune escape mechanisms; *c*) to evaluate possible novel immunotherapies.

Main Research Themes

1. Analysis of possible novel diagnostic and prognostic factors in cutaneous melanoma

This is a three years multidisciplinary study aiming to increase diagnostic and prognostic knowledge in the melanoma field. Particularly, the study will investigate novel possible biomarkers in diagnosis and progression of melanoma. Our detailed plan is as follows:

a) distinguish within "thin" melanomas some subsets with no/low progression potential from subsets with a high progression potential, with possible impact on the follow-up program and adjuvant therapy;
b) monitor changes of possible different "patterns" in time and/or in relation with specific therapeutical measures;
c) identify possible diagnostic and therapeutic targets, preferentially early developed, as novel molecular targets.

The multidisciplinary study will converge the expertise of dermatologists, general and plastic surgeons, oncologist, epidemiologist and basic researchers (expert in cellular biology, genetics, proteomics and metabolomics) toward a unique objective.

The synergism among these highly qualified subgroups may lead to important contributions to the advancement of knowledge in the melanoma field.

2. Technical improvements of sentinel node biopsy procedure using computer-assisted gamma probe with adjustable collimation

The Sentinel Node (SN) biopsy technique has revolutionized the surgical approach to melanoma patients. SN biopsy procedure is of growing importance for correct melanoma staging, early detection of nodal micrometastasis and proper enrollment of melanoma patients in trials of adjuvant therapy. The SN status is the most significant prognostic factor, exceeding any primary tumor feature. The success of the SN biopsy technique, which aims for the highest SN identification rate and the lowest number of false negatives, depends greatly on technical aspects and on the accuracy of the procedure. We performed the SN biopsy in 950 patients. We developed a computer-assisted gamma probe with adjustable collimation in collaboration with an Italian company. With this instrument, we achieved a 100% SN identification rate. Advantages of this instruments are: high capability to detect the SN signal when melanoma is located close to the SN site; possibility to adjust collimation during the procedure in relationship to the depth of the SN; high sensitivity; high spatial resolution; to calculate SN/background and SN/non-SN ratios; to calibrate energy windows in each patients; to record node counts. We found distant metastases in 2.0% of SN-negative patients and in 24% of SN-positive patients ($p < 0.001$). We found highly statistically significant differences between SN-negative and SN-positive patients in both3 year disease-free survival (86.3% *versus* 49.2%) and three year disease-specific survival (92.3% *versus* 7.1%) ($p < 0.001$).

At present, we are focusing our attention on the development of a *new model of forceps* specifically designed for SN harvest, with the aim to speed up the procedure and to harvest the SN without any traction or damage to the superficial nodal structures.

3. Antigens presenting system analysis on dendritic cells in primary and metastatic melanoma

Dendritic cells (DC) are specialized antigen presenting cells essential to generate primary immune responses against tumors. In the past years, several efforts have been made in order to exploit DC as natural adjuvant in cancer immunotherapy and some encouraging results have been obtained particularly in the melanoma field.

We have focused our interest on DC starting different projects. In collaboration with the Department of Dermatology of the University of Zurich, we have shown that skin DC, the first DC that cutaneous melanoma encounter during progression, are well equipped with all the components of the classical antigen presenting system and, notably, additionally express all members of the CD1 family, a novel antigen presenting system for lipid and glycolipid recognition. It is worth mentioning that melanoma express several kinds of gangliosides, a particular kind of glycolipids.

Continuing our study of the CD1 molecules on DC, we demonstrated that the CD1 system (CD1a, b, c and d), expressed by DC, is heavily down-regulated in melanoma metastasis. This phenomena was related to the production of IL-10, a well known immune suppressive cytokine. We have proposed this phenomenon as a novel immune escape mechanism adopted by metastatic melanoma.

Melanoma cells are able to generate several immune-escape mechanisms to circumvent the immune response. Understanding these mechanisms is crucial to design new immuno-therapeutical approaches. Therefore, we are currently extending our interest to DC located in the sentinel lymph node because there is evidence showing that DC have phenotypic and functional defects in the SN of cancer patients.

4. Maturation state of Langerhan dendritic cells in sentinel lymph nodes of melanoma patients

The sentinel lymph node represents the first node of the lymphatic draining systemfrom a tumor and is the site where mature DC are expected to present tumor antigens to naive T-cells generating cytotoxic T-cell responses against tumor.

Recently, it has been reported that not all the DC reaching lymphoid organs are mature, but certain types are phenotypically and functionally immature, and are very likely to maintain immune tolerance towards self-antigens. Furthermore, there is also evidence showing that DC have phenotypic and functional defects in the SN of cancer patients. Therefore, we extended our DC studies aiming to evaluate the maturation state of Langerhan cells in SLN. Interestingly, our preliminary results indicated that in some melanoma patients Langerhan cells in SN are not completely mature as evaluated by flow cytometry and confocal scanning microscopy studies. In line with these observations, Langerhan cells cultured for 24 hours in the presence of a maturation *stimuli* reached a full maturation profile. These preliminary data suggest that in some melanoma patients Langerhan cells migrate to the SL in an immature state likely to maintain immune tolerance towards self-antigens. These data, if confirmed and extended to functional aspects, may open the way for new strategies in *in situ* DC vaccination.

5. Plasmacytoid dendritic cell analysis in sentinel lymph nodes of melanoma patients

We are currently investigating the presence and function of others DC subsets, with particular attention to plasmacytoid dendritic cells (pDC), a DC subset endowed with the capacity to drive immunity or tolerance depending on the micro-environment. We have demonstrated that pDC are normally present in the SN of melanoma patients and accumulate in metastatic nodes. These pDC show an immunophenotype similar to that of blood pDC, which are in a resting state. Consistently, pDC do not produce type I interferon in SLN. These data support the tolerogenic role of pDC in cancer immunology. To further shed light on the role of pDC, our team is currently studying the expression of IDO, a key tolerogenic molecule, on pDC from melanoma SLN. The presence of a IDO-positive subset of tolerogenic DC has been shown in melanoma draining nodes, but their nature remained elusive. We have now shown that most of the IDO-positive DC correspond to the pDC, further supporting their role in cancer tolerance. This finding opens up new opportunities to design immunotherapy strategies by combining IDO inhibitor drugs in addition to TLR agonists.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
Trial 18991: adjuvant Peg-Intron treatment in stage III melanoma *versus* observation after regional lymph node dissection: a multicenter randomized phase III trial	2008	European Organization for Research and Treatment of Cancer (EORTC)	12
Trial 18961: postoperative adjuvant ganglioside GM2-KLH/QS-21 vaccination treatment *versus* observation after resection of primary cutaneous melanoma (AJCC stage II, T3-T4N0M0): a two-arm multicenter randomized phase III trial	2007	EORTC	33

Main Collaborations

With Units within ITT
- » ITT Core Research Laboratory (CRL)
- » Istituto per lo Studio e la Prevenzione Oncologica (ISPO), Firenze
- » Department of Dermatological Sciences, University of Florence
- » Department of Biochemistry, University of Florence
- » Department of Clinic Physiopathology, University of Florence
- » Department of Pathology, University of Florence
- » Department of Experimental Oncology and Pathology, University of Florence
- » Department of Pharmacology, University of Florence
- » Department of Clinical Physiopathology and Endocrinology, University of Florence

With other Italian and Foreign Institutions/Organizations
- » Italian Melanoma Intergroup (IMI)
- » Gruppo Italiano Polispecialistico per il Melanoma (GIPME)
- » Melanoma Group, EORTC

Publications

1. Urso C, Borgognoni L, Doria M, Tinacci G, Zini E: *Non-sentinel lymph node involvement in a patient with an atypical Spitz tumor and a positive sentinel node. Report of a case and review of the literature*. J Cutan Pathol 2009; 36: 586-90.
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DERMATOPATHOLOGY – PIGMENTED SKIN LESIONS

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Introduction

The "Dermatopathology – Pigmented Skin Lesions" Unit of the Pathology Department was established at Ospedale "Santa Maria Annunziata" in 1998.

The main interests of the Unit are: *a*) the histological diagnosis of melanocytic skin lesions and melanoma; *b*) the study of sentinel lymph node biopsy in melanocytic lesions; *c*) the morphology, diagnosis and classification of sweat gland tumors.

Main Research Theme

Studies on melanoma and cutaneous tumors

Main achievements:

a) *Dysplastic nevus*. Dysplastic nevus was studied between 1998 and 2001 with a detailed analysis of a large series of nevi with varying clinical appearance. In this study, we used histological parameters known to be diagnostic for "dysplastic nevus" for the analysis of melanocytic nevi. The study highlights that melanocytic nevi cannot be divided into two different classes of lesions, *i.e.* common and dysplastic nevi, but appear to constitute a continuous spectrum of forms in which the border between common lesions, not implying an increased melanoma risk, and "dysplastic" lesions, implying an increased risk, is arbitrary. Such results may indicate that the conclusions of studies on "dysplastic nevi" cannot be accepted, as they may be biased by the diagnostic selection of lesions.

b) *Histological diagnosis of melanoma*. The diagnostic value of suprabasal melanocytes has been under analysis since 1998 in the diagnosis of melanoma. Results from an analysis of a large series of melanomas and melanocytic nevi have led to the recognition of a histological feature, pseudoinfiltration, not infrequently present in benign melanocytic lesions, such as Spitz nevi, Reed nevi, recurrent nevi, childhood nevi, genital nevi, etc., which may mimic true pagetoid infiltration of melanoma and must be distinguished from it. A specific set of histological elements to distinguish pseudoinfiltration from pagetoid infiltration has been proposed. In pagetoid infiltration, the number of suprabasal melanocytes is generally high, and all the epidermal layers are usually involved. The phenomenon often seems to be multifocal or diffuse and is generally observed in both the central and peripheral portions of the lesion. Epidermal layers appear disconnected, destroyed, and eroded, and the contours of the epidermal spaces containing melanocytes are irregular and indented. In pseudoinfiltration, the number of suprabasal melanocytes is generally low, and only the basal and spinous layers are involved. The phenomenon seems to be focal or spatially limited and is generally observed in the central portion of the lesion. Epidermal layers appear displaced, compressed, but basically intact, and the contours of the epidermal spaces containing melanocytes are regular and smooth.

c) *Histological features of melanoma*. A multicentric study on the incidence in nevi of current diagnostic parameters used in the diagnosis of melanoma was performed in collaboration with eight Italian universities. The results showed that diagnostic parameters reputed useful in the diagnosis of malignant melanoma can be frequently found in benign melanocytic nevi. An additional multicentric study on the histological features of melanoma was performed in collaboration with seven Italian universities. The results have shown that the interobserver reproducibility of current histological parameters used in the diagnosis of melanoma is good, provided that an accurate definition of them is given. Thus, problems in the diagnosis of melanoma are not due to poor concordance.

d) *Sentinel lymph node biopsy in melanoma*. This procedure has been performed and studied since 1998. To date sentinel nodes from 650 patients have been analyzed.

e) *Sentinel lymph node biopsy in atypical Spitz tumors*. This procedure was studied in 12 patients with lesions labeled as atypical Spitz tumors: 4 showed lymph node deposits of atypical melanocytes (2 of which were aged < 2 years). Results suggested that so-called atypical Spitz tumors possess a relevant metastatic potential.

f) *Carcinomas of sweat glands*. Sweat gland carcinomas were studied by our Unit between 1998 and 2001. A large series of 60 of these rare tumors were studied (the second largest series in the world literature) to define the clinical and histological characteristics of such uncommon tumors. Results showed that the classification and the taxonomy of this chapter of pathology may need to be revised.

g) *Angiomatoid cellular blue nevus*. In 2005, a new previously unrecognized histological variant of the blue nevus was described.

h) *Pseudomyxoid melanoma*. In 2006, a new previously unrecognized histological variant of melanoma was described.

i) *Diagnostic value of current diagnostic parameters of melanoma*. Between 2006 and 2008, the efficacy of the histological criteria currently used in the diagnosis of melanoma were analyzed. We performed a quantitative analysis of 72 conventional (non-Spitzoid, non-desmoplastic) melanomas and 73 conventional melanocytic nevi, used as controls, for 13 histological diagnostic parameters. Results showed that all parameters, except poor circumscription, seemed to be significantly associated with melanoma ($P < 0.05$). Cytological atypia, dermal lymphocytic infiltrate, asymmetry, dimension > 6mm and absence of maturation showed high sensitivity (> 90%); absence of maturation, mitoses, necrosis, asymmetrical melanin, suprabasal melanocytes and melanin in deep cells showed high specificity (> 90%); irregular-confluent nests and predominating single melanocytes were poorly sensitive and poorly specific. We conclude that not all parameters were shown to have the same diagnostic value. Absence of maturation and, limited to melanomas < 2 mm, suprabasal melanocytes were the most discriminating (sensitive and specific) histological features. Cytological atypia, dimension > 6 mm, suprabasal melanocytes and mitoses were additionally reliable diagnostic features, showing relatively high sensitivity and relatively high specificity.

Current work:

a) *Atypical blue nevi*: Blue nevi represent a complex spectrum of lesions, extending from benign forms (common and cellular blue nevi) to malignant ones (malignant blue “nevus” or blue melanoma). While the extreme forms at the two ends of the spectrum are relatively well defined, the intermediate forms are often difficult to diagnose. This is because the histological border between benign and malignant lesions is ill-defined, among these, atypical blue nevi that are melanocytic lesions showing the classical characteristics of blue nevi mixed with one or more features commonly occurring in melanoma. This study is devoted to analyzing the histology of blue nevi and atypical blue nevi in a series of patients who have undergone surgery and/or sentinel node biopsy, in an attempt to establish the diagnostic threshold of malignant cases.

b) *Diagnostic evaluation of intranodal melanocytes*: Intranodal melanocytes often represent a challenging diagnostic problem, concerning the differentiation of benign from malignant melanocytic lesions. The occurrence of intranodal melanocytes in cutaneous borderline melanocytic lesions, such as atypical Spitz tumors or atypical blue nevi, poses further difficult problems, whether or not they are true nodal metastases or simply nodal nevi. This morphological, immunohistochemical and clinical study is aimed at clarifying this controversial subject.

Future work:

a) Relationship between number, dimension and location of melanoma metastases in sentinel lymph nodes and possible involvement of non-sentinel nodes.

b) Evaluation of histological parameters in Spitz nevi/tumors, atypical Spitz tumors and Spitzoid melanomas.

c) Geno-phenotypical evaluation of Spitz tumors.

d) Animal type melanoma and related lesions.

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d) Mandal RV, Murali R, Lundquist KF, et al: *Pigmented epithelioid melanocytoma: favorable outcome after 5-year follow-up*. Am J Surg Pathol 2009; 33(12): 1778-82.

Main Collaborations

With Units within ITT

» Plastic Surgery, Melanoma Referral Center, Ospedale "Santa Maria Annunziata", Firenze
» Department of Experimental Pathology and Oncology, University of Florence
» Dermatology Clinic, University of Florence
» Pathological Anatomy, Ospedale "Misericordia e Dolce", Prato

With other Italian and Foreign Institutions/Organizations

» Department of Dermatological Clinic, "La Sapienza" University, Roma
» Department of Dermatological Clinic, University of Genoa
» Department of Dermatological Clinic, "Tor Vergata" University, Roma
» Department of Dermatological Clinic, University of Turin
» Department of Human Pathology Clinic, University of Messina
» Dermatological Clinic, University of Bologna
» Department of Dermatological Clinic, University of Milan
» Department of Dermatological Clinic, University of Bari
» Pathological Anatomy, Ospedale di Benevento

Publications

1. Urso C, Borgognoni L, Doria M, et al: *Non-sentinel lymph node involvement in a patient with an atypical Spitz tumor and a positive sentinel node. Report of a case and review of the literature*. J Cutan Pathol 2009; 36: 586-90.
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7. Urso C: *Melanocytic lesions, Spitz tumors, and Don Ferrante's logic*. Am J Dermatopathol 2007; 29: 491-4.
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MEDICAL ONCOLOGY 1

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Team Members

The Medical Oncology Unit consists of eight board-certified physicians, twenty-eight nurses, one data manager, three phsyco-oncologists and fellows who provide comprehensive medical oncology care for patients with cancer

Introduction

Areas of expertise include diagnostic and treatment services for all cancers, outpatient and inpatient chemotherapy, pain and symptom control, and collaboration with all the members of the Department of Oncology.

Between 600 and 700 new diagnoses of cancer are made by the Medical Oncology Unit each year. Multidisciplinary participation in diagnosis and treatment ensures individually tailored, comprehensive and state-of-the-art care for each patient with appropriate input from all treating physicians and support personnel.
An active clinical research program is carried out in gastrointestinal cancer, lung cancer, breast cancer, supportive care and biomolecular target therapy. Since 2002, more than 50 research protocols have been started and now 22 are ongoing.
The service of psycho-oncology, which came into being along with ordinary hospital stays, has throughout the years become an integral part of the services offered to the oncology patient and, where requested, his family. This guarantees that every phase of his illness will be managed with both simple interventions and, when necessary, with a higher level of personalized services.

Main Research Themes

1. Gene expression as predictive markers of outcome in non-small cell lung cancer patients (stage IIIB with pleural effusion and stage IV) treated with chemotherapy

The study is ongoing and at the moment three patients have been enrolled and treated.

2. Prospective study of molecular prognostic factors and pharmacokinetic analysis in patients suffering from GIST treated with target therapy

The study is supported by a grant from Novartis and approved by our ethics committee. Enrollment is ongoing.

3. Metabolomic evaluation of predictive biomarkers of angiogenesis

This exploratory study is evaluating the presence of the metabolomic presence of angiogenesis biomarkers in the peripheral blood of patients with metastatic colorectal cancer treated with anti-angiogenetic drugs, such as bevacizumab. The aim of the study is early identification of patients that will respond to treatment.

4. Isolation and biomolecular characterization of Circulating Tumors Cells (CTCs) in patients with advanced colorectal cancer

We are evaluating if the number of CTCs isolated with the system ISET is correlated with prognosis in terms of progression-free survival and overall survival. We are also leading a biomolecular analysis on CTCs to determine k-Ras status, which is fundamental for choosing treatment in colorectal cancer.

Clinical Trials

1. Colorectal cancer

Description	Year	Sponsor	Number of patients recruited to date
Randomized, open, phase III study to evaluate the efficacy and safety of Avastin and capecitabine in combination *versus* capecitabine alone, as fist-line treatment in elderly patients with metastatic colorectal cancer	2009	Roche	4
Phase II, double-blind, placebo-controlled study on enzastaurin with 5-FU + bevacizumab (Avastin) as a maintenance regime after fist-line therapy for colorectal cancer	2009	Eli Lilly	6
Clinical study on the metastatic colorectal tumor: FOLFOX6 *versus* FOLFOX6 AFLIBERCEPT (VEGF Trap)	2009	Sanofy-Aventis	1
Phase II, double-blind, placebo-controlled study on enzastaurin with 5-FU + bevacizumab (Avastin) as a maintenance regime after first-line treatment for colorectal cancer	2009	Eli Lilly	6
An open-label, multicenter, randomized phase III study of second-line chemotherapy with or without bevacizumab in metastatic colorectal cancer patients who have received first-line chemotherapy + bevacizumab	2008	Gruppo Oncologico del Nord Ovest (GONO)	3
Randomized, phase III study to evaluate the safety and efficacy of Litz photodynamic therapy + chemotherapy *versus* chemotherapy alone after failure of first-line treatment in patients with colorectal cancer	2008	Ergomed	1
Randomized study to evaluate treatment duration with FOLFOX4 (three *versus* six months) ± bevacizumab as adjuvant therapy for patients with high risk stage II/III colon cancer	2007	GISCAD	10

2. Stomach cancer

Description	Year	Sponsor	Number of patients recruited to date
A phase II study of HER2 + advanced or metastatic gastric or esophageal or gastroesophageal junction adenocarcinoma treated with capecitabine + oxaliplatin with or without lapatinib	2009	Glaxo	3

3. Pancreatic cancer

Description	Year	Sponsor	Number of patients recruited to date
Study on the effect of erlotinib + gemcitabine in relation to the rash in patients with advanced pancreas cancer	2008	Roche	6

4. Breast cancer

Description	Year	Sponsor	Number of patients recruited to date
Phase III study on vinflunine in association with gemcitabine *versus* paclitaxel and gemcitabine in patients affected by locally recurring o metastatic, non-operable breast cancer, after adjuvant therapy based on anthracycline. Enrollment ongoing	2008	Pierre Fabre	1
Observational study on cardiac events in patients in the early stages of HER2-positive breast cancer treated with herceptin	2008	Roche – Quintiles	2

5. Epidemiological/Observational study

Description	Year	Sponsor	Number of patients recruited to date
DIREG_L_02814: multicenter, case-control, observational study on the epidemiology and risk factors for thromboembolic events in cancer patients and on the influence of VTE on patient outcome	2008	Sanofi-Aventis	10

Main Collaborations

With Units within ITT
- Nuclear Medicine Unit, AOU Careggi, Firenze
- General Surgery Units, AOU Careggi, Firenze
- Clinical Biochemistry Unit, AOU Careggi, Firenze
- Division of Orthopedic Oncological Surgery, AOU Careggi, Firenze
- Department of Radiotherapy, AOU Careggi, Firenze
- Department of Human Pathology and Oncology, AOU Careggi, Firenze
- Department of Experimental Pathology and Oncology, AOU Careggi, Firenze
- Department of Genetic Diagnosis, AOU Careggi, Firenze

With other Italian and Foreign Institutions/Organizations
- Gruppo Oncologico Italiano di Ricerca Clinica (GOIRC)
- Italian Group for the Study of Cancer of the Digestive System (GISCAD)

» European Organization for Research and Treatment of Cancer (EORTC)
» Pan-European Trials in Alimentary Tract Cancer (PETACC)

Publications

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MEDICAL ONCOLOGY 2

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Introduction

The research interest of the Unit, at the University of Florence, Faculty of Medicine and Surgery, has been focusing for several years on the study of molecular and cellular mechanisms of pleiotropic drug resistance to anticancer drugs (multidrug resistance phenotype or MDR1). With this phenotype, tumor cells survive and multiply even when confronted with chemotherapy. The impact of the development of such cell phenotypes goes well beyond drug resistance and involves several aspects of cell biology.

Pathogenic and therapeutic aspects of Hepatocellular Carcinoma (HCC), renal cancer, biliary cancer and colon cancer represent the clinical interest of the Unit, as well as new therapeutic approaches in non-small cell lung cancer.
Since 1966, the School of Specialization in Medical Oncology has been active at the AOU Careggi of Florence. The education network coordinated by Prof. Mazzanti included, in addition to the AOU Careggi, other branches of the Tuscan Health System: Azienda USL 4 Prato (Dr. Angelo Di Leo), Azienda USL 11 Empoli (Dr. Giammaria Fiorentini) and Azienda Sanitaria di Firenze (Dr. Luisa Fioretto).

Main Research Themes

1. Multidrug Resistance (MDR1) phenotype and apoptosis

Main achievements: The most interesting finding of our studies is that the MDR1 phenotype in human cancer cell lines determines the acquisition of an angiogenic phenotype. This means that patients, regardless of the therapy, are exposed to major risks. Because of an autocrine loop in the MDR1-positive cells, tumor cells acquire the capacity to produce and secrete large amounts of HGF in addition to VEGF that, in our model on HCC, are responsible for stimulating migration and proliferation of endothelial cells and also make these same cells highly resistant to various types of stress.

These phenomena, which have been described in various articles (2,3,6,7,8) may partly explain why the MDR1 phenotype in human tumors is associated with negative prognosis regardless of the drugs used for treatment. The MDR1-positive cancer cells become more resistant to the most commonly used antiproliferative drugs (anthracyclines, taxanes, vinca alkaloids, mitoxantrone, etc.), developing resistance also to hypoxia and to ionizing radiation. In addition, they acquire the capacity to intensely stimulate tumor angiogenesis. During these studies, we have shown that P-glycoprotein, the main actor of the MDR1 phenotype and one of the ABC transmembrane proteins, encoded by the MDR1 gene, is expressed not only in plasma membranes or in membranes of cell organelles, such as Golgi or endoplasmic reticulum, but also in mitochondria membrane. This finding could explain the relationship between MDR1 phenotype and resistance to apoptosis, as P-gp is in some manner involved in blocking the release of cytochrome c from mitochondria. With regards this point, we have also shown that celecoxib, a specific inhibitor of COX-2 activity, permits cytochrome C release from mitochondria, re-activating apoptosis. This effect is mediated by P-gp in tumor cells and could be a possible explanation for the controversial results on the utility of celecoxib in the treatment of some types of tumors (1). We have also studied the interrelationship between P-gp and other drugs used in chemotherapy, and also with herbs. We have found an interesting relationship that could affect the metabolism of drugs during chemotherapy in patients who are self prescribing treatment with herbs (5,9,10).

More recently, we enlarged our scientific interests to study a protein known as breast cancer resistance protein (BCRP) that is commonly overexpressed in drug resistant cancer cells. We have shown that BCRP, besides being expressed in plasma membranes, is also expressed in mitochondria, like P-gp. Its expression is not limited to MDR1-positive cancer cells however, but is also present in cancer cells that do not express a high degree of drug resistance. According to these data, it is possible to suggest for these proteins, particularly BCRP, are involved in the physiology of cancer cells, which was unforeseeable until two-three years ago (2).

Current work: We are currently trying to assess the role that BCRP may have in inducing resistance to apoptosis and anti-cancer drugs, and its physiological role in normal cells that express this protein and in MDR1 cancer cells.

2. Colorectal adenocarcinoma and the role of BCRP

Main achievements: We have shown that Colorectal Cancer (CRC) limited to the organ wall has a proteome that shows different features in the less abundant proteins than in healthy tissue from the same patient. In addition, we have also confirmed the hypothesis by Warburg that tumor tissue has a predilection for glycolysis because of the proteic pattern of tumor cells.

Current work: A study in which we are verifying whether the expression of BCRP varies according to distribution along the colon and according to different stages of cancer is ongoing. Since BCRP could be related to cancer related angiogenesis by interaction with the expression and activity of iNOS, we are conducting an estimation of the expression of BCRP behavior of in colon cancer and in normal mucosa of the same patients. Preliminary data confirmed that BCRP has a different distribution in the colon according to the site (more in the right parts of the colon and the lowest expression in rectal mucosa).

Future work: If the hypothesis we are working on turns out to be correct, we may be able to suggest that those patients who overexpressed BCRP in cancer tissue are not good candidates for cytotoxic chemotherapy as these cells are likely to be resistant to this treatment.

3. Hepatocellular carcinoma

Main achievements: We showed that HCC that develops in patients with liver HCV-positive cirrhosis is probably linked to the angiogenetic capacity of HCV. Later, we demonstrated that patients with liver cirrhosis due to HCV infection can be divided in two groups, which differ in the risk for developing HCC. Those with low liver angiogenetic activity are at low risk for developing liver cancer, whereas those with intense angiogenetic activity are at a much higher risk (1,4). These results supported the use of interferon in the treatment of HCV-positive patients and suggested possible new therapies (antiangiogenic drugs) in prevention, and maybe in treatment during the early phases of HCC development. Accordingly, it has been demonstrated that sorafenib, which has antiangiogenic properties, is an effective treatment of HCC.

Future work: We plan to investigate the role of new anti-tyrosine kinase drugs in the treatment of HCC, and if we should have positive results, to extend this to prophylaxis of HCC in HCV cirrhotics. In addition, we are trying to develop a new approach to treat P-gp overexpressing HCC, via a vaccine against HCC, exploiting the construction of monoclonal antibodies against P-gp (1).

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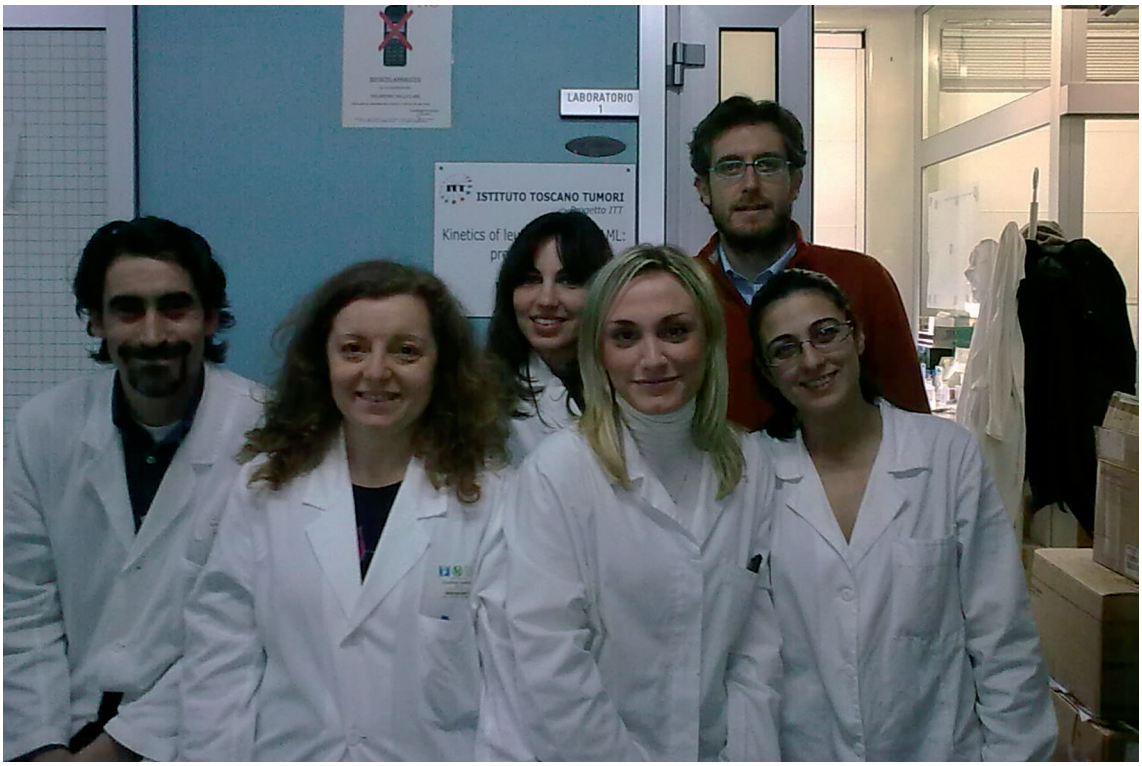
LEUKEMIA



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Introduction

Our Unit deals with biological characterization and therapeutic advances in the field of acute leukemias. The hematologists of the team collect clinical information from patients and enter data in the clinical data base. The Unit participates in multicenter clinical trials investigating novel therapeutic schedules and drugs aiming to improve the clinical outcome of patients.

The Unit works on advanced diagnostics of acute leukemias. Two main laboratories are involved in the project: the Flow Cytometry Lab and Molecular Genetics Lab. Integrating data allows prognostic stratification of patients and provides further understanding on the relationship between genotype and phenotype.

Main Research Theme

Clearance of leukemic blasts from Peripheral Blood (PBC) in Acute Myeloid Leukemia (AML): predictive value

Main achievements: The main target of our investigation was to evaluate if the clearance of blasts from PBC during induction therapy could be an early predictor of response and survival in AML patients. In order to do this, between 2004 and 2007 we carried out a daily quantitative assessment of peripheral blasts using Flow Cytometry (FC) during the conventional "3+7" induction cycle in a cohort of 61 adult patients with AML. PBC was expressed as the ratio between the baseline absolute blast count (day 1 of therapy) and the daily absolute blast count (day 2-8). PBC showed an excellent correlation with the response to induction therapy, both assessed by conventional morphology and FC. Specifically, we observed that PBC discriminated between responsive and refractory patients from day 2, although day 5 resulted as the most informative time point.

We then investigated PBC by RT-PCR for Wilms' Tumor 1 (WT1) gene expression on a cohort of 57 patients. PBC was expressed as a "WT1 ratio" (*i.e.* the ratio of WT1 copy number measured on day 1 and day 5 of therapy). The WT1 ratio was predictive of CR attainment assessed using conventional morphology. Furthermore, the WT1 ratio allowed estimation of the duration of CR among patients responding to the first cycle. Overall, our data show that PBC is a very early predictor of outcome in AML patients; as such, it could be exploited as a tool for customizing therapeutic strategy from the first days of treatment.

Current work: We have extended our research in a multicenter setting within the Northern Italian Leukemia Group (NILG) AML 02-06 protocol. NILG AML/02-06 protocol (http://www.nilg.it/index4.asp) is a multicenter, randomized trial in adult patients with AML with the aim of comparing a standard dose (ICE regimen) *versus* a High Dose (HDS) remission induction regimen. Furthermore, we have planned to include data on proliferative rate and apoptosis of leukemic blasts assessed using FC, in order to improve the predictive value of PBC on the major clinical endpoints.

The main aim of the project is to evaluate the predictive significance of PBC, as assessed by FC and PCR, on the rate of CR after induction therapy within the NILG AML/02-06 protocol.

The secondary aims are: *a*) to evaluate the predictive significance of PBC on Disease-Free (DFS) and Overall Survival (OS); *b*) to design a PBC-oriented treatment protocol for AML; *c*) to validate PBC as a surrogate endpoint of outcome in AML; *d*) to evaluate differences in PBC between the two induction arms of the randomized NILG AML/02-06 protocol; *e*) to investigate the correlation between data on the proliferative rate and apoptosis with PBC data, CR rate, DFS and OS.

Future work: Preliminary data from our experiences have demonstrated that PBC provides a prediction of CR achievement and duration in "real time" for each patient, being a sort of *in vivo* chemosensitivity test. The study of PBC is minimally invasive (it requires only two PB samples), relatively simple and reproducible, since it uses well-defined methods. The transfer of this information to clinical practice has two main applications.

a) *Customization of induction intensity*. At present, two main induction strategies are used for AML: *i*) standard induction, *ii*) intensified induction. Given the heterogeneity of the disease, each one of these strategies, if applied uniformly, is inappropriate. PBC analysis may provide an opportunity to customize induction treatment in AML patients. Specifically one could envisage starting treatment with standard induction. As soon as the PBC data predict poor response or duration of remission, the patient might be immediately switched to intensified induction and post-remission therapy. If instead the PBC data predict good response, one would of course continue with standard induction. Such an approach is expected to determine a high CR rate (at least comparable with the rate of an intensified induction), but with lower overall toxicity. Furthermore, the prognostic information

(correlation between PBC and survival) allows early planning of the subsequent phases of therapy, such as allogeneic transplantation, since timing of treatment is often crucial in AML. By adapting the therapy to the chemosensitivity of each individual patient, PBC could lead to an important improvement in clinical management of AML patients.

b) *PBC as a surrogate endpoint*. On the basis of the expected results, it could be possible to validate PBC as a surrogate endpoint in AML. Specifically, hypothesizing our results confirmed on a larger cohort of patients, we might define, for example, a cut-off value for PBC beyond whom the patient has a very high probability of survival at two years. Applying this model to different drugs or schedules, their efficacy could be evaluated by the rate of patients having a PBC higher than the cut-off, since this target, whatever the therapy has produced, correlates with good outcome. Since AML is quite a rare disease and clinical trials often need several years to obtain information when the primary (true) endpoint is survival, a validated, *in vivo* chemosensitivity test, able to predict CR rate and survival could shorten follow-up time and reduce the number of patients needed for a clinical trial. This approach could be performed in parallel with clinical trials addressing novel agents or schedules for AML treatment, anticipating and/or integrating the conventional efficacy indicators.

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Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
A multicentric, randomized trial in adult patients with AML, to compare: *a*) standard-dose *versus* high-dose remission induction regimen, and *b*) autologous blood stem cell transplantation *versus* autologous blood stem cell-supported, multi-cycle, high-dose chemotherapy program, within a risk-oriented post-remission strategy reserving allogeneic stem cell transplantation for high-risk cases. Protocol NILG-AML 02/06	2007	NILG	795
Treatment of adult acute lymphoblastic leukemia using a post-remission program whose intensity varies depending on the risk class defined on the basis of minimal residual disease	2000	NILG	280

Research Grants

Year	Funding Agency	Amount
2008	Cassa di Risparmio di Firenze	€ 30,000
2007	Istituto Toscano Tumori	€ 90,000

Main Collaborations

With other Italian and Foreign Institutions/Organizations

» NILG
» Laboratory of Hemopathology, Ospedale "Santa Maria della Misericordia", Perugia
» Servicio de Citometria, Centro de Investigacion del Cancer (CIC), Salamanca (Spain)

Publications

1. Bassan R, Spinelli O, Oldani E, et al: *Improved risk classification for risk-specific therapy based on the molecular study of minimal residual disease (MRD) in adult acute lymphoblastic leukemia (ALL)*. Blood 2009; 113: 4153-62.
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BREAST UNIT

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Lorenza Marotti, Data Manager

Introduction

From more than 30 years, the Unit has carried out an intensive activity not only from a clinical point of view, but also regarding teaching and research. The Unit is entirely dedicated to breast cancer care and treats about 600 new cases yearly.

A multidisciplinary approach is the base condition necessary to offer patients the best targeted care. Since 1990, the Unit has been a referring center for practical training in breast cancer care within the training program of the Italian School of Senology. Since 1999, the Unit has been the Regional Reference Centre for surgical treatment of breast diseases.
The Unit is an affiliate of the Department of the European Organisation for Research and Treatment of Cancer (EORTC).
The activities of the Unit consist mainly in clinical trials at national and international levels, in training breast cancer oncoplastic surgeons (5) and in identifying quality indicators in breast cancer diagnosis and care (1).

Main Research Theme

Breast Cancer

The Unit participates in trials covering different aspects related to the improvement of new technology in the loco-regional treatment of breast cancer.

Main achievements: Develop a minimally invasive surgical approach and partial breast irradiation; have the same information for staging and prognosis; limit morbidity and improve cosmesis.

Current work:

a) One of the most challenging trials is the EORTC AMAROS trial. The main objective of the trial is to prove an equivalent local/regional control with reduced morbidity, for patients with proven axillary lymph node metastasis using sentinel node biopsy, if treated with axillary radiotherapy instead of axillary lymph node dissection (a,b).
b) The Restore-2 trial will show the results of postsurgical treatment using adipose tissue-derived stem and regenerative cells in patients with functional and cosmetic breast deformity.
c) The IBIS II trial will determine if anastrozole is at least as effective as tamoxifen in local control and prevention of contralateral disease in women with locally excised ER or PgR-positive DCIS.
d) The study on Accelerated Partial Breast irradiation with IMRT is evaluating, in a randomized clinical trial, the possibility of treating the index quadrant in a selected group of patients with early stage breast cancer, compared to fractionated whole breast treatment (2).

Future work: One of the most promising trials is the EORTC POWER trial, a randomized phase III equivalence trial aimed at demonstrating less than 5% clinical axillary recurrence at five years for patients with proven metastasis using sentinel node biopsy and a low and intermediate risk for additional lymph node metastases, if axillary treatment is omitted.

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Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
Phase IV post marketing study of a CE Marked Medical Device, RESTORE-2: a clinical evaluation of adipose-derived regenerative cells in the treatment of patients with breast deformities post-segmental breast reception (lumpectomy) with or without radiation therapy	2008		8
International Breast Cancer Intervention Study (IBIS II): an international multicenter study of anastrozole in post-menopausal women at increased risk of breast cancer	2005		20
Phase III clinical trial: accelerated partial breast irradiation with IMRT. Postoperative radiotherapy on whole breast *versus* postoperative partial breast irradiation only on the excised quadrant by IMRT in patients with early breast cancer	2005		200
EORTC trial 10981-22023 After Mapping of the Axilla: Radiotherapy or Surgery (AMAROS). A phase III study comparing a complete axillary lymph node dissection with radiotherapy to the axilla in sentinel biopsy positive patients	2001		474

Main Collaborations

With Units within ITT
» All Medical Oncology Departments and Radiotherapy Units of ITT network
» Istituto per lo Studio e la Prevenzione Oncologica (ISPO), Firenze

With other Italian and Foreign Institutions/Organizations
» Istituto Europeo di Oncologia (IEO), Milano
» Breast Health Institute, Boston, Massachusetts (USA)
» European Society of Breast Cancer Specialists (EUSOMA)
» European Organisation for Research and Treatment of Cancer (EORTC)
» European Society of Surgical Oncology (ESSO)
» European Cancer Organisation (ECCO)

Publications

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GENERAL SURGERY

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Introduction

Our Research Unit started its activity around 1985 with the study of the functional disorders of the upper gastro-intestinal tract. Our study between 1985 and 2000 produced an International Patent (USA patent 4976265; 1990) concerning a device for detection of bile reflux (Bilitec), which has been commercialized for about ten years, first by Synectics and later by Medtronics, all over the world. During this period our research effort shifted on gastric and esophageal carcinogenesis. Later, we focused our attention on colonic and pancreatic carcinogenesis.

Main Research Themes

1. Circulating Tumor Cells (CTC) in colorectal cancer

RT-PCR was capable of detecting variable CEA levels in 49 of the 50 patients affected with colorectal cancer. CEA levels in the peripheral blood showed a significant decrease postoperatively. This study is now in progress with two aims:

- to evaluate the degree of correspondence between the results obtained by means of RT-PCR and those obtained by means of the Cell Search System;
- to compare the findings of CTC evaluation in the peripheral blood with those in the splanchnic blood.

2. Pathogenetic role of COX-2 in esophageal mucosal changes due to reflux and in esophageal adenocarcinogenetic sequence

The sequence of the events: gastroesophageal reflux, esophagitis, intestinal metaplasia (Barrett's esophagus), dysplasia, invasive cancer is widely accepted as the main adenocarcinogenetic pathway in the esophagus. In this pathway, COX-2 seems to play an important role due to its pro-inflammatory, angiogenetic and cell proliferation promoting effect. Thirty-three patients were enrolled in this study. In biopsies which were collected at routinely performed endoscopies, Prostaglandin E_2 and endothelial growth factor production, caspase activity and cell proliferative activity were evaluated. The involvement of COX-2 was shown in all steps of the sequence, even in the earliest ones (this represents the novelty of this study).

3. *HERG1* K+ channels as novel regulators of tumor development and progression and targets for therapy in esophageal adenocarcinoma

Our and other groups have demonstrated that K+ channels belonging to the *hERG1* family can be included in the list of ion channels mis/overexpressed in cancer cells and whose activity is involved in the regulation of tumor establishment and progression. *hERG1* proteins are encoded by the *herg1* (human eag-related gene1) gene. The *herg1* gene and the *hERG1* protein, as well as the related current I_{hERG}, are expressed in neoplastic cell lines, as well as in several types of primary human cancers: endometrial adenocarcinoma, acute myeloid and lymphoblastic lukemias, colorectal cancers, high grade astrocytomas, gastric cancers and precancerous gastric and esophageal lesions, such as Barrett's esophagus. Based on this background, we propose a project whose principal aims are:

- the definition of the genetic mechanisms leading to *herg1* overexpression in esophageal adenocarcinoma, with particular attention to duodenoesophageal reflux-related cancer, and the clinical relevance of such overexpression in terms of outcome and response to chemotherapy;
- the definition of the role exerted by *hERG1* in tumor establishment and progression using *in vivo* models;
- the use of drugs/tools capable of inhibiting *hERG1* channels as novel anticancer therapies in preclinical models.

Clinical Trial

Description	Year	Sponsor	Number of patients recruited to date
National multicentric trial "EndoAppendicectomy"	2009		281

Research Grants

Year	Funding Agency	Amount
2009	Ente Cassa di Risparmio	€ 20,000
2009	Regione Toscana	€ 22,000
2006	Ente Cassa di Risparmio	€ 80,000
2005	Ministero dell'Istruzione, dell'Università e della Ricerca – PRIN	€ 110,000
2004	Ente Cassa di Risparmio	€ 91,000
2003	Ente Cassa di Risparmio	€ 59,000

Main Collaborations

With Units within ITT

» Department of Human Pathology and Oncology, AOU Careggi, Firenze
» Department of Experimental Pathology and Oncology, AOU Careggi, Firenze
» Department of Preclinical and Clinical Pharmacology, AOU Careggi, Firenze

Publications

1. Neri B, Cipriani G, Grifoni R, Molinara E, Pantaleo P, Rangan S, et al: *Gemcitabine plus irinotecan as first-line weekly therapy in locally advanced and/or metastatic pancreatic cancer*. Oncol Res 2009; 17(11-12): 559-64.
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4. Bruno L, Nesi G, Nobili S, Veltri M, Girardi LR, Boni D, et al: *Postoperative chemotherapy in resected gastric cancer: results of a single center experience*. J Chemother 2008 Aug; 20(4): 497-502.
5. Taddei A, Castiglione F, Degl'Innocenti DR, et al: *NF2 expression levels of gastrointestinal stromal tumors: a quantitative real-time PCR study*. Tumori 2008; 94: 551-5.
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14. Freschi G, Landi L, Castagnoli A, Taddei A, Bechi P, Bucciarelli G: *Advanced thyroid carcinoma: an experience of 385 cases*. Eur J Surg Oncol 2006 Jun; 32(5): 577-82 [Epub 2006 Apr 27].
15. Gazzieri D, Trevisani M, Tarantini F, Bechi P, Masotti G, Gensini GF, et al: *Ethanol dilates coronary arteries and increases coronary flow via transient receptor potential vanilloid 1 and calcitonin gene-related peptide*. Cardiovasc Res 2006 Jun 1;70(3): 589-99 [Epub 2006 Mar 3].
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17. Lastraioli E, Taddei A, Messerini L, et al: *hERG1 channels in human esophagus: evidence for their aberrant expression in the malignant progression of Barrett's esophagus*. J Cell Physiol 2006; 209: 398-404.
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GENERAL AND ONCOLOGIC SURGERY

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Introduction

The primary activities of the General Surgery Department are in the area of oncology, including hepatobiliary pancreatic surgery, coloproctology, upper digestive tract (oncology and functionality) surgery, digestive tract endoscopy (diagnostic and therapeutic), advanced laparoscopic surgery, endocrine surgery and senology. Oncological follow-up of all pathologies treated is carried out in collaboration with the Division of Oncology. Day-Surgery and Outpatient Surgery are also performed. The Director and all members of the staff are regularly involved in scientific research and medical education activities in conjunction with the University of Florence and the principal surgical and oncological societies in Italy. This Surgical Department works together with the General Surgical Specialization School of the University of Florence, thus participating in the preparation of young surgeons, and it is one of the accredited centers for training in Laparoscopic Surgery of the ACOI School of Laparoscopy.

Main Research Themes

1. Dendrimeric peptides for targeted therapy

Main achievement: Targeting peptides on cancer cells.

Current work: Collecting samples of Gastrointestinal (GI) tumors.

Future work: Selecting samples of healthy and cancerous tissues by fluoroconjugated peptides.

2. hERG expression in GI tumors

Main achievement: hERG gene expression in malignant cells: clinical correlations and response to chemotherapy.

Current work: Correlation between hERG expression in colorectal cancer and survival.

Future work: Investigate hERG expression in gastric cancer.

3. Tumor antigen peptide-specific T-cells

Main achievement: Tumor antigen peptide-specific T-cells in gastric cancer.

Current work: Investigating tumor antigen peptide-specific T-cells in gastric cancer.

Future work: Tumor antigen peptide-specific T-cells in colorectal and pancreatic cancer.

Clinical Trial

Description	Year	Sponsor	Number of patients recruited to date
CHOLEGAS Study: multicentric, randomized, blinded, controlled trial of gastrectomy + prophylactic cholecystectomy *versus* gastrectomy only, in adults submitted to gastric cancer surgery with curative intent	2009	Gruppo Italiano di Ricerca sul Cancro Gastrico (GIRCG)	75 (out of 125)

Main Collaborations

With Units within ITT
» Medical Oncology Unit, AOU Careggi, Firenze
» Department of Radiotherapy, AOU Careggi, Firenze
» Department of General Pathology, University of Florence
» Department of Internal Medicine, University of Florence
» Department of Biotechnology, University of Siena

With other Italian and Foreign Institutions/Organizations
» GIRCG

Publications

1. Farsi M, Bernini M, Bencini L, et al: *The CHOLEGAS study: multicentric randomized, blinded, controlled trial of gastrectomy plus prophylactic cholecystectomy versus gastrectomy only, in adults submitted to gastric cancer surgery with curative intent*. Trials 2009; 10: 32.
2. Amedei A, Niccolai E, Bella CD, et al: *Characterization of tumor antigen peptide-specific T-cells isolated from the neoplastic tissue of patients with gastric adenocarcinoma*. Cancer Immunol Immunother 2009; 58: 1821-32.
3. Lastraioli E, Guasti L, Crociani O, et al: *Herg1 gene and HERG1 protein are overexpressed in colorectal cancers and regulate cell invasion of tumour cells.* Cancer Res 2004; 64: 606-11.

UROLOGY

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Simone Caroassai Grisanti, Resident in Urology
Leonardo Guidoni, Resident in Urology
Andrea Ringressi, Resident in Urology

Introduction

The Unit was established in 1992. Our main topics of interest include:

- surgical oncology;
- reconstructive surgery;
- endourology;
- renal transplantation;
- female urology;
- andrology;
- basic research.

Main Research Themes

1. Prostate cancer

- Biochemical relapse and overall/cancer-specific survival in pT3-4 patients.
- Early pelvic floor muscle training after radical prostatectomy.
- Role of duloxetine in pharmacological neuromodulation on pudendal nerves in patients receiving major pelvic surgery.
- Early pharmacological treatment in patients subjected to nerve-sparing radical prostatectomy.

2. Kidney cancer

- Quantitative analysis of carbonic anhydrase IX mRNA in renal cell carcinoma and its significance as an independent predictor of neoplastic progression and patient survival.
- Real-Time-PCR (RT-PCR) analysis of tankyrase in *renal cell carcinoma*.
- Chromosomal analysis, microsatellite instability and mutilation in sporadic *renal cell carcinoma*.
- Surgical treatment of caval neoplastic thrombosis.
- Surgery of advanced-metastatic renal cancer.
- Neoplastic disease of the native kidney in kidney transplant recipients.

3. Bladder tumors

- Identification and quantitative analysis of urinary methylated DNA by means of RT-PCR in patients with non-muscle invasive bladder cancer.
- Nerve- and seminal-sparing surgery in orthotopic sigmoid neobladder.

Main Collaborations

With Units within ITT

» Department of Critical Care Medicine and Surgery, University of Florence
» Radiotherapy Unit, AOU Careggi, Firenze

With other Italian and Foreign Institutions/Organizations

» Neuro Urology Unit, AOU Careggi, Firenze
» Lahey Clinic, Boston, Massachusetts (USA)

Publications

1. Neri B, Molinara E, Pantaleo P, et al: *Weekly administration of docetaxel and epirubicin as first-line treatment for hormone-refractory prostate carcinoma*. Oncol Res 2009; 17: 565-70.
2. Filocamo MT, Zanazzi M, Li Marzi V, et al: *Renal cell carcinoma of native kidney after renal transplantation: clinical relevance of early detection*. Transplant Proc 2009; 41: 4197-201.
3. Vinci S, Giannarini G, Selli C, et al: *Quantitative methylation analysis of BCL2, hTERT, and DAPK promoters in urine sediment for the detection of non-muscle-invasive urothelial carcinoma of the bladder: a prospective, two-center validation study*. Urol Oncol 2009 [Epub ahead of print].
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9. Girolami F, Passerini I, Gargano D, et al: *Microsatellite analysis of chromosome 3p region in sporadic renal cell carcinomas*. Pathol Oncol Res 2002; 8: 241-4.

PNEUMOLOGY

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Gabriella Fontanini, Pathologist
Florio Innocenti, Pneumologist
Mario Mascalchi, Radiologist
Alberto Mussi, Surgeon
Eugenio Paci, Epidemiologist
Giulia Picozzi, Radiologist
Alberto Janni, Surgeon

Introduction

The Research Unit for the lung cancer screening pilot study using with low-dose spiral CT was established in 2000. In 2004, the randomized, controlled ITALUNG-CT multicenter study started with collaboration

between Florence, Pisa and Pistoia screening centers. Our research activity consists mainly in clinical trials and translational research on lung cancer.
The Research Unit for Epidemiological study of the Epidermal Growth Factor Receptor (EGFR) was instituted in 2006 with the purpose of studying the epidemiological distribution of positive EGFR mutations in subjects with lung adenocarcinoma in Tuscany, regarding gender and smoking habits and different histological subtypes/patterns of adenocarcinoma.
Furthermore the ethics group of Careggi for palliative care (GRuppo Etico CAreggi LEniterapia – GRECALE) was instituted in 2003 with the purpose of studying the ethical issues of patients with chronic and lethal disease, to improve therapeutic alliance and patient communication, and to promote end of life decisions.

Main Research Themes

1. Screening of lung cancer with low dose spiral CT: randomized controlled trial (ITALUNG_CT)

In 1999, the ELCAP group published (a) the results from the early detection project of lung cancer in high risk subjects in New York, showing that the CT Scan was a sensitive tool for the identification of non-calcified pulmonary nodules and early lung cancer. A methodological debate was then open about the need to demonstrate a reduction in lung cancer mortality for the subjects invited to be screened with the CT Scan *versus* unscreened subjects or those screened with CXR by means of a Randomized Clinical Trial (RCT). The request for a comparative evaluation of mortality reduction by means of RCTs has also been confirmed after the recent publication of modeling results from the one arm studies carried out in Italy and in the US, where the model predicted any effect in terms of mortality reduction (a). The ITALUNG_CT RCT will contribute to the European evaluation on the efficacy of the CT Scan in lung cancer screening, pooling the data with other on-going RCTs which have a comparable study design (b).

Main achievements: Three-thousand two-hundred six smokers or ex-smokers, 55-64 years old, were enrolled. Baseline CT scan screening of 1,406 active arm subjects have already been completed; 21 lung cancers (stage I: 52.4%) were detected (detection rate 1.42%) (3). CT scans of the 1st, 2nd and 3rd annual repeat have also been completed; 17 lung cancers (stage I: 58.8%) were detected (detection of the 1st, 2nd and 3rd annual repeat was 0.28%, 0.49% and 0.35%); 2 interval cases were diagnosed during the observation period. The main histological type of detected lung cancer was adenocarcinoma (47.6% and 70.5% of baseline and repeat lung cancer). In the ITALUNG_CT, the RCT evaluated the diagnostic value of a grid of molecular genetic markers detectable in sputum and plasma samples of individuals enrolled in a lung cancer screening program (c).

The diagnostic value of a grid of molecular genetic markers detectable in sputum and plasma samples of individuals enrolled in a lung cancer screening program with low-dose CT has been evaluated. There was a highly statistically significant difference between the proportion of subjects with a negative baseline CT screening test, who were positive to allelic imbalance, and those with a Non-Calcified Nodule (NCN ≥ 5 mm). Allelic imbalance showed good performance for the screening of NCN ≥ 5mm (c).

Current work: Follow-up CT scan of the 3rd repeat.

Future work: Statistical analysis of mortality for lung cancer in active and control arms.

2. Epidemiological study of EGFR mutations in patients with lung adenocarcinoma from Tuscany

Main achievements: The samples from 411 patients with lung adenocarcinoma have been collected (the pathological features of each tumor were evaluated in 235 tumors), assessing mutations in exons 18 to 21 of the EGFR gene and in codons 12 and 13 of the K-RAS gene: 52/411 (12.6%) of these were positive for EGFR mutations; 67/411 (17.9%) were positive for K-RAS mutations. EGFR mutations were more frequently observed in females ($p < 0.0001$), in nonsmokers ($p = 0.005$) and in the presence of bronchioloalveolar features ($p = 0.0004$); K-RAS mutations were more frequent in males ($p = 0.0007$) and were associated with smoking habits ($p = 0.005$).

Future work: Although the data obtained have yet to be analyzed and confirmed with a larger number of patients, epidemiological study of the distribution of positive EGFR mutations in subjects with lung adenocarcinoma in Tuscany should provide useful information for targeted therapy.

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Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
ITALUNG_CT: screening of lung cancer with low dose spiral CT: randomized, controlled trial	2004	Regione Toscana and Ministero dell'Istruzione, dell'Università e della Ricerca – PRIN	3,206
EGFR study: epidemiological study of EGFR mutations in patients with lung adenocarcinoma in Tuscany	2004	Regione Toscana	411

Research Grants

Year	Funding Agency	Amount
2008	Regione Toscana	€ 350,000
2007	Regione Toscana	€ 454,472
2006	Regione Toscana	€ 454,472
2005	Regione Toscana	€ 454,472
2004	Regione Toscana	€ 454,472

Main Collaborations

With Units within ITT

» Pathology Units, Aziende USL, Regione Toscana
» Department of Nuclear Medicine, AOU Careggi, Firenze
» Thoracic Surgery Unit, AOU Careggi, Firenze
» Cytopathology Unit, Istituto per lo Studio e la Prevenzione Oncologica (ISPO), Firenze
» Descriptive and Clinical Epidemiology Unit, ISPO, Firenze
» Radiodiagnostic Section, Department of Clinical Physiopathology, University of Florence
» Department of Human Pathology and Oncology, University of Florence
» Radiology Department, Azienda Ospedaliero Universitaria Pisana (AOU Pisana)
» Thoracic Surgery Unit, Cardiopulmonary Department, AOU Pisana
» Radiology Department, AOU Pisana
» Pulmonary Unit, Cardiopulmonary Department, AOU Pisana
» Pathology Institute, University of Pisa
» Pneumology Department, Ospedale "Del Ceppo", Pistoia

Publications

1. Boldrini L, Alì G, Gisfredi S, et al: *Epidermal growth factor receptor and K-RAS mutations in 411 lung adenocarcinoma: a population-based prospective study*. Oncol Rep 2009; 22: 683-91.
2. Lopes Pegna A, Picozzi G: *Lung cancer screening update.* Curr Opin Pulm Med 2009; 15: 327-33.
3. Camiciottoli G, Cavigli E, Grassi L, et al: *Prevalence and correlates of pulmonary emphysema in smokers and former smokers. A densitometric study of participants in the ITALUNG trial.* Eur Radiol 2009; 19: 58-66.
4. Carozzi FM, Bisanzi S, Falini P, et al: *Molecular profile in body fluids in subjects enrolled in a randomised trial for lung cancer screening: perspectives of integrated strategies for early diagnosis.* Lung Cancer 2009 Jul; 29 [Epub ahead of print].
5. Lopes Pegna A, Picozzi G, Mascalchi M, et al: *Design, recruitment and baseline results of the ITALUNG trial for lung cancer screening with lowdose CT*. Lung Cancer 2008; 64: 34-40.
6. Diciotti S, Picozzi G, Falchini M, et al: *3-D segmentation algorithm of small lung nodules in spiral ct images*. IEEE Trans Inf Technol Biomed 2008; 12: 7-19.
7. Bach PB, Jett JR, Pastorino U, et al: *Computed tomography screening and lung cancer outcomes*. JAMA 2007; 297: 953-61.
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9. Mascalchi M, Belli G, Zappa M, et al: *Risk-benefit analysis of X-ray exposure associated with lung cancer screening in the Italung-CT trial*. Am J of Roentgenology 2006; 187: 421-9.
10. Shigematsu H, Lin L, Takahashi T, et al: *Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers*. J Natl Cancer Inst 2005; 97: 339-46.
11. Picozzi G, Paci E, Lopes Pegna A, et al: *Screening of lung cancer with low-dose spiral CT: results of a three year pilot study and design of the randomized controlled trial "Italung-CT"*. Radiol Med 2005; 109: 17-26.
12. Pao W, Miller V, Zakowski M, et al: *EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib*. Proc Natl Acad Sci USA 2004; 101: 13306-11.
13. Lynch TJ, Bell DW, Sordella R, et al: *Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small cell lung cancer to gefitinib*. N Engl J Med 2004; 350: 2129-39.
14. Paez JG, Janne PA, Lee JC, et al: *EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy*. Science 2004; 304: 1497-500.

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Introduction

The Histopathology Research Unit carries out intense scientific research activities entailing the histomorphological and biomolecular characterization of a wide range of human neoplasias.

Main Research Themes

1. Lymphangiogenesis in solid tumors: molecular mechanisms and clinical significance

Main achievements:

a) *Head and Neck Squamous Cell Carcinoma (HNSCC).* Tumor metastasis to regional lymph nodes via the lymphatic system represents the first step of dissemination in HNSCC and it serves as a major prognostic indicator of disease progression and as a guide for therapeutic strategies. We demonstrated that the number and relative area of intratumoral and peritumoral lymphatics (demonstrated by immunostaining for the lymphatic marker D2-40, Figure 1) was significantly higher in HNSCC cases with lymph node metastasis, although no correlation was found between tumor lymphangiogenesis and disease-free or overall survival (23). We also studied the correlation between lymphangiogenesis and inducible Nitric Oxide Synthase (iNOS) activity in HNSCCs and the possible involvement of the lymphangiogenic factor Vascular Endothelial Growth Factor (VEGF)-C. iNOS activity measured in specimens from the tumor periphery correlated strongly with both Lymphatic Vessel (LV) density and LV area. In addition, VEGF-C mRNA expression was significantly elevated in tumors with high iNOS activity, and VEGF-C expression correlated positively with the presence of lymph node metastases. In the human squamous carcinoma cell line, *in vitro*, exogenous and endogenous stimulation of the iNOS pathway led to up-regulation of VEGF-C, which was blocked by the NOS inhibitor. Taken together, our results indicate that iNOS activity may promote lymphangiogenesis and spread to lymph nodes in HNSCC, with the possible involvement of VEGF-C (15).

b) *Cutaneous melanoma.* Cutaneous melanoma spreads preferentially through the lymphatic route, and Sentinel Lymph Node (SLN) status is regarded as the most important predictor of survival. We demonstrated that the number and area of peritumorous and intratumorous lymphatics was significantly higher in melanomas associated with SLN metastasis than in non-metastatic melanomas. No significant difference in VEGF-C expression by neoplastic cells was shown between metastatic and non-metastatic melanomas. The intratumor LV area was the most significant predictor of SLN metastasis. Using multivariate analysis, peritumorous LV density was an independent variable affecting overall survival, whereas the intratumorous LV area approached significance. The tumor-associated lymphatic network constitutes a potential criterion in the selection of high risk patients for complementary treatment and a new target for anti-melanoma therapeutic strategies (16). As other solid tumors, melanomas are often infiltrated by host inflammatory cells, including those of the monocyte-macrophage lineage. There is increasing evidence to support the important contribution of monocytes/macrophages to tumor neoangiogenesis through production of VEGF, under the influence of certain factors of the tumor environment such as hypoxia and NO. Moreover, Tumor-Associated Macrophages (TAM) have been implicated in the formation of peritumoral lymphangiogenesis through production of VEGF-C and VEGF-D, specific growth factors for lymphatic endothelia. Macrophages use arginine either to synthesize NO through iNOS or to produce ornithine through arginase activity. Although the effects of NO are primarily cytotoxic, the production of ornithine, a precursor for polyamine synthesis, may promote tumor cell proliferation. Thus, the iNOS/arginase balance in TAMs may be crucial in tumor progression. We demonstrated that: *i*) the percentage of iNOS-positive TAMs was significantly higher in *in situ* and thin melanoma in comparison with more advanced, thicker tumors; *ii*) the percentage of arginase-positive TAMs did not change among the pT categories analyzed; *iii*) the percentage of iNOS-positive TAMs was greater than that of arginase-positive TAMs in peritumoral and intratumoral location of thin melanomas (pT1). Moreover, by using an i*n vitro* experimental protocol, represented by B16 murine melanoma cells co-cultivated with inflammatory macrophages, we found that melanoma cells stimulate iNOS expression and NO production in macrophages. In conclusion, our *in vivo* and *in vitro* results suggest that, mainly in early melanoma lesions, iNOS prevails over arginase in TAMs, a phenomenon possibly stimulated by contact with tumor cells. However, macrophages stimulated by murine melanoma cells secreted a level of NO compatible with anti-tumor activity only in the presence of IFNgamma (11).

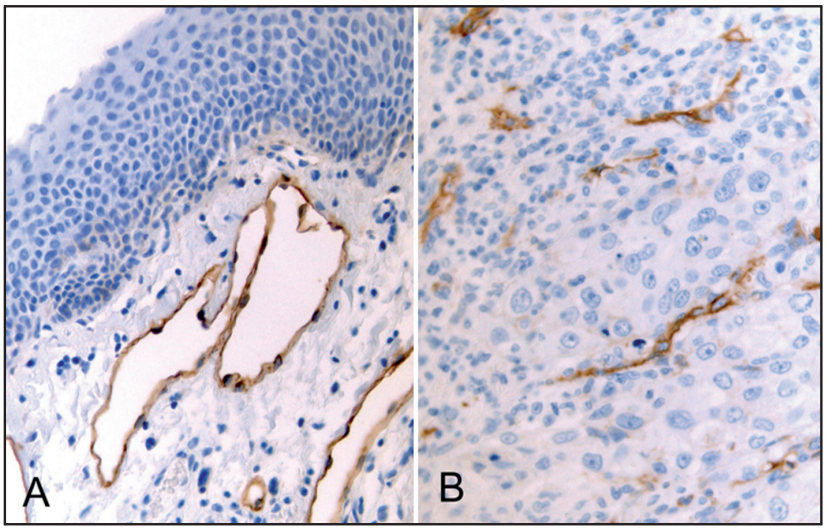

Figure 1 - Tumor lymphatic vessels stained detected with the monoclonal antibody D2-40 in HNSCC. A) markedly dilated vessels adjacent to the tumor. B) Collapsed lymphatics within the tumor

Current and future work:

a) *HNSCC*. We are developing an animal model to assess the role of different factors, including nitric oxide and VEGF-C, in regulating this process. We also plan to test the efficacy of inhibitors of iNOS in blocking lymphangiogenesis in this experimental model.

b) *Cutaneous melanoma*. At present we are focusing on understanding the biological and possible prognostic significance of TAM through the assessment of the quantity of infiltrating macrophages in a larger series of melanoma cases, along with the evaluation of their activation status by determining a panel of macrophage bioactive mediators.

2. Immunohistochemical markers for cancer diagnosis and classification

Main achievements: Even though a large number of immunohistochemical markers have proven to be valuable in the differential diagnosis between mesothelioma and carcinoma, no single antibody with absolute sensitivity and specificity has yet been identified. We evaluated the expression of a series of available muscle markers in both epithelioid pleural mesothelioma and pulmonary adenocarcinoma and found h-caldesmon (a specific marker for smooth muscle tumors) to be a highly sensitive and specific marker for the positive diagnosis of epithelioid pleural mesotheliomas (Figure 2). (17) These results prompted us to investigate the value of h-caldesmon in differentiating peritoneal epithelioid mesothelioma from papillary serous carcinoma of the ovary involving the peritoneum. Both tumors show overlapping morphological features and differential diagnosis may be very difficult. Our results indicated h-caldesmon, calretinin, Ber-Ep4 and estrogen receptors as the markers with the best performance in differentiating epithelioid peritoneal mesothelioma from papillary serous carcinoma of the ovary (10).

Current and future work: The distinction between mesothelial reactive hyperplasia and epithelioid mesothelioma is a well known diagnostic challenge for pathologists, especially when dealing with small biopsy specimens. No specific immunohistochemical marker has been found to be useful in this differential diagnosis. Ion channels regulate a broad range of cellular activities and alterations in their function have been reported in different pathologies, including cancer. The aberrant expression of potassium channels encoded by the human ether-a-go-go related gene 1 (hERG1 channels) has been demonstrated in many types of human cancer. The aim of the current study is to evaluate the expression of hERG1 protein in a series of epithelioid pleural mesotheliomas and reactive pleural mesothelial hyperplasias.

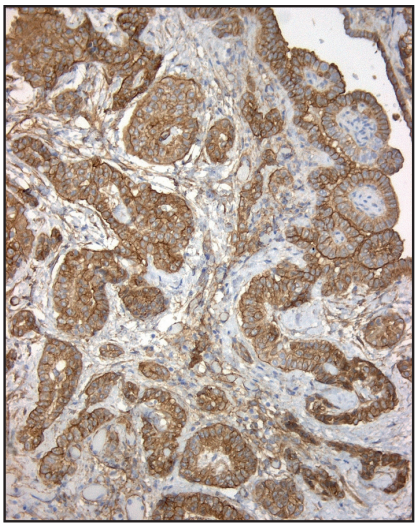

Figure 2 - Strong and diffuse cytoplasmic h-caldesmon expression in a case of epithelioid mesothelioma

3. Pathological and biomolecular prognostic factors in solid human tumors

Main achievements: We evaluated a wide spectrum of pathological parameters in patients with lymph node-negative colorectal cancer (stage IIA) in order to identify a subset of patients at high risk for tumor recurrence. The incidence and the prognostic significance of occult tumor cells in the lymph nodes of colorectal cancer patients were investigated. We found that isolated tumor cells (metastatic tumors measuring 0.2 mm or less) showed no effect on survival. On the contrary, micrometastases (metastatic tumors measuring more than 0.2 mm, but less than 0.2 cm.) showed some impact on prognosis, but their effect was diluted by other factors, such as pattern of tumor growth and level of tumor extramural spread (14). The combination of tumor growth pattern (expanding or infiltrating) and the extent of tumor spread beyond the muscularis propria (≤ 5 mm or > 5 mm) allowed us to identify patients with low (expanding growth and extramural infiltration ≤ 5 mm) or high risk (infiltrating growth and extramural infiltration > 5 mm) of tumor recurrence (12). We studied, as biomolecular prognostic factors, the role of hERG channels in the progression of colorectal cancer. The *hERG1* gene and the hERG1 protein are expressed in colon cancer cell lines, and the activity of hERG channels modulates colon cancer cell invasiveness; both the *hERG1* gene and hERG1 protein were expressed in a high percentage of primary human colorectal cancers, with the highest incidence occurring in metastatic cancers, whereas no expression could be detected in either normal colonic mucosa or in adenomas (29).

Current and future work: Our current work is focused on evaluation of more biomolecular prognostic factors in solid tumors, in particular: *a*) expression of antiapoptotic (Bcl2, BclXL) and proapoptotic factors (Bax, Bak) in preoperative biopsies of rectal cancer performed before chemoradiotherapy; *b*) mutations in the Epidermal Growth Factor Receptor (EGFR) in non-small cell lung cancer. EGFR has become a potential target for various antitumor strategies, the aim of our current study is to evaluate the efficacy of EGFR tyrosine kinase inhibitors in a population of patients with advanced lung adenocarcinoma and with EGFR gene mutations.

Research Grants

Year	Funding Agency	Amount
2009	Ente Cassa di Risparmio di Firenze	€ 140,000
2008	Monte dei Paschi di Siena	€ 30,000
2008	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) – PRIN 2008	€ 56,544
2008	Istituto Toscano Tumori	€ 78,500
2008	Ente Cassa di Risparmio di Firenze	€ 50,000
2007	MIUR – PRIN 2007	€ 52,800
2007	Ente Cassa di Risparmio di Firenze	€ 134,000
2005	MIUR – PRIN	€ 20,200
2005	3M Italia	€ 10,000
2004	MIUR – PRIN	€ 60,800
2004	Ente Cassa di Risparmio di Firenze	€ 150,000
2003-2004	Novartis	€ 8,000
2003	Ente Cassa di Risparmio di Firenze	€ 172,000

Main Collaborations

With Units within ITT

» Other Departments of the University of Florence
» Other Departments/Units of AOU Careggi, Firenze
» Department of Human Pathology and Oncology, University of Siena
» Department of Preventive Medicine and Anatomic Pathology Unit, Azienda USL 11 Empoli

With other Italian and Foreign Institutions/Organizations

» Department of Experimental Pharmacology, University of Naples
» Department of Biomedical Sciences and Human Oncology, University of Turin
» Pathology Unit, Ospedale Civile, Treviso
» Pathology Unit, University of Parma
» Department of Histopathology, Royal Surrey County Hospital, Guilford (UK)
» Melanoma Unit, Department of Dermatology, Hospital Clínic, Barcelona (Spain)
» Institut Fédératif de Recherche 50, Faculté de Médecine Pasteur, Nice (France)
» Department of Pathology, Hospital Clínic, Barcelona (Spain)
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» Department of Pathology, Free University, Amsterdam (The Netherlands)
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» Department of Pharmacology and Therapeutics, University of Calgary (Canada)
» Optical and Biomedical Engineering Laboratory, University of Western Australia
» Department of Histopathology, Flinders University of South Australia

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HEREDITARY ENDOCRINE TUMORS

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Laura Masi
Alberto Falchetti

Introduction

The Regional Referral Center for Hereditary Endocrine Tumors was officially approved and recognized as a Regional Referral Center in April 1999. During the years, its role in assistance and genetic diagnosis has increased significantly, acting as, in particular, a pole of attraction for the sector of Multiple Endocrine type 1 and 2 Neoplasms (MEN1 and MEN2) not only within the metropolitan area of Florence and the Regione Toscana, but also nationally.

In 1991, the Italian Register of Multiple Endocrine Neoplasms (RINEM) was officially established with headquarters in Florence, and at the Regional Referral Center for Hereditary Endocrine Tumors since

1999. Since then, we have collected reports on over 200 familial cases of MEN1, more than 50 sporadic cases of MEN1, over 200 familial cases of MEN2 and 20 sporadic forms of MEN2. The RINEM is constantly updated by the Center itself.
Therefore, the Center has been dealing with the genetic and hereditary aspects of endocrine tumors for years and the specific experiences acquired have made it a Center of proven value, representing a model of interaction between clinical and basic applied research in this sector, as witnessed by the important contribution it has made in the drawing up of the international guidelines for diagnosis and management of Multiple Endocrine Neoplasms.
It has therefore been possible to lay the foundations for the birth of the Italian Association of type 1 and 2 Multiple Endocrine Neoplasms (AIMEN).
Our activities take place in different sectors:

- outpatient activity that also includes genetic counseling;
- Day-Hospital admittance, with the most reliable diagnostic tests given;
- clinical trials with new antiproliferative drugs;
- consultation with other hospitals;
- laboratory activity for genetic diagnosis of endocrine tumors.

Main Research Themes

1. Study of tumorigenesis in MEN1 and in the sporadic counterpart of tumors

Main achievements: The study of the expression of mRNA of MEN1 in fibroblasts in three patients compared to the "healthy" control, conducted by Northern blot and confirmed by qRT-PCR, demonstrates a significant reduction in the expression of mRNA of MEN1 in those affected compared to "healthy" subjects. The analysis of protein expression, Western blot, is, however, in disagreement with mRNA expression. The menin "wild-type" protein and the mutated proteins of the patients (expected in a number of copies reduced by about 50%) are indeed expressed at the same level. We have therefore hypothesized a molecular "compensation" mechanism by the "wild-type" alleles on which we are presently working.

Current work: To confirm this hypothesis, we have designed both a synthetic siRNA that would cut both the MEN1 alleles, and a synthetic ribozyme that cuts in a specific way both the "wild-type" allele and the mutated allele in patient fibroblasts. Indeed, the silencing of both MEN1 alleles (analyzed with qRT-PCR) induces inhibition of the menin protein (analyzed with Western blot), while silencing of the mutated allele (analyzed with qRT-PCR) induces the expression of the menin protein (analyzed with Western blot). This fact would confirm our hypothesis that sees the "wild-type" menin protein compensated for the "loss" of the mutated copy. At present, we are verifying, through "RNA gel retardation assay", if at the base of this mechanism there is an interaction between menin protein and mRNA menin.

2. Study of the profiles of expression of microRNAs involved in MEN1, MEN2, VHL, FHH syndromes and other forms of endocrine tumors

Main achievements: The micro-RNAs are small, non-codifying RNAs, of 19-24 nucleotides, which negatively regulate the gene expression of various genes involved in many biological processes. Different groups have studied the role of microRNA expression in tissues of patients affected by different tumors, demonstrating that microRNAs are expressed in a differential manner in normal and tumor tissues. These differences are tumor-specific and, in some cases, are correlated with a specific prognosis. We have fine-tuned the "nylon microarray" technique for the study of the profiles of microRNA gene expression in cells and tissues of MEN1 patients. This technique allows a sensitive and reproducible

study of the expression levels of microRNAs, characterized to date, present in enriched fractions of small RNAs coming from different tissues and primary cultures of MEN1 patients.

Current work: We have analyzed the loss of heterozygosity for MEN1 alleles in constitutive DNA and at the somatic level, parathyroid adenomas, of MEN1 patients, and compared them with the expression of some microRNAs that result involved in neuroendocrine pathologies.

Future work: The understanding of this molecular mechanism should allow the development of a gene therapy based on the cell endogenic property, activating the oncosuppressor in the face of losing one of its alleles. A strategy we would like to use calls for the synthesis of trans-slicing ribozymes. Trans-splicing ribozymes derive from theintrons of group I normally present in the cell in nature. RNA trans-splicing are RNA molecules with the capacity to cut specific sequences of mRNA and subsequently tie the two exonic ends both *in vitro* and *in vivo*. Just as for the catalyzed splicing of the introns in group I, the substratum of the trans-splicing ribozymes is the localized phosphodiesteric tie after uridine at the mRNA target level. The group I trans-splicing RNA ribozymes bind to a specific target sequence of mRNA through the pairing between the exon sequence and the guide sequence of the ribozyme itself (IGS). This IGS sequence defines the specificity of the ribozyme and can be modified so as to pair with any sequence desired. In fact, by modifying the IGS sequence the ribozyme can be engineered to recognize any sequence found downstream from the IGS. This sequence, localized in terminal 3', can potentially be substituted by any nucleotide sequence without altering the functionality of the ribozyme itself. This ability of the RNA trans-splicing ribozymes to cut and paste the mRNA sequences classifies them as possible therapeutic applications in the repair of mutated mRNA (trans-splicing RNA repairing). In fact, the design of trans-splicing RNA repairing ribozymes specific for the mutated mRNA could allow us to obtain the aimed removal of the altered region and its substitution with the "wild-type" sequence. The advantage of the use of trans-splicing RNA repairing in the treatment of monogenic diseases lies in the fact, since it is capable of modifying mRNA in the post-transcriptional phase, that their application permits the maintenance of the natural regulation of the gene transcription. Furthermore, it is possible to induce the expression of the modified ribozymes only in the appropriate cells through the use of vectors that allow its expression of the ribozyme transcript. Finally, it is possible to think of designing the ribozyme in order to make it specific for correcting any mutation.

Confirmation of the involvement of specific microRNAs in neuroendocrine pathologies, hereditary and not, opens up the possibility to the development of nylon-microarrays that allow an accurate evaluation of the expression profiles of these microRNAs with a diagnostic and prognostic value.

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ENDOCRINOLOGY: ADRENAL PATHOLOGY

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Introduction

This research group has been working for years on dysfunctional and neoplastic pathologies of the adrenal glands, carrying out clinical and basic research on neoplastic pathologies of the adrenal cortex (Adrenocortical Carcinoma – ACC), the adrenal medulla (pheochromocytoma – PHEO and the

extra-adrenal chromaffin tissue (paraganglioma – PGL). The research is carried out within the Department of Clinical Physiopathology of the University of Florence and the Department of Biomedicine. In the Laboratory of Endocrinology and Clinical Biochemistry there are ample facilities for the genetic and molecular biological studies.

Main Research Themes

1. Genetics and biology of pheochromocytoma/paraganglioma

Main achievement: We have studied the molecular as well as the genetic characteristics of sporadic and familiar PHEOs and PGLs. We have documented, for the first time in Italy, the presence of a founding effect in eight families living in the area of Florence, Pistoia and Prato affected by the PGL1 syndrome. We are extending the genetic characterization to all patients affected by PHEO/PGL, discovering many mutations which have not yet been described in the literature, as well as familial cases with unusual phenotypical manifestations.

Five hundred and one patients affected by PHEO/PGL have been genotyped for all the discovered susceptibility genes, demonstrating an overall incidence of familial cases in about 25% of patients.

Future work: Very recently, two new susceptibility genes, SDH5 and TMEM127, have been discovered. The analysis of these genes will be extended to all our old and new patients. We are at present participating in a very large international study coordinated by Prof. Patricia Dahia, San Antonio, Texas, on the incidence of familial cases due to mutations on the newly discovered susceptibility gene, TMEM127.

2. Mitochondrial succinodehydrogenase and neuroendocrine cancer

Germline mutations of SDH subunit encoding genes are responsible for the occurrence of tumors, such as PHEO and PGL. At present, no clue exists regarding the molecular mechanisms by which the mutations lead to neoplasia.

Main achievement: We have recently validated the yeast model as a relevant tool to investigate the functional consequences of SDH subunit mutations in patients affected by PGL syndromes. We showed that a novel germline missense SDHB mutation (C191Y) in a patient affected by a glomus tumor inactivates SDH activity both in yeast and in the patient's tumor tissue, and that C191 residue plays a crucial role in the enzymatic activity of the catalytic complex SDHA/SDHB (SDH1/SDH2). The SDHB missense mutation leads to an increased sensitivity to oxidative stress and to mtDNA mutability (Figure 1). This model might be of clinical relevance to establish the pathogenic significance of novel mutations or even rare polymorphisms.

Current work: We are currently investigating whether the different SDH subunit mutations may account for the different biochemical and functional alterations in tumor tissues.

We are evaluating SDH and Citrate Synthase (CS) activity in PHEO/PGL tissues, obtained at surgery, with or without mutations for SDHB, SDHC and SDHD. Moreover, using both immunohistochemistry and Western blot analysis, mitochondrial number and morphology in tumors, both SDH mutated and wild type, are analyzed and compared to mitochondria present in healthy tissues.

As dynamic parameters, such as mitochondrial membrane potential, ROS production and apoptosis sensitivity following drug stimulation can be properly evaluated only in living cells, we have recently stably transfected a human neuroblastoma cell line with either the correspondent wild type or the mutated constructs corresponding to the most representative SDH variations found in our patients.

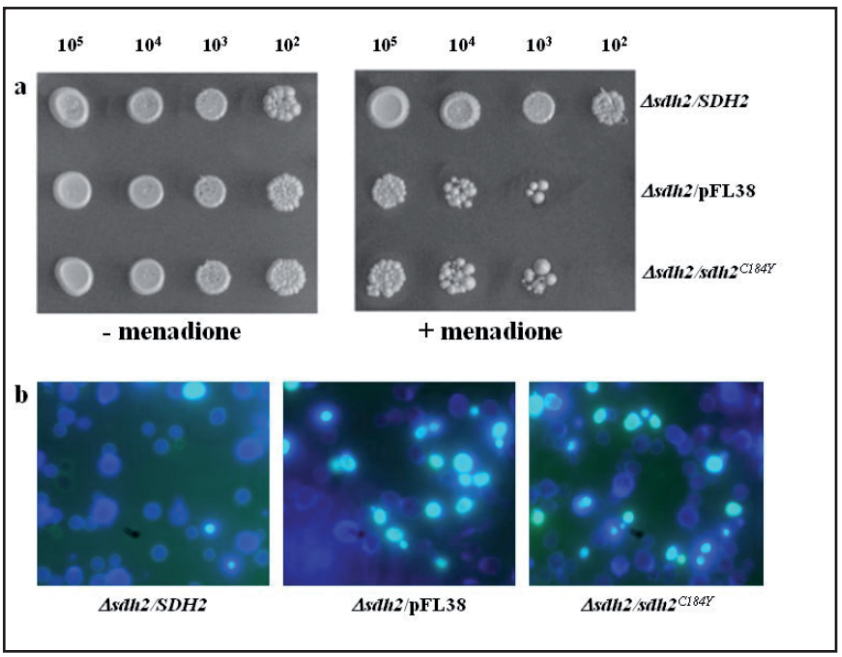

Figure 1 - Increased sensitivity to oxidative stress in SDHB mutated strain. *a*) Menadione sensitivity. Equal amounts of serial dilution of cells from exponentially grown cultures (10^5, 10^4, 10^3, 10^2 cells) were grown on YNB plates supplemented with 2% glucose with or without 20 μM menadione (right and left panel respectively). The growth was scored after five days of incubation at 28 °C control plates and after ten days for menadione plates. *b*) ROS accumulation in Δsdh2 strain transformed with wild type SDH2, the sole plasmid pFL38 and the $sdh2^{C184Y}$ mutant allele after dihydrorhodamine 123 staining. From (3)

Since the goal of this project is to understand the role of the different SDH mutations in PHEO/PGL development, we are studying *in vivo* proliferation, invasiveness and migration potential of neuroblastoma cells transfected with SDH mutations.

Future work: By means of a multidisciplinary approach based on immunohistochemistry, molecular, and cellular biology, we will evaluate the functional impact of different SDH mutations on expression of hypoxia-induced factors, cell proliferation, invasion and migration, and apoptosis sensitivity of SDH-mutated neuroblastoma cell lines. In addition, the development of xenograft animal models of PHEO/PGL, obtained by subcutaneous and intracardiac inoculation of neuroblastoma cells transfected with different SDH mutations, will enable us to investigate *in vivo* proliferation, invasiveness and migration potential of such cells.

3. Genetics and biology of adrenocortical carcinoma

ACC is a rare and aggressive endocrine tumor with a poor prognosis, characterized by radio/chemotherapy resistance. Early diagnosis followed by total surgical tumor resection is the only valuable option for ACC cure. Prognosis depends on the tumor stage at surgery: mean survival rate at five years ranges between 16 and 38%; however, the high frequency of metastatic disease reduces the survival to less than 10%. Mitotane is the only adrenal-specific pharmacological treatment available for advanced ACC. However, its activity seems mainly due to its cytotoxic effects on both normal and tumor adrenocortical cells, and the molecular mechanism of action of this drug is still poorly understood. The development of new, efficacious and specific drugs able to specifically interfere with ACC is mandatory, but requires a better knowledge of the mechanisms underlying malignant tumor transformation and aggressiveness obtained by both *in vivo* and *in vitro* studies.

Main achievement: Among the putative novel drugs under study for their anticancer properties, we have recently demonstrated the anti-neoplastic properties of a rosiglitazone (RGZ), an anti-diabetic insulin sensitizer drug used in type 2 diabetes treatment. In particular, RGZ interferes with cancer

growth by blocking *in vitro* cell proliferation/migration and inducing cell differentiation/apoptosis in the ACC cell line, H295R, through repression of the IGF1R intracellular downstream signaling, namely ERK and PI3K pathways, responsible for supporting ACC cell growth (4). Moreover, we have shown that oral administration of RGZ (5 mg/kg/day) results in a significant delay in tumor growth (Figure 2) in a mouse xenograft ACC model, obtained by subcutaneous injection of the human ACC H295R cell line in athymic mice, by reducing angiogenic, vascular, proliferation and anti-apoptotic gene expression in the tumor. These findings suggest a potential role for RGZ as a novel and promising adjuvant therapy after surgical removal of ACC, alone or in combination with mitotane to obtain more efficacious antitumor results.

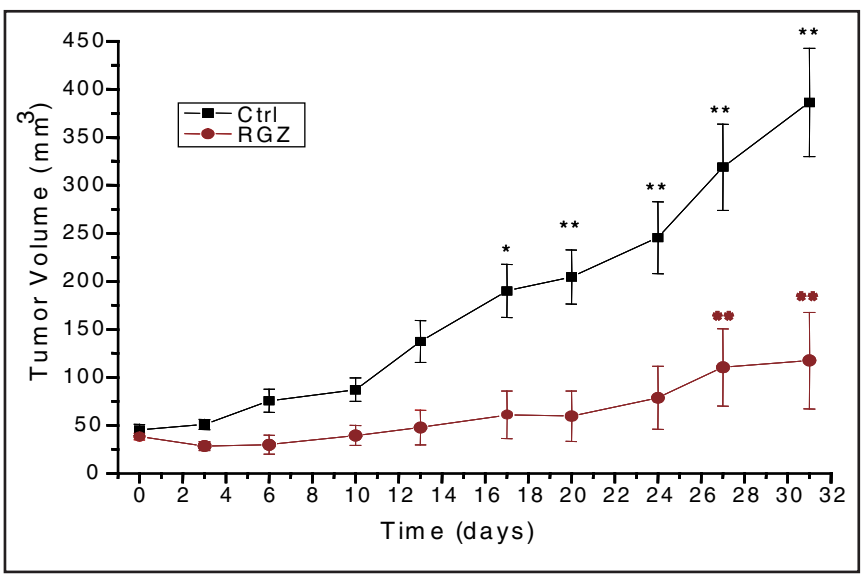

Figure 2 - RGZ reduces tumor volume in ACC xenografts. Growth curves of xenograft ACC. Values represent mean + SE of measured tumor volume over time in control (Ctrl) group (filled squares, n = 16) and in the 5 mg/kg/day RGZ orally-treated group (filled circles, n = 17). Data have been pooled from a total of 8-9 mice/group of mice treated in two independent experiments. P < 0.001 between Ctrl and RGZ groups for all points starting from day 3. * P < 0.005, ** P < 0.001 *versus* day 0. From (1)

On clinical ground, we have participated in the First International Randomized trial in locally advanced and Metastatic Adrenocortical Carcinoma Treatment (FIRM-ACT) comparing the efficacy of two different treatments (etoposide, doxorubicin, cisplatin and mitotane *versus* streptozotocin and mitotane). The trial closed at the end of 2009. We are waiting for the results.

Current work: At present, we had applied to participate in the ADIUVO trial, an international trial assessing the efficacy of adjuvant mitotane therapy in patients with ACC diagnosed as disease-free after surgery. The local ethical committee has denied our participation, considering the contract by HRA (the pharmaceutical Company supplying the drug) with the leading Center (Torino) unacceptable for a non-profit study.

From the bench side of ACC studies, we are currently trying to characterize putative ACC biomarkers by analyzing, through a multitasking proteomic approach, adrenal tissue samples from patients with ACC and Adrenal Adenoma (ADE) to compare to data obtained from normal adrenal tissues. In particular, such biomarkers will be combined with already consolidated parameters characterizing ACC, to obtain more discriminating prognostic and diagnostic factors.

Future work: Future projects are aimed to evaluate the role of specific cell signaling mechanisms and molecules, identified as ACC-specific biomarkers by tissue screening and *in vitro* cell functional studies on H295R cell lines, in the development, progression and invasiveness of the adrenocortical cancer. Moreover, such markers will be evaluated as possible targets for RGZ and mitotane anti-cancer treatment both in *in vitro* cell models and *in vivo* xenograft mouse models.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
"Assessment of the role of adjuvant therapy in ACC patients" to evaluate the role of adjuvant therapy in ACC patients (ongoing)	2009	Non-profit study	Waiting for admission
"FIRM ACT" which compares the efficacy between two different treatments (etoposide, doxorubicin, cisplatin and mitotane *versus* streptozotocin and mitotane)	2004	None	300 in total 2 patients from our Center

Research Grants

Year	Funding Agency	Amount
2009	Departmental grant	€ 2,244
2008	Istituto Toscano Tumori (ITT)	€ 118,500
2008	Departmental grant	€ 2,536
2007	ITT	€ 132,000
2007	Departmental grant	€ 2,582
2006	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) – COFIN	€ 30,000
2006	Departmental grant	€ 2,555
2006	Ente Cassa di Risparmio	€ 25,000
2005	Departmental grant	€ 3,069
2005	Ministry funds for Interlink Project 2004-2006	€ 30,000
2005	Ente Cassa Risparmio	€ 50,000
2004	Departmental grant	€ 4,738
2004	MIUR – COFIN	€ 45,000
2003	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 30,000
2003	Departmental grant	€ 2,335

Main Collaborations

With Units within ITT

» Department of Oncology, AOU Careggi, Firenze
» General Surgery Unit, AOU Careggi, Firenze
» Department of Pharmacology, University of Florence

With other Italian and Foreign Institutions/Organizations

» Endocrinology and Medicine, University of Padua
» Department of Genetics, Biology of Micro-organisms, Anthropology and Evolution, University of Parma

- Department of Endocrinology and Diabetes, University of Wuerzburg (Germany)
- Department of Endocrinology of Cochin Hospital, Faculté de Médecine René Descartes, Université Paris 5 (France)
- Hypertension Unit, Hôpital Européen Georges Pompidou, Hôpitaux de Paris and Faculté de Médecine René Descartes, Université Paris 5 (France)
- Medizinische Klinik – Innenstadt, Ludwig-Maximilians University, Munich (Germany)
- Department of Medicine, The Medical School, University of Birmingham (UK)
- The Radboud University Nijmegen Medical Centre (The Netherlands)
- National Institute of Health (NIH), Bethesda, Maryland (USA)
- Department of Medicine, Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, Texas (USA)

Publications

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CLINICAL BIOCHEMISTRY

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Principal Investigator

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Serena Vinci, PhD Student
Nicola Pratesi, PhD Student
Irene Mancini, Research Fellow

Introduction

Our Research Unit is mainly involved in the development and clinical validation of new potential biomarkers for the most common human solid cancers, such as breast, colon, lung, bladder, kidney, prostate, melanoma, etc. These studies aim to evaluate molecular targets either in primary tissues or in biofluids, such as blood or urine. The main area of interest is pointed toward the identification and quantification of genetic and epigenetic biomarkers with the use of innovative techniques. To this purpose, our Unit is particularly involved in the development of new strategies for the accurate and sensitive detection of target sequences in complex matrices. In addition, our research Unit has special competences in the techniques of measuring gene expression.

Main Research Themes

1. Pharmacogenetics

- Evaluate genetic variations that can influence response to classical anti-cancer treatments like:
  – platinum derivatives;
  – irinotecan;
  – taxanes;
  – methotrexate;
  – fluoropyrimidines;
  – radiotherapy.
- Research of somatic mutations in cancers that can influence the response to target-oriented therapy (*i.e.* mutations of *EGFR*, *KRAS*, *BRAF*, *c-KIT*) with highly sensitive detection techniques.

2. New techniques for highly sensitive detection of somatic mutation in cancer tissues

- Optimization of pre-screening techniques, such as High Resolution Melting.
- Enrichment of mutated alleles, through Cold-PCR.

3. Methylation of gene promoters with potential antitumor activity and action of demethylating drugs

- Identification of CpG methylate islands in promotion of genes with potential antiproliferative activity.
- *In vitro* experiments of reactivation of expression of demethylating drugs.
- Characterization of the degree of methylation of the same sequences of human tumors and corresponding control tissues.
- Innovative techniques for detection of methylated DNA with quantitative procedures (Figure 1).

4. Measurement of expression of genes involved in tumor progression using real time RT-PCR

- Measure the expression of mRNA and miRNA involved in the development and progression of different types of tumors: lung, breast, colon, bladder, kidney, prostate, adrenal, neuroblastoma.
- Study of the modulation of expression of the same mRNA and miRNA in *in vitro* experiments.
- Evaluation of gene-profiling experiments.
- Development and standardization of new methodological approaches (Figure 2).

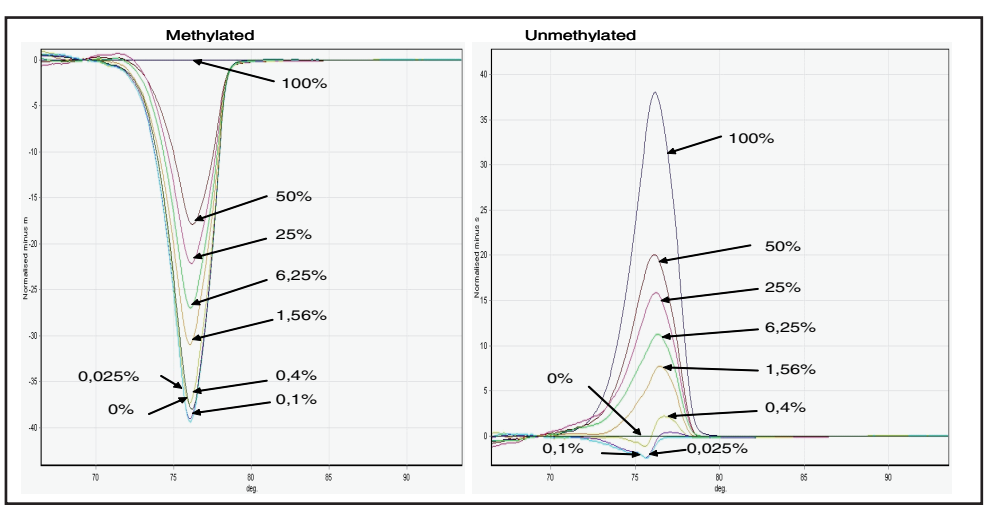

Figure 1 – Quantitative evaluation of DNA methylation by optimization of a differential-high resolution melt analysis protocol

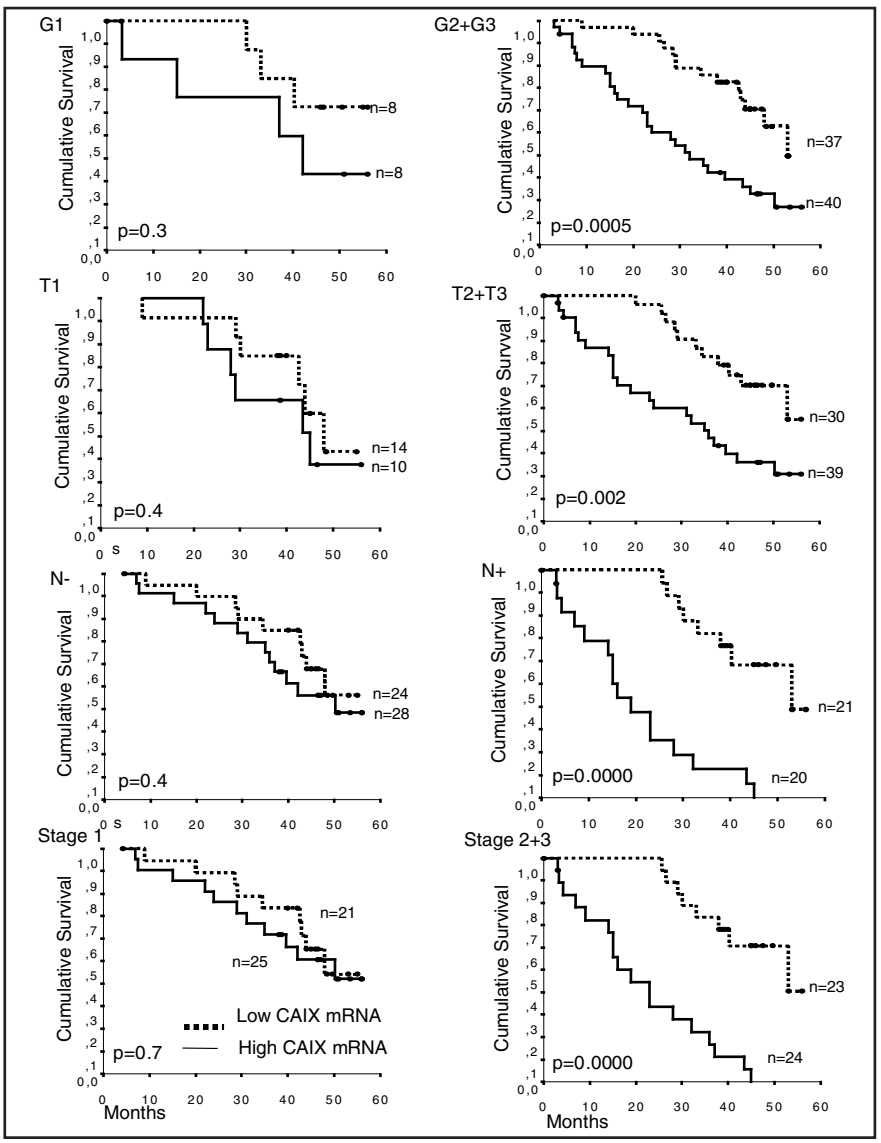

Figure 2 - Alternative splicing variants of carbonic anhydrase IX in human non-small cell lung cancer

5. Identification of a genetic multimarker panel for the detection of cancer-derived DNA in plasma of melanoma patients

- Measurement of total DNA in human plasma.
- Measurement of DNA integrity-index.
- Real-time measurement of BRAF-mutated alleles.
- Methylation-specific real-time PCR for the detection of abnormally methylated genes.

Research Grants

Year	Funding Agency	Amount
2009	Istituto Toscano Tumori	€ 181,000
2008-2010	Ministero della Salute	€ 100,000
2007-2009	Ministero dell'Istruzione, dell'Università e della Ricerca – PRIN	€ 48,000
2007-2009	Ministero della Salute	€ 30,000

Main Collaborations

With Units within ITT

» Endocrinology Unit, AOU Careggi, Firenze
» Medical Oncology Unit, AOU Careggi, Firenze
» Radiotherapy Unit, AOU Careggi, Firenze
» Breast Unit, AOU Careggi, Firenze
» Surgery Units, AOU Careggi, Firenze

With other Italian and Foreign Institutions/Organizations

» Istituto Nazionale Tumori (INT), Milano
» Urology Surgery, University of Pisa
» Istituto Tumori "Giovanni Paolo II", Bari
» Laboratory of Clinical Molecular Biology, Ospedale "San Raffaele", Milano

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Lisa Simi, Researcher

Introduction

The Clinical Biochemistry research group is recognized internationally for its achievement in the following fields of research: chemiluminescence, immunochemistry, hormonal assay, molecular diagnostics with real-time PCR, circulating tumor cells, quality control.

Main Research Themes

1. Molecular diagnostics: development of molecular diagnostics for pharmacogenomics

SNPs could account for interindividual variation in the activity of metabolizing enzymes then affecting the extent of pro-drug activation and, as a result, the efficacy of chemo- and radiotherapy treatment. The SNPs investigated are selected on the basis of the assigned patient's therapy and are analyzed following the reported pattern:

- *fluoropyrimidine response*: GSTP1, TSER (TYMS),MTHFR (2 SNPs);
- *cisplatinum response*: GSTP1,XRCC1,ERCC1 (2 SNPs);
- *radiotherapy*: GSTP1, XRCC1,XRCC3, RAD51.

2. Circulating tumor cells: CTC analysis is performed using ISET technology

An application of the studies under development is on melanoma (both cutaneous and uvela melanoma), as summarized in Figure 1.

3. Real-time PCR

Methods for detection of genetic alterations are developed based on sequencing, pyrosequencing, real-time PCR and high resolution melting (see Publications).

4. Oncology

Studies are ongoing in this Unit in colon, breast, head and neck and prostate cancers in collaboration with the Medical Oncology Unit under Prof. Francesco Di Costanzo of the AOU Careggi, and Prof. Giampaolo Biti of the Radiotherapy Unit of the University of Florence.

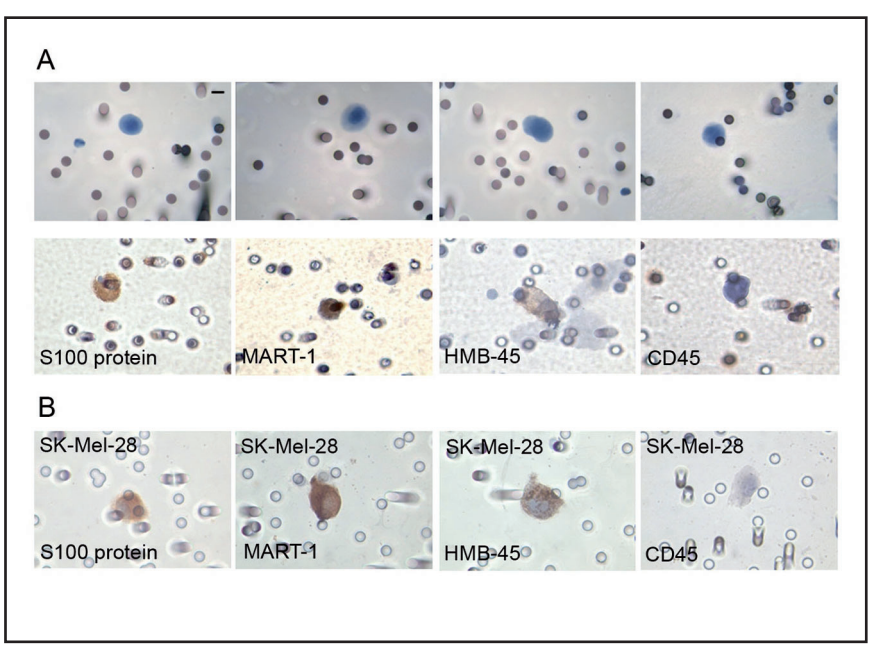

Figure 1 - Circulating melanoma cells detected by Isolation by Size of Epithelial Tumor cell (ISET). A) Neoplastic cells fulfill criteria for circulating tumor cells, including: *a*) cell size ≥16 μm; *b*) nucleo-cytoplasmic ratio ≥ 50%; *c*) irregular nuclear shape; *d*) hyperchromatic nucleus; and *e*) basophilic cytoplasm (upper row, original magnification x63, haematoxylin and eosin stain). Immunohistochemical analyses with anti-S100 protein, MART-1, HMB-45 antibodies identify circulating melanoma cells as weakly to moderately stained cells. CD45 is negative (lower row, original magnification x63). B) Melanoma cell lines (SK-Mel-28) immunostained in artificial samples with S100 protein, MART-1, HMB-45 and CD45. Note that the intensity of the immunostaining is stronger and more diffuse. (Original magnification x63; scale bar 10 μm). From De Giorgi V, Pinzani P, Salvianti F, Panelos J, Paglierani M, Janoska A, et al: *Application of a filtration and isolation by size technique to the detection of circulating tumor cells in cutaneous melanoma*. J Invest Dermatol 2010; In Press

5. Quality control in molecular diagnostics

This Unit is deeply involved in the implementation of several programs on external quality for the EU-granted SPIDIA Project (see www.spidia.eu) and the activities of the IFCC (M. Pazzagli is chair of the IFCC Committee of Molecular Diagnostics: see www.ifcc.org).

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b) Pantel K, Brakenhoff RH, Brandt B: *Detection, clinical relevance and specific biological properties of disseminating tumour cells*. Nat Rev Cancer 2008 May; 8(5): 329-40.
c) Alunni-Fabbroni M, Sandri MT: *Circulating tumor cells in clinical practice: methods of detection and possible characterization*. Methods 2010 Apr; 50(4): 289-97.

Research Grants

Year	Funding Agency	Amount
2008	European Union	€ 1,245,000
2008	Ministero dell'Istruzione, dell'Università e della Ricerca – PRIN	€ 50,000
2004	European Union	€ 739,000

Main Collaborations

With Units within ITT
» Breast Unit, AOU Careggi, Firenze
» Department of Human Pathology, AOU Careggi, Firenze
» Department of Oncology, AOU Careggi, Firenze
» Department of Obstetrics and Gynecology, AOU Careggi, Firenze
» Medical Oncology Unit, Azienda USL 4 Prato

With other Italian and Foreign Institutions/Organizations
» Istituto Tumori "Giovanni Paolo II", Bari
» Heidelberg University (Germany)
» Rotterdam University (The Netherlands)

Publications

1. Rousseau F, Gancberg D, Schimmel H, et al: *Considerations for the development of a reference method for sequencing of haploid DNA. An opinion paper on behalf of the IFCC Committee on Molecular Diagnostics, International Federation of Clinical Chemistry and Laboratory Medicine*. Clin Chem Lab Med 2009; 47(11): 1343-50.
2. Verderio P, Ramsden SC, Orlando C, et al: *External quality assessment schemes for real-time PCR: a statistical procedure to corrective actions*. Clin Chem Lab Med 2008; 46(5): 717-21.
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ONCOLOGICAL GYNECOLOGY

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Ketty Tavella, Gynecologist
Elena Pecchioli, Gynecologist
Monica Masini, Nurse

Introduction

This Unit's activity includes the medical treatment of all gynecological malignancies, including mammary pathology.
The Unit shares its laboratories with a Surgical Unit and, in addition, also houses the Regional Reference Center for trophoblastic and gestational tumors.
Together we collaborate on verification of fertility in the oncological patients, and a special service for menopausal oncological patients is also provided.

Main Research Themes

1. Biomolecular prognostic factors in gynecological malignancies

Expression of the retinoblastoma-related gene Rb2/p130 in endometrial carcinoma; HER-2/neu and Bcl-2, COX-2 and preoperative CA-125, angiogenesis, tumor cell proliferation, P-glycoprotein, Microvessel Density (MDV), tumor-infiltrating gamma/delta T-lymphocytes, P161NK4 overexpression and Vascular Endothelial Growth Factor (VEGF) in ovarian carcinoma; HER-2/neu oncogene in uterine carcinosarcomas; cyclooxygenase-2, proto-oncogene c-KIT and estrogen and progesterone receptors in uterine leiomyosarcoma.

2. Clinical evaluation of the latest anticancer drugs

Abagovomab maintenance therapy in patients with epithelial ovarian cancer, capecitabine and vinorelbine in metastatic breast cancer, six courses of paclitaxel in patients with advanced epithelial ovarian cancer in complete response after six courses of paclitaxel/platinum-based chemotherapy, high dose methotrexate in gestational trophoblastic neoplasia.

3. Innovative diagnostic research in gynecological oncology

Diagnostic role of 2-[18-F] deoxyglucose (2-[18-F]FDG) Positron Emission Tomography (PET) in the restaging of ovarian cancer, bioluminescence *in vitro* chemosensitivity and c-erb-2 amplification in human ovarian, multidrug resistance in ovarian cancer (comparing an immunocytochemical study and ATP-tumor chemosensitivity assay), the utility of CDX-2 in distinguishing between primary and secondary (intestinal) mucinous ovarian carcinoma.

4. Side effects of pharmaceuticals

Antiemetic prophylactic treatment for cancer chemotherapy-induced nausea and vomiting. Prophylaxis of venous thromboembolism in oncological surgery and chemotherapy.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
Randomized, phase II GOIRC study on oral administration of capecitabine and vinorelbine in combination compared to their sequential use in metastatic breast cancer	2009		
A randomized, double blind, placebo-controlled, multicentric trial of abagovomab maintenance therapy in patients with epithelial ovarian cancer after complete response to first-line chemotherapy	2006		
A multinational, multicenter, randomized, double-blind, parallel group, placebo-controlled clinical trial to investigate safety and efficacy of tibolone (Org OD14) in women with climacteric symptoms and a history of breast cancer	2005		

Description	Year	Sponsor	Number of patients recruited to date
Prospective observational study on the impact of anemia on the management of cancer patients over 65 years of age who undergo chemotherapy	2004		

Main Collaborations

With Units within ITT

» Department of Anatomy and Pathology, AOU Careggi, Firenze
» Department of Nuclear Medicine, AOU Careggi, Firenze
» Department of Radiology, AOU Careggi, Firenze
» Department of Pharmacology, AOU Careggi, Firenze
» Department of Radiotherapy, AOU Careggi, Firenze
» Istituto per lo Studio e la Prevenzione Oncologica (ISPO), Firenze

Publications

1. Pecorelli S, Favalli G, Gadducci A, et al; After 6 Italian Cooperative Group: *Phase III trial of observation versus six courses of paclitaxel in patients with advanced epithelial ovarian cancer in complete response after six courses of paclitaxel/platinum-based chemotherapy: final results of the After-6 protocol 1*. J Clin Oncol 2009 Oct; 27(28): 4642-8.
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ONCOLOGICAL ORTHOPEDICS

Unit Address

Oncological and Reconstructive Orthopedics Unit
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Davide Matera, Orthopedic Surgeon
Nicola Mondanelli, Orthopedic Surgeon
Guido Scoccianti, Orthopedic Surgeon

Introduction

The Unit, created in January 1994, is devoted to the treatment of tumors of the musculoskeletal system (bone and soft tissues; primary or metastatic disease) and to reconstructive orthopedic surgery. Specialized techniques are used to approach these diseases which require special reconstructive procedures after oncological resection, such as megaprostheses, massive allografts, microsurgical soft

tissue flaps and vascularized bone grafts. Some innovative procedures were developed in these fields (a new megaprostheses system; special reconstructive techniques involving a combination of massive allografts and microsurgical autografts, or a combination of massive allografts and prostheses).
The experience acquired in reconstruction of wide bone and soft tissue defects in oncological surgery, is also used to approach difficult orthopedic cases, like wide bone defects following trauma, or prosthetic joint replacement surgery.

Main Research Themes

1. Orthopedic techniques in regenerative medicine

Main achievement: Several studies were performed in the area of stem cells, with regard to their application in orthopedic surgery.

Current work: In order to enhance tissue regeneration and healing, we have tried to obtain a graft with osteconductive, inductive and osteogenic properties using mesenchymal stem cells from the iliac crest and/or autologous growth factors obtained from peripheral blood. Since 2000, 190 patients have been treated using different combinations of mesenchymal stem cells, growth factors and scaffolds in benign bone tumors or post-traumatic pseudoarthrosis with encouraging results in about 90% of the cases.

Future work: Development of a protocol for stem cell and growth factor application in orthopedic diseases. Development and testing on new scaffolds. Optimization of stem cells and growth factor accrual procedures.

2. Microsurgical bone reconstruction, alone or in combination with massive allografts

Main achievement: A new bone reconstruction technique was introduced by the main investigator, using a vascularized fibula graft combined to a massive allograft for intercalary reconstruction or arthrodesis after bone resection for tumors of the limbs. A series of more than 100 cases were observed in our Unit. In children, a special procedure, epiphyseal growth plate transplantation, was used, obtaining a growing graft in the upper limb.

Current work: Improvement of the technique is currently being addressed by optimizing osteosynthesis devices and postsurgical protocols.

Future work: Association of these procedures with regenerative medicine techniques and reconstruction of vascularized articular surfaces.

3. Treatment of bone metastases

Main achievement: During recent decades, the life expectancy of patients affected by metastatic carcinoma has improved considerably due to advances in chemotherapy, immunotherapy, hormonal treatment and radiotherapy. In order to standardize the surgical treatment of bone metastases, we developed a classification system for metastatic patients (divided into four classes) and a flow chart for treatment choice.

Current work: This classification was approved by the Italian Society of Orthopedics and Traumatology and published as guidelines for the treatment of bone metastases.

Future work: Developing a regional database of bone metastases and a network of different centers involved in their treatment to optimize treatment of these patients.

4. Treatment of soft tissue sarcomas

Main achievement: Despite the low-incidence of this pathology in the general population, vast experience was acquired with more than 1,000 cases treated in our Center. Wide or radical resection is mandatory for local control of the disease and this often makes special reconstructive techniques necessary (particularly microsurgical free o local flaps). In particular cases, free vascularized and innervated flaps are used to obtain a functional reconstruction of muscular units. Limb salvage success was more than 90% in our series.

Current work: Optimizing indications, timing and modalities of combined treatment using surgery, chemotherapy and radiation therapy. Guidelines for the treatment of soft tissue sarcomas have been recently developed and published by the Italian Orthopedics and Traumatology Society.

Future work: Development of new neoadjuvant or adjuvant treatments, involving new gene or molecular therapy.

5. Bone allografts and bone banking

Main achievement: Massive bone allografts are an important reconstructive option after large surgical bone resections. They afford immediate bone stability and bone stock reconstitution, without morbidity of donor sites, like in autologous grafts, and without main immunological reactions. More than 250 massive allografts were implanted (alone or combined with other techniques) by our Unit. A collaboration with a local Bone Bank (Careggi) was developed and our surgeons participate in graft harvesting.

Current work: Improving of the technique is currently addressed by optimizing allograft harvesting, choice, preparation and implantation.

Future work: Custom tailoring of the allografts in a presurgical set to reduce surgical times. Optimizing imaging techniques for the choice of allografts in order to achieve the best allograft match for recipient site.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
ISG/AIEOP EW-1: protocol of neoadjuvant treatment for patients affected by non-metastatic Ewing/PNET, phase III	2009	Italian Sarcoma Group (ISG) and Associazione Italiana Ematologia e Oncologia Pediatrica (AIEOP)	
ISG/A/Oss: observational study for patients affected by non-metastatic osteosarcoma of the limbs	2007	ISG	
ISG: localized high risk soft tissue sarcomas of limbs and superficial trunk; combined treatment with adjuvant chemotherapy, three to five cycles	2003	ISG	
ISG/OS-1: evaluation of the efficacy of ifosfamide in neoadjuvant treatment of non-metastatic osteosarcoma of the limbs	2000	ISG	

Description	Year	Sponsor	Number of patients recruited to date
ISG/SSG IV: protocol of treatment for patients affected by high-risk Ewing/PNET sarcoma	1999	ISG/Scandinavian Sarcoma Group (SSG)	
ISG/SSG III: protocol of neoadjuvant treatment for patients affected by non-metastatic Ewing/ PNET sarcoma	1999	ISG/SSG	

Research Grants

Year	Funding Agency	Amount
2006	Ospedale "S. Corona", Pietra Ligure (Savona)	€ 55,000
2004	RITA	€ 50,000

Main Collaborations

With Units within ITT

» Pediatric Hematology-Oncology Unit, Azienda Ospedaliero Universitaria Meyer, Firenze
» Radiotherapy Unit, Medical Oncology Unit, Reconstructive Microsurgery Unit, Vascular Surgery Unit, General Surgery Unit, Hematology Unit, Thoracic Surgery Unit, Urologic Surgery Unit, AOU Careggi, Firenze
» Medical Oncology Unit, Azienda Ospedaliero Universitaria Senese
» Medical Oncology Unit, Pediatric Oncology Unit, Radiotherapy Unit, Azienda Ospedaliero Universitaria Pisana

With other Italian and Foreign Institutions/Organizations

» Istituto Nazionale Tumori (INT), Milano
» Centro Riferimento Oncologico (CRO), Aviano (Pordenone)
» Istituto Ortopedico Rizzoli, Bologna
» University of Hamburg (Germany)
» University of Innsbruck (Austria)

Publications

1. Abed YY, Beltrami G, Campanacci DA, Innocenti M, Scoccianti G, Capanna R: *Biological reconstruction after resection of bone tumours around the knee: long-term follow-up*. J Bone Joint Surg Br 2009; 91: 1366-72.
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5. Pakos EE, Nearchou AD, Grimer RJ, et al: *Prognostic factors and outcomes for osteosarcoma: an international collaboration.* Eur J Cancer 2009; 45: 2367-75.
6. Capanna R, De Biase P, Sensi L: *Minimally invasive techniques for treatment of metastatic cancer*. Orthopade 2009; 38: 343-7.
7. Palomba A, Beltrami G, Campanacci D, Capanna R, Franchi A: *Atypical organizing haematoma/seroma following treatment for soft tissue sarcoma.* Histopathology 2009; 54: 505-7.
8. Innocenti M, Abed YY, Beltrami G, Delcroix L, Balatri A, Capanna R: *Quadriceps muscle reconstruction with free functioning latissimus dorsi muscle flap after oncological resection.* Microsurgery 2009; 29: 189-98.
9. Campanacci DA, Matera D, Franchi A, Capanna R: *Synovial chondrosarcoma of the hip: report of two cases and literature review.* Chir Organi Mov 2008; 92: 139-44.
10. Campanacci DA, Scoccianti G, Beltrami G, Mugnaini M, Capanna R: *Ankle arthrodesis with bone graft after distal tibia resection for bone tumors.* Foot Ankle Int 2008; 29: 1031-7.
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12. Kanakaris NK, Calori GM, Verdonk R, et al: *Application of BMP-7 to tibial non-unions: a 3-year multicenter experience.* Injury 2008; 39 Suppl 2: S83-9.
13. Tognarini I, Sorace S, Zonefrati R, et al: *In vitro differentiation of human mesenchymal stem cells on Ti6Al4V surfaces.* Biomaterials 2008; 29: 809-24.
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GENETIC DIAGNOSTICS

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Matteo Benelli, Bioinformatic Researcher

Introduction

The Genetic Diagnostics Unit offers cytogenetic and molecular diagnosis of hereditary diseases, performing both prenatal and postnatal tests. The laboratory is a regional reference center for the diagnosis and prevention of cystic fibrosis, Anderson-Fabry disease, amyloidosis, maculopathies, hypertrophic cardiomyopathy and muscular dystrophy. It is also involved in the genetic characterization of patients affected by hemophilia, mental retardation, hereditary deafness, hereditary hemochromatosis

and genetic syndromes related to numerical or structural chromosomal alterations. In addition, the Genetic Diagnostics Unit is involved in genetic investigation of hematological malignancies, performing cytogenetic analysis in patients referred from the Hematology Unit of Florence. The main research activities of the Genetic Diagnostics Unit are focused on Gastric Cancer (GC), pancreatic cancer, multiple myeloma and sinonasal intestinal-type adenocarcinoma. The Genetic Diagnostics Unit is also involved in research projects concerning pharmacogenetics, as well as in bioinformatics analysis. The activity of the bioinformatic team is mainly focused on the development of: *a*) new algorithms able to analyze a variety of genome scanning technology data (microarray, array-CGH, SNP array and massively parallel sequencing); *b*) novel statistical models able to integrate genome scanning technology data to reconstruct gene regulatory networks. The team cooperates with researchers from national and international institutes and health centers and gives bioinformatic support aimed to provide deep insight into the huge amounts of data produced by these technologies.

Main Research Themes

1. Gene expression profile and therapeutic implication in GC

Main achievements: From clinical overview to translational research. The general goal of this project is to identify molecular parameters that can predict prognosis and response to treatment, improving the management of GC. The patients enrolled in this study are referred from the Siena and Bibbiena surgical centers (Laboratory of Translational Research, Section of Surgical Oncology, Department of Human Pathology and Oncology, Siena; Surgery Unit, Ospedale di Bibbiena, Arezzo). The project is coordinated by Prof. Franco Roviello (University of Siena) and it represents the collaborative efforts developed also in cooperation with the Cancer Genetic Unit, University of Porto, and with the Surgical Oncology Unit, University of Verona.

Current work: In Italy, differences in GC prevalence have been observed in regional distribution, Tuscany (Siena and Casentino-Arezzo) showing a higher incidence with respect to other regions. Considering these areas, Roviello et al have recently described the first E-cadherin germline mutation in an Italian family. On the other hand, the Genetic Diagnostics Unit laboratory has analyzed markers for LOH and microsatellite instability on chromosomes 1q, 3p, 5p, 6p, 8p, 8q, 15q, 17p and 18.

Future work: We are investigating the role of somatic E-cadherin mutations as well as E-cadherin promoter hypermethylation (a,b) in a large series of GC patients, and the role of EGFR activation in GC progression. In addition, we are analyzing GC patients using Array-based Comparative Genomic Hybridization (A-CGH), to obtain a wider molecular characterization of GC. The obtained results will be correlated with the clinical parameters of the GC patients. In particular, specific copy number alterations detected by CGH-array can be associated with clinical outcome and directly related to sequence information to aid in the identification of genes, with predictive and prognostic potential.

2. Molecular characterization of sinonasal intestinal-type adenocarcinoma of leather and wood workers for screening and early diagnosis

Main achievements: Intestinal Type Adenocarcinoma (ITAC) of the sinonasal tract is characterized by a strict correlation with exposures to wood and leather dusts (c) and the high mortality is mainly due to local invasion, while distant spread is a rare event. The strategy of the present study is to obtain a wider molecular characterization of ITAC of the sinonasal tract, using A-CGH and expression analysis, in order to understand the mechanisms implicated in the development of these tumors.

Current work: The group of Prof. Marco Santucci (University of Florence) has contributed to the characterization of the morphological and immunohistochemical profile of ITAC with several studies. More recently, they focused on the characterization of the phenotype of sinonasal ITAC in comparison with that of colonic adenocarcinoma. They found that most ITACs are characterized by the simultaneous expression of cytokeratin 7 and 20, while most colonic adenocarcinomas are characterized by the expression of cytokeratin 20 only (d).

Future work: The study is organized in the following steps: *a*) identification of the molecular profile of ITAC using the microarray technique on prospectively collected samples (at least 10 per year); *b*) identification of chromosomal alterations specifically associated with ITAC using A-CGH on prospectively and retrospectively collected samples (approximately 100 cases); *c*) characterization of the immunoprofile of ITAC with particular reference to the expression of oncogenes and mucin secretory products using the tissue microarray technique on paraffin embedded samples (approximately 100 cases); *d*) correlation of the molecular profile of ITAC with work exposure to identify a specific profile associated with tumors arising in wood and leather workers.

3. Pharmacogenetics

Current work: Gene expression as predictive markers of outcome in Non-Small Cell Lung Cancer (NSCLC) patients (stage IIB with pleural effusion and stage IV) treated with chemotherapy. GOIRC randomized phase trial. The project is carried out in collaboration with the Medical Oncology Unit (AOU Careggi). The aim of the study is to assess if it is possible to optimize first-line chemotherapy in NSCLC using biological and genetic markers. Based on the evidence that RRM1 and ERCC1 expression may result in chemoresistance, the aims of our phase II trial are: *a*) evaluate ERCC1, RRM1 single nucleotide polymorphisms, able to suggest a possible correlation between genetic characteristics and patient' response to platinum-based therapy, in the histological specimens or biopsies of patients with NSCLC stage IIIB-IV at diagnosis; *b*) evaluate if *a priori* selection based on patient genetics can be made and can lead to the administration of the most suitable treatment which should result in the best possible outcome.

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d) Franchi A, Massi D, Palomba A, et al: *CDX-2, cytokeratin 7 and cytokeratin 20 immunohistochemical expression in the differential diagnosis of primary adenocarcinomas of the sinonasal tract*. Virchows Archiv 2004; 445: 63-7.

Research Grants

Year	Funding Agency	Amount
2009-2011	Istituto Toscano Tumori (ITT)	€ 78.500
2009	Sanofi-Aventis	€ 120.000
2008-2011	ITT	€ 270.000
2008	Agenzia Italiana del Farmaco (AIFA)	€ 18.000

Main Collaborations

With Units within ITT

» Medical Oncology Unit, AOU Careggi, Firenze
» Department of Human Pathology and Oncology, University of Florence
» Department of Human Pathology and Oncology, University of Siena
» Department of Critical Care Medicine/Surgery, University of Florence
» Surgery Unit, Azienda USL 8 Arezzo

With other Italian and Foreign Institutions/Organizations

» Infantile Neuropsychiatric Unit, AOU Careggi, Firenze
» Italy Muscular and Neurodegenerative Disease Unit, Istituto "Giannina Gaslini", Genova
» Laboratory of Genetics, Ente Ospedaliero Ospedali "Galliera", Genova
» Surgical Oncology Unit, University of Verona
» Cancer Genetic Unit, University of Porto (Portugal)
» Wigler Lab of Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (USA)

Publications

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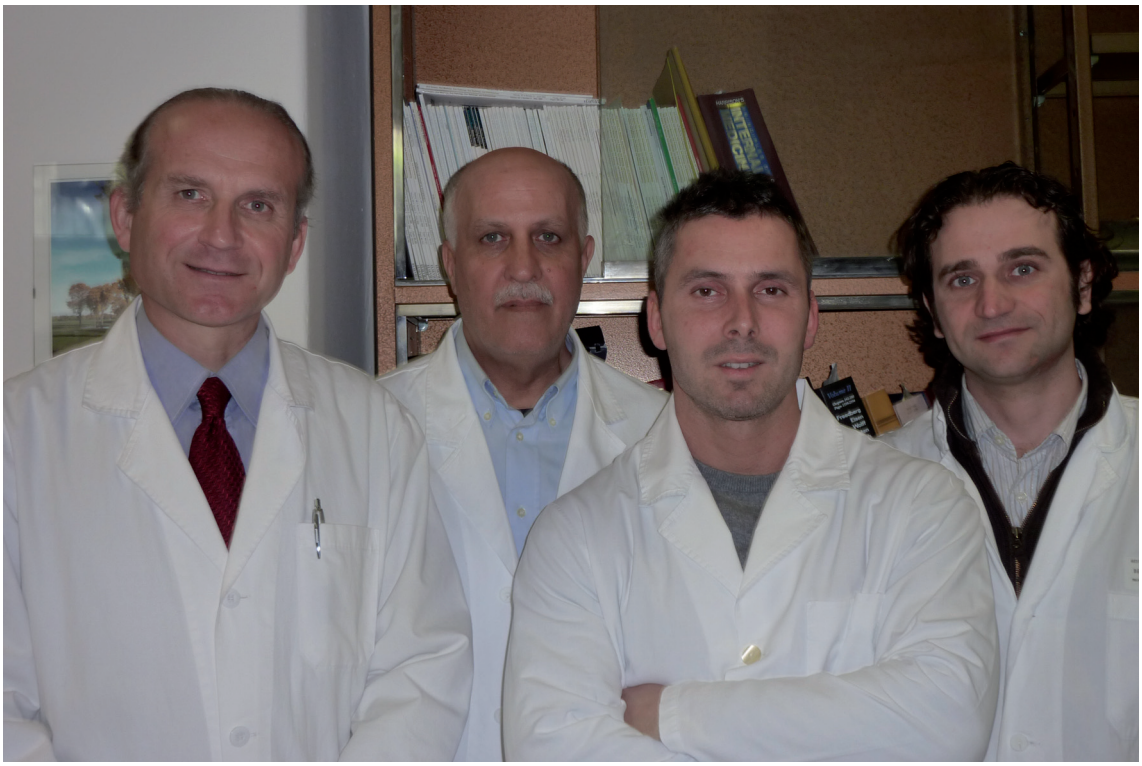
ORAL NEOPLASTIC DISEASES



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Introduction

Instituted in 1992, the Unit received regional and national recognition as a Reference Center in March 2002. The main activity of the Unit consists in the clinical evaluation of patients referred from various Departments, such as Hematology, Medical Oncology, Bone Marrow Transplantation, Kidney Transplantation, Internal Medicine, the Department of ENT, Immunology, Infectious Diseases and the Azienda Ospedaliero Universitaria Meyer. The most frequent diseases diagnosed are: oral cancer and precancerous lesions, infections and allied lesions in immunocompromised patients, HIV-related oral tumors, oral lesions (infections, tumors and GVH lesions) in bone marrow transplantation patients, oral

manifestations of chemotherapy and radiation treatment, autoimmune oral diseases, salivary diseases (including tumors) and jaw bone and soft tissue lesions and tumors. As part of the Reference Center, there is an immunopathology and immunohistochemistry laboratory.
Our research activity consists mainly in clinical and histopathological characterization of the oral diseases. Over the last three years, we have concentrated on:

- bisphosphonate-related osteonecrosis of the jaw;
- early diagnosis of oral cancer;
- oral drugs reactions;
- oral infections and tumors in HIV-infected patients and other immunocompromised patients;
- clinical evaluation and characterization of oral precancerous conditions (lichen planus) and lesions (leukoplakia/erythroplakia);
- immunopathogenesis and precancerous significance of oral lichen planus.

Main Research Themes

1. Immunopathogenesis of oral lichen planus

Analysis, using a sophisticated system (Luminex), of the complex cytokine network which regulates the development of oral lichen planus. This study is in progress.

2. Identification of molecular markers that may predict progression of oral leukoplakia to cancer

Analysis of 60 oral cases of dysplastic oral leukoplakia. This study evaluation is in progress.

Main Collaborations

With Units within ITT

» Pathology Unit, AOU Careggi, Firenze

With other Italian and Foreign Institutions/Organizations

» Department of Pathology, Oral Pathology, University of Chicago-Medical Center (USA)

Publications

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CHEMOTHERAPY

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Stefania Nobili, Research Scientist
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Cristina Napoli, Research Fellow
Gabriele Perrone, Research Fellow

Introduction

The oncological research activity of the Chemotherapy Unit is carried out in the Organizational Departmental Structure of Oncological Pharmacology, part of the Department of Integrated Oncological Treatment of AOU Careggi, and in the Anticancer Chemotherapy Section (laboratory) of the Department of Pharmacology of University of Florence.

The Organizational Departmental Structure of Oncological Pharmacology (headed by Prof. Teresita Mazzei) carries out clinical oncology, oncological research and treatment. The Structure include two permanent position physicians and two physicians who are obtaining their specialization degrees in

Oncology. The research activities and medical assistance include chemotherapy and medical oncology, mainly as treatment for gastrointestinal, breast, lung, gynecological and urological cancers.
The Anticancer Chemotherapy Section (headed by Prof. Enrico Mini) is involved in basic and translational oncological research in the field of chemotherapy and oncological pharmacology. The research team includes two physicians (permanent position), two research scientists and three research fellows. The personnel involved perform studies on clinical, molecular and biochemical pharmacology of anticancer agents.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
The MARTHA (SICOG 0803) trial phase: maintenance and reinduction chemotherapy with Avastin in metastatic colon cancer	2009-2014		
Three or six colon adjuvant: randomized study to evaluate treatment duration of the FOLFOX4 (three *versus* six months) ± bevacizumab as adjuvant treatment in patients with high risk stage II/III colon cancer	2008-2015		
Adjuvant treatment of fully resected stage III colon cancer with FOLFOX4 *versus* FOLFOX4 + cetuximab	2006-2011		
Randomized phase III study of metastatic colorectal carcinoma: chemo-immunotherapy GOLFIG regimen *versus* standard FOLFOX4-GOLFIG2 chemotherapy	2006-2009		
A randomized phase II trial testing the efficacy of three bevacizumab-containing first-line regimens for metastatic colorectal cancer	2006-2008		
Pharmacogenetics UGT1A1 of irinotecan in association with continuous infusion of 5FU (FOLFIRI) in metastatic colorectal cancer	2006-2008		
Prospective and retrospective, multicenter, non-randomized open pharmcogenomic study to identify noted and newly identified determinants predicting toxicity and efficacy of chemotherapy in colorectal carcinoma patients	2005-2008		

Research Grants

Year	Funding Agency	Amount
2009-2010	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) – PRIN	
2009	Cassa di Risparmio di Firenze	

Year	Funding Agency	Amount
2008	Cassa di Risparmio di Firenze	
2007-ongoing	Associazione Giacomo Onlus	
2007	Cassa di Risparmio di Firenze	
2006-2007	Eli Lilly	
2006-2007	MIUR – PRIN (two projects)	
2005-2007	Associazione Italiana per la Ricerca sul Cancro (AIRC), Regione Toscana	
2005-2006	Cassa di Risparmio di Firenze	
2004-2006	Sanofi-Synthelabo	
2004-2006	Eli Lilly	
2004-2006	Aventis-Pfizer	

Main Collaborations

With Units within ITT

» Toxicology and Environmental Carcinogenesis Unit, Department of Pharmacology, University of Florence
» Surgery Division, Department of Clinical Physiopathology, University of Florence
» Division of Medical Genetics, Department of Clinical Physiopathology, University of Florence
» Department of Human Pathology and Oncology, University of Florence
» Surgery Division, Department of Medical and Surgical Critical Care, University of Florence
» Department of Pathology and Experimental Oncology, University of Florence
» Department of Inorganic Chemistry, University of Florence
» Department of Pharmaceutical Sciences, University of Florence
» Pharmacology Division, Department of Internal Medicine, University of Pisa

With other Italian and Foreign Institutions/Organizations

» Clinical Pharmacology Division, Centro di Riferimento Oncologico (CRO), Aviano (Pordenone)
» Istituto di Ricerche Farmacologiche "Mario Negri", Milano
» Gruppo Oncologico Chirurgico Cooperativo Italiano (GOCCI)
» Gruppo Italiano per lo Studio dei Carcinomi dell'Apparato Digerente (GISCAD)
» Department of Oncology, The Queen's University of Belfast (UK)
» European Organization for Research and Treatment of Cancer (EORTC)
» Pan-European Trials in Adjuvant Colon Cancer (PETACC) Group
» Fédération Francophone de Cancérologie Digestive (FFCD)

Publications

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NORMAL AND LEUKEMIC HEMATOPOIETIC STEM CELLS

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Introduction

The Unit has been engaged in the following studies:

1. Control of maintenance and expansion *in vitro* of Hematopoietic Stem or Progenitor Cells (HSCs, HPCs) in severely hypoxic environments; modulation in hypoxia of the action of stem cell-active cytokines (first publication in the field: 1987).

2. Selection of Leukemia Stem or Progenitor Cells (LSCs, LPCs) in hypoxia; characterization of LSC adaptation to chronic hypoxia; drug-resistance of hypoxia-selected Chronic Myeloid Leukemia (CML) LSCs (first publication in the field: 2006).
3. Apoptotic response of Acute Myeloid Leukemia (AML) cells to hypoxia; epigenetic modifications induced in AML cells by hypoxia and/or inhibitors of histone deacetylases; involvement of TRAIL in hypoxia-induced apoptosis (first publication in the field: 2003).
4. Role of Macrophage Colony-Stimulating Factor (MCSF) and Mitogen-Activated Protein Kinases (MAPK) in survival, proliferation, adhesion and activation of macrophages and cancer epithelial cells (first publication in the field: 1991).

Main Research Themes

1. Control of maintenance and expansion *in vitro* of HSCs or HPCs in severe hypoxia

Main achievements:

a) Demonstration that incubation in severe hypoxia selects HSCs, enhancing their maintenance *in vitro*, and that hypoxia is a characteristic feature of the microenvironment typical of the "stem cell niche" (1993-2000).

b) Development of an innovative method, the *Culture Repopulating Ability* (CRA) assay to measure *in vitro* the capacity of Short-Term Repopulating (STR)-HSCs to reconstitute hematopoiesis *in vivo*; the CRA assay is a simple and economic method suitable to assess the effects of drugs or other treatments on human HSCs (2000).

c) The demonstration that HSCs can cycle in hypoxia and that a limited degree of HSC cycling (one replication cycle) in hypoxia, but not normoxia, enhances HSC self-renewal and consequently the generation of HPCs from HSCs. The demonstration that pushing hematopoiesis with IL3 induces HSCs to cycle more than once and thereby lose self-renewal (15).

Current and future work: Development and testing of the efficiency of an improved system for the expansion of HSCs and HPCs *in vitro* based on cell incubation in hypoxia. This system would include three separate phases for HSC selection and amplification in severe hypoxia and HPC generation/clonal expansion in mild hypoxia or normoxia. Different combinations of the so-called *Stem Cell-Active Factors* (SCAF: Flt-L, Kit-L, TPo, IL6, VEGF) will be used during HSC amplification, and combinations of hematopoietic stimulators, such as IL3, during clonal expansion. The effects of different glucose concentrations in culture medium and of 0.1% *versus* 1% O2 during HSC selection will also be compared.

2. Role of hypoxia in the selection and maintenance of LSC or LPC

Main achievements:

a) Adaptation of the CRA assay (see above) to the detection of LSCs in the activated (*standard*-CRA) or quiescent (*extended*-CRA) types; this enables a simple and efficient testing of drugs with regard to their specific effects on LSC maintenance (6).

b) Demonstration that the BCR/Abl oncogenetic protein, responsible for the autonomous growth of CML, is suppressed in hypoxia-resistant CML cells (8,16).

c) Demonstration that incubation in severe hypoxia selects LSC from different types of leukemias, confirming that LSCs, like HSCs, are hypoxia-adapted (6,8).

d) Demonstration that hypoxia-adapted LSC of CML are maintained in a BCR/Abl-independent fashion and resistant to Imatinib Mesylate (IM), a BCR/Abl inhibitor (8).

Current work: We are trying to understand why and how in CML cells the expression of BCR/Abl protein is down-regulated in hypoxia, determining whether the maintenance of hypoxia-adapted LSCs require BCR/Abl suppression and whether the mechanism of suppression is transcriptional or post-translational. The role of BCR/Abl protein expression in the regulation of LSC/LPC balance is being addressed. Hypoxia-induced BCR/Abl accumulation in the nucleus and its relevance to LSC selection or LPC maintenance are also being evaluated. Finally, the sensitivity of BCR/Abl-independent, IM-resistant LSC to drugs of other classes than IM itself is being tested.

Future work: To undertake the characterization of mechanisms enabling the BCR/Abl-independent maintenance of LSC in hypoxia. It will first be tried to establish whether this maintenance is antagonized by the inhibition of BCR/Abl nuclear translocation or by boosting LSC clonal expansion (IL3), or enhanced by stimuli competing with this expansion (SCAF, see Theme 1). The relevance of signaling pathways in LSC maintenance (MAPK; VEGF; PI3K>Akt>mTOR; bmi-1, STAT5, etc.) and the interference of the relative inhibitors with this maintenance will then be tested. Special emphasis will be given at this stage to the use of primary cells explanted from CML patients, rather than stabilized cell lines.

3. Apoptotic response of AML cells to inhibitors of histone deacetylases (HDAC) or hypoxia

Main achievements:

a) Demonstration that treatment with the HDAC inhibitor (HDACi) sodium butyrate or D1 restores histone acetylation inhibits proliferation and triggers apoptosis in AML cells. However, only in the so-called *Core Binding Factor* (CBF)-AML were these HDACi found to induce terminal granulocytic maturation, removing the maturation block due to constitutive HDAC recruitment. This points to the possible use of sodium butyrate or D1 as monotherapy to treat CBF-AML cases (11).

b) Demonstration that the JNK inhibitor SP600125, but not inhibitors of other MAPK, enhances the D1-induced apoptosis in an additive fashion. In non-CBF AML cell lines, where D1 is ineffective, SP600125 alone significantly induced apoptosis and synergized with D1, pointing to the combined administration of the two drugs as a promising tool for AML treatment (7).

c) Demonstration that the HDACi ITF2357 (Givinostat) selectively blocks proliferation and induces apoptosis in CBF-AML, indicating that ITF2357 is a very potent anti-leukemic agent, to be used especially at low doses to treat AML subtypes involving abnormal HDAC activity (4).

Current work: Characterization of the effects of HDACi of different chemical families on CBF-AML cells, trying to detect different patterns of induced acetylation in lysine residues of H4 and H3 histones, and to establish the kinetics of this induction. Characterization of the effects of hypoxia on AML cells, the mechanism underlying their apoptotic response to hypoxia and the role in this response of the extrinsic apoptotic pathway and TRAIL.

Future work: To compare two HDACi of different chemical families, possibly with different kinetics and patterns of histone acetylation (see Current work) in order to evaluate their different capacity to induce, in CBF-AML cells, the expression of AML1 target genes (such as CEB-Pa, *fms*/MCSF.R, IL3).

4. Role of MCSF and MAPK in survival, proliferation, adhesion and activation of stromal or cancer epithelial cells

Main achievements:

a) Demonstration that MCSF induces tyrosine phosphorylation of FAK in macrophages, and that FAK interferes with MCSF-induced cell spreading and adhesion in a Src-dependent fashion, while MAPK ERK1/2 and JNK inhibit this effect (9).

b) Demonstration of critical modulation of MEK1/2 and ERK1/2 activation in macrophages based on the MCSF dose; while mitogenic MCSF doses enhanced, 100-fold lower doses reduced ERK1/2 phosphorylation as well as cell proliferation; this was the first report that the same growth factor,

depending on its dose, can exert opposite effects on cell proliferation by switching ERK1/2 signaling on or off (12).

c) Demonstration that ERK5 mediates the proliferative response of macrophages to MCSF and that Src family kinases connect ERK5 to this response (3).

d) Demonstration that ERK5 inhibits PDGF-induced proliferation and stimulates PDGF-induced motility in hepatic stellate cells (5).

e) Demonstration that macrophage activators trans-down-modulate the expression of MCSF receptors (MCSF.R) in macrophages, that this effect is directed to block MCSF-dependent signals antagonizing activation rather than to inhibit macrophage proliferation, and that the modulation is operated by *Tumor Necrosis Factor*-a-*Converting Enzyme* (17).

Current and future work: We are engaged in transferring the experience accumulated in the above-mentioned studies to the characterization of the role of MAPK- and MCSF-dependent signals (activation of ERK5 and MCSF.R, in particular) in survival, proliferation and adhesion of hepatocellular and breast carcinoma cells. We believe these signals to be good candidates as targets of anti-cancer therapy. Immortalized cell lines will be used in preliminary studies and data obtained will then be confirmed with cell or tissue explants of the above-mentioned neoplasias, as well as in mouse models.

Research Grants

Year	Funding Agency	Amount
2009-2011	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 50,000/year
2009-2010	Istituto Toscano Tumori	€ 93,000
2008-2010	Federazione Italiana per la Ricerca sul Cancro	€ 50,000
2007-2008	Associazione Italiana per la Lotta contro le Leucemie e i Linfomi	€ 20,000
2003-2005	Istituto Superiore di Sanità	€ 88,000

Main Collaborations

With Units within ITT

» ITT Core Research Laboratory (CRL)
» Department of Internal Medicine, AOU Careggi, Firenze
» Medical Oncology Unit, Azienda USL 4 Prato
» Hematology Unit, AOU Careggi, Firenze

With other Italian and Foreign Institutions/Organizations

» Laboratoire d'Hématopoïèse Normale et Pathologique, Université Victor Segalen – Bordeaux 2 (France)
» Etablissement Français du Sang Aquitaine-Limousin, Bordeaux (France)
» Institut Für Mikrobiologie Und Genetik, Wiener Biozentrum, Universität Wien (Austria)
» Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Yeshiva University, New York (USA)

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EXPERIMENTAL TOXICOLOGY AND CARCINOGENESIS

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Introduction

The team is currently active in the application of genomic methods in gastrointestinal carcinogenesis (colon cancer) in experimental animal systems and human studies; additional programs include the study of DNA damage in normal circulating and cancer cells.

Main Research Themes

1. Experimental studies on colon carcinogenesis

Main achievement: Discovery of the earliest pre-neoplastic lesions in the colon, named Mucin Deprived Foci (MDF).

Current work: Effect of foods with different polyphenol levels on early pre-neoplastic lesions in rodent models.

Future work: Further characterization of the genomic, proteomic and structural alteration of MDF and development of early diagnostic markers for colon cancer.

2. Intestinal inflammation and colon carcinogenesis

Main achievement: Discovery of the anti-inflammatory and cancer inhibiting effects of food containing high levels of polyphenols.

Current work: Effect of polyphenol-rich foods on the inflammatory processes in the colon.

Future work: Characterization of the relationship between low level inflammation, gene expression and cancer in rodent models (HLA-B27 transgenic rats; DSS chronic administration in conventional rats).

3. DNA oxidation damage

Main achievement: Discovery of the effects of food polyphenols on tissue oxidation damage. Determination of basal level of eukaryotic cell DNA oxidation damage.

Current work: Effect of food components on DNA damage.

Future work: Use of the comet assay for assessing chemotherapeutic agents in mammary cancer. Correlation between DNA oxidation damage and mutation rate.

4. Characterization of sporadic colorectal cancer with genomic methods

Main achievement: Development of platforms of microarrays for the assessment of gene expression and development of biostatistic methods for the analysis of genomic data in humans.

Current work: Development of genomic markers for the prediction of colon cancer prognosis.

Future work: Development of methods for analyzing gene expression data. Application of genomic data to the characterization of human sporadic colon cancer.

5. Ageing in *Saccharomyces cerevisiae* and human cells *in vitro*

Main achievement: Characterization of the effects of resveratrol on cell oxidative damage and ageing.

Current work: Correlation between cellular effects of resveratrol and genomic regulation in human cells and yeast.

Future work: Development of system biology analysis to correlate the genomic regulation of *Saccharomyces cerevisiae* and mammalian cells in conditions of cellular stress.

Clinical Trial

Description	Year	Sponsor	Number of patients recruited to date
Effect of gastroprotected butyrate preparations on the colon	2009	Promefarm	40

Research Grants

Year	Funding Agency	Amount
2009	Istituto Toscano Tumori	€ 106,000
2003-2010	Genomics of Dendritic Cells, Network of Excellence (DCTERA)	
2003-2010	Nutrigenomics, Network of Excellence (NUGO)	€ 1,215,000

Main Collaborations

With Units within ITT

» Genetics and Gene Transfer in Oncology Unit, ITT Core Research Laboratory (CRL), Firenze
» Medical Oncology Unit, Azienda USL 4 Prato

Publications

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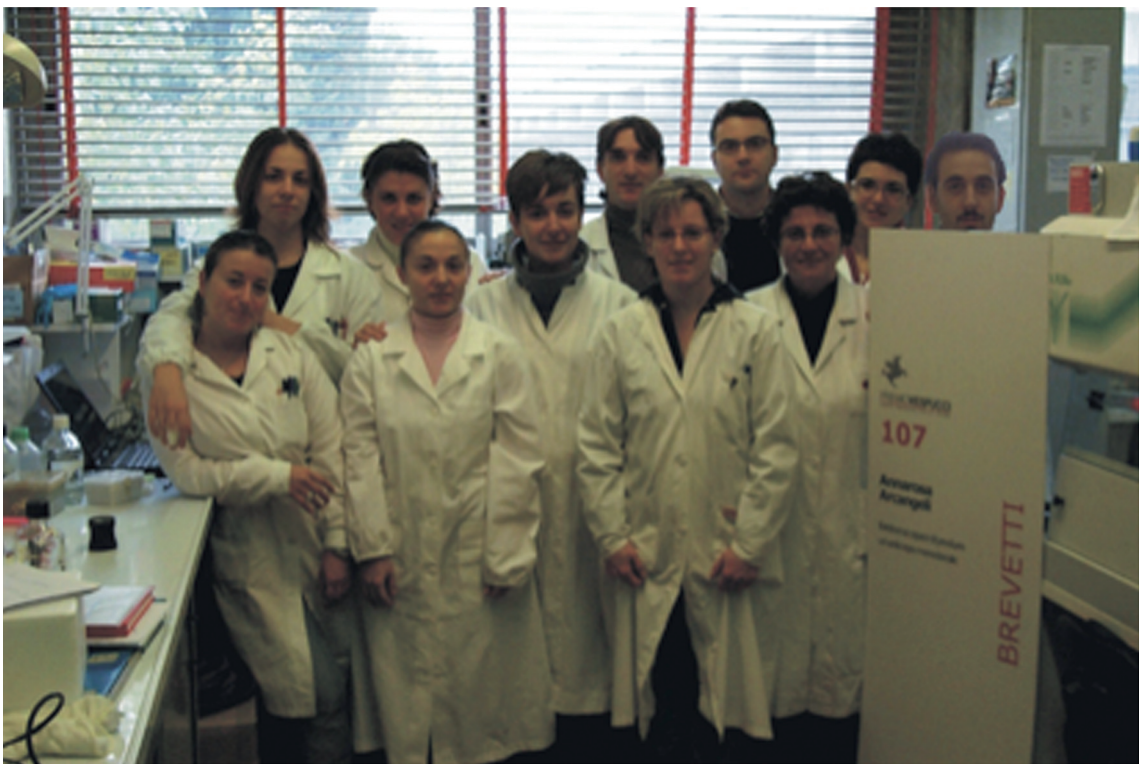
ION CHANNELS AND CANCER



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Introduction

The Unit's research is performed both at the Department of Experimental Pathology and Oncology and at the Centro Stabulazione Animali da Laboratorio (Ce.S.A.L.). This Facility contains a brand-new section fully equipped for the maintenance and manipulation of transgenic and nude mice (L.I.Ge.M.A.).

The Unit is involved in studies aimed at determining the role of ion channels in tumor establishment and progression. In particular, our group has demonstrated that K^+ channels belonging to the hERG1 family are frequently mis/overexpressed in several human cancers. Moreover, the activity of these channels contributes to the regulation of various components of neoplastic growth, including proliferation, apoptosis, acquisition of an invasive phenotype, regulation of VEGF secretion and hence, tumor angiogenesis.

Main Research Themes

Ion channels and transporters control many facets of cancer cell biology (a-c) and blocking the activity of these impairs tumor cell growth *in vitro* and *in vivo*. This new paradigm, emerging over the last two decades, has opened up new opportunities for pharmaceutical research in oncology (a-c). The contribution of our group to this field includes the demonstration that K_v11.1 (hERG1) channels are aberrantly expressed in several human cancers where they control different aspects of neoplastic cell biology, such as proliferation and apoptosis of leukemic cells, acquisition of an invasive phenotype in Acute Myeloid Leukemias (AMLs) and Colorectal Cancers (CRCs), and regulation of VEGF secretion in brain and Gastrointestinal (GI) tumors (b). hERG1-dependent effects were shown *in vitro* and, more recently, *in vivo*. In preclinical models of both leukemia and CRC, hERG1 overexpression confers a higher malignancy to neoplastic cells. It is likely that hERG1 can cause such diverse effects because it modulates different intracellular signaling cascades. These pathways are often activated by the formation of membrane complexes between hERG1 and integrin receptors, which can also recruit growth factor and chemokine receptors (d,e).

1. hERG1 expression in gastric cancer, CRC and acute leukemias: correlation with clinico-pathological features and response to therapy

Main achievements:

a) *CRC*. We previously demonstrated that the hERG1 protein is expressed in CRC and can be easily detected by Immunohistochemistry (IHC) in primary human CRC samples. Using the mAb anti-hERG1 produced in Dr. Arcangeli's lab and patented by the University of Florence (FI2006A000008), we carried out a study on a cohort of 189 patients. In this study, we also determined the expression of several hypoxia/angiogenesis markers: VEGF, Glut1, CA IX, HIF-1. A statistically significant inverse correlation ($P = 0.004$) emerged between hERG1 and Glut1. Moreover, analysis of Overall Survival (OS) at two years, using a multivariate analysis (through the multivariate Cox proportional hazards regression model) adjusted for Duke's stages showed that patients with high hERG1 and concomitant low Glut1 expression had poorer survival ($P = 0.036$).

b) *Gastric cancer.* A study performed on 360 specimens of both advanced and early gastric cancers, as well as on 31 specimens of gastric dysplasias showed: *i*) a statistically significant correlation between hERG1 expression in neoplastic tissues compared to normal gastric mucosa ($p < 0.0001$); *ii*) a statistically significant correlation between hERG1 expression and Laurèn's intestinal type carcinomas ($p < 0.001$); *iii*) a statistically significant correlation between hERG1 and VEGF expression ($p < 0.0001$); *iv*) a statistically significant correlation between hERG1 expression in dysplasias and progression towards cancer ($P = 0.046$).

c) *AMLs*. In the course of a study evaluating the functional significance of the FLT-1/hERG1B/b_1 signaling complex in AML, the quantitative expression of hERG1 transcripts was analyzed by Real Time-PCR (RQ-PCR) on Peripheral Blood (PB) mononuclear cells from 61 patients with *de novo* AML. AML cases examined showed an increased level in hERG1 transcript (mean value = 3.29E+01 ±1.81E+01),

and an even greater increase in the level of hERG1b (mean value = 5.11E+04 ± 5.18E+03). The correlation between hERG1 expression and clinical features and outcome was analyzed in 42 of the AML patients enrolled in the study, who were undergoing standard chemotherapy. Patients were identified as either $hERG1^+$ (n = 26) or $hERG1^-$ (n = 16). It emerged that: *i*) Complete Remission (CR) was obtained in 61% of $hERG1^+$ *versus* 82% in $hERG1^-$ patients (p = 0.222); *ii*) the percentage of relapses was 79% in $hERG1^+$ *versus* 21% in $hERG1^-$ patients (p = 0.035); *iii*) the median time to relapse was 11.9 in $hERG1^+$ *versus* 25.3 months in $hERG1^-$ patients (p = 0.014). Finally, the OS in the same cohort of patients was analyzed: the median OS of $hERG1^+$ AML patients was 12 months, compared with 23 months for $hERG1^-$ (p = 0.026).

d) *Acute Lymphoblastic Leukemias (ALL)*. Despite the success in the treatment of pediatric ALL, a subset of patients remains refractory to chemotherapy. It has been reported that bone Marrow Mesenchymal Cells (MSCs) can contribute to drug resistance in leukemic cells. At the moment, no molecular marker can be used to predict chemoresistance at the onset of the disease, while the response to chemotherapy is monitored by determining the Minimal Residual Disease (MRD) at day 78 after the beginning of induction therapy. We have recently identified a novel and important mechanism by which MSCs in the bone marrow microenvironment protect ALL cells from chemotherapy. Central to this protection is a plasma membrane complex on leukemic cells consisting of hERG1 potassium channels, the beta1 integrin subunit and the SDF-1a receptor CXCR4. hERG1 channels were the only proteins within the complex that directly mediated the delivery of cell survival signals leading to chemoresistance. Besides the possibility of overcoming chemoresistance in ALL through the use of hERG1 blockers, we determined that the expression level of the hERG1 protein correlates with *in vitro* chemoresistance. In particular, the level of hERG1 expression, determined by measuring the Median Fluorescence Intensity (MFI) index by flow cytometry, is directly correlated with the protective effect of MSCs on leukemia cells treated with doxorubicin.

Current and future work: On the basis of the results obtained so far and presented above, the analysis of hERG1 expression will be continued in the four types of cancer as follows:

a) *Validation and standardization of methods for predicting outcome, and stratify therapeutic options in patients with CRC based upon hERG1 expression*. Based on the data reported above, the main objective of the present task is the validation of the diagnostic and prognostic value of hERG1 channels in conjunction with Glut1 as potential biomarkers for CRC. This will be evaluated through *i*) an IHC and biomolecular (RQ-PCR) analysis on biobanked, archival samples from patients whose clinical characteristics and outcome are documented. Moreover, since hERG1 is not the only K^+ channel aberrantly expressed in CRC, it is plausible that, in addition to hERG1, a K^+ channel profile can be used as a prognostic marker in CRC. Hence, *ii*) we propose to perform a microarray gene expression and miRNA profile of K^+ channels in fresh CRC samples. These data will also lead to the design *iii*) of novel imaging tools for hERG1 to be potentially used in early diagnosis, stratification and monitoring of the course of CRC and the response to therapy.

b) *Validation of flow cytometry assay to use hERG1 channels as predictors of chemoresistance in acute leukemias*. We propose to validate the hERG1 MFI detection, along with the evaluation of chemoresistance *in vitro* (index 1) and MRD +78, to predict chemoresistance *in vivo* in pediatric patients with ALL and AML. In a first phase, we plan to analyze 150 patients whose Bone Marrow (BM) leukemic cells have been collected and stored at the Department of Pediatrics, Oncohematology Laboratory, University of Padova. The same protocol could be also applied to adult patients.

2. Characterization of the molecular mechanisms leading to hERG1 gene overexpression and to the differential expression of the hERG1 splice variants in tumors

Main achievements: In the past years, we have provided evidence that the hERG1 gene (and the corresponding protein) is constantly overexpressed in various types of tumor cell lines. In addition, we

have shown that tumor cells often express the hERG1 alternative transcript hERG1b along with full-length hERG1 RNA. Another variant was identified and cloned from tumor cells in our laboratory, named $hERG1_{BUSO}$ (Accession number AJ609614). On the whole, four splice variants of the hERG1 gene have been discovered so far: hERG1, hERG1b, $hERG1_{USO}$ and $hERG1_{BUSO}$. We have also characterized the functions of such variants in cancer cells: *a*) the hERG1 protein is apparently involved in the regulation of tumor cell invasion through a physical and functional link with integrins; *b*) the hERG1B variant is significantly overexpressed during the S-phase of the cell cycle in tumor cells and is apparently involved in the regulation of tumor cell entry into the proliferative state; *c*) the two USO-containing isoforms are expressed in several normal and neoplastic human tissues and cells, but do not give detectable hERG1 currents, since they are retained intracellularly. The USO-containing isoforms form heterotetramers with both hERG1 and hERG1B *in vitro* and *in vivo*, and in this way can regulate trafficking of these hERG1 proteins, thus modulating the intensity of hERG1 currents. This modulatory mechanism, although not exclusive, is characteristic of tumor cells that highly express $hERG1B_{USO}$. Another aim of this task is the evaluation if differences in methylation pattern could be responsible for the variable expression of the hERG1 protein in colon and gastric primary tumors and cell lines. To this purpose, we used bisulfite conversion-based methods. We have selected two different hERG1 DNA sequences, one located in a region comprising the hERG1 promoter and one at the beginning of the coding sequence respectively, and we searched for a potential CpG island. We found two putative areas to be targeted: one around the hERG1 promoter and the other in the coding sequence of the gene of interest. We are going to set up the optimal conditions to standardize the protocol to avoid false positive/negative results.

3. Characterization of the macromolecular signaling complexes centered on hERG1 channels

Main achievements: hERG1 channel activity modulates *a*) cell proliferation and apoptosis, which in turn mediates chemoresistance in leukemias; *b*) the acquisition of an invasive phenotype, which confers malignancy to AMLs and CRCs; *c*) the regulation of VEGF secretion with a clear impact to cancer progression in tumors of the brain and of the GI tract. hERG1 can induce such diverse effects since it triggers and modulates intracellular signaling cascades (c). This role depends on the formation, on the plasma membrane of tumor cells, of macromolecular complexes with integrin receptors. Such complexes often also comprise growth factor and chemokine receptors. Inside the complex, the link between hERG1 and integrins is two-fold: integrins, mainly the beta1 subunit, can activate hERG1. Conversely, the channel, once activated by integrins, can modulate signaling pathways downstream to integrin receptors. Hence, the targeting and, possibly, blocking of such complexes may represent an alternative strategy to inhibit hERG1 channel activity in cancer cells. Fundamental to designing such strategy is our recent demonstration that the beta1 subunit of integrins and the hERG1 protein directly interact on the plasma membrane of cancer cells; this was obtained performing FRET experiments using fluorochrome-tagged recombinant proteins. The experiments clearly indicated that the two proteins directly interact to form a plasma membrane complex characterized by an intermolecular distance between the two proteins lower than 4 nm.

Current and future work:

a) *Design and testing of inhibiting peptides which may unlock the hERG1/integrin complex*. Preliminary to the accomplishment of this subtask is the identification of the epitopes of the two proteins which are involved in the complex. Recent FRET and immunoprecipitation experiments have identified in the cytosolic C-terminus of beta1 and the cytosolic C terminus of hERG1, the regions of the two proteins involved in complex formation. Hence, we will perform pull down experiments using the appropriate domains of beta1 and hERG1 protein which have been identified as mediating complex formation, fused with the GST protein and tested with lysates obtained from HEK 293 cells (to bind the GST-linked domain of the hERG1 protein) and from HEK 293 cells stably transfected with the *hERG1* cDNA (HEK-hERG1 cells) to bind the GST-linked domain of the beta 1 integrin. GST-linked

functional domains of the hERG1 protein are already available in our laboratory. Once identified, the epitopes mediating the beta1/hERG1 interaction, inhibiting peptides will be synthesized and tested.

b) *Production of bifunctional antibodies targeting beta1 and hERG1 extracellular domains*. Bivalent and bispecific antibodies are emerging as tools showing many practical applications, including immunodiagnosis and therapy. One of such bi-specific antibody fragment is the diabody. Instead of single polypeptide chain with four domains, diabodies are dimers, each chain comprising two domains. Each chain alone is incapable of binding to antigen, but associates with the other chain to form a bispecific diabody. We will produce bispecific diabodies against the beta 1 integrin and the hERG1 channel starting from purified total RNA extracted from hybridomas secerning the hERG1-Mab and anti beta1, TS2/16, antibodies. To prove that both antigen-binding sites are located on the same bispecific fragment, we will use a bridge-ELISA by binding one specificity to the solid phase antigen and the other in solution. If we are successful in finding a proper diabody, we will test the ability of such a molecule to recognize both the beta1 and hERG1 protein in living cells, as well as to inhibit integrin-dependent signaling.

4. Studies of the anticancer effect of drugs which inhibit hERG1 expression/activity

Main achievements: We have recently identified a novel mechanism by which MSCs in the bone marrow microenvironment protect ALL cells from chemotherapy. Central to this protection is a plasma membrane complex on leukemic cells consisting of hERG1 potassium channels, the beta1 integrin subunit and the SDF-1a receptor CXCR4. hERG1 channels were the only proteins within the complex that directly mediated the delivery of cell survival signals leading to chemoresistance. In murine models, including mice injected with corticosteroid-resistant cells, treatment with hERG1 blockers significantly increased the rate of apoptosis of leukemic cells in BM and reduced leukemic infiltration of peripheral organs. These data suggest that inhibitors of hERG1 activity would be useful in remission retrieval therapy for patients with relapsed ALL. Besides the classical class III antiarrhythmics, we identified other compounds with potent anti-hERG1 effects and anti-leukemic activity *in vitro*. Two of these, sertindole and erythromycin, should not carry any serious risk of cardiotoxicity and therefore would be promising candidates for inclusion in clinical trials.

We have also provided evidence that hERG1 channels are overexpressed in human cancers of the GI tract, where they contribute to perform the angiogenic switch. In fact, hERG1 channels regulate VEGF-A expression and VEGF-A secretion *in vitro*, through a hypoxia-independent process, which is triggered by integrins on the plasma membrane and converge to the regulation of PI3/Akt and, in turn, HIF-1 activity. This mechanism is also operative *in vivo*, so that the treatment with hERG1 blockers decreases the vascularization and growth of tumor masses obtained after injection of hERG1-expressing GI cancer cells into immunodeficient mice.

Current and future work: Design and testing of non-cardiotoxic hERG1 blockers.

a) *In vivo tests of non-torsadogenic hERG1 blockers in conjunction with classical chemotherapeutic drugs in a leukemia mouse model*. We will test the two hERG1 blockers, erythromycin and sertindole (in a first phase), *in vivo* in a leukemia mouse model. Such a model consists of NOD-SCID mice injected with ALL cells transduced with the firefly luciferase gene (NALM6-luc and 380-luc). Molecular imaging of the bioluminescence produced by luc-expressing ALL cells will permit non-invasive detection of leukemic engraftment in the mice; this will allow us to better evaluate disease progression, define the time and dose of treatment and, finally, have a more precise evaluation of the response to treatment.

b) *In vivo test of non-torsadogenic hERG1 blockers along with antiangiogenesis drugs on a colon cancer mouse model*. We have produced an orthotopic murine model of CRC, where CRC cells from the HCT116 cell line are injected into the coecal wall, to better mirror the human disease. Once visualized, the effective establishment of HCT116 cell growth in coecum (3-7 days) mice will be

treated with the following drugs: erythromycin or sertindole alone or in association with: *i*) the murine analogue of bevacizumab (A4.6.1) (100 mg i.p., twice weekly); *ii*) PTK 787 (100 mg/kg, p.o. for four days). The effect of treatment on the angiogenic pathway will be evaluated by both IHC and RQ-PCR. The same type of experiments will also be performed in transgenic mouse models. Complementary to the pharmacological approach, we will use a molecular approach to decrease hERG1 expression in CRC cells, in order to have a model which not only confirms the pharmacological data, but also could represent an alternative therapeutical approach. To this purpose, we will make use of hERG1-shRNAs transduced in CRC cells by lentiviral vectors, a method we have successfully applied (see L.I.Ge.M.A.). In order to study the function of hERG1 *in vivo* we will use a different lentiviral vector produced by using the plasmid pGIPZ shRNAmir (Open Biosystems), in which a GFP reporter and shRNAmir are part of a bicistronic transcript, allowing the visualization of the silenced cells. Using this approach, we will investigate the effect of the post-transcriptional suppression of hERG1 *in vivo*, by monitoring tumor growth and disease progression as above.

c) *Biophysical test of novel compounds useful to block hERG1 channels in cancer cells*: *background and preliminary results*. The aim of the present task is to identify and test drugs that behave as hERG1 blockers, but can be used in clinics in the future due to being devoid of cardiotoxicity and more specific for the hERG1 channels expressed in cancer cells. We will test, in a first phase, the biophysical properties and the effects on cancer cells *in vitro* of roscovitine, several derivatives from dofetilide and peptide toxins. In a second phase, any other drug we can obtain from different pharmaceutical companies will be tested. We will analyze hERG1 currents by patch clamp using whole cell configuration. Parallel experiments will be performed to test the biological activity of such compounds *in vitro* on proliferation, apoptosis, invasion of tumor cells. In the case that any of the tested drug have the characteristics (method of block, preference for the block on tumor hERG isoform(s), inactivation dependence), it will be tested in one of the above preclinical models.

5. Analysis of the oncogenic role and the molecular steps driven by the overexpression of hERG1/hERG1b genes in the development of cancer through the study of genetically modified (over- and misexpressing) mice

Main achievements: See L.I.Ge.M.A. page 407.

Current and future work:

a) *CRC*. To confirm and better analyze the effect of hERG1 overexpression, a greater number of mice of different ages will be examined. In these mice the occurrence of alterations in the rate and pattern of cell proliferation will be studied using anti Ki-67 Ab. The presence and position of hERG1-expressing cells in the crypts will be determined. The degree of differentiation will be studied by testing the specific binding of three different lectins and performing Alcian Blu/PAS staining to assess the presence of goblet cells. In addition, the effects of hERG1 overexpression on the susceptibility to carcinogenic agents will be evaluated by inoculating different doses of azoxymethane i.p. into transgenic mice and analyzing the development of aberrant foci crypts after five weeks of treatment. Founder mice treated in the same manner will be used as controls. Another approach will also be followed in order to demonstrate whether hERG1 channel overexpression contributes to tumor progression. To this purpose, hERG1-expressing mice will be mated with mice harboring genetic alterations causing the development of intestinal tumors, like MUC2 -/- mice or Apc1638 +/- mice. In these mice, it has been shown that the additional alteration of other genes, such as p21/waf1, enhances tumor formation. After assessing hERG1-expression in the developing Apc 1638 +/- / hERG1mice (or MUC2 -/-/hERG1 mice), the tumor malignancy (in terms of size, frequency and histology) compared to controls (Apc +/- or MUC 2 -/-) mice will be evaluated. Moreover, tumor local invasion, as well as the development of hepatic and lung metastases, will be analyzed.

b) *Acute leukemias*. To create a genetic model of AML, the founder mice (F1B) described above will be mated with mice expressing Cre in the myeloid lineage, recombination being under the control of the cathepsin G (CG) gene promoter. hCG/Cre mice will be produced by microinjection of a vector containing the hCG promoter and the coding sequence of Cre. In the next generation of mice, hERG1 should be expressed only in myeloid cells, especially promyelocytes. This possibility will be tested performing an IHC on the BM, using the anti-6xHis Ab (see above) in conjunction with myeloid-specific markers (CD15, CD45). The possibility that such mice spontaneously develop a leukemic or pre-leukemic disease will be tested: the standard hematological parameters will be monitored and the cellular morphology will be analyzed in BM and PB smears. Splenic, hepatic morphology and BM architecture will be evaluated. Any alteration in myelopoiesis will be studied by FC using standard myelocyte lineage differentiation markers (c-kit, Mac-1, Gr-1 as in 26), and clonogenic assay. Many transgenic models of leukemia often develop disease only after a very long time. Therefore, the possibility of inducing leukemia by subjecting the mice to sublethal irradiation will also be tested. To produce a genetic model of B-ALL, the production of mice overexpressing hERG1b, as well as hERG1b KO mice will be necessary. We have obtained hERG1b KO mice from Dr. H. Duff (University of Calgary, Canada); we have mated heterozygous mice and screened for homozygous mice, by PCR on tail DNA. We now have homozygous mice of different ages in which any alterations in the myeloid and lymphoid differentiation pattern will be evaluated by histological examination (as above) and FC. Myeloid differentiation will be analyzed as above, while the pattern of differentiation in the B lymphocyte lineage will be determined though the following markers: CD45R, 19, 24, AA4.1. To develop mice overexpressing hERG1b, we will make use of the same RAGE strategy used so far and explained above, after substituting the hERG1b cDNA, in place of the hERG1 cDNA, in the targeting vector. In this case, founder mice will be mated with mice expressing Cre in the B lineage cells, the recombination being under the control of the Cd19 gene promoter (C.129P2-Cd19$^{tm1(cre)Cgn}$/; Jackson Lab.). The analysis of double transgenic mice will be performed as explained above.

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Research Grants

Year	Funding Agency	Amount
2009-2011	Istituto Toscano Tumori	€ 193,000
2007-2010	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 160,000
2007-2009	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR)	€ 54,714
2006-2009	Association for International Cancer Research	€ 182,000
2006-2009	Associazione "Noi per Voi" Onlus	€ 75,000
2004-2006	AIRC	€ 120,000

Main Collaborations

With Units within ITT

» General Surgery and Surgical Oncology Units, AOU Careggi, Firenze
» Medical Oncology Unit, AOU Careggi, Firenze
» Surgical Pathology Unit, AOU Careggi, Firenze
» Pediatric Hematology-Oncology and Hemotransplantation Units, Azienda Ospedaliero Universitaria Meyer, Firenze

With other Italian and Foreign Institutions/Organizations

» Division of Surgery, University of Verona
» Department of Biotechnology and Biosciences, "Bicocca" University of Milan
» Department of Genetics, Biology and Biochemistry, University of Turin
» Department of Internal Medicine, University of Turin
» Laboratory of Pediatric Oncohematology, University of Padua
» Medical Research Council Centre (MRC), Cambridge (UK)
» Department of Cell Physiology and Pharmacology, University of Leicester (UK)
» Center of Health Sciences and College of Osteopathic Medicine, Oklahoma State University, Tulsa (USA)
» Forsyth Center for Regenerative and Developmental Biology, Boston (USA)
» Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver (Canada)
» University of Calgary (Canada)

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TUMOR BIOLOGY AND CANCER STEM CELLS

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Introduction

The interests of the Unit are focused on cancer research and, in particular, on the mechanisms controlling cell growth and differentiation and their alterations leading to neoplastic transformation. Over the last decade, the attention has been focused on the role of electric fields and ion channels in cell signaling at the plasma membrane level, also extending the study to the Neuroblastoma model.

More recently, the interest of the Unit has been directed towards the identification and characterization of the stem cell compartment responsible for the renewal and diffusion of cancer, with particular

attention focused on the neuroblastoma. A new approach to the characterization of cancer clones at various stages of the neoplastic progression was first introduced in these studies, based on the precise quantitative measurements of the ion currents expressed simultaneously on single cells. More recently, studies are being carried out on the final strategy of cancer stem cells necessary for growth and survival in restricted environments. These studies are identifying the very essential metabolic pathways of highly anaplastic tumors.

Main Research Themes

1. Cancer stem cells

- Identification and biophysical characterization.
  Main achievements: We recently described the cell renewal of neuroblastomas, identifying the stem cell compartments driving the spontaneous or growth factor-induced development. Stem cell characterization was obtained in these studies by integrating immunochemical procedures with electrophysiological measurements of ion currents mediated by channels simultaneously expressed on single cell surfaces (Figure 1). This approach led to the introduction of the new parameter, *Electrophysiological Cluster of Differentiation* (ECD), which proved crucial for protocols aimed at purging the stem cell compartment by exposing tumor complex populations to hypoxia and/or antiblastic treatments.
  Current work: We are pursuing the identification and characterization of cancer stem cells in various neoplasias, including colon carcinomas and leukemia.
  Future work: Isolation of the highest stem cell compartment in tumors featuring experimental protocols capable of eradicating this population which is responsible for minimal residual disease.

2. Biophysical features of cancer cells

- The biological meaning of the cancer cell membrane depolarization.
- Ion channels and electrophysiological profile of cancer cells.
  This research has been directed towards the integration of electrical and biochemical signals in tumor cell commitment to differentiation, providing new a biological bases for differentiation cancer therapy, and unraveling the action mechanism of widely used inducers of tumor cell differentiation (the so-

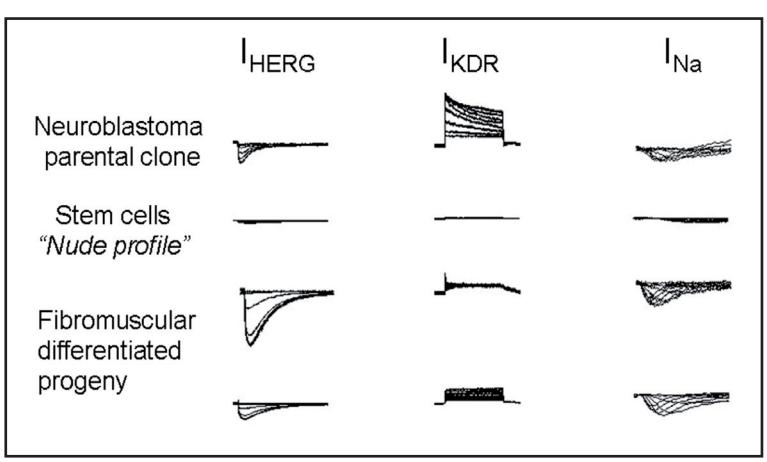

Figure 1 - Electrophysiological Cluster of Differentiation

called “hybrid polar compounds”, the prototypes of which are dimethyl-sulfoxide and hexamethylene-bisacetamide). The role of the cell membrane electrostatic profile in the regulation of cancer cell growth and differentiation has been explored by studying the role of potassium channels in governing the resting potential (Vrest) and related cellular ionic homeostasis, showing how in tumors of different histogenesis, this potential is controlled by hERG channels, the biophysical properties of which appear suitable for conferring selective advantages to tumor cells in a hypoxic environment.

3. Cancer cells metabolism

- The anaerobic metabolic asset and its role in dormant cancer.
- Hypoxia and drug resistance of cancer cells and its role in the genomic instability of the tumor. The main results obtained in this field consist in the identification of the role of glycolysis and mitochondrial respiration in the regulation of a metabolic check-point controlling cancer stem cell recruitment into growth and their survival in the dormant state.

Research Grants

Year	Funding Agency	Amount
2008-2010	Cassa di Risparmio di Firenze	€ 30,000 /year
2004-2007	Cassa di Risparmio di Firenze (Promelab)	€ 100,000 /year
2004-2006	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 50,000 /year
2004	Ministero dell’Istruzione, dell’Università e della Ricerca (MIUR)	€ 30,000
2002-2004	University of Florence	€ 10,000
2001-2003	AIRC	€ 50,000 /year

Main Collaborations

With Units within ITT

» Department of Experimental Pathology and Oncology, University of Florence
» Department of Internal Medicine, University of Florence

With other Italian and Foreign Institutions/Organizations

» University of Zurich (Switzerland)

Publications

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BASIC MECHANISMS OF ANGIOGENESIS AND ANTI-ANGIOGENESIS

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Introduction

This Unit moved towards the molecular basis of protease-dependent cancer cell invasion in 1985 describing, in parallel with two other groups, the receptor for urokinase Plasminogen Activator (uPAR) and its expression in malignant cells. The study of uPAR in cancer, endothelial and inflammatory cells, focused on its role in invasion and chemotaxis, and led to a patent on uPAR antisense to inhibit uPAR-dependent invasion. The fibrinolytic system was then studied in Systemic Sclerosis (SSc), a fibrotic and anti-angiogenetic

disease, in order to identify anti-angiogenic mechanisms to be corrected in SSc and to be utilized to control cancer angiogenesis. TGFbeta, the main cause for SSc-fibrosis, stimulates angiogenesis in a uPAR-dependent fashion, which is inhibited by anti-TGFbeta peptides. Since tumors undergo a "TGFbeta-switch" characterized by epithelial-mesenchymal transition and an increase in vascularization, we are studying the possibility of using anti-TGFbeta peptides to control these critical phases in tumor progression.

Main Research Themes

1. Role of uPAR in cancer spreading and cancer angiogenesis

Main achievements: The existence and differential expression of uPAR in cancer cells was first described by Prof. Del Rosso's group in 1985 (a). The importance of the receptor as an invasion factor became immediately evident, given its location on the invading pole of cancer cells, where its ligand (uPA) initiates a proteolytic cascade with the eventual activation of metalloprotease zymogens. Del Rosso's group provided the first evidence of a proteolytic-independent activity of uPA in stimulating chemotaxis of uPAR-expressing cells (b). Along with many other research teams, the group in Florence identified other proteolysis-independent roles of uPAR, such as adhesion to vitronectin, induction of uPA autocriny, interaction with integrins. All these characteristics made uPAR a suitable molecule to be targeted in anti-cancer therapy. The group patented a uPAR antisense oligonucleotide, which resulted efficient in preventing cancer cell spreading and metastasis *in vitro* and *in vivo* (c).

Current work: Control of uPAR in cancer cell spreading and cancer angiogenesis is currently being investigated by inducing an external uPAR truncation by proteolytic enzymes (such as MMP12) able to cleave the first uPAR domain, thereby preventing both initiation of the proteolytic cascade and uPAR interaction with adhesion molecules (d).

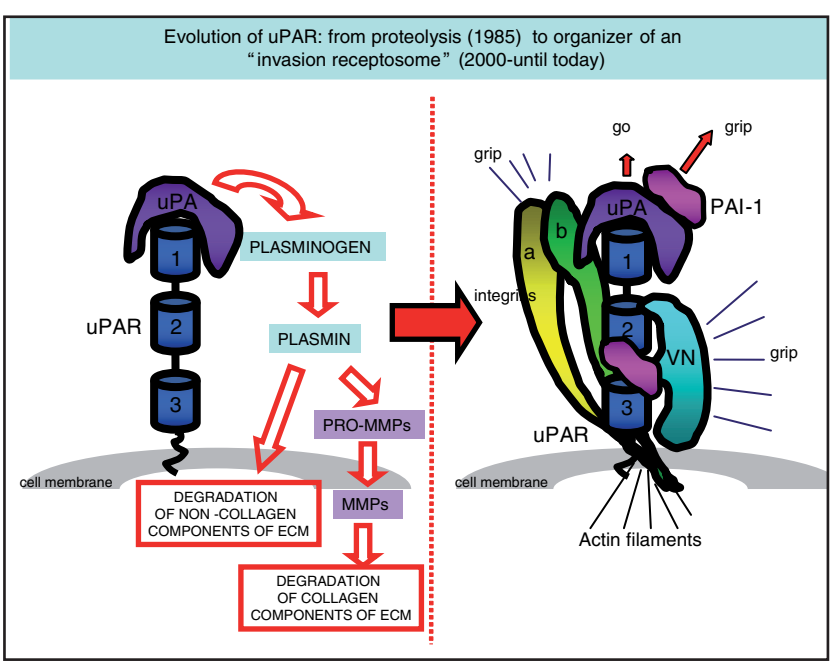

Figure 1 - The left side of the figure shows the first identified role of uPAR, based on the initial description of its functions: activation of the proteolytic cascade leading to ECM degradation performed by plasmin and/or by plasmin-activated pro-MMPs. The right side shows the modern view of uPAR as an organizer of the "grip-and-go" cycles of cell migration: by interaction with integrins and vitronectin uPAR provides the "grip" required for cell adhesion and the "go" provided by uPAR-driven proteolysis. These features identify a uPAR-receptosome that is going to become increasingly complex, along with the discovery of new uPAR properties

Future work: Previous studies on SSc led the group to consider TGFb and its role in angiogenesis and in inducing overexpression of uPAR. Therefore, we will develop tumor cell lines able to conditionally express an active form of TGFb, under the control of a tetracycline-dependent promoter (tet-off). These cell lines will be xenografted into nude mice in order to simulate the so-called "TGFb-switch" occurring during carcinogenesis. The transfected cell lines will be employed to assess the effects of TGFb on many parameters of carcinogenesis, such as angiogenesis (performed by both resident endothelial cells and by endothelial cell precursors), cell motility/plasticity and cell invasion. The efficacy of peptide TGFb inhibitors will be assessed both *in vitro* and *in vivo*.

2. Mechanisms of anti-angiogenesis in SSc

Main achievements: Vascular alterations, eventuating into vessel atrophy, precede the accumulation of fibrotic tissue in the skin and in internal organs. The ensuing tissue hypoxia is not followed by suitable angiogenesis, thus allowing to identify SSc as a natural model system for the study of anti-angiogenesis. We have studied microvascular endothelial cells isolated from the skin of normal and SSc subjects (N-MVEC and SSc-MVEC), identifying over production of MMP12 in SSc-MVEC, as well as other alterations able to account for insufficient angiogenesis (7, 9, 10, 13, 22).

Current work: We are currently using gain/loss of function approaches (transfection of anti-angiogenic MMP12 and pentraxin-3 in N-MVEC and silencing of the same molecules in SSc-MVEC), in order to inhibit angiogenic responses in normal endothelial cells and to promote angiogenesis in SSc endothelial cells. Such approaches restore the angiogenic properties of SSc-MVEC even *in vivo* (e).

Future work: We are aiming to construct a plasmid bearing siRNA against anti-angiogenic MMP12 and PTX3, to be inserted within lentiviral vectors. Such a construct will be delivered to tight-skin mice, which exhibit a SSc-like accumulation of fibrotic tissue, as well as vascular defects, in order to induce an increase in defective vascularization of the heart.

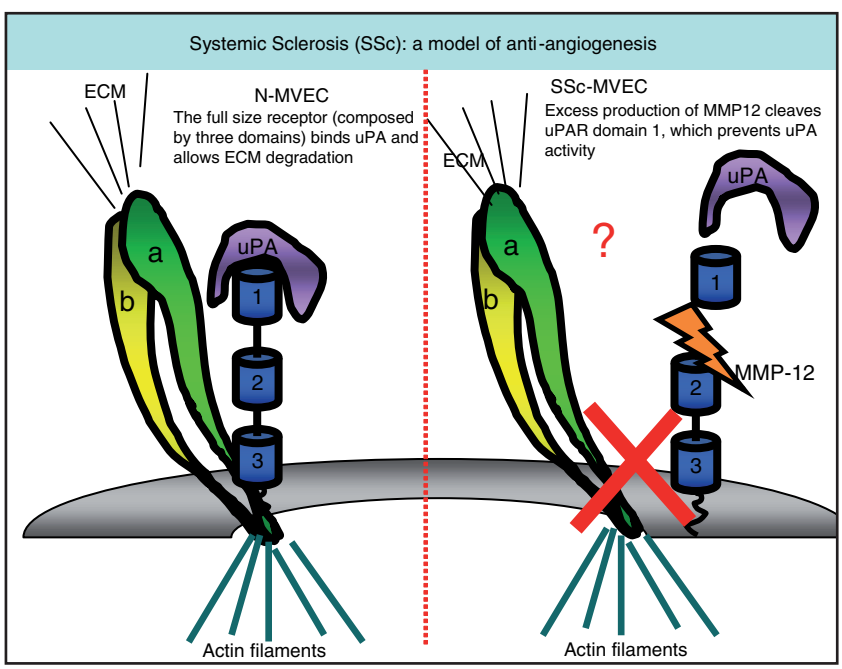

Figure 2 - The study of SSc endothelial cells led to identification of several anti-angiogenic mechanisms. One of the most important is based on SS-MVEC hyper-production of MMP12, a matrix-metalloprotease which cleaves uPAR, thereby inhibiting its "grip ang go" properties. Additionally, MMP-12-mediated uPAR cleavage also impairs uPAR-dependent interactions with integrins and the subsequent integrin-dependent actin stress fibers assembly

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Research Grants

Year	Funding Agency	Amount
2009-2010	Toscana Life Science	€ 80,000
2009	DIGNA (Spain)	€ 55,000
2007-2009	Ente Cassa di Risparmio di Firenze	€144,000
2007	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) – PRIN	€ 50,000
2006-2008	TRB (Switzerland)	€ 45,000
2005	MIUR – PRIN	€ 40,000
2003-2009	Ateneo (ex-60%)	€ 30,000
2003	MIUR-PRIN	€ 35,000

Main Collaborations

With Units within ITT

» Cell Adhesion and Motility in the Regulation of Metastatic Activity, University of Florence

With other Italian and Foreign Institutions/Organizations

» Regional Referral Center for Thrombosis, Azienda Ospadaliero Universitaria Careggi, Firenze
» Istituto Multimedica Castellanza, Varese
» University of Toledo, Ohio (USA)
» New York University, New York (USA)

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L.I.Ge.M.A.

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Introduction

L.I.Ge.M.A. is a brand new section located inside the Animal Facility of the University of Florence (Ce.S.A.L.) and it is fully equipped for the manipulation of TG mice. The section is composed of four rooms: a microinjection room, a sterile room fully equipped for the culture and transfection of ES cells, a

molecular biology laboratory and a room devoted to the housing of the Transgenic (TG) mice. L.I.Ge.M.A. is sponsored by the University of Florence, the Istituto Toscano Tumori and the Azienda Ospedaliero Universitaria Careggi. The facility offers several different services for the production and analysis of TG mice (production of vectors, microinjection into oocytes and blastocysts, culture and transfection of ES cells, production and screening of chimeras, etc.). L.I.Ge.M.A. also offers services for experiments to be performed on nude and NOD-SCID mice. These services, in addition to the maintenance of the mice in germ-free conditions, also include: injection (both subcutaneous, s.c. and intravenous, i.v.) of tumor cells into nu/nu or NOD-SCID mice; treatment with anticancer drugs; morphological analysis of tumor masses; morphological and cytofluorimetric analysis of leukemias. More recently, L.I.Ge.M.A. initiated a service for the production of polyclonal and monoclonal antibodies, as well as for their genetic manipulation, also ensuing production of small chain variable Fragments (scFv) and diabodies. Ongoing projects will also provide humanization of well established mouse monoclonal antibodies.

Main Research Themes

1. Fully accomplished projects

a) *Production of TG mice*:

i) *Production of TG mice overexpressing the herg1 gene in the intestinal mucosa*. The RAGE strategy was used to develop a TG model for the selective overexpression of herg1. This strategy has been effectively used in the study and in the production of animal models of human cancer based on the Cre/LoxP system. In the RAGE strategy, a DNA sequence floxed by two LoxPs in the same orientation is inserted in the transgene between the promoter and the gene to be activated. The inserted sequence acts as a transcriptional stop, preventing the transcription of the gene of interest until, by means of Cre-mediated recombination, through mating TG mice with Cre expressing mice, this sequence is eliminated. We produced a vector for the overexpression of the herg1 gene. The vector was microinjected into FVB oocytes using standard procedures. Among the numerous pups obtained, two (F1A and F1B) resulted to be TG. Both the putative founders showed germline transmission of the transgene. After establishing the two founder lines the number of transgene copies integrated and the level of expression of the reporter gene and of herg1 were characterized. Southern experiments have shown that F1A had only one copy of the vector while F1B had four copies. In addition, both lines did not express herg1 at all, and F1B expressed the reported gene at a high level. We therefore decided to cross such founder mice with mice expressing Cre in the intestinal mucosa, the recombination being under the control of the FABP gene promoter. As of now, we have identified two double TG mice from the resulting pups. These mice were sacrificed at three months of age, and the effective recombination *in vivo* was verified. An IHC analysis was performed on tissues expressing (small intestine, cecum, colon and bladder) and not expressing (lung) Cre, using anti-6xHis Ab; tissues obtained from a herg1 TG mouse (not activated by Cre) of the same age were used as controls. Such experiments showed that hERG1 is indeed expressed in the same tissues where Cre is expressed, with almost no background in other tissues. Moreover, both double TG mice showed polyps at the level of the cecum.

ii) *Production of Tg mice overexpressing the Arp gene* as a model of hepatic fibrosis, as an experimental model for studying the role of the nuclear receptor ARP in hepatic fibrosis. Two founders for the construct of interest were produced after microinjection of DNA in FVB oocytes, using standard procedures.

iii) *Production of Tg mice overexpressing the ET4 gene* under the control of the rat prostate-specific probasin promoter, as an experimental model of prostate cancer. Three founders for the

construct of interest were produced after microinjection of DNA in FVB oocytes, using standard procedures.

iv) *Production of Tg mice overexpressing the MET gene under the control of its own promoter*, as an experimental model to assess whether overexpression of MET alone in epithelium induces overexpression of HGF/SF in stroma and other tissues, and whether overexpression of MET in dominant tissues causes tumorigenesis. These mice could represent a model for testing new therapies against MET. The construct of interest was produced by the researcher, and microinjection in FVB oocytes, using standard procedures, led to the production of four different founder mice.

v) *Production of Tg mice overexpressing the HGF/SF gene under the control of its own promoter*, as an experimental model to assess whether overexpression of HGF/SF alone in the stroma induces overexpression of MET in epithelia and other tissues, and whether overexpression of HGF/SF in dominant tissues causes tumorigenesis. These mice could represent a model for testing new therapies against MET. The construct of interest was produced by the researcher, and microinjection in FVB oocytes, using standard procedures, led to the production of two different founder mice.

vi) *Production of Tg mice overexpressing the IL17A gene under the control of the CMV promoter*. The injection of this construct did not lead to the production of any transgenic mouse, even after many microinjections, probably due to the embryonic lethality of high ubiquitous IL17A expression.

vii) *Production of Knock-Out (KO) mice for the VEGFR3 gene*, as an experimental model of several human cancers. A clone of ES cells was injected in FVB blastocysts and five chimeras were obtained.

b) *Isolation and culture of ES cells* isolated from blastocyst of myc+/- e myc -/- mice as an experimental model of different human tumors.

c) *Injection of human Acute Myeloid Leukemia (AML) cells into NOD-SCID immunodeficient mice*. *In vivo* studies on AML. During the last year we performed an *in vivo* study to test whether the FLT-1/hERG1/b_1 complex regulates leukemia cell malignancy. We used a repopulation assay in NOD-SCID mice, applying two different approaches: *i*) inoculation of herg1$^-$ and herg1$^+$ leukemia blasts from primary AML; *ii*) inoculation of HL60 cells and of HL60 overexpressing herg1 (hERG1-HL60). It emerged that: *i*) the BM engraftment (measured determining the amount of human (h) CD45+ cells by flow cytometry) was roughly the same irrespective of herg1 expression; *ii*) the efficiency of the bloodstream invasion (measured determining the amount of hCD45+ cells) was significantly higher in herg1$^+$ compared to herg1$^-$ blasts, as well as in hERG1-HL60 compared to HL60 cells; *iii*) BM angiogenesis (determined by E&E staining, as well as through IHC with anti-mCD34) was significantly higher in mice inoculated with either herg1$^+$ blasts or hERG1-HL60 cells. IHC with anti-hMHCI antibodies showed an increased density of undifferentiated leukemic cells and a concomitant decrease in endogenous hemopoiesis in the BM of mice injected with either herg1$^+$ blasts or hERG1-HL60 cells; *iv*) only mice injected with herg1$^+$ blasts and hERG1-HL60 cells displayed a substantial hepatic and splenic invasion (determined by E&E staining, and IHC with anti-hMHCI antibody). On the whole, these data strongly indicated that herg1 overexpression confers a higher malignancy to AML blasts, since it contributes to the exit of leukemia blasts into the bloodstream with subsequent invasion of extramedullary sites.

d) *Injection of human AML HL60 cells overexpressing the ICRE gene into NOD-SCID immunodeficient mice*. The inducible cyclic AMP (cAMP) early repressor (ICER) and cAMP Response Element-Binding protein (CREB) are transcriptional regulators of the cAMP-mediated signaling pathway. CREB has been demonstrated to be upregulated in the majority of childhood leukemias, contributing to disease progression, whereas ICER, its endogenous repressor, was found to be downregulated. Our research focus has been the function of restored ICER expression. NOD-SCID mice were used in all the experiments. All mice were kept in microinsulator cages supplied with sterile food

and water. The mice were inoculated with 100 µl of cells suspended in culture medium. HL60 cells and ICER-HL60 were injected at a concentration of 5 × 106 cells/mouse. Mice were sacrificed three weeks after inoculation. To determine BM angiogenesis, BM sections were stained with anti-CD34 antibodies. In addition to endothelial cells, scattered myeloid blasts were also strongly positive for CD34. These cells were easily recognized by their morphology and served as internal controls to verify the adequacy of staining. For each countable microvessel the outline was identified and traced and different morphometric parameters were estimated. It emerged that ICER decreases the ability of HL60 to invade the extramedullary sites and to promote bone marrow angiogenesis in non-obese diabetic/severe combined immunodeficient mice, demonstrating its potential effects on tumor progression.

e) *Subcutaneous (s.c.) injection of Endometrial Adenocarcinoma (EC) cells overexpressing the LH receptor into nu/nu mice*. The aim of this project was production of preclinical mouse models in which to test the efficacy of treatment with Gn-RH analogs. In particular, we will use: *i*) nu/nu immunodeficient mice s.c. injected with Hec1A cells transfected with and, hence, overexpressing LH/hCG-R; *ii*) TG mice which over express LH/hCG-R in the endometrium in an age-dependent manner.

We performed *in vivo* experiments using athymic nu/nu mice. These mice show severe deficiencies in reproductive function: In particular, we studied the correlation between LH/hCG-R expression and cell growth *in vivo* by injecting EC cell lines overexpressing LH/hCG-R s.c. into nude mice and treating the mice with hCG. The tumor masses were measured every three days with a caliber, and the volumes recorded.

f) *Production and use of an anti-hERG1 Monoclonal Antibody (MoAb)*. We developed a MoAb directed against an extracellular loop of the hERG1 protein. We showed that this MoAb can be used in flow cytometry in living, unpermeabilized, cells. This MoAb has recently been patented by the University of Florence

2. Ongoing projects

a) *Production of Knock-in mice expressing the A90V mutation in the perforin protein as a model of "hemophagocytic lymphohistiocytosis".*

b) *Production of Knock-in mice, expressing a mutated form of the Nav1.1 α subunit.*

c) *Studies of the anticancer effect of drugs and molecular tools which inhibit hERG1 expression/activity in preclinical models*.

d) *In vivo experiments on nu/nu mice*. Male, 4-6 weeks aged, nu/nu mice (from Harlan) were injected with human epithelial cell lines that express the herg1 gene at different levels: HEK 293 mock-transfected (HEK-mock), HEK 293 transfected with the herg1 gene (HEK-hERG1), the colorectal cancer cell lines H630, HCT8 and HCT116. Mice were sacrificed three (for colorectal cancer cell lines) or four (for HEK cells) weeks after injection. It emerged that: a) cells that express a higher amount of hERG1 give rise to greater subcutaneous masses (HEK-mock = 0.006 mm^3; HEK-hERG1 = 0.041 mm^3, $p < 0.0001$); these masses also show a higher intratumoral angiogenesis (evidenced by H&E staining and by IHC with anti-VEGF and anti-mCD34 antibodies); b) cells that express a higher amount of hERG1 give rise to lung micrometastases, evidenced by IHC with anti-hMHC antibody.

e) *Establishment of a novel orthotopic implant in the colon*. Animals were anesthetized with Avertin and inoculated with tumor cells (10^6 cells in 100ml) using a catheter. Through the same catheter, the colonic mucosa had been previously washed and treated with trypsin, to allow for better implant of the tumor cells. After four weeks, a neoplastic mass developed at the level of sigma; the mass was explanted and its neoplastic histology was determined.

f) *In vivo use of hERG1 blockers: Way*. The *in vivo* toxicity of Way was determined on CD1 mice. Way was injected i.v. at the final concentrations of 60-600 mM (the control group received sterile Ringer-

solution). Toxicity was evaluated: no significant variation in any of the parameters examined was observed at any of the doses used.

g) *Injection of human Acute Lymphoid Leukemia (ALL) cell lines into NOD-SCID immunodeficient mice for evaluation of drug effects which inhibit hERG1*.

h) *Lentiviruses*.

i) *Subcutaneous and orthotopic injection of EC cells overexpressing the LH receptor into nu/nu mice, and treatment with hrLH*. The aim of this project is to study the role of LH/hCG-R in tumor invasion and metastasis *in vivo* by orthotopical implanting.

3. Permanent projects

L.I.Ge.M.A. provides service during the entire time that the animals stay at Ce.S.A.L. and L.I.Ge.M.A. has worked in such a way for two different customers: mice genotyping.

Research Grant

Year	Funding Agency	Amount
2005	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 5,000

Publications

See Ion Channels and Cancer Unit page 387.

CANCER SUSCEPTIBILITY

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Introduction

The Unit has been in practice since 2001. The Unit's interests are clinical and research activities as well as Medical Genetics teaching at the Faculty of Medicine of the University of Florence. The Training Program in Medical Genetics of the University of Florence is carried out by the Unit's staff. The clinical activities concern counseling for prenatal diagnosis, infertility and hereditary tumors, with mutation screening for the main genes involved in familial colorectal cancer, breast cancer and cutaneous

melanoma. The topic of the Unit's research activity is focused on the role of genes predisposing to these hereditary tumors. The medical genetics laboratory is equipped with facilities and instruments necessary to carry out clinical work and research in this field.

Main Research Themes

1. Unclassified sequence variants (VUS) in genes predisposing to colorectal cancer: assessment of pathogenicity by a multifactorial approach

Variant of Unknown Significance (VUS) in cancer-predisposing genes represents a major challenge for genetic counseling. This is a growing issue since the number of VUS identified in the clinical setting is steadily increasing with the widespread availability of genetic testing. A number of different clinical and biological parameters can be assessed for the purpose of classifying mutations. However, no single factor is usually sufficient to obtain a definite classification. Accurate analysis of VUS is important both for detailed understanding of the biochemical mechanisms of gene/protein function and to improve the clinical utility of cancer genetic testing (a,b).

Main achievements: Patients with VUS in (Mismatch Repair (MMR) genes and *MUTYH* have been selected. So far, 42 VUS in MMR genes have been identified: 16 *MLH1* (3 of which are present in > 2 independent families), 12 *MSH2* (2 detected in 2 families), 5 *MSH6* and 9 *PMS2* (3 in > 2 families). Based on the localization within the gene sequence and/or the predicted effect on transcription/translation, the above VUS can be classified in the following way: *a*) 12 intronic; *b*) 21 missense substitutions; *c*) 3 in-frame deletions of single amino acids; *d*) 6 exonic silent nucleotide substitutions. Biological samples from normal tissue (blood leukocytes and/or intestinal mucosa) for DNA/RNA investigation have already been obtained, for a total of 18 RNA samples and 46 DNA samples (probands + relatives). Moreover, 46 tumor samples (paraffin blocks) for investigation of molecular markers are also available. For *MUTYH*, 1 intronic and 6 exonic VUS have been detected: 5 are present as monoallelic changes in 7 unrelated patients/families, while 2 are concurrent with a clearly deleterious variant in 2 unrelated patients/families. Ten blood leukocyte and 5 paraffin-embedded tumor samples from the probands and relatives have already been collected for DNA/RNA investigations.

Current work:

a) Determination of novel *MUTYH* mutation mechanisms. Screening of the UTR regions has already been performed on 16 patients who were found to be simple heterozygotes for *MUTYH* VUS or for the common Caucasian mutations p.Tyr179Cys and p.Gly396Asp.
b) Analysis of co-segregation of VUS within families and the concurrence of *bona fide* deleterious mutations.
c) Investigation of molecular and histochemical markers of MMR and *MUTYH* deficiencies in tumor samples.
d) *In silico* analysis. At present, we are preparing sequence alignments for *MUTYH* and MMR genes. Preliminary analyses have been conducted on the set of identified *MUTYH* VUS.
e) Transcript analysis. Analysis of abnormal splicing patterns on cDNA from peripheral leukocytes is in progress for VUS with a high *in silico* score predicting alteration of the splicing regulatory mechanisms.

Future work:

Evaluate the pathogenetic role of gene VUS in the MMR and *MUTYH* genes involved in susceptibility to colorectal cancer, by pursuing the following specific aims:

a) estimate of the odds of pathogenicity by means of a co-segregation analysis of the variant with the phenotype and evaluation of the concurrent presence of *bona fide* deleterious mutations in families where VUS has been ascertained;
b) estimate of pathogenicity based on evaluation of molecular and histochemical markers of MMR and base excision repair deficiency in tumor samples;
c) *in silico* prediction of the potential effects of VUS on RNA processing and on the function of the encoded proteins;
d) estimate of pathogenicity based on *in vivo* and *ex vivo* transcript analyses for the detection of alterations in RNA splicing and/or expression;
e) estimate of odds of pathogenicity based on the integrated Bayesian Analysis of all parameters considered in the above aims.

2. Assessment of the genetic origin of the common Caucasian *MUTYH* mutations

MUTYH-associated polyposis (MAP) is an autosomal, recessive, inherited disorder characterized by a high risk of polyposis and colorectal carcinoma. Although MAP-causing mutations are distributed across the *MUTYH* locus, a few population-specific mutations have been reported. The majority of changes found in the *MUTYH* gene are missense variants; among these, p.Y179C and p.G396D represent approximately 75% of *MUTYH* mutations found in Caucasians (c,d). The high frequency of these two mutations in this area has been undertaken to determine whether common haplotypes can be recognized. Caucasian populations could result from either recurrent *de novo* mutational events or from founder effects.

Main achievement: A preliminary study to assess whether the high frequency (~75%) of the two common Caucasian mutations is due to a founder effect has been carried out. To this aim, so far 22 families with these two mutations at the homozygous and heterozygous state have been collected and analysis of linked microsatellites and single nucleotide polymorphisms (SNPs) in the *MUTYH* gene have been performed.

Current work: This study is ongoing on 26 individuals belonging to 15 families segregating the p.Tyr179Cys (n = 9) and the p.Gly 396Asp (n = 6) mutations. Five microsatellites and 4 intragenic SNPs have already been tested. The microsatellites, derived from Marshfield and/or Généthon sex-average genetics map (http://www.ncbi.nlm.nih.gov/mapview/), are located upstream and downstream of the *MUTYH* gene on the 1p34.3. The 4 SNPs have been chosen using dbSNP (http://www.ncbi.nlm.nih.gov/sites/entrez) and are located in intron 1, near the 5'-UTR and in exons 2 and 12, respectively. 22 Caucasian nuclear, cancer-free families were tested to estimate control allele and haplotype frequencies.

Future work:

a) Collect and extend the analysis to more *MUTYH* families with p.Y179C and p.G396D mutations.
b) Manually construct haplotypes to minimize the number of recombinations.
c) Compare the distributions of allelic frequencies in normal and mutated chromosomes by χ^2 and two-tailed Fisher's exact tests.
d) Estimate the age of the two mutations.

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Research Grants

Year	Funding Agency	Amount
2008-2009	Istituto Toscano Tumori	€ 70,000
2007-2008	Lega Italiana per la Lotta contro i Tumori (LILT)	€ 9,000
2005-2006	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 100,000
2003-2004	Ministero dell'Istruzione, dell'Università e della Ricerca	€ 110,000

Main Collaborations

With other Italian and Foreign Institutions/Organizations
» Centro di Riferimento Oncologico (CRO), Aviano (Pordenone)
» "Cattolica" University, Roma
» Ospedale "Casa Sollievo della Sofferenza", San Giovanni Rotondo (Foggia)
» University of Modena and Reggio Emilia
» International Agency for Research on Cancer (IARC), Lyon (France)

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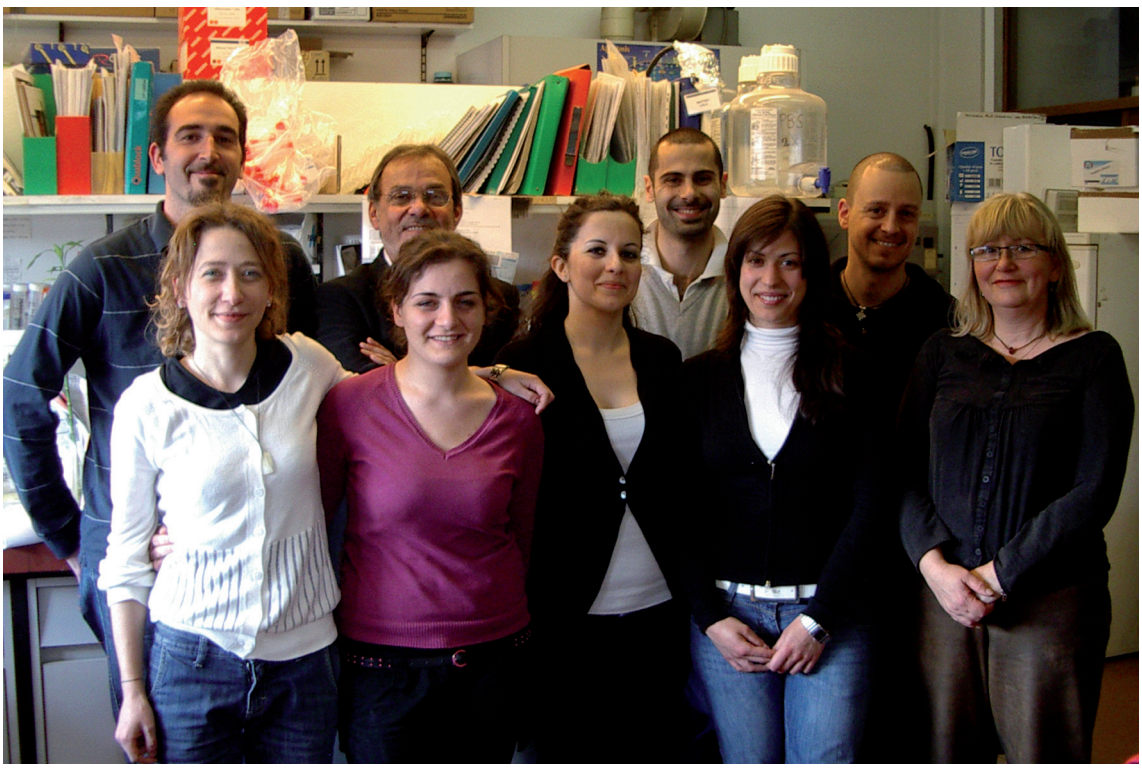
APOPTOSIS DEREGULATION IN CANCER



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Introduction

Sergio Capaccioli started his cancer research focusing on alterations in tRNAs and aminoacyl-tRNA synthetases in cancer and successively on the impact of oxidative metabolism in cancer cell growth. In 1991, he founded a Research Unit aimed to study alterations in gene expression in cancer by means of antisense strategies. Following his research on apoptosis at the Imperial Cancer Research Fund of London in 1994, his Unit started studying mechanisms underlying apoptosis deregulation in cancer and degenerative diseases, focusing on post-transcriptional control. Among some of the more remarkable achievements are the demonstration that *Bcl-2* expression is controlled at a post-transcriptional level by complex RNA/protein interactions, which are disrupted in cancer, and the evidence of CoQ10 anti-apoptotic activity. The interest aroused by these topics led to two international meetings, six patents and a novel therapeutic drug. Recently, the Unit founded the Phoenix/ONLUS Stem Cell Foundation (www.stemphoenix.org).

Main Research Themes

1. Post-transcriptional control of gene expression, its alteration in human diseases and their possible therapeutic targeting

Main achievements: For almost two decades, our research has focused on post-transcriptional modulation of gene expression by antisense strategies both to assess their phenotypic effects and to find innovative gene therapeutics (10). Using antisense tools, we assessed the neurological effects of blocking the Shaker-like Kv1.1 potassium channel, and reverted the neoplastic phenotype by targeting *P170*, uPAR and *Bcl-2* mRNAs. We also demonstrated that cellular transfection with fragmented DNA or with synthetic DNA segments was able to commit cells to apoptosis via activation of p53 by mimicking the effects of genotoxic agents. By modulating *c-Myc* and *Bcl-2,* we demonstrated their opposite effects in, respectively, lowering and enhancing the apoptosis threshold of cancer cells in response to genotoxic agents and in shifting apoptosis to necrosis via a new syncretic process of cell death we named “aponecrosis”.

Current work: Focusing on post-transcriptional control of cancer gene expression, based on interactions among mRNA AU-Rich Elements (AREs) and relevant ARE-binding Proteins (AUBPs). Our main current task is identification and characterization of possible disruption of this mechanism in cancer as a consequence of competitions among deregulated AUBPs. We are especially focusing on *HIF-1*α, *Bcl-2*, uPAR and *VEGF,* which share highly homolog AREs and are therefore the main candidates for a possible co-deregulation.

Future work: Our main future work is exogenous modulation of the above genes by very peculiar ARE targeting tools endowed with a potential post-transcriptional gene therapeutic property.

2. The post-transcriptional operon of *Bcl-2* and its alteration in cancer

Main achievements: In the attempt to unravel the mechanisms of *Bcl-2* overexpression in t(14;18) follicular lymphomas/leukemias by specifically targeting their hybrid *Bcl-2*/*IgH* mRNA with antisense oligonucleotides or ribozymes – used both as a molecular research tool and as a possible source of innovative cancer therapeutics – we identified a non-coding RNA that causes overexpression of *Bcl-2* by overlapping a negative regulative element harbored in its mRNA (a). This leaded us to first discover that *Bcl-2* is controlled at a post-transcriptional level by complex interactions among an ARE located in its mRNA and multiple AUBPs. Among these, we have discovered AUF1, Tino, *Bcl-2* itself, HuR and ζ-Crystallin (b), and demonstrated that their deregulation results in *Bcl-2* overexpression in cancer (Figure 1).

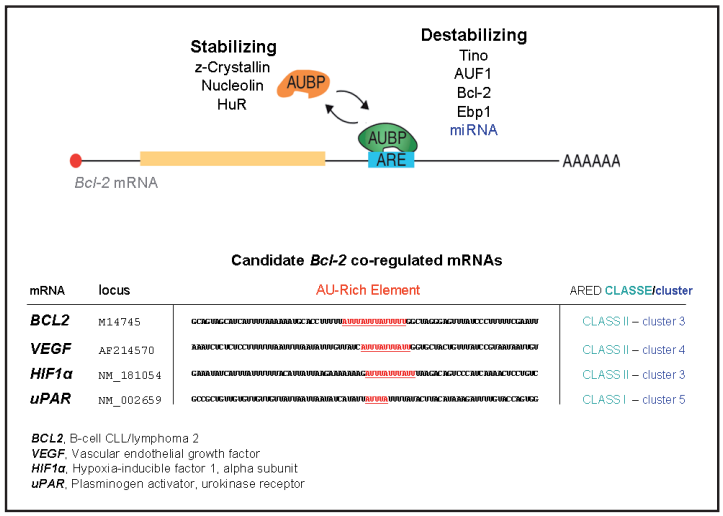

Figure 1 - Bcl-2 mRNA post-transcriptional regulators

Current work: Characterizing the functional activity of *Bcl-2* AUBP Tino, firstly discovered in our lab, whose expression – based on our experimental results and sequence orthology data – is blocked by the translational inhibitor quaking. Analysis of Tino target mRNAs, using *in vivo* and *in vitro* complementary microarray-based strategies followed by computational analyses, has revealed that Tino belongs to a circuitry of mRNA trans-acting factors, playing a key role in the post-transcriptional control of gene expression during cell fate determination and differentiation and possibly in oncogenesis.

Future work: As the best experimental model to assess the role of the *Tino* gene, we are preparing conditional *Tino* knockout mice, which will be generated at the Institut Clinique de la Souris, France, in collaboration with Prof. Marc Billaud (Université de Lyon).

3. Identification of new cancer-specific diagnostic markers and/or therapeutic targets of melanoma

Main achievements: In this recently started project, we have thus far screened different melanocyte lesions for tetraspanin CD63. This protein is overexpressed in the early phases of melanoma onset but disappears in invasive and metastasizing melanomas. This confirms CD63 both as an invasion and metastasis suppressor gene and a new melanoma diagnostic/prognostic marker.

Current work: We are now exploring the mechanisms by which CD63 inhibits cancer invasion and metastasis, focusing on its possible interactions with some invasion/metastasis related genes.

Future work: Screening melanocyte lesions for tetraspanin TSPAN8, reported to be expressed in the late stages of melanomas and their metastasis, and microRNA profiling as a possible therapeutic target. Furthermore, in collaboration with Prof. Piero Dolara (University of Florence), we will apply the high-throughput array-based comparative genomic hybridization technology to analyze the entire genome of normal and dysplastic melanocytic nevi, compared to normal neighboring tissue, in order to identify possible mutations as early markers/targets of neoplastic transformation.

4. Evaluation of new molecular therapies for treatment of severe human retinopathies

Main achievements: We have demonstrated the high effectiveness of CoQ10 in its quite new role of mPTP closer demonstrated in our lab (21), and of peculiar *Bcl-2* ARE antisense oligoribonucleotides (4) in inhibiting *Bcl-2* down-regulation by stabilizing its mRNA and thereby in preventing apoptosis of

ganglion cells and retinal pigmented epithelial cells both *in vitro* and *in vivo* in experimental models of retinal damage. Both of these have been patented. The ability of CoQ10 applied to the cornea to quantitatively reach the retina resulted in a new pharmaceutical product, namely CoQ10 collyrium (Visufarma).

Current work: Extending our research in this area to find new antiangiogenetic molecules for treatment of the numerous retinopathies sustained by neoangiogenesis, including productive macular degeneration, diabetic retinopathy and retinitis pigmentosa. We have designed an anti-uPAR antisense oligonucleotide, whose parenteral application in mice dramatically reduced retinal angiogenesis in response to hypoxia (Figure 2).

Future work: We will design a pharmaceutical preparation of this oligonucleotide and test its delivery as eye drops.

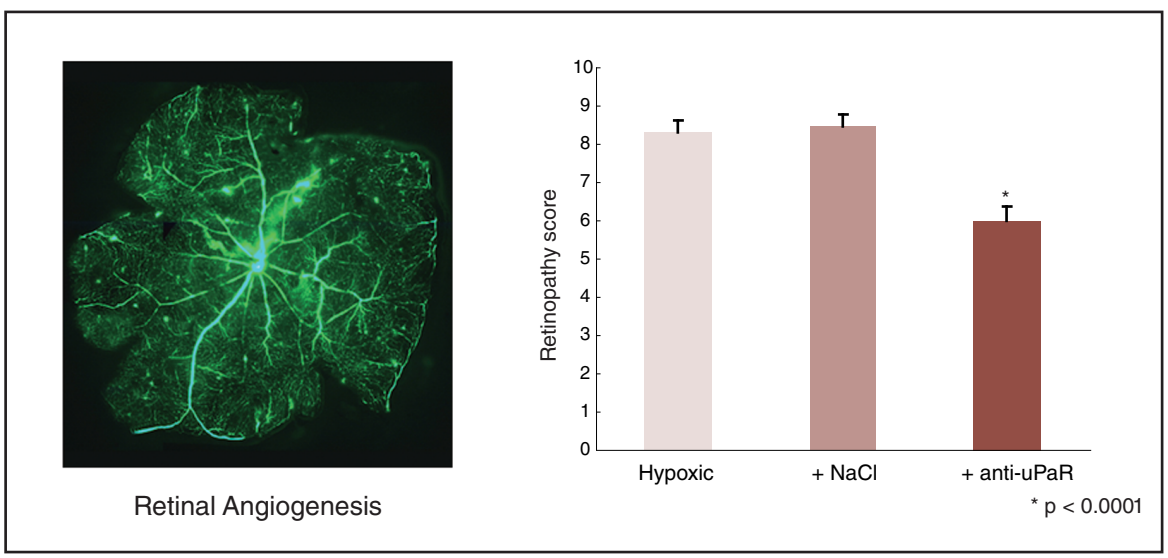

Figure 2 - Anti-uPAR antisense oligonucleotide treatment in a model of hypoxia induced retinopathy: improvement of retinopathy score

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Research Grants

Year	Funding Agency	Amount
2009	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 50,000
2008-2009	Fondazione Cassa di Risparmio di Lucca	€ 30,000
2008	AIRC	€ 50,000

Year	Funding Agency	Amount
2007-2009	Ministero dell'Istruzione, dell'Università e della Ricerca – PRIN	€ 30,000
2007	AIRC	€ 30,000
2006	AIRC	€ 30,000
2006	Visufarma SpA	€ 90,000
2005-2009	Ente Cassa di Risparmio di Firenze	€ 100,000
2005	AIRC	€ 30,000

Main Collaborations

With Units within ITT

» Regional Melanoma Referral Center, Azienda Sanitaria di Firenze (ASF)
» Dermatopathology and Pigmented Skin Lesions Unit, ASF
» Department of Dermatological Sciences, University of Florence
» Department of Pharmacology, University of Florence

With other Italian and Foreign Institutions/Organizations

» Laboratory of Bioinorganic Chemistry, University of Florence
» Department of Clinical Physiopathology, ASA Research Division, University of Florence
» Center for Research, Transfer and High Education, DENOThe, University of Florence
» Department of Biology, University of Pisa
» Istituto per la Sintesi Organica e la Fotoreattività, Consiglio Nazionale Ricerche (CNR), Bologna
» Laboratory for Orthopedic Pathophysiology and Regenerative Medicine, Istituto Ortopedico "Rizzoli", Bologna
» Translational Oncogenomics Unit, Istituto Nazionale Tumori "Regina Elena", Roma
» Centre de Biologie du Développement, Université Paul Sabatier, Toulouse (France)
» University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey (USA)
» National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland (USA)
» Laboratoire de Génétique, Signalisation et Cancer, Université Claude Bernard, Lyon (France)

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CELL ADHESION, CELL MOTILITY AND METASTASIS

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Introduction

Paola Chiarugi and her team have been studying the structure-function relationship of tyrosine phosphatases for 10 years. Their studies have contributed towards the elucidation of the action mechanism of these enzymes using mutagenesis techniques and also to the definition of their role in the control of cell proliferation, adhesion and motility. More recently, the interests of the Unit have been directed towards the redox regulation of oxidant-sensitive proteins during cell proliferation, cell adhesion to Extracellular Matrix (ECM) and plasticity of cell motility during tumor cell invasion.

Main Research Themes

1. Reactive Oxygen Species as intracellular messengers driving cell migration and survival

Reactive Oxygen Species (ROS) include oxygen metabolites, such as superoxide anions and hydrogen peroxide, which are essential mediators of cell signaling. They are generated as by-products of normal aerobic metabolism or as second messengers in various transduction pathways. Several lines of evidence demonstrate that NADPH oxidase is specifically involved in the generation of ROS by means of soluble growth factors, while 5-lipoxygenase is a source of ROS during the synthesis of leukotrienes. Transient fluctuations in ROS provide important regulatory functions, and a number of defense systems have evolved to fight ROS accumulation. Unfortunately, these defense mechanisms are not always adequate for counteracting ROS production, resulting in a state of oxidative stress, implicated in a wide variety of disease processes including cancer.

Our contribution to redox biology deals with the demonstration that ROS act on intracellular proteins by modulating their functions via reversible oxidation. Thiols, by virtue of their ability to be reversibly oxidized, are recognized as key targets of oxidative stress, and they therefore act as redox-sensitive switches. Target proteins for ROS include Protein Tyrosine Phosphatases (PTPs) and Protein Tyrosine Kinases (PTKs). We have reported the reversible oxidation among PTPs, firstly for PTP1B during EGF signaling, and secondly for low Mw (LMW)-PTP during PDGF stimulation and integrin signaling. More recently, we and also other authors have reported reversible oxidation during growth factor signaling for SHP2 and PTEN. H2O2 can also activate protein kinases and, among these, PTKs.

Nevertheless, PTK activation appears to be essentially due to two mechanisms: *a*) direct cysteine oxidation; *b*) concomitant inhibition of PTPs, that indirectly leads to sustained activation of PTKs. In particular, we recently reported the relevance of Src redox regulation for anchorage-dependent growth. We also described how the tyrosine kinase c-Src is oxidized in response to ECM adhesion and that this leads to an enhancement of its kinase activity. The oxidation/activation is probably due to an S-S bond between Cys245 and Cys487, thus leading to the achievement of the full activation of the Src kinase during cell/ECM contact.

In addition, an increase in Focal Adhesion Kinase (FAK) tyrosine phosphorylation owing to oxidative stress has been described in different cell lines. We have also reported how the integrin-dependent ROS burst culminates in a down-regulation of a FAK phosphatase, namely LMW-PTP. The redox regulation of FAK leads to ERK and Src phosphorylation through the inhibition of LMW-PTP, ultimately affecting focal adhesion formation and cell spreading. The model that we recently proposed describes how Rac-mediated ROS production during anchorage-dependent cell growth results in the inhibition of LMW-PTP and an increase in the phosphorylation/activation of its targets, p190Rho-GAP, Src and FAK. This signal leads to the down-regulation of Rho activity, accounting for Rac-induced formation of membrane ruffles during integrin-mediated cell spreading. These findings, together with those concerning FAK regulation, define a key role for the redox regulation of LMW-PTP in the redox-dependent control of actin cytoskeleton during cell adhesion and proliferation. Finally, we have reported how the redox regulation of SHP2, another PTP, influences anchorage-dependent cell growth.

Joint integrin/RTK signaling is required for cell proliferation, survival and migration. Several mechanisms ensure that integrin and RTK signals are properly integrated in the anchorage-dependent cell. Formation of integrin/GF-receptor complexes may lead to three kinds of signals: concerted, integrin-dependent RTK activation and GF-dependent integrin activation. In the first case, signals triggered by GF receptors and those induced by integrin engagement might follow parallel pathways, with additive activation of converging signaling cascades. This collaboration can exploit membrane-proximal transducers, such as FAK, that act as signaling scaffolds to keep the complex together. This coordinated activity of FAK is

highlighted by the observation that fibroblasts lacking FAK are refractory to PDGF and EGF-dependent migration, and this defect is overcome by the re-expression of a functional FAK. In the second case, integrin engagement can lead to adhesion-dependent, ligand-independent activation of integrin-associated GF receptors. At least for the EGF-R/alpha$_v$-integrin complex, this type of collaboration is assured by Src and involves the recruitment of the adaptor protein p120Cas.

In contrast to untransformed cells, GF autocrine and anchorage-independent growth are a fundamental feature of cancer cells. Indeed, cell transformation results in alterations in serum- and adhesion-dependent cell growth, loss of contact inhibition, and changes in adhesiveness and motility. It has been noted that ROS production is increased in cancer cells and these oxidants are thought to play multiple roles in tumor initiation, progression and maintenance. In particular, excess ROS production associated with cell transformation could release co-stimulatory and deregulated signals which are normally and transiently triggered by cell/ECM interaction. Accordingly, constitutive overexpression of active oncogenic Rac-1 or Ras in non-transformed adherent cells confers these cells with the ability to grow in the absence of ECM contact.

Oxygen radicals have been implicated in a number of conditions ranging from aging to atherosclerosis. Little is known with regard to how ROS, like superoxide and hydrogen peroxide, are regulated in cells and what specific aspects of cellular activity might be influenced by the physiological and pathophysiological levels of these molecules. We are attempting to use a molecular biological approach to address these issues. It is our hope that we can gain a broader and more complete understanding of how cellular ROS are regulated. In addition, we are attempting to identify the direct molecular targets of cellular oxidants. Finally, we are hopeful that this molecular understanding will lead to new insights into diseases in which oxidant stress plays a role, and help in the design of new therapeutic agents. Cancer cells can generate constitutively high levels of ROS, which are thought to promote cell proliferation, cell motility, invasion and angiogenesis, all of which are prerequisites for tumor metastasis. We are now focusing our interest on developing redox-based drugs with a selective potential to kill cancer cells. If the redox make-up of the cancer cell is distinctly different from that of healthy cells, it might be possible to employ redox-activated agents to selectively target the cancer.

Many cancer cells exhibit a disturbed intracellular redox balance, making them distinctly different from their “healthy” counterparts, and several attempts have been made to use this naturally occurring oxidative stress to selectively kill cancer cells. Within this context, we are particularly interested in redox catalysts, *i.e.* drugs that enhance the toxicity of ROS. Biochemical research into catalysts able to generate or interconvert ROS is still in its infancy. Nevertheless, recent progress has been made with compounds mimicking the redox behavior of SOD and GPx. Such enzyme mimics exhibit a catalytic activity similar to their parent enzyme, but show a different substrate specificity. This leads to ROS-based catalysis distinctly different from the “normal” SOD or GPx reactions (hydrogen peroxide and hydroxyl radicals from superoxide), and *in vivo* it can result in cancer cell death owing to increased toxicity of ROS. Importantly, the compounds used in these studies were highly specific for cancer cells, and did not have a major effect on healthy cells, *i.e.* in the absence of elevated intracellular ROS concentrations. Our future plan involves the use of some of these drugs in collaboration with C. Jacob in Germany in our redox-dependent model of carcinogenesis.

In addition, resistance to detachment-induced apoptosis, a process commonly referred to as *anoikis*, is emerging as a hallmark of metastatic malignancies, mainly because it can ensure anchorage-independent growth and survival during organ colonization. Moreover, sustained oxidative stress has been associated with several steps of carcinogenesis, including transformation and achievement of a motile mesenchymal phenotype. Here, we demonstrate that metastatic prostate carcinoma cells, undergoing a constitutive deregulated production of ROS due to sustained activation of 5-lipoxygenase, lack suicidal pathways in response to lack of a matrix contact. These amplified and persistent redox signals leads to oxidation and activation of the kinase Src, thereby sustaining a ligand-independent

phosphorylation of the epidermal growth factor receptor. This leads to chronic activation of pro-survival signals, culminating in degradation of the pro-apoptotic protein Bim, thereby promoting cell survival even in absence of proper adhesion. *Anoikis* sensitivity to prostate metastatic cells is restored with antioxidant intervention or genetic manipulation of the redox-mediated pro-survival pathway. Exposure to a pro-oxidant environment strongly increases *anoikis* resistance as well in non-transformed prostate epithelial cells. Hence, our results allow new insight into the etiology of the molecular mechanisms granting *anoikis* resistance to prostate aggressive cancers, opening new avenues in pharmacological intervention for antioxidant-sensitive invasive tumors.

Finally, we have explored the role of redox signaling during hypoxia. It is largely accepted that hypoxia is a crucial aspect for the growth of solid tumors, including melanoma; however, the role of Hypoxia Inducible Factors (HIFs) in metastasis dissemination is still poorly understood. Recent data implicate mitochondrial ROS production observed in mild hypoxia (1-3% O_2) in HIF-1α stabilization. In contrast to expectations, limited oxygen increases mitochondrial ROS rather than diminishing them. ROS, in turn, inactivate prolyl hydroxylases, through oxidation of the ferrous ion that is essential for their catalytic mechanism, and hence stabilize HIF-1α. Vitamin C has been shown to decrease HIF-1 levels by preventing the oxidation of the catalytic ferrous ion. In keeping with this, it has been recently reported that the anti-tumorigenic effect of antioxidants, such as N-acetyl cysteine and vitamin C in murine models of Myc-mediated tumorigenesis, are indeed HIF-1-dependent.

We observed that human melanoma Hs29-4T cells respond to hypoxia through a deregulation of mitochondrial release of ROS by the electron transfer chain complex III. These ROS are mandatory to stabilize HIF-1α, the master transcriptional regulator of the hypoxic response. Following hypoxia, Hs29-4T cells undergo redox- and HIF-1α-dependent overexpression and activation of the Met proto-oncogene, which drives the complex motogenic escape program. Silencing analyses revealed a definite hierarchy of this process, in which mitochondrial ROS drive HIF-1α stabilization, while the Met proto-oncogene is the final effector arm in the activation of the metastatic program. The axis mitochondrial ROS/HIF-1α/Met overexpression is a molecular program crucial for spreading and motility of melanoma cells, for forming capillary-like structures by vasculogenic mimicry, a known strategy adopted by several cancers to help dissemination of metastases at a distance, as well as for invading and colonizing lungs with metastases. Hence, we propose that hypoxia-driven ROS act as a primary driving force to elicit an invasive program exploited by aggressive melanoma cells to escape from a hypoxically hostile and pro-oxidant environment.

2. The involvement of Eph/ephrins in plasticity of cell motility and metastasis dissemination

Considering the high incidence and mortality due to metastatic cancers, it is critical to understand the mechanisms behind metastasis and identify new targets for therapy. In recent years, two broad mechanisms for metastasis have received significant attention: plasticity in cell motility, *i.e.* transition towards a different motile phenotype, and tumor microenvironment interactions. The achievement of the migratory feature is a prerequisite of metastases and a hallmark of malignant tumor progression. The change in adhesive preferences of cancer cells that mediate their reciprocal interaction with the ECM and neighboring stromal cells is a crucial event in the acquisition of metastatic properties. Recently, several key advances have challenged the view of cancer cell motility indicating two essential milestones: first, that the gene expression-based motile phenotype is determined very early on during cancer development and second, that cancer cells display different types of cell motility among which mesenchymal, collective and amoeboid motility.

It is estimated that a third of carcinomas undergoes an Epithelial -Mesenchymal Transition (EMT), and after the transition uses mesenchymal motility to spread. EMT is sustained by a great change in the gene-expression pattern, mainly driven by AP1 and SMAD2 transcription factors and Snail/slug transcription repressors. EMT is accompanied by a reduction or loss in E-cadherin and the re-expression of N-cadherin, which appears to enhance the motility of various tumor cell types.

a) Our laboratory has attempted to elucidate the molecular mechanism via which Eph receptors affect cell adhesion and migration during embryogenesis and oncogenesis. We have investigated the molecular cues elicited by ephrinA1 in carcinoma cells and their motile phenotype. Ephrin kinases and their ephrin ligands transduce the repulsion of cells in axon guidance, migration, invasiveness and tumor growth, exerting a negative signaling on cell proliferation and adhesion. A key role of their kinase activity has been confirmed by mutant kinase inactive receptors which shift the cellular response from repulsion to adhesion. Our studies were aimed at investigating the role of tyrosine phosphorylation of EphA2 kinase on repulsive cues. We recently demonstrated that LMW-PTP, by means of dephosphorylation of EphA2 kinase, negatively regulates the ephrinA1-mediated repulsive response, cell proliferation, cell adhesion and spreading, and the formation of retraction fibers, thereby confirming the relevance of the net level of tyrosine phosphorylation of Eph receptors. A second approach to this focus is the study of a single tyrosine substitution of EphA2 on the ephrinA1-mediated repulsive response, cell adhesion and spreading. The integration of both PTP-mediated and tyrosine mutant-mediated studies allowed us to draw a comprehensive picture of the tyrosine kinase dependent and independent responses. Our ongoing studies show evidence that ephrinA1 inhibits integrin mediated adhesion through a redox-based Rac1 signaling, thus releasing previously engaged cell-matrix constraints. We demonstrated that the release of these constraints is a key hallmark of ephrin-elicited motility and invasive properties of Ephs-expressing carcinomas.

b) In addition to these studies, we focused our interest on the second milestone of cell movement: the release of cell-ECM constraints. Again, we investigated the role of ephrinA1 as a motile factor. Interactions linking the Eph receptor tyrosine kinase and ephrin ligands transduce short-range repulsive signals regulating several motile biological processes, including axon pathfinding, angiogenesis and tumor growth. These ephrin-induced effects are believed to be mediated by alterations in actin dynamics and cytoskeleton reorganization. The members of the small Rho GTPase family elicit various effects on actin structures and are probably involved in Eph receptor-induced actin modulation. In particular, some ephrin ligands lead to a decrease in integrin-mediated cell adhesion and spreading.

We have reported that the ability of ephrinA1 to inhibit cell adhesion and spreading in prostatic carcinoma cells is strictly dependent on the decrease in the activity of the small GTPase Rac1. Given the recognized role of Rac-driven redox signaling for the integrin function, reported to play an essential role in focal adhesion formation and in the overall organization of actin cytoskeleton, we investigated the possible involvement of oxidants in ephrinA1/EphA2 signaling. We provided evidence that ROS are an integration point of the ephrinA1/integrin interplay. We identified a redox circuitry in which the ephrinA1-mediated inhibition of Rac1 leads to negative regulation of integrin redox signaling, affecting the activity of the tyrosine phosphatase LMW-PTP. The enzyme in turn actively dephosphorylates its substrate p190RhoGAP, finally leading to RhoA activation. Taken together, our data suggest a redox-based Rac-dependent upregulation of Rho activity, concurring with the inhibitory effect elicited by ephrinA1 on integrin-mediated adhesion strength.

c) Finally, we demonstrated that EphA2 re-expression in B16 murine melanoma cells, that use a defined mesenchymal invasion strategy, converts their migration style from mesenchymal to amoeboid-like, conferring a plasticity in tumor cell invasiveness. Indeed, in response to re-expression and activation of EphA2, melanoma cells activate a non-proteolytic invasive program which proceeds through the activation of cytoskeleton motility, the retraction of cell protrusions, a Rho-mediated rounding of the cell body, and squeezing among the 3D matrix, giving rise to successful lung and peritoneal lymph node metastases. Our results suggest that, among the redundant mechanisms operating in tumor cells to penetrate the anatomical barriers of host tissues, EphA2 plays a pivotal role in the adaptive switch in the migration pattern and mechanism, defining and distinguishing tumor cell invasion strategies. Thus, targeting EphA2 might represent a future approach for the therapy of cancer dissemination.

3. The role of reactive stroma in cancer progression

During primary tumor formation, the cells in a carcinoma engage a multifaceted collection of mesenchymal cells, jointly forming the tumor-associated stroma. As tumor progression proceeds, the stromal cells create a "reactive stroma" that releases a variety of signals that induce changes in carcinoma cell phenotype (a). Tumor-associated host cells are themselves invasive and some of them arrive at the site of metastasis ahead of the cancer cells, thereby facilitating the invasion process.

We are interested in examining the nature of the signals that are released by activated stroma and serve to induce EMT, an epigenetic profound change in cell phenotype that causes immotile epithelial cells to acquire traits such as motility, invasiveness, and resistance to apoptosis or the ability to adapt to environmental changes and continue to invade successfully. These signals serve to induce expression of a series of transcription factors that are capable, in turn, of inducing the plasticity of cell motility (a).

In the EMT process, the cells lose their epithelial characteristics, including their polarity and specialized cell-cell contacts, and acquire a mesenchymal migratory behavior, allowing them to move away from their original site towards remote locations. EMT illustrates the differentiation plasticity during development, but is commonly exploited by cancer cells to invade and metastatize (b). We observed that mesenchymal motility is characterized by elongated and polarized cell morphology, which depends upon ECM proteolysis of the moving cells that, through production of matrix metalloproteinases (MMPs), thereby generate a "path". This leads to activation of Rac1 at the leading edge of the cell, and to inhibition of RhoA GTPase.

For our studies, we used as models human prostatic carcinoma cells, such as PC3, Ln-CaP, DU-145, as well as RWPE1-NB11 and PNT-1 non-transformed immortalized human epithelial prostatic cell lines. After setting the molecular basis of EMT in these cell lines in the presence of CAFs, we translated our knowledge into prostate carcinoma specimens obtained through our collaboration with the Urology Unit of the University of Florence. We also achieved from these collaborators information on *in vivo* aggressiveness and follow-up (short and medium term) of patients bearing these cancers. CAFs have been recovered from these specimens, as well as from benign prostate hyperplasia, and tested in culture for their migratory/invasive properties.

Our results concerning the bidirectional liaison established between prostate carcinoma cells and stromal fibroblasts, strongly affecting both fibroblasts activation and carcinoma aggressiveness, lead to two major conclusions: *a*) orthotopic fibroblasts activated by soluble factors released by cancer cells promote a MMP-driven EMT in carcinoma cells; *b*) EMT induced by CAFs not only endows cells with migratory and invasive capabilities allowing development of spontaneous lung metastases, but also leads to the achievement of stem-cell properties.

We clarified that CAFs are key determinants in the malignant progression of cancer, and qualified their functional contribution as extremely powerful in terms of metastatic dissemination. We reported that CAF stimulation results in a clear EMT of cancer cells, which acquire a proteolysis-based motility, as well as a short tumor latency, high rate of tumor growth and incidence (Figure 1). The spur given by CAF co-injection is extremely powerful as it elicits spontaneous lung metastases of tumor cells. To our knowledge, this is the first observation of spontaneous metastases of human prostate carcinoma cells injected in an heterotopic site in mouse models.

Moreover, analysis of the mutual interplay between prostate carcinoma cells and CAFs revealed a mandatory role of carcinoma-derived interleukin-6 in fibroblast activation. We observed that CAFs, isolated from human aggressive prostate carcinoma, are sensitive to *in vitro* IL-6 stimulation and their response is very similar to PCa-AFs in terms of EMT and invasiveness elicited in PC3 cells. Therefore, it is conceivable that *in vivo* the population of CAFs which escort the tumor mass is composed of multiple phenotypes, including MFs and IL-6-sensitive fibroblasts. Indeed, we found among CAFs

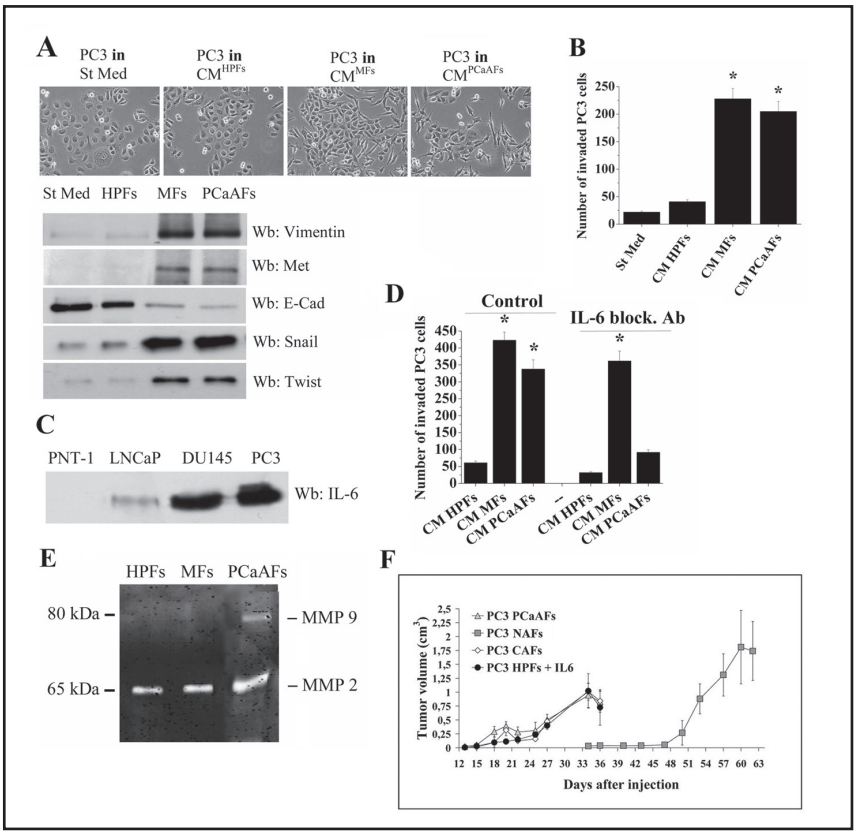

Figure 1 - CAFs enhance PC3 cells aggressive properties. A) Conditioned Media (CM) derived from MFs and PCaAFs induce on PC3 cells morphological changes and marker regulation typical of EMT. B) Human activated flbroblasts enhance PC3 cells invasiveness as evaluated by a Boyden assay in the presence of matrigel coating. C) Expression of IL-6 augments in prostate carcinoma cells with increased aggressiveness with respect to untransformed cells. D) PC3 cells were treated with CM from activated fibroblasts (MFs, PCaAFs), in the presence or absence of IL-6 blocking antibodies, highlighting the mandatory role of IL-6 in PC3-mediated fibroblast activation. E) PCaAFs produce active MMPs with respect to MFs as revealed by zimography assay. F) Xenograft growth in SCID bg/bg mice of PC3 cells subcutaneously injected with activated fibroblasts

extracted by prostate carcinoma biopsies a mixed population which respond to both IL-6 and TGF-β, suggesting co-existence of phenotypes resembling MFs and PCa-AFs. We can speculate that different CAF populations can affect different steps of tumor development, as well as that early and late phases of cancer progression may have different effects on the activation of intratumoral fibroblasts. This idea is further confirmed by the absence of EMT in non-transformed prostate epithelial cells or by the inability of LNCaP cells, caused by less aggressive prostate cancers, to activate fibroblasts. Hence, IL-6 is a powerful activating signal for stromal fibroblasts, as indicated by its ability to mimic the complexity of stimuli acting *in vivo* on CAFs.

In turn, activated fibroblasts, through secretion of metalloproteases (MMPs), elicit in cancer cells a clear EMT, as well as an enhancement of tumor growth and development of spontaneous metastases. Our findings reveal that CAF-mediated EMT in PC3 cells shows a decrease in E-cadherin, an increase in Snail, vimentin and Met and the use of proteolysis-based invasiveness. Interestingly, CAF-mediated EMT, peculiarity in our experimental model, is engaged by MMPs secreted by activated fibroblasts. To our knowledge, only one report has thus far indicated the forced expression or exogenous addition of MMP-3 to facilitate the transition.In addition, MMP-2 and MMP-9 have been shown to cleave E-cadherin, which leads to its internalization and relocalization of transcriptionally active β-catenin, to the nucleus, inducing EMT (c).

Additionally, we have explored the possibility that cancer stem cells, self-renewing, tumor-seeding cells found in several solid tumors, may be a cancer subpopulation endowed with a particular type of cell plasticity in their motility. Weinberg's lab first reported that breast cancer cells undergoing EMT gain many of the properties of adult stem cells (d), a finding which has been also proposed for prostate

carcinoma cells (e). In this light, we demonstrated that the interraction between stromal fibroblasts and prostate carcinoma cells, eliciting a clear EMT in carcinoma cells, leads to an increase in $CD44^{high}$/$CD24^{low}$-ratio and CD133 expression, as well as to the enhancement of clonogenicity, self renewal and tumorigenic properties. In addition, prostate cancer cells undergoing CAF-mediated EMT generate $CD44^{high}/CD24^{low}$ xenografts and, more importantly, spontaneous lung metastases (Figure 2). The latter result is extremely provocative as it supports the notion that, while EMT plays a role in the generation of high-grade invasive cells with stem cell-like properties, the same stem cell population is responsible for metastatic dissemination.

Therefore, we propose a correlation between cells undergoing CAF-mediated EMT and cancer stemness in terms of their ability to spread and reconstitute metastatic tumors. In this context cancer stemness, and by extension metastatic dissemination, is directly induced by fibroblasts of the tumor microenvironment. Control or suppression of CAF-mediated EMT may serve as a basis for the development of therapies that target tumor growth and dissemination in secondary organs.

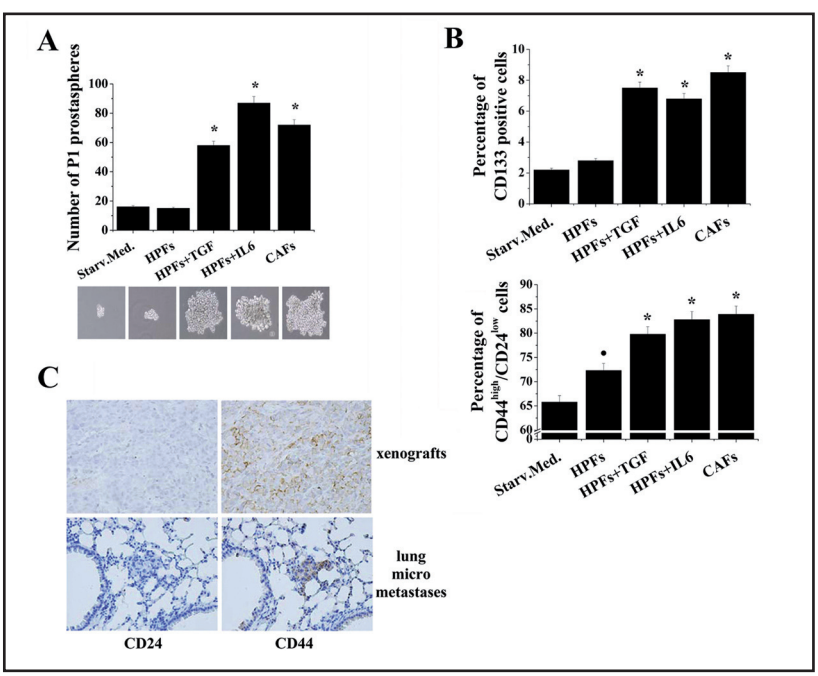

Figure 2 – CAFs, PCa-AFs and MFs induce cancer celi stemness. A) PCS cells were incubated with CM from HPFs, stimulated with TGF-β or IL-6, or with CM from CAFs. More numerous and larger P1 spheres were obtained from PC3 cells treated with CM from activated fibroblasts. B) PC3 cells treated as in A were analysed for expression of the cell-surface marker FITC-CD133 (upper panel) or FITC-CD44 and PE-CD24 (lower panel) by means of FACS analysis. Activated fibroblasts strongly enhance the percentage of both CD133-positive and CD44high/CD24low populations in PC3 cells. C) Paraffin-embedded tissue sections from xenografts and lung micrometastases obtained by PC3/CAFs co-injection in SCID bg/bg-mice were immunostained with CD44 and CD24 antibodies

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Research Grants

Year	Funding Agency	Amount
2009	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 70,000
2008-2010	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) – PRIN	€ 44,000
2008-2010	Istituto Toscano Tumori	€ 98,000
2008-2010	Association Française contre les Myopathies	€ 75,000
2008	Ente Cassa di Risparmio di Firenze	€ 20,000
2006-2008	AIRC	€ 150,000
2005	Ente Cassa di Risparmio di Firenze	€ 100,000
2005	Consorzio Interuniversitario Biotecnologie	€ 40,000
2004-2007	Regione Toscana, Tresor Project	€ 750,000
2004-2006	MIUR – PRIN	€ 91,500

Main Collaborations

With Units within ITT

» Department of Experimental Pathology and Oncology, University of Florence
» Department of Clinical Physiopathology, University of Florence
» Department of Organic Chemistry, University of Florence

With other Italian and Foreign Institutions/Organizations

» Istituto Nazionale Tumori (INT), Milano
» Institute for Cancer Research and Treatment, University of Turin
» University of Dresda (Germany)
» "Monash" University, Melbourne (Australia)

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VASCULAR PHARMACOLOGY

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Introduction

The Vascular Pharmacology Unit is interested in the study of the cellular and molecular mechanisms of vascular remodeling, such as the angiogenic process and the hyperplasia and hypertrophy of vascular smooth muscle cells, events characteristic of some pathological situations (atherosclerosis and restenosis). In the past, this group was mainly involved in the mechanisms involved in tumor angiogenesis by exploring the role of nitric oxide in this process. The cellular mechanisms of some

anti-angiogenic molecules, such as linomide, have also been investigated. In an effort to understand the molecular and cellular mechanisms of vascular remodeling, the role of intracellular mitogens on either endothelial or vascular smooth muscle cell proliferation and the role of hypoxia on vascular cell functions have been investigated. Recently, the characterization of circulating human stem cells and their *in vitro* differentiation in vascular and Dendritic Cells (DC), and the interaction between DC and vascular cells has been investigated.

Main Research Theme

Characterization of circulating DC and monocyte-derived DC (MoDC) in Colorectal Cancer (CRC) patients

Main achievement: We have demonstrated that patients with metastasis had a higher number of circulating DCs but less monocyte-derived DCs (MoDCs) compared to those without metastasis. Surgically-treated patients showed less circulating DCs and MoDCs compared to those who were not treated surgically. Chemotherapy-treated patients had fewer circulating DCs and a compromised ability to obtain MoDCs compared to untreated patients (Figure 1).

Future work: Enroll a greater number of CRC patients in the study in order to:

a) evaluate different DC subsets in CRC tissue which might highlight important antigen uptake mechanisms performed by the different DCs; characterization of the different DC subsets circulating in peripheral blood of CRC patients to get insight into the initiation of the immunoresponse;
b) functionally characterize MoDCs;
c) apply advanced optoelectronic techniques to study the morphology and function of DCs in patients affected by CRC, as well as autofluorescence pattern of tissue specimens to develop a new method for tumor diagnosis and prognosis.

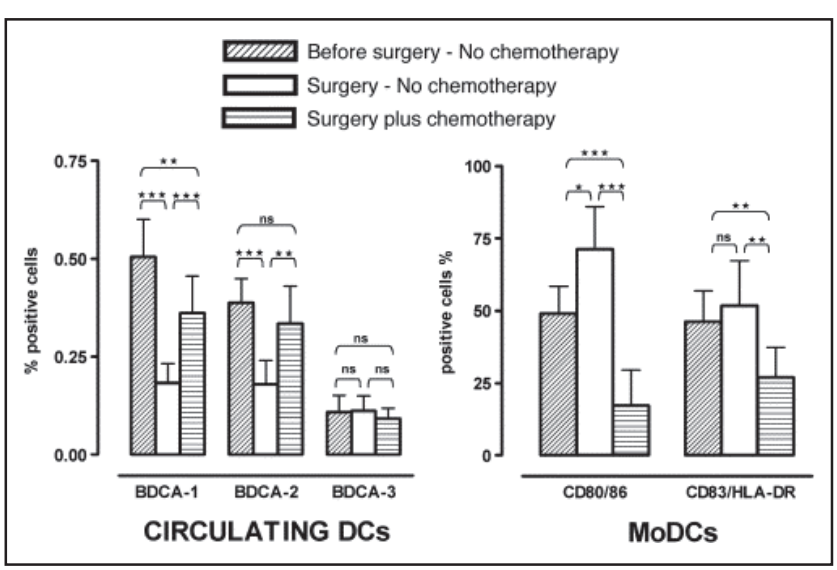

Figure 1 - Effect of different clinical characteristics of CRC patients on circulating DCs and MoDCs. Flow cytometric analysis was performed on tumor-bearing CRC patients without any therapy, CRC patients who underwent only tumor excision and CRC patients who underwent both surgery and chemotherapy. *P < 0.05; **P < 0.01; ***P < 0.001 for before surgery-no chemotherapy *versus* surgery-no chemotherapy and surgery plus chemotherapy

Research Grants

Year	Funding Agency	Amount
2009	Angelini – ACRAT	€ 30,000
2007-2009	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) – PRIN	€ 29,207
2003-2005	MIUR – PRIN	€ 52,900
2001-2004	MIUR – FIRB	€ 246,135
2001-2003	MIUR – PRIN	€ 53,712

Main Collaborations

With other Italian and Foreign Institutions/Organizations

» Deparment of Internal Medicine, University of Florence
» Deparment of Pharmacology, University of Florence
» Department of Critical Medicine and Surgery, University of Florence
» Department of Dermatological Sciences, University of Florence
» Center of Excellence in Optronics (CEO), Firenze
» Department of Biochemistry, University of Bologna
» Istituto Nazionale per le Ricerche Cardiovascolari (INRC), Bologna
» Department of Clinical and Experimental Medicine, University of Perugia

Publications

1. Bellik L, Gerlini G, Parenti A, et al: *Role of conventional treatments on circulating and monocyte-derived dendritic cells in colorectal cancer*. Clin Immunol 2006; 121: 74-80.
2. Bellik L, Ledda F, Parenti A: *Morphological and phenotypical characterization of human endothelial progenitor cells in an early stage of differentiation*. FEBS Letters 2005; 579: 2731-6.
3. Bellik L, Vinci MC, Filippi S, Ledda F, Parenti A: *Intracellular pathways triggered by the selective Flt-1-agonist placental growth factor in vascular smooth muscle cells exposed to hypoxia*. Br J Pharmacol 2005; 146: 568-75.
4. Parenti A, Bellik L, Brogelli L, Filippi S, Donnini A, Ledda F: *Endogenous VEGF-A is responsible for the mitogenic effect of monocyte chemotactic protein-1 on vascular smooth muscle cells*. Am J Physiol 2004; 286: 1978-84.
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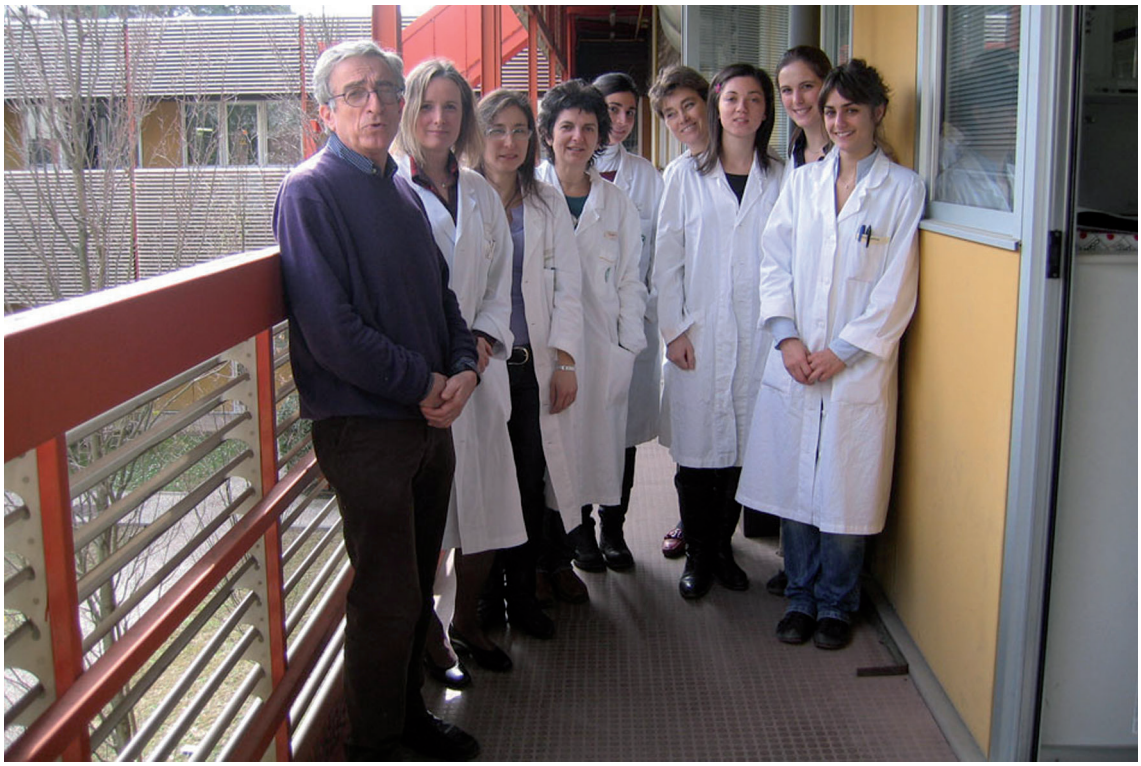
ENDOCRINOLOGY AND ANDROLOGY



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Claudia Giachini, PhD Student
Francesca Nuti, PhD
Lara Tamburino, PhD Student
Ilaria Laface, Fellow

Introduction

Our Unit started to work on prostate pathology early in the 1990s. Initial studies were dedicated to the role of growth factors on proliferation of benign prostate hypertrophic cells. Later on, the Unit began to work on prostate carcinoma. In particular, our studies in this area were aimed at identifying factors that

cooperate with androgens on the progression and invasion of the cancer cell, focusing on the role of the androgen receptor and its interaction with growth factor receptors.

In the last few years, we have been studying the genetic features of testis cancer (carcinomas and teratomas). Currently, we are studying the expression of seladin-1, a possible marker for the progression or chemoresistance of these types of cancers. Moreover, we initiated a study aimed to define the role of stromal *versus* epithelial androgen receptors in prostate cancer.

Main Research Themes

1. The role of androgens in the invasion of prostate carcinoma cells

Main achievements: Most studies were conducted in available human prostate carcinoma cell lines, which are characterized by a different invasion ability, and in a cell line obtained in the lab after transfection with a functional androgen receptor. We have shown that expression of a functional androgen receptor in the cells limits the invasion properties *in vitro* through modulation of integrin a6b4 expression and through an interaction with the receptor of the epidermal growth factor, which limits its downstream signaling, in particular PI3K activation.

We have also studied the role of possible therapeutic agents, such as the EGF receptor inhibitor IRESSA and the vitamin D analog BXL629 on growth and invasion properties of carcinoma cell lines. Both agents were able to inhibit proliferation and invasion ability of the cells, through inhibition of PI3K signaling.

Other recent projects concern: *i*) development of *in vitro* models to investigate the role of neuroendocrine differentiation of prostate cancer cells in progression (a) and *ii*) the role of the gene TMPRSS2:ERG (2) and seladin-1 (3) in the progression of prostate cancer.

Current work: The study of the role of stromal and epithelial expression of the androgen receptor in the progression of prostate cancer.

2. Study on mechanisms of chemoresistance in testis cancer

Main achievement: Studies on testis cancer cell lines and on tumor specimens identified in seladin-1, a possible marker of chemoresistance (1).

Current work: The role of TSPY1 gene dosage in testis cancer.

Reference

a) Marchiani S, Tamburrino L, Nesi G, et al: *Androgen-responsive and -unresponsive prostate cancer cell lines respond differently to stimuli inducing neuroendocrine differentiation*. Int J Androl 2010 [Epub ahead of print].

Research Grants

Year	Funding Agency	Amount
2008	Istituto Toscano Tumori	
2007-2009	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 15,000
2007	Ente Cassa di Risparmio di Firenze	€ 30,000
2006	Ministero dell'Istruzione, dell'Università e della Ricerca – PRIN	€ 70,300

Main Collaborations

With Units within ITT

» Istituto per lo Studio e la Prevenzione Oncologica (ISPO), Firenze
» Department of Pathological Anatomy, Azienda Ospedaliero Universitaria Careggi, Firenze

With other Italian Institutions/Organizations

» Department of General Pathology, University of Naples
» Endocrinology Institute, University of Milan

Publications

1. Nuti F, Luciani P, Marinari E, et al: *Seladin-1 and testicular germ cell tumors: new insights into cisplatin responsiveness*. J Pathol 2009; 219: 491-500.
2. Bonaccorsi L, Nesi G, Nuti F, et al: *Persistence of expression of the TMPRSS2:ERG fusion gene after pre-surgery androgen ablation may be associated with early prostate specific antigen relapse of prostate cancer: preliminary results*. J Endocrinol Invest 2009; 32: 590-6.
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5. Bonaccorsi L, Nosi D, Muratori M, Formigli L, Forti G, Baldi E: *Altered endocytosis of epidermal growth factor receptor in androgen receptor positive prostate cancer cell lines*. J Mol Endocrinol 2007; 38: 51-66.
6. Marchiani S, Bonaccorsi L, Ferruzzi P, et al: *The vitamin D analogue BXL-628 inhibits growth factor-stimulated proliferation and invasion of DU145 prostate cancer cells*. J Cancer Res Clin Oncol 2006; 132: 408-16.
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11. Bonaccorsi L, Muratori M, Carloni V, et al: *The androgen receptor associates with the epidermal growth factor receptor in androgen-sensitive prostate cancer cells*. Steroids 2004; 69: 549-52.
12. Bonaccorsi L, Marchiani S, Muratori M, Forti G, Baldi E: *Gefitinib ("IRESSA", ZD1839) inhibits EGF-induced invasion in prostate cancer cells by suppressing PI3 K/AKT activation*. J Cancer Res Clin Oncol 2004; 130: 604-14.
13. Gelmini S, Tricarico C, Petrone L, et al: *Real-time RT-PCR for the measurement of prostate-specific antigen mRNA expression in benign hyperplasia and adenocarcinoma of prostate*. Clin Chem Lab Med 2003; 41: 261-5.
14. Baldi E, Bonaccorsi L, Forti G: *Androgen receptor: good guy or bad guy in prostate cancer invasion?* Endocrinology 2003; 144: 2901. Review.
15. Bonaccorsi L, Muratori M, Carloni V, et al: *Androgen receptor and prostate cancer invasion*. Int J Androl 2003; 26: 21-5. Review.
16. Rosini P, Bonaccorsi L, Baldi E, et al: *Androgen receptor expression induces FGF2, FGF-binding protein production, and FGF2 release in prostate carcinoma cells: role of FGF2 in growth, survival, and androgen receptor down-modulation*. Prostate 2002; 53: 310-21.
17. Crescioli C, Maggi M, Luconi M, et al: *Vitamin D3 analogue inhibits keratinocyte growth factor signaling and induces apoptosis in human prostate cancer cells*. Prostate 2002; 50: 15-26.
18. Granchi S, Brocchi S, Bonaccorsi L, et al: *Endothelin-1 production by prostate cancer cell lines is up-regulated by factors involved in cancer progression and down-regulated by androgens*. Prostate 2001; 49: 267-77.
19. Gelmini S, Tricarico C, Vona G, et al: *Real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) for the measurement of prostate-specific antigen mRNA in the peripheral blood of patients with prostate carcinoma using the taqman detection system*. Clin Chem Lab Med 2001; 39: 385-91.

GLYCOVACCINES AND MOLECULAR RECOGNITION OF CARBOHYDRATES

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Martina Cacciarini, Organic Chemist
Oscar Francesconi, PostDoc
Grabriele Gabrielli, PhD Student

Introduction

In 2000, Prof. Cristina Nativi, along with a PhD Student, started a new research project on sulfur-containing glycoconjugates and glycomimetics, relying on her experience in sulfur and carbohydrate chemistry. Nowadays, the group is involved in several projects concerning the development of oligosaccharides for production of conjugate vaccines. In 2002, a collaboration with Dr. Stefano Roelens enlarged the interests of the group towards molecular recognition of carbohydrates. Currently, several students are involved in the development of new synthetic receptors for biological applications.

Main Research Themes

1. Glycovaccines

a) Design and synthesis of hydrolytically stable mimetics of gangliosides to employ in the biological treatment of tumors.
b) Carbohydrate-based antivirals.
c) Mucin-type glycomimetic scaffolds.

Main achievements:

a) Design and synthesis of a stable mimetic of the antigen GM-3 ganglioside lactone. Glycoconjugates able to raise immune response *in vivo* have been developed. Biological and immunological tests are still in progress.
b) Synthesis of carbohydrate-containing molecules designed to interact with HA. Biological tests under evaluation.
c) A galacto-containing mimetic has been synthesized. The latter is recognized *in vitro* by lectins. NMR studies confirmed the chair conformation of the carbohydrate portion. Multivalent platforms for a multi-topic presentation of the epitope are in preparation.

Future work:

a) Production of mAb after immunization of mice with GM-3 ganglioside mimetic; development of multivalent systems for better presentation of the antigen and a more efficient stimulation of the mouse immune system.
b) Biological tests *versus* viral and human NA.
c) Development of multivalent rafts to interact with mucins and activate an immuno-response.

2. Molecular recognition of carbohydrates, in collaboration with Dr. Stefano Roelens, Consiglio Nazionale delle Ricerche (CNR) – IMC

Development of synthetic receptors for molecular recognition of mono- and disaccharides of the glycocalyx involved in molecular recognition/infections.

Main achievements: Selective synthetic receptors have been designed and synthesized to be able to lock mannose residues. Microbiological tests showed effective interactions between the receptors and pathogens covered by mannose.

Future work: Development of nanostructures decorated with our receptor to investigate possible multivalent interactions with Dendritic Cells (DC).

Reference

Ardá A, Venturi C, Nativi C, et al: *A chiral pyrrolic tripodal receptor enantioselectively recognizes beta-mannose and beta-mannosides*. Chem Eur 2010; 16: 414-8.

Research Grants

Year	Funding Agency	Amount
2009	Istituto Toscano Tumori	€ 100,000
2008	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) – PRIN	€ 57,700
2008	Ente Cassa di Risparmio di Firenze	€ 60,000
2007	ITT	€ 50,000
2007	Ente Cassa di Risparmio di Firenze	€ 80,000
2006	MIUR – PRIN	€ 54,000
2006	Ente Cassa di Risparmio di Firenze	€ 36,000
2004	MIUR – PRIN	€ 55,000

Main Collaborations

With Units within ITT

» Department of Pathology, University of Florence
» Department of Nuclear Medicine, University of Florence
» Department of Anatomy, University of Florence
» Centro Interdipartimentale di Spettrometria di Massa (CISM), University of Florence

With other Italian and Foreign Institutions/Organizations

» Department of Organic Chemistry, University of Pavia
» Department of Virology, CNR, Pavia
» Department of Immunology, Istituto "Giannina Gaslini", Genova
» Department of Biomedical and Biotechnology Sciences, University of Brescia
» Centre of Biological Investigations (CSIC), Madrid (Spain)
» Department of Chemistry and Biochemistry, University of Berne (Switzerland)
» Group of Nanomedicine, Department of Chemistry, University of Grenoble (France)

Publications

1. Jimenez-Barbero J, Dragoni E, Venturi C, et al: *Alpha-o-linked glycopeptide mimetics: synthesis, conformation analysis and interactions with viscumin, a galactoside-binding model lectin*. Chem Eur J 2009; 15: 10423-31.
2. Dragoni E, Calderone V, Fragai M, Juiswal R, Luchinat C, Nativi C: *Biotin-tagged probes for MMP expression and activation: design, synthesis, and binding properties*. Bioconjugate Chem 2009; 20: 719-27.
3. Ristori S, Di Cola E, Lunghi C, Nativi C, Richichi B: *Structural studies of liposomes loaded with a GM-3 lactone analogue for the targeting of tumour epitopes*. Biochem Biophy Acta 2009; 1788: 2518-25.
4. Altamura M, Dragoni E, Infantino AS, et al: *Cyclic glycopeptidomimetics through a versatile sugar-based scaffold*. Bioorg Med Chem Lett 2009; 19: 3841-4.
5. Johansson EMV, Crusz SA, Kiolomiets E, et al: *Inhibition and dispersion of pseudomonas aeruginosa biofilms by glycopeptide dendrimers targeting the fucose-specific lectin LecB*. Chem Biol 2008; 15: 1249-57.
6. Nativi C, Roelens S: *1-n diols*. In Clayden J (ed.): *Science of synthesis. Compounds with one saturated carbon-heteroatom bond*. Stuttgart, Thieme Verlag, 2008; vol. 36, 757-98.

7. Nativi C, Cacciarini M, Francesconi O, Vacca A, Moneti G, Ienco A, et al: *Pyrrolic tripodal receptors effectively recognizing monosaccharides. Affinity assessment through a generalized binding descriptor*. J Am Chem Soc 2007; 129: 4377-85.
8. Nativi C, Cacciarini M, Francesconi O, Moneti G, Roelens S: *A β–mannoside selective pyrrolic tripodal receptor*. Organic Lett 2007; 9: 4685-88.
9. Toma L, Di Cola E, Ienco A, et al: *Synthesis, conformational studies, binding assessment and liposome insertion of a thioether-bridged mimetic of the antigen GM-3 ganglioside lactone*. Chem Bio Chem 2007; 8: 1646-9.
10. Cacciarini M, Menichetti S, Nativi C, Richichi B: *The hetero Diels-Alder approach to carbohydrate-containing molecular scaffolding*. Curr Org Synth 2007; 41: 47-57.
11. Francesconi O, Ienco A, Moneti G, Nativi C, Roelens S: *A self-assembled pyrrolic cage receptor specifically recognizes β-glucopyranosides.* Angew Chem Int Ed 2006; 45: 6693-6.
12. Calderone V, Fragai M, Luchinat C, Nativi C, Richichi B, Roelens S: *A high affinity carbohydrate-containing inhibitor of matrix metalloproteinases*. Chem Med Chem 2006; 1: 598-601.
13. Fragai M, Nativi C, Richichi B, Venturi C: *Design in silico, synthesis and binding evaluation of a carbohydrate-based scaffold for structurally novel inhibitors of matrix metalloproteinases*. Chem Bio Chem 2005; 6: 1345-9.
14. Romani A, Menichetti S, Arapitas P, Nativi C, Turchetti B, Buzzoni P: *O-methylglucogalloyl ester: synthesis and evaluation of their antimycotic activity*. Biorg Med Chem Lett 2005; 15: 4000-3.
15. Venturi F, Venturi C, Liguori F, Cacciarini M, Montalbano M, Nativi C: *A new scaffold for the stereoselective synthesis of a-o-linked glycopeptido mimetics*. J Org Chem 2004; 69: 6153-5.
16. Vacca A, Nativi C, Cacciarini M, Pergoli R, Roelens S: *A new tripodal receptor for molecular recognition of monosaccharides. A paradigm for assessing glycosides binding affinities and selectivities by ^{1}H-NMR spectroscopy*. J Am Chem Soc 2004; 126: 16456-65.
17. Capozzi G, Catelani G, D'Andrea F, Menichetti S, Nativi C: *Conformational evaluation of some 4-deoxyhex-4-enopyranose derivatives and their use in the preparation of a previously undescribed class of 3-thio-L-sorbopyranosides and their 6-C-methoxy analogues*. Carbohydrate Res 2003; 338: 123-32.
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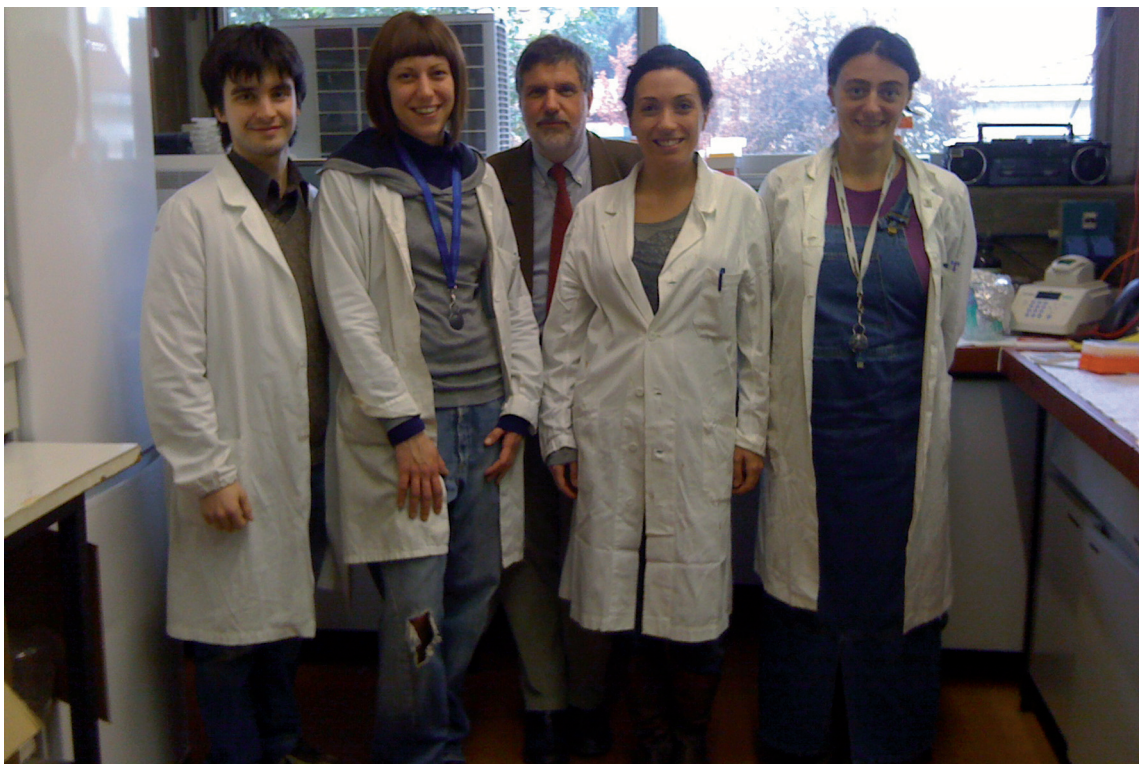
BIOTECHNOLOGY



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Laura Pietrovito, Research Fellow

Introduction

This Research Group has been working for many years on the mechanisms of tumorigenesis, using mainly *in vitro* models. Recently, our research has focused on the study of the oncogenes involved in the genesis and development of various types of human cancers. Moreover, in collaboration with Paola Chiarugi, a study is being performed on the involvement of the EphA2 tyrosine kinase receptor, which is up-regulated in a variety of cancers. In particular, its role in the mechanisms of cell motility and metastasis has been investigated (3,4).

Main Research Theme

The tumorigenetic role of Low Molecular Weight Phosphotyrosine-Phosphatase (LMW-PTP)

Main achievement: We have recently performed studies on a wide range of human tumors, investigating the expression of LMW-PTP. The results (8) indicate a significant increase in the expression of LMW-PTP in tumor tissue compared to healthy tissue, for a wide array of human tumors including breast (twice the mean increase), bladder (1.8 x), kidney (1.5 x) and colon (1.8 x). The results are shown in Figure 1.

Moreover, a higher expression of LMW-PTP is significantly correlated with a worse prognosis and less chance of survival (especially for colon cancer). We have also carried out experiments in animal models, examining colorectal tumors induced by specific mutagens in rat colon. The results (2) confirmed that overexpression of LMW-PTP is significantly higher in tumor tissue. In addition, carcinomas express higher levels of the protein with respect to adenomas.

Current work: Through the use of various cell models and techniques of modulation of genetic expression and proteomic and transcriptomic analyses, we intend to clarify the precise role of this gene in tumorigenesis, especially in colorectal cancer. We will try to identify gene products which can be a direct substrate of LMW-PTP or which are influenced by its overexpression in tumor tissue. We are also studying the structure of the LMW-PTP promoter, since we have solid indications that a single point mutation, originating in carcinoma cells, may be responsible for the overexpression of this gene in tumors. Indeed, with the use of a reporter gene, it was possible to assess that a single point mutation is able to double gene expression in several cell lines of different origins. Our current model is that a repressor is normally active on this gene: the observed point mutation may decrease its affinity to a specific element in the promoter, enhancing gene expression. We are now starting a promoter sequence analysis in a wide range of tumor samples in order to assess the correlation between this mutation and the overexpression of LMW-PTP in tumor.

Future work: The precise understanding of the tumorigenic mechanisms induced by increased LMLW-PTP expression are the final aim of this research project. Results of proteomic research can open new scenarios regarding the low concentrations of this phosphatase. Moreover, analysis of the promoter region of the LMW-PTP gene should allow us to identify the specific factors regulating gene expression.

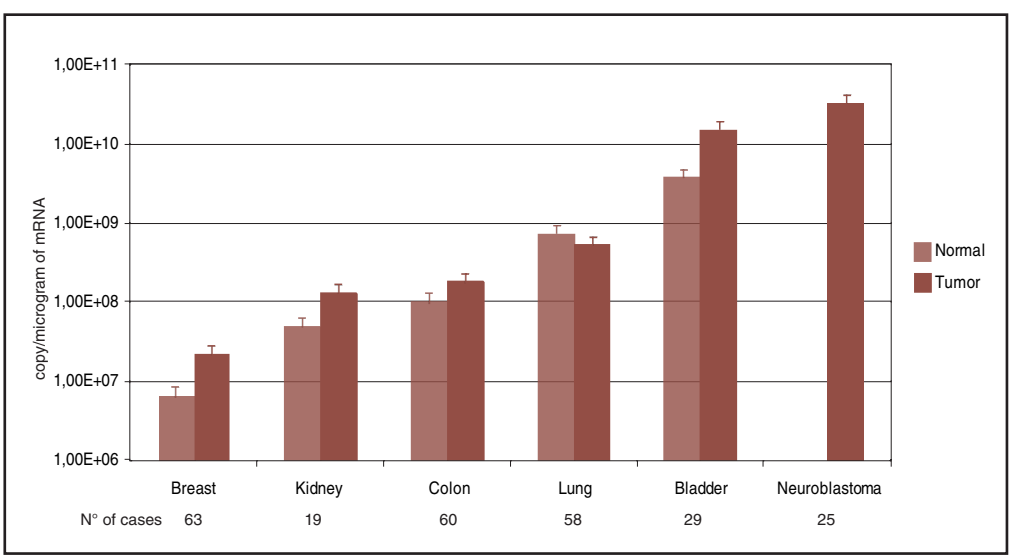

Figure 1 - Expression of LMW-PTP in healthy and tumor tissue. Data are presented in semilogarithmic scale

Research Grants

Year	Funding Agency	Amount
2008-2011	European Union	€ 350,000 (for all the University of Florence Units)
2008-2010	Istituto Toscano Tumori	€ 102,000
2003-2006	Pharmamar Madrid	€ 42,000

Main Collaborations

With Units within ITT

» Other Departments of the University of Florence

With other Italian and Foreign Institutions/Organizations

» Hospitex Diagnostics Company, Sesto Fiorentino (Firenze)
» Institut Pasteur, Paris (France)
» National Centre for Sensor Research (NCSR), Dublin City University (Ireland)

Publications

1. Taddei ML, Parri M, Angelucci A, et al: *Kinase-dependent and -independent roles of EphA2 in the regulation of prostate cancer invasion and metastasis*. Am J Pathol 2009; 174: 1492-503.
2. Marzocchini R, Malentacchi F, Biagini M, et al: *The expression of low molecular weight protein tyrosine phosphatase is up-regulated in 1,2-dimethylhydrazine-induced colon tumours in rats*. Int J Cancer 2008; 122: 1675-8.
3. Taddei ML, Parri M, Mello T, et al: *Integrin-mediated cell adhesion and spreading engage different sources of reactive oxygen species*. Antioxid Redox Signal 2007; 9: 469-81.
4. Parri M, Buricchi F, Giannoni E, et al: *EphrinA1 activates a Src/focal adhesion kinase-mediated motility response leading to rho-dependent actino/myosin contractility*. J Biol Chem 2007; 282: 19619-28.
5. Fiaschi T, Cozzi G, Raugei G, Formigli L, Ramponi G, Chiarugi P: *Redox regulation of beta-actin during integrin-mediated cell adhesion*. J Biol Chem 2006; 281: 22983-91.
6. Giannoni E, Raugei G, Chiarugi P, Ramponi G: *A novel redox-based switch: LMW-PTP oxidation enhances Grb2 binding and leads to ERK activation*. Biochem Biophys Res Commun 2006; 348: 367-73.
7. Taddei ML, Chiarugi P, Cuevas C, Ramponi G, Raugei G: *Oxidation and inactivation of low molecular weight protein tyrosine phosphatase by the anticancer drug Aplidin*. Int J Cancer 2006; 118: 2082-8.
8. Malentacchi F, Marzocchini R, Gelmini S, et al: *Up-regulated expression of low molecular weight protein tyrosine phosphatases in different human cancers*. Biochem Biophys Res Commun 2005; 334: 875-83.
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10. Chiarugi P, Taddei ML, Schiavone N: *LMW-PTP is a positive regulator of tumor onset and growth*. Oncogene 2004; 23: 3905-14.
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12. Chiarugi P, Cirri P, Taddei ML, et al: *New perspectives in PDGF receptor downregulation: the main role of phosphotyrosine phosphatases*. J Cell Sci 2002; 115: 2219-32.
13. Chiarugi P, Cirri P, Taddei ML, et al: *Insight into the role of low molecular weight phosphotyrosine phosphatase (LMW-PTP) on platelet-derived growth factor receptor (PDGF-r) signaling. LMW-PTP controls PDGF-r kinase activity through TYR-857 dephosphorylation*. J Biol Chem 2002; 277: 37331-8.
14. Raugei G, Ramponi G, Chiarugi P: *Low molecular weight protein tyrosine phosphatases: small, but smart*. Cell Mol Life Sci 2002; 59: 941-9.
15. Taddei ML, Chiarugi P, Cirri P, et al: *Beta-catenin interacts with low-molecular-weight protein tyrosine phosphatase leading to cadherin-mediated cell-cell adhesion increase*. Cancer Res 2002; 62: 6489-99.
16. Chiarugi P, Fiaschi T, Taddei ML, et al: *Two vicinal cysteines confer a peculiar redox regulation to low molecular weight protein tyrosine phosphatase in response to platelet-derived growth factor receptor stimulation*. J Biol Chem 2001; 276: 33478-87.
17. Fiaschi T, Chiarugi P, Buricchi F, et al: *Low molecular weight protein-tyrosine phosphatase is involved in growth inhibition during cell differentiation*. J Biol Chem 2001; 276: 49156-63.

INTERNAL MEDICINE

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Introduction

This Research Unit studies metastasizing mechanisms involving the genes responsible for the suppression of cellular motility. In particular, we have unveiled the role of tetraspanin CD81-phosphoinositide 4 kinase complex as a suppressor of cellular motility. We have discovered that CD81 functions as a metastasis suppressor gene.

Main Research Theme

Cell and molecular biology of carcinoma progression and metastasis

Main achievement: Specifically, the mechanisms by which some cell surface molecules impact tumor cell invasion and metastasis is being investigated. The goal is to decipher the complex mechanisms by which these molecules impact the behavior of cancer cells in colonizing distant sites, with the ultimate goal of developing specific drugs which could interfere with the processes by which metastatic tumor cells invade and ultimately colonize distant organs.

Recent research efforts have focused on tetraspanin CD81, a molecule that belongs to the tetraspanin family. Tetraspanins are a large family of membrane proteins that are implicated in cell fusion, differentiation and tumor invasion. Specifically, tetraspanin CD81 has been involved in cell proliferation but the mechanism is unknown. Research has been focused on the regulation and function of CD81 in tumors. The Unit previously discovered a novel mechanism of ERK/MAPKinase activation during tumor cell proliferation. Further focus on the role of CD81 brought about the discovery of a new type of intracellular vesicles that are involved in remodeling cellular cortex and ultimately in controlling metastatic cell motility. CD81 might inhibit hepatoma cell motility by sequestering alpha-actinin-4 in CD81-enriched vesicles with subsequent remodeling of the actin cytoskeleton. Studies on the role of tetraspanin CD81 as a metastasis suppressor gene have become clear through unraveling biochemical processes that mediate the steps of the metastatic cascade in liver cancer (2).

Future work: Our research now addresses the role of cell fusion. Cell fusion may play a fundamental role in cancer development. The ability to fuse is not limited to a particular cell or tumor type and can occur between tumor cells as well as between a tumor and a normal cell. The ultimate goal of our studies is to unveil the mechanisms of cancer cell fusion, in particular, addressing the role of the tetraspanin/ADAM10-related multi-protein complex in promoting fusion of cancer cells and therefore contributing to their epigenetic malignant progression both *in vitro* and *in vivo*.

The results of these studies will provide important new information on some uncharacterized mechanisms in the progression of cancer. The information derived from this study may have two relevant immediate consequences in the development of more efficient pharmacological strategies against cancer. Firstly, the possibility of targeting hidden actions of multi-task metalloproteases, such as ADAM10, and particularly its contribution to cancer cell fusion. Secondly, a better understanding of the biology of fused cancer cells, will contribute to better use of cytotoxic drugs in the management of cancer.

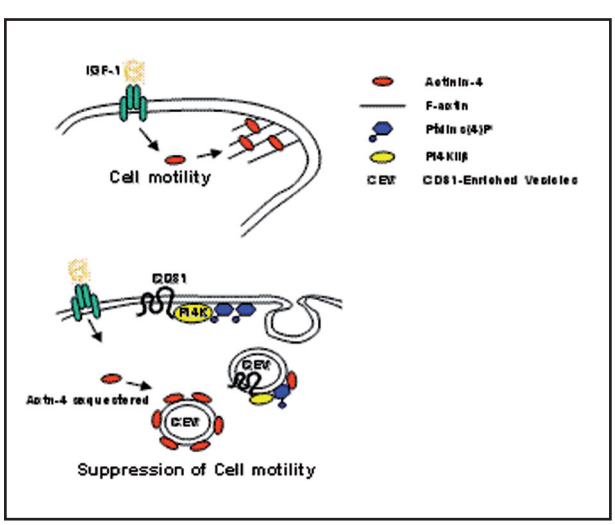

Figure 1 - Model for CD81-PI4KIIβ complex in cell motility suppression. Upon IGF-1 stimulation, the CD81-PI4KIIβ complex promotes the formation of CD81-Enriched Vesicles (CEV) that recruit and sequester actinin-4, subtracting this protein to the actin-bundling process and lead to the arrest of cell motility. The function of CEV is dictated by the presence of PI4KIIβ

Research Grant

Year	Funding Agency	Amount
2004-2006	Ministero dell'Istruzione, dell'Università e della Ricerca – PRIN	€ 56,000

Main Collaboration

With other Italian and Foreign Institutions/Organizations

» Dana Farber Cancer Center, Boston (USA)

Publications

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PEDIATRIC HEMATOLOGY-ONCOLOGY

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Anna Maria Buccoliero, Pathologist
Franco Bambi, Head Transfusion Medicine

Introduction

The Department takes care of diagnosis and treatment of patients with cancer of any type, aged from 0-18 years. It also cares for selected young adults with cancer types which are typical of the pediatric age.
All patients are enrolled in current therapeutic trials run by cooperative bodies, such as the Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) or the International Society of Pediatric Oncology (SIOP).
The Department is also the national referral center for histiocytosis in children and adults.

Main Research Themes

1. Histiocytosis

The Head of the Department chairs the International Registry for Familial Hemophagocytic Lymphohistiocytosis (FHL), a congenital immune deficiency with an invariably fatal outcome. For clinical diagnosis validation and for genetic analysis, data and blood samples are referred from Italy and other countries (England, Spain, Eastern Europe, South America).

The cooperative study for Langerhans Cell Histiocytosis (LCH), an intriguing disease with an unclear origin and regarded as cancer by many investigators, is also chaired by the Principal Investigator (PI). To address this issue, research is also conducted in the field of genetics, immunity and pathogenesis of the disease.

The PI chairs the therapeutic trials for FHL and LCH in Italy.

2. Brain tumors

The team serves as a Referral Unit for over 40 new cases per year. Research programs are ongoing along several lines, including modification of the blood brain barrier selectivity to allow accumulation of chemotherapy agents inside the brain tumor tissue.

Experimental animal models for brain tumor are being prepared to investigate novel therapeutic approaches. Expression of selected molecules, receptor or oncogenes are also being investigated in brain tumor tissues.

3. Childhood leukemia

The PI serves as a national clinical coordinator for the front-line treatment protocol AIEOP-BFM-ALL 2000, and as a national coordinator of the EsPhALL protocol for treatment of Ph+ childhood Acute Lymphoblastic Leukemia (ALL); he is member of the international cooperative working group on childhood ALL, also called the “Ponte di Legno Group”, which carries on research on selected, rare subgroup of ALL. In this setting, he has performed the largest worldwide clinical study on patients with childhood Ph+ ALL (NEJM, 2000).

4. HSC Transplantation

Hematopoietic stem cell transplantation is performed from match related, unrelated or alternative donors including cord-blood for treatment of malignancies or to correct inborn errors, including immune deficiencies.

Research Grants

Year	Funding Agency	Amount
2008	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 50,000
2007-2009	AIRC	€ 70,000
2004	Ministero della Salute – Ricerca finalizzata	€ 120,000
	European Union	€ 152,700

Main Collaborations

With Units within ITT

» Department of Neurosciences, AOU Meyer, Firenze
» Oncological and Reconstructive Orthopedics Unit, Azienda Ospedaliero Universitaria Careggi (AOU Careggi), Firenze
» Department of Pediatric Ophthalmology, AOU Careggi, Firenze

With other Italian and Foreign Institutions/Organizations

» Pharmacology Institute, University of Florence
» Istituto Nazionale per la Ricerca sul Cancro (IST), Genova
» Istituto "Giannina Gaslini", Genova
» Department of Pediatrics, University of Padua
» Istituto Nazionale Tumori (INT), Milano
» University of Lyon (France)
» Karolinska Institut, Stockholm (Sweden)
» St. Jude Children's Research Hospital (SJCRH) Memphis, Tennessee (USA)
» Children's Hospital Boston, Harvard University, Massachusetts (USA)

Publications

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PEDIATRIC BRAIN TUMORS

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Introduction

There are many types of brain tumors in children. The more common types of brain tumors in children are astrocytomas, medulloblastomas, ependymomas, germ cell tumors and brainstem gliomas. For most of these tumors the gold standard treatment is surgery; however, they can also be sensitive to chemotherapy and/or radiotherapy.

We are one of the first centers in Italy to provide care for brain tumors using a multidisciplinary team of people from many health fields, referred to as the Neuro-oncology Team.

The mission of the Neuro-oncology Team is to provide the best possible patient-focused care and state-of-the-art treatment for patients with pediatric brain tumors. Members of the Neuro-oncology Team include experts in these categories: Neurosurgery, Pediatric Oncology, Radiation Oncology, Neuropathology, Neuroradiology.

Main Research Themes

1. Pediatric brain tumors: molecular biology

a) *Analysis of expression pattern of chemoresistance-related genes in pediatric brain tumors*. Chemotherapy in solid tumors is poorly effective, partly due to intrinsic or acquired drug resistance of tumor cells which leads to unsatisfactory outcome. Resistance to chemotherapy in pediatric brain tumors is complex and may involve multiple mechanisms. These may include DNA repair systems, efflux of cytotoxic agents by transmembrane transport proteins, DNA damage accumulations, enhanced detoxification of alkylating agents.

We are investigating the expression of several chemoresistance-related genes, alkylated repair protein alkB homolog 2 and 3 (ALKBH2, ALKBH3), O-6-methylguanine-DNA methyltransferase (MGMT), P-glycoprotein (MDR), Multidrug Resistance Protein (MRP) 1, Topoisomerase-2α (TOPO-2α) and Glutathione-S-Transferase α (GST-α) in malignant pediatric brain tumors. Up to now, we have analyzed eight histologically identified samples: four anaplastic ependymomas, one ganglioglioma, one glioblastoma (GBM), one medulloblastoma and two non-neoplastic brain tissues as normal controls. The different expression of the target genes normalized to 18S rRNA were calculated using the DD*C*t method, DD*C*T = (*C*T,Target - *C*T,Endo)Goi - (*C*T,Target 2 *C*T,Endo). The expression levels of each of the analyzed genes were generally higher in brain tumors than in non-neoplastic brain tissues. Alkbh2 and Alkbh3 had a similar expression pattern in normal tissue, while almost all the tumors showed a higher Alkbh2 expression. The expression profile was similar for MGMT and MDR; samples with a high expression of MGMT showed high expression of MDR and, in some cases, also GSTP. MRP was expressed at low levels in the controls; all samples, particularly the medulloblastoma, showed increased expression. Anaplastic astrocytomas and medulloblastoma showed an increased expression in the GSTP gene. TOPO-2α is expressed at very low levels in normal samples and all neoplastic samples, except ependymoma, showed a marked increment. MRP expression was detected in normal samples and at higher levels in tumors.

Our work represents a study on expression patterns of the genes possibly related to drug resistance in pediatric brain tumors.

b) *Analysis of microRNA expression profile in pediatric brain tumors*. MicroRNAs are single-stranded RNA molecules of 19-24 nucleotides in length that do not encode proteins. They do, however, regulate post-transcriptional genetic expression through imperfectly pairing with sites in the 3'-UTR of their target mRNAs, leading to cleavage of mRNAs, or directly inhibiting translation processes. Several data indicate microRNAs are key regulatory molecules of biological mechanism, such as cellular differentiation, cellular proliferation, apoptosis, development, anti-viral defense and tumorigenesis.

Recently, studies have shown distinct patterns in microRNA expression in adult GBM and have indicated that microRNAs play a role in the genesis of glioma, including cell proliferation, invasion, glioma stem cell behavior, and angiogenesis.

We have studied a set of five microRNA (miR-21, miR-7, miR-124, miR-137 and miR-128) using quantitative RT-PCR in paraffin-embedded tissue of ten pediatric GBMs. Our results showed overexpression of miR-21 in all samples, while the others microRNAs (miR-7, miR-124, miR-137 and miR-128) showed low expression *versus* our control (the brain biopsy of a pediatric patient who underwent brain surgery for any reason other than the presence of tumor). Our data suggest that upregulation of miR-21 and downregulation of the other miRs investigated may contribute to GBM. These preliminary data seem similar to those reported in the literature concerning case studies on adult GBM.

In the future, we want to extend the series of pediatric GBM to validate the accuracy of our data and we would like to compare quantitatively the differences in the expression profile, a set of five microRNA in pediatric GBM, against those in adulthood.

c) *Role of β-adrenergic receptors in pediatric brain tumor neoangiogenesis*. Microvessel β-adrenergic receptors have been well characterized in human and animal brain. They seem to sub-serve the regulation of capillary function in both physiological and pathological conditions. Three subtypes have been distinguished: β1, β2 and β3-adrenergic receptors. Brain tumors are supplied by vessels that differ from those supplying normal cerebral tissue in various structural and functional parameters. In order to study the characteristics of brain tumor microcirculation, we investigated the presence of β-adrenergic receptors in different types of highly vascularized pediatric tumors (3 anaplastic ependymoma, 6 Medulloblastoma and 3 high grade glioma, HGG) using qRT-PCR and immunohistochemistry of β-adrenergic receptors (β1, 2 and 3). The preliminary expression data indicates the presence of β1 and β2 receptors and the absence of β3 receptor expression in all types of pediatric tumors in our study *versus* our control (Universal Human Reference RNA, Stratagene). Our study showed the feedback of β1 and β2 receptor expression in microvascularization and could represent a main mechanism in regulation of tumor vascularization. Thus, beta blockers alone or in combination with chemotherapeutic agents could play a relevant role in blocking neoangiogenesis in pediatric brain tumors.

2. Pediatric brain tumors: Preclinical study

Sub-chronic morphine administration facilitates doxorubicin penetration into the brain of the rat. A prerequisite for the efficacy of an anti-neoplastic agent is that it reaches the tumor at a therapeutic concentration. For brain tumors, this mechanism is complicated by the presence of the Blood Brain Barrier (BBB), which is a major physical and physiological hurdle for the delivery of chemotherapics into the brain. On the basis of previous results that demonstrated the ability of morphine to generate severe, yet fully reversible disruption of the barrier, we attempted analyzing the crossing of doxorubicin into the brain in a rat model.

We carried out a quantitative analysis of doxorubicin (12 mg/kg, i.p.) using mass spectrometry in rats after co-administration with morphine (10 mg/kg, i.p.). All animal manipulations were carried out according to the European Community guidelines for animal care (DL 116/92, application of the European Communities Council Directive 86/609/EEC). We analyzed the safety of this combination by detecting doxorubicin levels in heart and kidney, the plasma LDH activity and tissue lipid peroxidation.

The level of doxorubicin was significantly higher in all brain areas of rats treated with morphine than in the controls. We found significant statistical differences in doxorubicin levels of treated rats compared to the control groups ($P < 0.001$). No difference in LDH activity was found between rats treated with doxorubicin alone and rats also treated with morphine. Lipid peroxidation was not statistically different among the treated and control groups.

This result will permit us to generate new chemotherapy approaches for treatment of brain tumors or other neurological malignancies by administration of agents that usually do not cross the BBB.

3. Pediatric brain tumors: therapy

a) *Intracavitary chemotherapy (Gliadel®) and daily, oral, low-dose etoposide for recurrent anaplastic ependymoma*. Anaplastic ependymoma is associated with a higher incidence of tumor recurrence and its prognosis still remains unsatisfactory. A characteristic of anaplastic ependymoma is recurrence at the primary site and just a small number of patients present evidence of dissemination. Beyond surgery, novel approaches that aim to improve the outcome and quality of life are now available. Intracavitary chemotherapy has considerable clinical implications in the treatment of malignant brain tumors. This approach allows us to bypass the BBB, obtaining a high concentration of BCNU into the tumor bed, protecting the drug from early degradation and minimizing systemic side effects and toxicity. Beyond the resistance of anaplastic ependymoma, a high local concentration of carmustine in association with low-dose chemotherapy could determine efficiency in prolonging survival.
We expected to establish the feasibility of administering low-dose oral etoposide in combination with intracavitary carmustine (BCNU) wafers (Gliadel®) implantation at the gross total resection for achieving synergistic treatment in children affected by recurrent anaplastic ependymoma. All patients had to present a Karnofsky Performance Scale (KPS) score > 80%. Our preliminary data on a few patients showed that the therapy was safe and well tolerated without any post-surgery complications in all patients. After BCNU wafer implantation, all patients achieved radiological and clinical stabilization for a mean period of three months. However, the patients relapsed after a few months as a brain MRI control examination showed. This multimodal approach was not effective for treatment of refractory tumors, such as anaplastic ependymoma. However the association of intracavitary with systemic therapy could be a useful method for controlling this aggressive disease.

b) *The role of transventricular neuroendoscopy in the management of brain tumor-related hydrocephalus in a pediatric population*. In the last decade, neuroendoscopy has been increasingly used in the management of intraventricular tumors (lateral and third ventricle) for resolution of the mass effect on the visual pathway and control of hydrocephalus. Neuroendoscopy shows great versatility in every intraventricular cystic or solid lesion associated to hydrocephalus. The same procedure can be used for controlling hydrocephalus associated with posterior fossa tumor. We also use neuroendoscopy for biopsy sampling of ventricle lesions, and we consider it an efficient, simple and low-risk procedure. We adopt this treatment in all patients with signs and symptoms of hydrocephalus (headache, vomiting and diplopia) due to obstruction of the cerebral aqueduct (pineal tumor) as well. In patients with hydrocephalus caused by posterior fossa tumors, we prefer to perform as a first step an endoscopic third ventriculostomy, finalized to reducing acute brain hypertension. Endoscopy can be used alone to achieve gross total removal or marsupialization of cystic tumors, sometimes in association with additional therapies, such as microsurgery, radiosurgery or intracavitary drug administration. The aim of our study is to assess the reliability of neuroendoscopy in the management of hydrocephalus secondary to brain tumor in a pediatric population.

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Clinical Trial

Description	Year	Sponsor	Number of patients recruited to date
Temozolomide and weekly vinorelbine (Navelbine®) for therapy of relapsed medulloblastoma. Mono-institutional study (submitted to Bio-ethical Board)	2009		

Research Grants

Year	Funding Agency	Amount
2008-2010	Associazione “Noi per Voi” Onlus	
2008-2010	Associazione Italiana per la Ricerca sul Cancro (AIRC)	
2006	Ministero dell’Istruzione, dell’Università e della Ricerca (MIUR)	
2006	AOU Careggi, Firenze	
2005	MIUR	

Main Collaborations

With Units within ITT
» Pediatric Hematology-Oncology Unit, AOU Meyer, Firenze
» Department of Clinical Physiopathology, AOU Careggi, Firenze
» Department of Pathology and Oncology, AOU Careggi, Firenze
» Department of Pharmacology, University of Florence

With other Italian and Foreign Institutions/Organizations
» Istituto Nazionale Tumori (INT), Milano

Publications

1. Spacca B, Amasio ME, Giordano F, et al: *Surgical management of congenital median perisellar transsphenoidal encephaloceles with an extracranial approach: a series of 6 cases.* Neurosurgery 2009; 65: 1140-5.
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CELL THERAPY

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Introduction

Cellular therapies are increasingly being explored for both regenerative stem cell therapy and immunotherapy T, NK cells; Mesenchymal Stem Cells (MSC) purposes. The Cellular Therapy Laboratory was specifically developed to provide access to cellular therapies for Meyer Hospital, being the pediatric

care referral center in Tuscany. This program was designed to comply with international regulations of good laboratory and manufacturing practices (GLP, GMP).
Research activity is currently focusing on the use of partially matched family donors for the treatment of malignancy, and standardizing of procedures for *in vitro* MSC expansion and their phenotypical evaluation.

Main Research Theme

Development of-GMP compliant conditions for MSC culture

a) Isolation and expansion of MSC with serum-free media according to GMP criteria

Under appropriate conditions MSC may differentiate *in vitro* into one of many cell phenotypes. The ability of MSC to differentiate into several tissues after engraftment has been confirmed *in vivo* following systemic injection. It is necessary to expand and characterize human MSC in animal-serum-free media to improve the compliance of the MSC infusions in patients to Good Clinical Practices (GCP).

For safety reasons, medical practitioners are compelled to avoid the use of animal serum to expand the cells for therapeutic purposes in humans, while serum-free media or human serum and plasma can be used.

To move from the preclinical findings of any study dealing with MSC to a clinical setting it is mandatory to produce the cells in the appropriate manner.

In order to reach this target, the laboratory of the Transfusion Center is provided of a controlled contamination area consistent with European GMP standards. The facility is characterized by an A/B grade area for sterile processing and by a C grade area for less crucial procedures with a PCL2 biohazard level. A continuous particle monitoring system is already present, as established by the recent revision of the European GMP.

Main achievements: Platelets play a fundamental role in hemostasis and tissue repair and are a safe and readily available source of growth factors for cell culture. In collaboration with the Laboratory of Cell Biology and Advanced Cancer Therapies, University of Modena, a protocol for MSC *ex-vivo* expansion with autologous Platelet-Rich Plasma (PRP) has been established.

Current work: Currently a collaborative effort is underway to establish a protocol for the culture and expansion of MSC according to European GMP standards for clinical use.

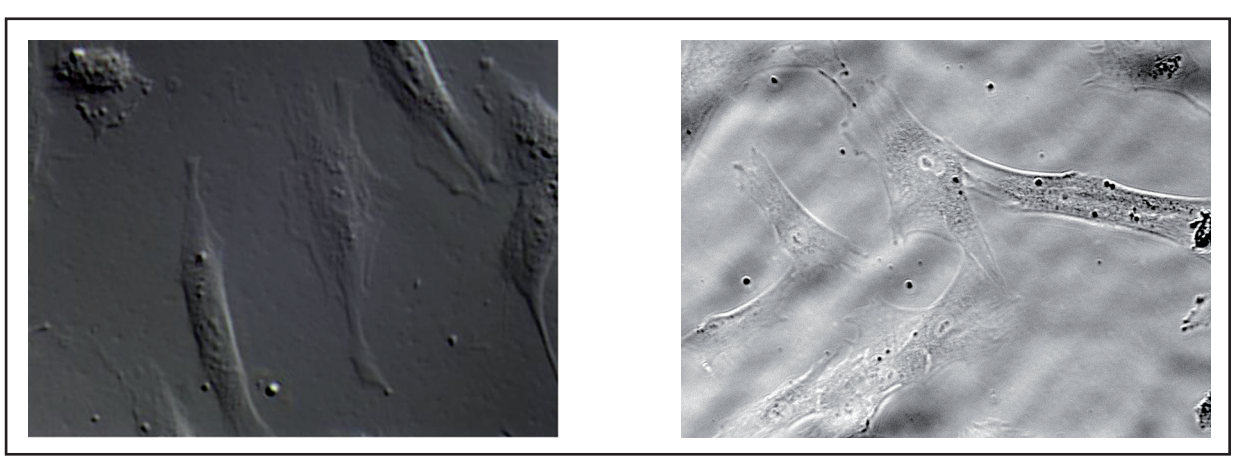

Figure 1 - Mesenchymal Stem Cells (MSC)

Future work: The serum-free MSC and serum-dependent MSC will be tested analyzing telomere length and telomerase activity in cultures by real-time PCR. These parameters are well established markers for long-lived cells and generally ensure the plasticity of MSC. In addition, *in vitro* plasticity of serum-free MSC will be tested using the standard protocol for bone cartilage and fat differentiation. Similarly, *in vivo* ability of a multi-tissue engraftment will be tested in the SCID model.

Once a protocol for the *ex-vivo* expansion of MSC has been established with PRP, according to GMP standards for clinical use, it will be validated in a clinical setting.

b) Development of a tissue-engineered construct to improve allograft integration for osteosarcoma patients

Osteosarcoma is a primary malignant tumor of the skeleton that occurs mainly in young patients.

Before chemotherapy was introduced, all patients with osteosarcoma were treated by amputation and almost all patients died within a year from diagnosis. Today the treatment is pre- and postoperative chemotherapy associated with surgery and the percentage of patients cured varies between 60% and 70%. Surgery is conservative (limb salvage) in more than 90% of patients and most of them require reconstruction.

Massive bone allografts are used with increased frequency in reconstructive surgery to replace missing bone parts, such as critical size defects. The effectiveness of the procedure depends on healing time and the type of graft host integration. Although most massive allografts have long-term success, 25% of reconstructions fails. To improve the integration of the graft, a tissue-engineered construct can be used. A tissue-engineered construct can be composed of stem cells, growth factors and biodegradable biomaterial.

Main achievements: MSC can differentiate into various cells of mesenchymal origin, among them bone. MSC have been shown to be effective in preclinical studies to regenerate bone in massive defects.

Current work: The composition and the safety of a tissue-engineered construct are currently under study. It is important that the biomaterial utilized is biodegradable and compatible with the differentiation of the MSC toward the osteogenic lineage. The Cell Therapy Laboratory is also involved in the European project REBORNE (see clinical trials).

Clinical Trial

Description	Year	Sponsor	Number of patients recruited to date
Project REBORNE for “Regenerating bone defects using new biomedical engineering approaches”	2008	European Union	

Research Grant

Year	Funding Agency	Amount
2009-2010	Associazione “Noi per Voi” Onlus	

Main Collaborations

With Units within ITT
» Oncological Orthopedics Unit, Azienda Ospedaliero Universitaria Careggi (AOU Careggi), Firenze
» Department of Experimental Pathology and Oncology, University of Florence

With other Italian and Foreign Institutions/Organizations
» Laboratory of Cell Biology, University of Modena and Reggio Emilia
» Laboratory of Bone Tissue Regeneration, Istituti Ortopedici Rizzoli, Bologna
» Laboratory of Cell Therapies Cardiocentro Ticino, Lugano (Switzerland)
» Etablissement Français du Sang, Bordeaux (France)

Publications

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OCCUPATIONAL AND ENVIRONMENTAL EPIDEMIOLOGY

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Introduction

The Unit is involved in:

1. Health monitoring and registration.
2. Surveillance studies on environmental cancer risks and lifestyles.
3. Surveillance studies on occupational risks.
4. Analytical studies on malignancies related to occupations, environment and lifestyles.
5. Intervention studies.

Main Research Themes

1. Health monitoring and registration

a) Regional Mortality Register (RMR): The RMR has been collecting and processing mortality data for the whole of Tuscany since 1987. About 41,000 death certificates are collected every year. Yearly, the RMR produces mortality rates (crude, age, sex and area specific, and standardized to the European population) and temporal and geographical trends.
The RMR cooperates with the Tuscan Tumor Register on performing survival analyses of cancer cases, with the Regional registers of occupational cancers and work related diseases and accidents. The RMR is involved in the mortality follow-up of cohort studies.

b) Regional Mesothelioma Register (COR): The COR is part of the National Register of Mesotheliomas. The Unit registered more than 1,000 mesothelioma cases diagnosed in the Tuscan population since 1988. Interviews to determine any possible history of asbestos exposure are conducted. The COR contributes to epidemiological studies on survival, latency evaluation, asbestos impact and incidence/mortality prediction.

c) Regional Sino-nasal Cancer Register: The Regional Registry of Sino-nasal Cancer has been active since 2005, estimating the incidence of sino-nasal cancer in Tuscany. Interviews to determine possible occupational exposure to wood and leather dusts are conducted. The Unit is involved in the implementation of the National Sino-nasal Cancer Register.

2. Surveillance studies on environmental cancer risks and lifestyles

The Unit is involved in *a*) the regional study on indoor pollution in schools; *b*) the regional project on UV solar Risk in outdoor workers aimed at studying the UV exposure in workplaces and behaviours of outdoor workers and the frequency of cutaneous effects (photocheratitis, erythema, precancerous lesions, non melanocitic skin cancer); *c*) the national longitudinal study on mortality and morbidity of Florentine families involved in the international ISAAC study; d) the regional longitudinal SIDRIAT study, funded by ITT, on the existing SIDRIA cohort that will allow to follow-up more than 37,000 residents from 13 to 75 years old enrolled 7-15 year ago, aiming to study the predictors of quit attempts and cessation among adult smokers, and the effects of household smoking habits on adolescents' initiation, before and after the introduction of the Italian smoking ban.

Unit is also conducting studies on passive smoking in workplaces and restoration places (bars, restaurants, discos), in cooperation with a national study 'group, aiming to evaluate the impact of the Italian Low banning tobacco smoking from public places and workplaces.

3. Surveillance studies on occupational risks

The Unit is involved in the Longitudinal Tuscan Study of socio-economic inequalities, coordinated by the Department of Statistics of the University of Florence and in the Occupational Cancer Monitoring

(OCCAM) project, coordinated by the National Cancer Institute (INT) in Milan in cooperation with the National Institute for Occupational Safety and Prevention (ISPESL).

4. Analytical studies

a) Cohort studies on workers involved in the relevant regional productive activities are conducted, in particular in occupations entailing exposure to solvents, asbestos, pesticides and silica.

b) Case control studies on malignancies related to occupations, environment and lifestyles: the Unit coordinates the "Multicenter Italian Case-Control Study on Haematolymphopoietic Malignancies and Exposure to Pesticides and Solvents". The Unit participates to the network InterLymph (http://epi.grants.cancer.gov/InterLymph/exec.html). Risk of cancer associated to lifestyles or environmental and occupational exposures is under investigation and is involved in the multicenter study "Gene-environment interaction in etiology of non-Hodgkin Lymphomas" coordinated by the University of Cagliari.
The Unit is involved in the SETIL study (Multicenter Case-Control Study on Leukemia, Non-Hodgkin's Lymphoma and Neuroblastoma Etiology in Children).

c) The Unit participates in the multinational case control study on brain tumors (MOBYKIDS) diagnosed in young people aimed at investigating the relation between brain tumors and electromagnetic field exposure from mobile phones and others sources of radio frequencies (recruitment of about 3,300 cases are expected).

5. Intervention studies

The Unit developed an intervention procedure regarding motivation to quit smoking addressed to women attending the service on the prevention of cervical cancer. The Unit is conducting a Worksite Health Promotion Intervention on smoking, diet and physical activity with the aim of measuring changes in behavioral risk factors after the delivery of the intervention.
The Unit is involved in the evaluation of national projects on educational interventions in children and adolescents on smoking.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
SPRINT study: randomized national multicenter trial on the efficacy of counseling addressed to women attending cervical cancer screening in reducing smoking prevalence and increasing motivation to quit	2009	Ministero della Salute	499
"Da noi non si fuma" study: randomized regional multicentric trial on the efficacy of an intervention procedure aimed at motivating women with children to live in a smoking free home and to drive a smoking free car	2009	Istituto Toscano Tumori	The recruitment will begin in June 2010

Research Grants

Year	Funding Agency	Amount
2009	Ministero della Salute/ISPESL	€ 94,000
2009	ITT	€ 176,000
2009	Regione Toscana	€ 95,000
2008	Regione Toscana	€ 78,000
2008	ITT	€ 60,000
2008	Ministero della Salute/Regione Toscana	€ 142,857
2008	Azienda Sanitaria di Firenze (ASF)	€ 73,500
2007	Regione Toscana	€ 55,800
2007	Regione Toscana	€ 120,000
2005	ISPESL	€ 64,557

Main Collaborations

With Units within ITT

» Pediatric Hematology-Oncology Unit, Azienda Ospedaliero Universitaria Meyer, Firenze
» Hematology Unit, Azienda Ospedaliero Universitaria Careggi (AOU Careggi), Firenze
» Radiotherapy Unit, AOU Careggi, Firenze
» Department of Pathology, AOU Careggi, Firenze
» Dermatology Unit, ASF
» Department of Dermatology, University of Florence

With other Italian and Foreign Institutions/Organizations

» CPO-Piemonte, Azienda Ospedaliera “San Giovanni Battista”, Torino
» Agenzia di Sanità Pubblica (ASP Lazio), Roma
» Centro Riferimento Oncologico (CRO), Aviano (Pordenone)
» Istituto Superiore di Sanità (ISS), Roma
» Istituto Nazionale Tumori (INT), Milano
» Istituto Nazionale per la Ricerca sul Cancro (IST), Genoa
» Istituto Superiore per la Prevenzione e la Sicurezza sul Lavoro (ISPELS), Roma
» Occupational Health Services, Regione Toscana, Firenze
» Public Health Agency, Barcelona (Spain)
» Imperial College, London (UK)
» Department of Work Environment University of Massachusetts, Lowell, Massachusetts (USA)
» InterLymph, USA (http://epi.grants.cancer.gov/InterLymph/exec.html)

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CLINICAL AND DESCRIPTIVE EPIDEMIOLOGY

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Introduction

The Unit is organized into three areas of activity and research, namely:

1. Cancer monitoring and epidemiological evaluation.
2. Epidemiological evaluation of screening programs and study of new technologies for early diagnosis.
3. Epidemiological evaluation of end of life care.

Main Research Themes

1. Cancer monitoring and epidemiological evaluation

The Tuscan Tumor Register (RTT) is a regional initiative that supplies epidemiological information on incidence, mortality (according to the RMR data), survival, time trends and prevalence of cancer.

Active since 1985, the RTT collects information on all cancer cases occurring in the resident population of the Local Health Units of Florence, Empoli and Prato, the RTT area (about 8,000 new cases are registered every year). The feasibility of the extension of cancer registration to the whole Tuscan Region has been tested and positively completed.

RTT data are used for national and international projects on cancer incidence, prevalence and survival. The RTT is a member of the Italian Association of Tumor Registers (AIRT), the European Network and the International Cancer Register Association. Data are published in the International Agency for Research on Cancer (IARC) publication *Cancer in Five Continents*. RTT data contribute to the AIRTUM database, a centralized archive which collects data from all the Italian cancer registries. Such an archive is under the responsibility of the Unit and is thus managed by the Unit. The AIRTUM database, which collects more than 1,800,000 cancer cases, is used for collaborative studies on different cancer epidemiology issues published in peer-reviewed papers, and on the Airtum website (www.registri-tumori.it), *e.g.* trends during 1998-2005, incidence and mortality update 2003-2005, childhood cancer, cancer among Italian women, survival, incidence, mortality and estimates. At the moment, a collaborative study on cancer prevalence is ongoing and an update on cancer survival is planned for the next year.

2. Epidemiological evaluation of screening programs (breast, cervix and colorectal) and study of new technologies for early diagnosis

The Unit is the headquarters of the National Observatory for Screening Monitoring headed by the Ministry of Health to support and evaluate the progress of Italian Screening programs for cervical, colorectal and breast cancers.

The Unit deals with the evaluation of new technologies by participating in experimental and clinical studies, on breast, lung, colorectal and prostate cancers and HPV in cervical cancer.

3. Epidemiological evaluation of end of life care

The Unit has been participating since the year 2000 in several international and national studies on end of life care. Furthermore, the Unit has contributed to the monitoring of the development of palliative care services in the Region of Tuscany, developing facilities for the collection of a minimum data set. For the period 2008-2010 the Unit was involved in three national studies on the implementation of the Liverpool Care Pathways in the Hospital (cluster randomized trial), the development of a national network of sentinel GPs for monitoring the quality of end of life care, and the description of palliative sedation in the residential care setting.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
Randomized study of the efficacy in reducing lung cancer mortality (ITALUNG)	2004	None	1,406
Multicenter study: evaluation of the efficacy of once-in-a-life sigmoidoscopy in reducing the incidence of colorectal cancer (CRC). SCORE1	1996	None	9,999 (1,596 in Arezzo)
New Technologies for Cervical Cancer (NTCC)	1996	None	94,670 (15,600 in Florence)
European Randomized Study on Prostate Cancer (Italian Center)	1995	None	162,243 (14,517 in Florence)
The use of commercially available personal UV-meters does cause less safe tanning habits: a randomized-controlled trial	1995	None	91

Research Grants

Year	Funding Agency	Amount
2009	ASR Abruzzo	€ 26,000
2009	Istituto Nazionale Tumori (INT) Milano	€ 75,000
2008	Regione Toscana	€ 370,000
2008	Regione Emilia Romagna	€ 47,000
2008	Istituto Nazionale per la Ricerca sul Cancro (IST) Genova	€ 10,000
2007	CPO-Piemonte	€ 22,000
2007	Ministero della Salute	€ 460,000
2007	Lega Italiana per la Lotta contro i Tumori (LILT)	€ 25,000
2007	Ministero della Salute, Progetto Integrato Oncologia	€ 310,000
2007	IST Genova	€ 71,340
2007	Regione Toscana	€ 451,472
2006	GlaxoSmithKline	€ 6,000
2006	Regione Toscana	€ 451,472
2006	Ministero della Salute	€ 220,000
2005	LILT	€ 60,339
2005	Regione Toscana	€ 451,472

Main Collaborations

With Units within ITT

» Dermatology Unit, Azienda Sanitaria di Firenze
» Dermatology Unit, University of Florence

With other Italian and Foreign Institutions/Organizations

- CPO-Piemonte, Azienda Ospedaliera "San Giovanni Battista", Torino
- Istituto Oncologico Veneto (IOV), Padova
- Centro Riferimento Oncologico (CRO), Aviano (Pordenone)
- Istituto Oncologico Romagnolo (IOR), Forlì (Forlì-Cesena)
- Agenzia di Sanità Pubblica (ASP Lazio), Roma
- Istituto Superiore di Sanità (ISS), Roma
- INT, Milano
- IST, Genova
- Vrije Universiteit, Brussels (Belgium)
- VU University Medical Center, EMGO Institute, Amsterdam (The Netherlands)
- Erasmus University, Rotterdam (The Netherlands)

Publications

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MOLECULAR AND NUTRITIONAL EPIDEMIOLOGY

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Introduction

The main aim of the Unit's activities is the identification of cancer risk factors in the general population and in high-risk subgroups, using specific etiological studies and biological markers of exposure, early damage and individual susceptibility. Results are contributing and will contribute to plan public health campaigns aimed to the primary prevention of cancer in the general population and in high-risk subgroups.

The Unit activities include a series of research projects, briefly grouped in the following areas:

1. Molecular and nutritional cancer epidemiology.
2. Cancer genetic epidemiology.
3. Epidemiology of stomach cancer, a tumor with high mortality/incidence rates in Tuscany.
4. Intervention studies in the field of cancer primary prevention.
5. Development and management of biological repositories and food composition databases.
6. Development and management of computer programs for the collection and analysis of data on dietary habits and physical activity.

Main Research Themes

1. Molecular and nutritional cancer epidemiology

The main aim of this research field is the identification of cancer risk factors linked to lifestyle, environmental exposures, medical history and individual susceptibility.

Main sub-projects:

a) The EPIC Study: A molecular epidemiology prospective project on diet, genetic susceptibility and cancer risk, based on a large local cohort enrolled in Florence (over 13,500 adult residents) as a section of the European Investigation into Cancer and Nutrition conducted in Italy and other nine countries and coordinated by IARC-WHO.

b) Dietary and lifestyle determinants of mammographic patterns and breast cancer risk in the EPIC-Florence cohort.

c) ENVIROGENOMARKERS - EU 7FP (Genomics and biomarkers of environmental exposures in breast cancer and lymphoma).

d) Evaluation of cancer risk in patients affected with Inflammatory Bowel Disease (IBD) based on the large Florence population-based cohort established in 1978-1992.

2. Cancer genetic epidemiology

Aim of this research field is the assessment of the role of individual predisposition in developing cancer, the identification of high-risk familial groups (particularly for breast and gastrointestinal tumors), and the identification of environmental exposures that can modulate cancer risk in carriers of constitutional alterations, or in members of high-risk families. Large well characterised consecutive series of cancer cases are available. Main sub-projects:

a) Molecular and genetic epidemiology of female breast cancer.

b) Molecular and genetic epidemiology of ovarian and/or female breast cancer in the frame of the Tuscany Cancer Family Project in close collaboration with the Universities of Florence and Pisa.

c) Molecular and genetic epidemiology of male breast cancer.

3. Epidemiology of stomach cancer, a tumor with high mortality/incidence rates in Tuscany

Aim of this research field is to evaluate the role of specific factors (diet, lifestyle, environmental exposures, infections, individual susceptibility) in developing gastric cancer in specific areas of the Tuscany with higher standardised mortality/incidence rates. Main sub-projects:

a) Molecular and nutritional epidemiology of gastric cancer on well characterized series with fresh frozen surgical specimens, and from high-risk area (Mugello and Casentino projects).
b) EUR-GAST (within the European project EPIC: Environmental factors, *Helicobacter pylori* infection, genetic susceptibility and gastric cancer risk in the European population).

4. Studies in the field of the primary prevention of cancer

Intervention studies are conducted in the field of primary prevention and are aimed at the reduction of cancer risk by diet and lifestyle changes, as well as chemoprevention studies. Measurement of biomarkers of intermediate biologic damage will be used for the validation of results in terms of efficacy. Main sub-projects:

a) PIO (Programma Integrato di Oncologia, Ministero della Salute - Regione Toscana: "Cancer Prevention: development of research based prevention models"; coordinator D. Palli, ISPO; website: www.pio-prevenzione-tumori.it).
b) The Massa-Viareggio project in a high mortality area of Tuscany.
c) A physical activity and dietary two year-intervention trial to reduce mammographic breast density in post-menopausal women at high risk of breast cancer.

5. Development and management of biorepositories and databases

a) A Biological Bank has been developed in 1992 when the EPIC project started, and is equipped with freezers and liquid nitrogen tanks for all molecular projects.
b) Database of Food Composition for Epidemiological Studies in Italy, is regularly updated for nutritional epidemiology studies in Italy and related to EPIC-Europe.

6. Programmes for the collection and analysis of Life-Style information on dietary habits and physical activity

Programmes have been developed to transform information on consumption frequencies of various foods in intake estimates of different nutrients and contaminants on the basis of composition tables, and are open for updates and modification according to the needs of projects with different tools for data collection and conversion tables.

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Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
DAMA project: a physical activity and dietary two-year intervention trial to reduce mammographic breast density, a strong risk factor for breast cancer, in post-menopausal women	2009	Istituto Toscano Tumori (ITT) and Lega Italiana per la Lotta contro i Tumori (LILT)	200

Research Grants

Year	Funding Agency	Amount
2009	European Union	€ 235,200
2009	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 100,000
2008	ITT	€ 300,000
2007	European Union	€ 159,494
2007	LILT	€ 50,000
2007	PIO Ministero della Salute	€ 488,282
2006	Regione Toscana	€ 27,000
2006	European Union	€ 97,369
2005	European Union	€ 249,480
2003-2008	AIRC	€ 760,000

Main Collaborations

With Units within ITT

» Breast Surgery Unit, Department of General Surgery, Department of Radiotherapy, Medical Genetics Institute, Department of Pathology, Azienda Ospedaliero Universitaria Careggi and University of Florence
» Medical Genetics Institute, Azienda Ospedaliero Universitaria Pisana and University of Pisa
» Department of Surgery, Department of Pathology, Ospedale “Santa Maria Annunziata”, Department of General Surgery, Ospedale “Nuovo del Mugello”, Azienda Sanitaria di Firenze

With other Italian and Foreign Institutions/Organizations

» Department of Experimental Medicine, “La Sapienza” University, Roma
» Department of Environment and Primary Prevention, Istituto Superiore di Sanità (ISS), Roma
» Department of Genetics, Biology and Biochemistry, University of Turin
» Epidemiology Unit, Istituto Nazionale Tumori (INT), Milano
» Department of Clinical and Experimental Medicine, “Federico II” University, Napoli
» Cancer Registry, Azienda Ospedaliera Civile “M.P. Arezzo”, Ragusa
» International Agency for Research on Cancer (IARC-WHO), Lyon (France)
» Department of Epidemiology, Catalan Institute of Oncology, Barcelona (Spain)
» Department Epidemiology and Public Health, Imperial College, London (UK)
» Cancer Research and Epidemiology Unit, University of Oxford (UK)
» MRC Cancer Epidemiology Unit, University of Cambridge (UK)
» National Hellenic Research Foundation, Athens (Greece)
» German Institute of Human Nutrition (DIFE), Potsdam-Rehbrücke (Germany)
» Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht (The Netherlands)

Publications

1. Palli D, Sera F, Giovannelli L, et al: *Environmental ozone exposure and oxidative DNA damage in adult residents of Florence, Italy*. Environ Pollut 2009; 157: 1521-5.
2. Vannucchi AM, Masala G, Antonioli E, et al: *Increased risk of lymphoid neoplasms in patients with Philadelphia chromosome-negative myeloproliferative neoplasms*. Cancer Epidemiol Biomarkers Prev 2009; 18: 2068-73.
3. Ottini L, Rizzolo P, Zanna I, et al: *BRCA1/BRCA2 mutation status and clinical-pathologic features of 108 male breast cancer cases from Tuscany: a population-based study in Central Italy*. Breast Cancer Res Treat 2009; 116: 577-86.
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5. Masala G, Bendinelli B, Versari D, et al: *Anthropometric and dietary determinants of blood pressure in over 7000 Mediterranean women: the European prospective investigation into cancer and nutrition-Florence cohort*. Journal of Hypertension 2008; 26: 2112-20.
6. Palli D, Saieva C, Munnia A, et al: *DNA adducts and PM(10) exposure in traffic-exposed workers and urban residents from the EPIC-Florence City study*. Sci Total Environ 2008; 403: 105-12.
7. Falchetti M, Saieva C, Lupi R, et al: *Gastric cancer with high-level microsatellite instability: target gene mutations, clinicopathologic features, and long-term survival*. Human Pathology 2008; 39: 925-32.
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9. Palli D, Falchetti M, Masala G, et al: *Association between the BRCA2 N372H variant and male breast cancer risk: a population-based case-control study in Tuscany, Central Italy*. BMC Cancer 2007; 7: 170.
10. Masala G, Ceroti M, Pala V, et al: *A dietary pattern rich in olive oil and raw vegetables is associated with lower mortality in Italian elderly subjects*. Br J Nutr 2007; 98: 406-15.
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ANALYTICAL AND BIOMOLECULAR CYTOLOGY

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Introduction

This Unit was established in 2002 and is involved in routine practice and research activity concerning diagnostic and prognostic biomarkers for cancer prevention. It is also involved in the development and management of biological banks.
The major research activity are:

1. Diagnostic molecular oncology: this section is involved in studies concerning specific genetic changes in carcinogenesis and their possible role in early diagnosis of cancer. Another sector of research activity concerns the application of molecular tests and their cost/efficacy comparison in the screening and early diagnosis of uterine cervical, colorectal, lung, prostate and bladder cancer.
2. Cancer risk factor: the mission of this section is to understand host response and carcinogenesis risk on genetic-environmental bases. Specific aims are to develop and validate intermediate biomarkers of cancer risk, as phenotypic markers of genetic susceptibility to cancer and cancer outcome, and to identify low penetrant traits in cancer susceptibility genes and gene-environment interactions affecting sporadic cancer risk and carcinogenesis in humans, considering the influence of dietary factors.

Main Research Themes

1. Human Papilloma Virus (HPV) Studies

a) HPV prevalence and risk factor in a cohort of Tuscan women, aged 18-24: the aim of the study is to evaluate the rate of prevalence, incidence, acquisition and clearance of HPV types in young women (18-24) and the risk factors correlated with such events. We are completing the 24-month Follow-up (FU). Two additional FU rounds (30 and 36 months) beyond the analysis of results.

b) Identifying HPV infection at high risk of progression to cervical cancer: the study of the molecular features associated with intraepithelial lesions and the evaluation of the potential advantage in CIN 2+ detection using this molecular marker in triage with HPV testing, instead of cytology. We are currently proceeding with the collection of samples. HR HPV-positive samples will be assessed: viral load, HPV integration, DNA methylation and E6/E7 overexpression. On blood samples of patients referred to colposcopy: Tumor specific T-cell immunity.

c) Using a self sampling device for the diagnosis of HPV in women not responding to an invitation to participate in a screening program for cervical cancer: the aim of this study is to evaluate the offer to women not responding to an invitation to participate in a cervical screening program to use instead a self-administered test for the diagnosis of HPV infection, in terms of acceptance by the users. Data analysis.

2. Project of integrated program “Risk prediction models, biomarkers predictivity, improving equity in cancer screening”: DNA stool triage and colorectal cancer screening

Purpose of this study was to evaluate the sensitivity and Negative Predictive Value (NPV) of molecular tests compared with CT, and the cost-effectiveness ratio of this triage strategy will be estimated based on the number of CTs potentially avoided. We are currently proceeding with the collection of samples and we have completed the molecular analysis (LDNA, DNA Quantification and MSI) on 150 samples. Molecular analysis on all 250 samples and data analysis.

3. Project of integrated program “Risk prediction models, biomarker predictivity, improving equity in cancer screening”: lung cancer screening through low dosage CT Scan and studies on the use of biomarker that are complementary to radiological testing

To assess the contribution of early biomarkers to lung cancer screening with a Low Dose CT Scan. A panel of biomarkers (microsatellite alterations, DNA plasma quantification, SNP) on plasma and sputum samples collected from on-going studies (ITALUNG_CT, I-ELCAP, Rome Branch) will be tested. A large biological bank will be available for further evaluation of the natural history of lung cancer as the follow-up of the subjects.

4. Oxyradical derived malondialdehyde DNA adducts, individual susceptibility and diet in colon and breast cancer

To assess the contribution of oxidative stress and oxidative DNA damage in the etiology of colon-rectum and breast cancers. We are analyzing the levels of malondialdehyde-dG adducts in cancer patients compared to controls, considering life-style habits. Our study will permit the identification of a specific pattern of DNA adducts to be used in cancer prevention studies.

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b) Cuzick J, Szarewski A, Cubie H, et al: *Management of women who test positive for high-risk types of human Papilloma virus: the HART study*. Lancet 2003; 362: 1871-6.
c) Henschke CL, McCauley DI, Yankelevitz DF, et al: *Early lung cancer action project: a summary of the findings on baseline screening*. Oncologist 2001; 6: 147-52.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
New Technologies for Cervical Cancer Screening: randomized controlled trial	2001-2012	European Union and Ministero della Salute	94,370

Research Grants

Year	Funding Agency	Amount
2009	Ministero della Salute	€ 49,000
2009	Istituto Toscano Tumori (ITT)	€ 60,000
2008	ITT	€ 210,000

Year	Funding Agency	Amount
2007	Ministero della Salute, Programma Integrato Oncologia	€ 200,000
2007	GlaxoSmithKline	€ 360,000
2007	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 150,000
2003	AIRC	€ 60,000

Main Collaborations

With Units within ITT
- Pathology Unit, Azienda USL 11 Empoli
- Pathology Unit, Azienda USL 12 Viareggio
- Pathology Unit, Azienda USL 8 Arezzo
- Clinical Trials Coordinating Center, ITT Core Research Laboratory, Firenze
- Pharmacology and Toxicology Unit, University of Florence

With other Italian and Foreign Institutions/Organizations
- Department of Public Health Care, University of Florence
- Registro Tumori del Veneto, Istituto Oncologico Veneto (IOV), Padova
- Istituto Superiore di Sanità (ISS), Roma
- CPO-Piemonte, Azienda Ospedaliera "San Giovanni Battista", Torino
- Agenzia di Sanità Pubblica (ASP Lazio)
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- University of Liverpool (UK)
- Institute of Cancer Research, London (UK)
- Department of Epidemiology and Biostatistics, Imperial College, London (UK)
- University of Maastricht (The Netherlands)
- International Agency for Research on Cancer, Lyon (France)
- Catalan Institute of Oncology, Barcelona (Spain)
- WHO Europe Reference HPV Laboratory, Geneva (Switzerland)
- CDC Atlanta, Georgia (USA)
- Northeastern University, Boston, Massachusetts (USA)
- National Cancer Institute, Bangkok (Thailand)

Publications

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SENOLOGY

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Introduction

The Unit, created in 1994, is mainly devoted to the diagnosis of self-referred women. The Unit is fully digitized and provided with modern diagnostic equipment. The activity is organized with the aim of promoting multidisciplinary cooperation and, in most cases, to achieve a final diagnosis in a single session.

The Unit includes follow-up a service, with about 3,500 breast cancer patients periodically invited for physical examination and mammography, and a section dedicated to women with high familial risk.

Research activity, often in cooperation with other Italian breast centers, is mainly addressed to the development of diagnostic protocols, validation of main imaging and needle biopsy techniques. The first Italian mammographic unit with direct magnification as well as the first Italian breast stereotaxic system were installed at the ISPO. The Unit has also contributed to the diagnostic evaluation of several technologies (*e.g.* thermography, measurements of the skin's electric potentials, computer-assisted diagnosis, etc.).
ISPO's contribution to the debate on the efficacy of instrumental follow-up in breast cancer, based mainly on a multicenter randomized clinical trial of over 1,200 patients, is well known at a national and an international level.

Main Research Themes

1. Technology assessment

Main achievement: Role of computer-aided detection in digital mammography; ultrasonography in dense breast; advantage and costs of real time reading in mammographic screening; role of fine needle aspiration cytology and core needle biopsy; costs of digital mammography.

Current work: Needle sampling of the breast, comparison between Fine Needle Cytology (FNAC) and Core Needle Biopsy (CNB).

Future work: Study the accuracy of galactography, a retrospective study.

2. Chemoprevention

Main achievement: TAM Study.

Current work: HOT Study.

Future work: TAM-01 Study.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
PIO-PROGETTO 5: a phase III, randomized trial to evaluate the effect of low-dose tamoxifen in women with intraepithelial neoplasia of the breast	2008-2010	Ministero della Salute	Recruitment not started yet
HOT Study: a phase III, randomized, multicenter trial to evaluate the efficacy of low-dose tamoxifen (*versus* placebo) as a preventive agent in menopausal women with HRT	2002-2008	Regione Toscana, Istituto Europeo di Oncologia	~140

Main Collaborations

With Units within ITT
» Breast Surgery Unit, Azienda Ospedaliero Universitaria Careggi (AOU Careggi), Firenze
» Genetics Unit, AOU Careggi, Firenze
» Breast Surgery Unit, Azienda Sanitaria di Firenze (ASF)

With other Italian and Foreign Institutions/Organizations
» Istituto Europeo di Oncologia (IEO), Milano
» Ente Ospedaliero Ospedali "Galliera", Genova

Publications

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ONCOLOGICAL SCREENING

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Introduction

The Screening Unit has been involved in population screening programs since 1964 when cervical cancer screening was implemented. During the 1970s, the first population mammographic screening program in Europe was launched and in the 1980s the fecal occult blood assay was introduced as a screening test. Several papers reporting the results of these activities and the evidence assessing oncological screening efficacy have been published in cooperation with other ISPO Units and external institutions. Since then, the Unit plays a reference role both at an Italian and an international level contributing to the definition of

guidelines on oncological screening. Moreover, ISPO has been appointed the Regional Reference Centre (CRR) for oncological prevention: a quality assurance program in the Tuscan Region is guaranteed by the use of quality indicator evaluations (a yearly Regional Report submitted over the last 10 years) and by periodic site-visits to each screening program which are carried out by a multidisciplinary team of experts.

Main Research Themes

1. Effectiveness studies and health technology assessment in oncological screening

Main achievements: The Unit reinforces the international literature in demonstrating that digital technology is comparable to conventional radiology in mammographic screening. Data are also consistent with organizational optimization and cost reduction.

Within a multicenter study, the high diagnostic accuracy of HPV as a primary screening test (New Technologies for Cervival Cancer Screening – NTCC) and the feasibility of HPV as a triage test in current practice had been assessed.

The correlation between Immunological Fecal Occult Blood Test (I-FOBT) cut-offs and sample number and colorectal cancer and adenoma detection were investigated. Cost evaluation at different compliance rates was also carried out. The diagnostic performance of CT colonography as a complementary test when complete endoscopy is not feasible was assessed.

Current work: Updating of a follow-up on digital and conventional mammography cohorts from a study on annual mammography screening for women aged 40-45 years (Eurotrial) and of using ultrasound in dense breasts (RiBES) is ongoing.

A national, multicenter, cost analysis on organized and opportunistic breast cancer screening is also ongoing.

The Unit is involved in a pilot study on HPV as a primary screening test to assess organizational impact, acceptance by women and economic sustainability.

Interval cancer evaluation according to different I-FOBT cut-offs and sample numbers is actually ongoing, as well as studies on hemoglobin stability in fecal samples according to seasonal variations.

Future work: Cost-effectiveness studies on mammography screening will be planned. Quantitative breast density measurements will be attempted to homogenize clinical assessment and to stratify women for further tailored preventive intervention. Diagnostic performance and interval cancer evaluations will be assessed according to quality imaging and monitor calibrations. Clinical performance, subject compliance and organizational impact of CT colonography and optical colonoscopy as primary screening tests will be evaluated in a randomized control trial. A secondary study will be carried out to evaluate compliance to CT colonography in I-FOBT-positive subjects refusing endoscopy.

In colorectal cancer screening, narrow banding imaging and auto-florescence will be assessed to evaluate the increase in clinician diagnostic accuracy, if any, in identifying flat lesions in I-FOBT-positive subjects and in risk increased familial subjects.

2. Research on population attitudes and compliance to screening, and on new organizational models

Main achievements: Communication strategies are of primary interest in order to increase compliance to screening. Effort to ameliorate participation was accomplished by elaborating and testing screening

materials (invitation letters, brochure) in focus groups with screening personnel and subjects eligible for screening. The Unit was involved in the national Inter-screening work group on communication and contributed to the editing of "100 questions on HPV" and "100 questions on colorectal cancer screening", and to planning the Tuscan regional communicative campaign on oncological screening in 2006-2007. Within a collaborative study, narrative medicine was explored in breast cancer care: a multimedia tool "La parola riconosciuta" reporting interviews by women affected by breast cancer. This was disseminated to facilitate daily work and communication within breast units.

Current work: Within the pilot study on the HPV screening program, implementation of differential communicative strategies is on-going with particular focus on the emotional impact of this new strategy. Moreover, a study (self-sampling) concerning a self-HPV testing device sent to the homes of eligible women has the purpose of increasing compliance in subjects usually not respondent to an invitation, while mailing of the I-FOBT kit to both previous attendees and non-responders aims to test a new model which applies test delivery, as well as facilitating the participation of eligible people.

Future work: A social marketing approach to evaluate non-attendance determinants in oncological screening will be implemented. According to the principle of narrative medicine, semi-structured interviews will facilitate the comprehension of the individual motivations of test-positive subjects in refusing diagnostic assessment in cervical and colorectal cancer screening, and new strategies for improving the attendance of this subject group will be studied. The results will promote the proposal of new communication strategies tailored to local and individual needs.
Finally, to evaluate if economic barriers could affect participation in mammography screening, social costs will be assessed in organized and opportunistic settings.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
Self sampling	2008	Agenzia di Sanità Pubblica (ASP Lazio)	2,300
RiBES	2003	Azienda Sanitaria Locale (ASL) Milano	6,816
Eurotrial	2000	Ministero della Salute	43,294

Research Grants

Year	Funding Agency	Amount
2009	Agenzia Regionale di Sanità (ARS) Abruzzo	€ 200,000
2008	ARS Abruzzo	€ 154,000
2008	ASP Lazio	€ 71,132
2003	ASL Milano	€ 63,351

Main Collaborations

With Units within ITT
» Screening Units Aziende USL Regione Toscana
» Breast Surgery Unit, Azienda Ospedaliero Universitaria Careggi (AOU Careggi), Firenze
» Department of Pathology, AOU Careggi, Firenze
» Azienda Sanitaria di Firenze

With other Italian and Foreign Institutions/Organizations
» ASL Milano
» CPO-Piemonte, Azienda Ospedaliera "San Giovanni Battista", Torino
» Agenzia di Sanità Pubblica (ASP Lazio), Roma
» Istituto Oncologico Veneto (IOV), Padova
» Azienda USL Modena
» Azienda USL 2 Umbria, Perugia
» Azienda Sanitaria Locale di Potenza (ASP)
» Centro di Riferimento Oncologico della Basilicata (CROB), Rionero in Vulture (Potenza)
» Azienda USL Piacenza
» Azienda USL Bologna

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10. Ronco G, Giorgi-Rossi P, Carozzi F, et al; NTCC Working Group: *Results at recruitment from a randomized controlled trial comparing human papillomavirus testing alone with conventional cytology as the primary cervical cancer screening test.* J Natl Cancer Inst 2008; 100: 492-501.
11. Zorzi M, Falcini F, Fedato C, et al: *Screening for colorectal cancer in Italy: 2006 survey*. Epidemiol Prev 2008; 32: 55-68.

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Introduction

The ISPO Biostatistics Unit founded in 2004 as the joint effort between the Medical School of the University of Florence, ISPO and Careggi Hospital; it collaborates with the Department of Statistics "Giuseppe Parenti" to which its academic staff belongs. The Unit's main activities are training and research.
The Unit offers courses on medical statistics, biostatistics and clinical epidemiology for the School of Medicine and the School of Science (degrees in Biology and Biotechnology). It is also a partner for the Master's in Epidemiology at the University of Turin, the Master's in Biostatistics at the Universities of Bologna and Florence, the PhD Program in Applied Statistics and the PhD Program in Nursing at the University of Florence.
The Unit has carried out research in Cancer Epidemiology and related statistical methods, Clinical Oncology, Environmental Epidemiology and Surveillance, Social Epidemiology and Psychiatry, Basic Sciences and Genomics.

Main Research Themes

1. Probabilistic decision models and health technology assessment

Governance of prevention programs requires development of prediction tools that allow to simulate many alternative scenarios by appropriate mathematical and probabilistic modeling. It uses information on disease, treatment effectiveness, prognostic factors and clinical guidelines.

The Unit leads a project on "Cancer Mass Screenings" and in the Integrated Oncology Project (Ministry of Health) it assessed the impact of preventable factors using the "Global Burden of Disease" methodology.

In 2009-2010, the Unit was further funded by the Ministry of Health for the projects "Statistical Models for Predicting the Impact of HPV Vaccination", "Cost-Effectiveness Analysis of Four Strategies for Cervical Screening" and "Cost-Effective Analysis of Mass Screening Programs for Breast Cancer". The Istituto Toscano Tumori (ITT) funded a three-year project "Statistical Models for Cancer Screening".

2. Statistical methods for functional genomics and high throughput analysis

Functional genomics is a rapidly evolving area for which convergence of different disciplines is required. The Unit was the principal investigator on two national projects, "Statistical Analysis of Gene Expression Data" (PRIN 2003) and "Statistical Methods in System Biology" (PRIN 2005) (with the universities of Turin, Milan, San Raffaele – Milan, Padua, Udine). The Unit was a partner in the Italy-France Galileo project 2004/2005 on "Experimental Designs in Functional Genomics" (with Genopole Evry, UMR INRA and AgroParisTech). Currently, it is a partner in the Network of Excellence NuGO (EU VI Framework) and the NuGO PPS Study (www.nugo.org). Research streams are: Bayesian approaches to functional genomics studies; meta-analysis and survival analysis to integrate gene expression profiles with prognostic factors; Bayesian approaches to the identification of gene regulatory networks and pathways.

3. Clinical trials and clinical epidemiology

The Unit hosted the ITT Clinical Trials Coordinating Center during its set-up. Currently a strong collaboration on statistical methodology has been established. The Unit takes care of statistical aspects of clinical trials and it has partnerships in Agenzia Italiana del Farmaco (AIFA)-funded projects (University of Florence, Consiglio Nazionale delle Ricerche – CNR, Fondazione Santa Lucia) and with

Formas. The Unit collaborates on several clinical epidemiological studies within the Azienda Ospedaliero Universitaria Careggi (AOU Careggi) and Azienda Ospedaliero Universitaria Meyer (AOU Meyer), the University of Florence, the University of Modena and Reggio Emilia and the University of Naples.

The Unit provides statistical expertise to the Clinical Risk Management and Patient Safety Centre of the DG Health, Region of Tuscany within the framework of the 2009-2010 strategic project "Study of the Incidence of Adverse Events in Italian Hospitals of the NHS".

Research Grants

Year	Funding Agency	Amount
2009	ITT	€ 190,000
2009	Regione Toscana	€ 120,000
2008	World Health Organization (WHO)	€ 70,000
2008	Regione Lombardia and University of Milan	€ 140,000
2007-2008	Ministero dell'Istruzione, dell'Università e della Ricerca – PRIN	€ 170,000
2007	European Union	€ 140,000
2007	Agenzia Italiana del Farmaco (AIFA)	€ 100,000
2006-2009	Ministero della Salute	€ 300,000

Main Collaborations

With Units within ITT
- Medical Oncology Unit, Azienda USL 4 Prato
- AOU Careggi, Firenze
- AOU Meyer, Firenze
- Clinical Trial Coordinating Center, ITT Core Research Laboratory, Firenze

With other Italian and Foreign Institutions/Organizations
- Istituto Nazionale Tumori (INT), Milano
- Fondazione Ca' Grande, University of Milan
- Fondazione IFOM, Milano
- "Cattolica" University, Roma
- University of Turin
- University of Udine
- University of Cagliari
- University of Naples
- University of Messina
- CNR, Pisa
- CNR, Palermo
- London School of Hygiene and Tropical Medicine (UK)
- University of Manchester (UK)
- European Nutrigenomics Organisation (NuGO) (UK)

» University of Rotterdam (The Netherlands)
» University of Basque Country (Spain)
» Tel Aviv University (Israel)
» Ben Gurion University of the Negev (Israel)
» Harvard University (USA)

Publications

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Introduction

The Unit’s activity is essentially dedicated to the treatment of solid tumors, including gastrointestinal cancer, breast cancer, liver tumors, ovarian cancer and primary and metastatic cancer of the liver. The Unit developed locoregional therapies, jointly with the Surgical Department, such as isolated limb perfusion in melanoma patients and hyperthermic abdominal perfusion after cytoreduction in ovarian and Gastrointestinal (GI) cancers.

The Unit's activity also consists in providing innovative cancer therapies, such as new preloaded microsphere drugs administered intra-arterially in primary and metastatic liver cancer, intraperitoneal chemotherapy in ovarian cancer, combined therapies (hyperthermia and chemotherapy) for solid tumors.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
CRM 197: phase I-II evaluation of subcutaneous CRM 197 in patients with advanced melanoma (AM)	2007-2009	Private sponsor	20
TARGeted Intraoperative Radiotherapy (TARGIT): international randomized trial in breast cancer	2002-2010	TARGIT Trail Operations Group. Centre for Clinical Science and Technology, London	2,800 treated in 22 Centers around the world

Main Collaborations

With Units within ITT
» Aziende USL, Regione Toscana

With other Italian and Foreign Institutions/Organizations
» Istituto Nazionale Tumori (INT), Milano
» Istituto Europeo di Oncologia (IEO), Milano
» Istituto Oncologico Veneto (IOV), Padova
» Istituto Oncologico Romagnolo (IOR), Forlì
» Centro di Riferimento Oncologico (CRO), Aviano (Pordenone)
» Rotterdam Cancer Center (The Netherlands)
» Polythecnique de Paris (France)
» Washington Cancer Center (USA)
» National Cancer Institute, Bethesda (USA)

Publications

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2. Fiorentini G, Filippeschi M, Turrisi G, et al: *Advanced cancer of the ovary: intraperitoneal chemotherapy as a new therapeutical option*. Vivo 2009; 23: 317-21.
3. Fiorentini G, Aliberti C, Del Conte A, et al: *Intra-arterial hepatic chemoembolization (TACE) of liver metastases from ocular melanoma with slow-release irinotecan-eluting beads. Early results of a phase II clinical study*. Vivo 2009; 23: 131-7.

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11. Fiorentini G, Cantore M, Rossi S, et al: *Hepatic arterial chemotherapy in combination with systemic chemotherapy compared with hepatic arterial chemotherapy alone for liver metastases from colorectal cancer: results of a multicentric randomized study*. Vivo 2006; 20: 707-9.
12. Aliberti C, Tilli M, Benea G, Fiorentini G: *Trans-arterial chemoembolization (TACE) of liver metastases from colorectal cancer using irinotecan-eluting beads: preliminary results*. Anticancer Res 2006; 26: 3793-5.
13. Peters S, Voelter V, Zografos L, et al: *Intra-arterial hepatic fotemustine for the treatment of liver metastases from uveal melanoma: experience in 101 patients*. Ann Oncol 2006; 17: 578-83.
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Viareggio Area

MEDICAL ONCOLOGY

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Chiara Valsuani, Fellow
Cheti Puccetti, Data Manager
Barbara Buralli, Psycho-Oncologist

Introduction

Our principal activity is dedicated to the delivery of the most up-to-date and highest quality of cancer treatment to patients. Our main fields of interest are breast, lung, colorectal and urogynecological cancers. Our research activities consist of mainly clinical trials and translational research.

Main Research Themes

1. From bench to bedside translational research

Molecular biology applied to the identification of new prognostic and predictive markers. Cell cycle control/alteration in cancer, molecules and signal transduction in cancer. In particular, the role of the dystroglycan complex (protein and mRNA level) as a prognostic marker in renal cell carcinoma, prostate and gastric cancers. The relationship between dystroglycan expression (protein and mRNA level) and sunitinib/sorafenib treatment response in advanced renal cell carcinoma. Modulation of docetaxel sensitivity in prostate cancer cell lines by overexpression of dystroglycan. Evaluation of predictive and/or prognostic significance of p53 in advanced breast cancer treated with taxane therapy. Evaluation of endocrine-resistance markers in adjuvant therapy of breast cancer. Evaluation of new predictive variables in advanced hepatocarcinoma. Relationship between chemotherapy in advanced lung cancer and expression of biological parameters (mRNA).

2. Clinical research

Active (past and present) participation in more than 30 phase II-III GCP clinical trials, to date. Self-ideation and management of phase II GCT clinical trials. Evaluation of the effect of fulvestrant on the lipid profile and others estrogen target systems in advanced breast cancer patients. Modulation of VEGF circulating serum levels by fulvestrant hormonal treatment in metastatic breast cancer patients. Management of patients with advanced lung cancer: evaluation of fractionated dose of oral vinorelbine.

Clinical Trials

1. Breast cancer

Description	Year	Sponsor	Number of patients recruited to date
CARA Study: international study to evaluate the impact of the administration of educational material on the compliance and persistence of treatment with aromatase inhibitors in post-menopausal women at the beginning stages of hormone-sensitive breast cancer	2009	AstraZeneca	19
Studio GIM 8: randomized, factorial design study comparing fulvestrant ± lapatinib ± aromatase inhibitors in patients with progressive breast cancer after aromatase inhibition therapy	2008	Oncotech	0
Study Beatrice BO20289: an international, multicenter, open-label, two-arm, phase III trial of adjuvant bevacizumab in triple negative breast cancer	2007	Roche	6
Spontaneous study: evaluation of the lipid profile in patients being treated with fulvestrant	2006	AUSL 12 Versilia	19
Study JAVLOR L00070 IN 303 BO: phase III study with vinflunine combined with gemcitabine *versus* paclitaxel combined with gemcitabine in patients affected by locally advanced or metastatic breast cancer after adjuvant therapy with anthracycline	2006	Pierre-Fabre	5

Description	Year	Sponsor	Number of patients recruited to date
Study ALTTO: adjuvant pivotal trial of herceptin, lapatinib or dual inhibition of ErB2 in patients with breast cancer	2006	GlaxoSmithKline	2
Study GIM 3 (FATA): phase III study comparing anastrozole, letrozole and exemestane and three sequential strategies (two years of therapy with tamoxifen followed by three years with aromatase inhibitors) *versus* the up-front strategy (five years of therapy with aromatase inhibitors) in adjuvant treatment of hormone-responsive breast cancer in post-menopausal women	2006	Oncotech	35
Study GIM 4: study on duration of treatment with adjuvant letrozole in menopausal women with breast cancer; long course *versus* short course	2005	Oncotech	33
Studio GIM 5: adjuvant therapy with letrozole following tamoxifen. Study of the correlation between the CYO19 gene and the efficacy of letrozole in post-menopausal women with breast tumors	2005	Oncotech	4
Study (CZOL446EIT14): multicenter, randomized, prospective, comparative two-arm study to evaluate the efficacy of zolendronic acid (every three months *versus* every four months) in patients with bone metastasis in breast cancer treated with zolendronic acid for approximately one year	2005	Novartis	5
Spontaneous study HOT: combination of trastuzumab, docetaxel and oxaliplatin in breast metastasis with overexpression of the HER2 receptor: phase II clinical study			

2. Lung cancer

Description	Year	Sponsor	Number of patients recruited to date
Study NEXT: open, randomized, multinational, phase IIIb trial evaluating the activity and safety of cetuximab, 250 mg/m2 weekly and 500 mg/m2 every two weeks, as maintenance therapy after platinum-based chemotherapy in combination with cetuximab as first-line treatment for subjects with advanced non-small cell lung cancer (NSCLC)	2009	Merck	8
Study A4061039: randomized, phase II study of cisplatin/pemetrexed with or without axitinib as first-line treatment for patients with non-squamous NSCLC	2009	Pfizer	1
Study EPICICLIN: epidemiological study to describe NSCLC clinical management patterns in Europe. Lung-epiciclin	2009	AstraZeneca	9

Description	Year	Sponsor	Number of patients recruited to date
Study H3E-EW–S124: double-blind, phase III study on pemetrexed + best supportive care *versus* placebo + best supportive care as maintenance treatment immediately following induction therapy with pemetrexed + cisplatin in patients affected by advanced/metastatic, NSCLC with a non-squamous histology	2008	Eli Lilly	15
Study Elderly NEXT: multicenter, phase II study of sequential chemotherapy with cisplatin/gemcitabine followed by docetaxel in elderly patients affected with advanced NSCLC	2008	AUSL 6 Livorno	2
Study FARM6PMFJM: multicenter, phase III randomized study of cisplatin and etoposide with or without bevacizumab as first-line treatment in extensive stage (ED) small cell lung cancer (SCLC)	2008	Gruppo Oncologico Italiano di Ricerca Clinica (GOIRC)	3
Study GOIRC 02/2006: a phase II, randomized trial on pemetrexed *versus* pemetrexed and carboplatin as an advanced study, second-line chemotherapy in NSCLC	2006	GOIRC	24
Study FORTIS-M: a phase III, randomized, double-blind, placebo-controlled study of oral talactoferrin in addition to best supportive care in patients with NSCLC who have failed two or more prior treatment regimens			

3. Colorectal cancer

Description	Year	Sponsor	Number of patients recruited to date
Study TRIBE: a phase III, randomized trial on FOLFOXIRI + bevacizumab *versus* FOLFIRI + bevacizumab as first-line treatment for metastatic colorectal cancer	2008	AUSL6 Livorno	9
Study Bebyp: an open-label, multicenter, randomized phase III study of second-line chemotherapy with or without bevacizumab in metastatic colorectal cancer patients who have received first-line chemotherapy + bevacizumab	2007	AUSL6 Livorno	9
Study TOSCA-GISCAD: a randomized trial investigating the role of the FOLFOX4 regimen duration (three *versus* six months) and bevacizumab as adjuvant therapy for patients with stage II/III colon cancer	2007	Gruppo Italiano per lo Studio dei Carcinomi dell'Apparato Digerente (GISCAD)	19

4. Kidney cancer

Description	Year	Sponsor	Number of patients recruited to date
Study GIR 2 Medical Optimization of Torisel (MOTOR): multicentric, phase II evaluation of torisel as II line treatment for metastatic RCC patients progressing after cytokine therapy, tyrosine kinase, or angiogenesis inhibitors	2009	Oncotech	2
Study GIR 1: sunitinib either before or after cytoreductive nephrectomy. A phase II trial in patients with metastatic renal cell carcinoma	2008	Oncotech	2
Spontaneous study: evaluate the expression of dystroglycan in patients with kidney cancer: analysis of its role as a prognostic factor			
Spontaneous study: use of inhaled interleukine-2 combined with subcutaneous interleukine-2 in patients with pulmonary metastasis from kidney cancer; evaluation of current treatment			
Study CRAD001: compassionate use with RAD001 (everolimus) in patient with Metastatic Renal Cell Carcinoma (MRCC)			

5. Gastric cancer

Description	Year	Sponsor	Number of patients recruited to date
Spontaneous study: neoadjuvant chemotherapy with docetaxel and oxaliplatin treatment in patients with locally advanced neoplastic patients			

6. Prostate cancer

Description	Year	Sponsor	Number of patients recruited to date
Study A618112: a multicenter, randomized, double blind, phase III study of sunitinib + prednisone *versus* prednisone in patients with progressive metastatic hormone-refractory prostate cancer after failure of a docetaxel-based chemotherapy regimen	2008	Pfizer	2

7. Hepatocellular Carcinoma (HCC)

Description	Year	Sponsor	Number of patients recruited to date
Study GIDEON: global investigation of therapeutic decisions in HCC and of its tratment with sorafenib	2008	Bayer-Healthcare AG	3

8. Psycho-oncology

Description	Year	Sponsor	Number of patients recruited to date
Spontaneous study: evaluation of psychological distress in patients recovering in the Medical Oncology Unit; the bases for intervention			

Main Collaborations

With Units within ITT
» Medical Oncology Unit, Azienda USL 6 Livorno
» Medical Oncology Unit, Azienda USL 5 Pisa
» Medical Oncology Unit, Azienda USL 2 Lucca

With other Italian and Foreign Institutions/Organizations
» Centro Ricerche Oncologiche Giovanni XXIII, "Cattolica" University, Roma
» Istituto Nazionale per la Ricerca sul Cancro (IST), Genova

Publications

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2. Sgambato A, Camerini A, Collecchi P, et al: *Cyclin E correlates with manganese superoxide dismutase expression and predicts survival in early breast cancer patients receiving adjuvant epirubicin-based chemotherapy*. Cancer Sci 2009; 100: 1026-33.
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7. Tibaldi C, Vasile E, Antonuzzo A, et al: *First line chemotherapy with planned sequential administration of gemcitabine followed by docetaxel in elderly advanced non-small-cell lung cancer patients: a multicenter phase II study*. Br J Cancer 2008; 98: 558-62.
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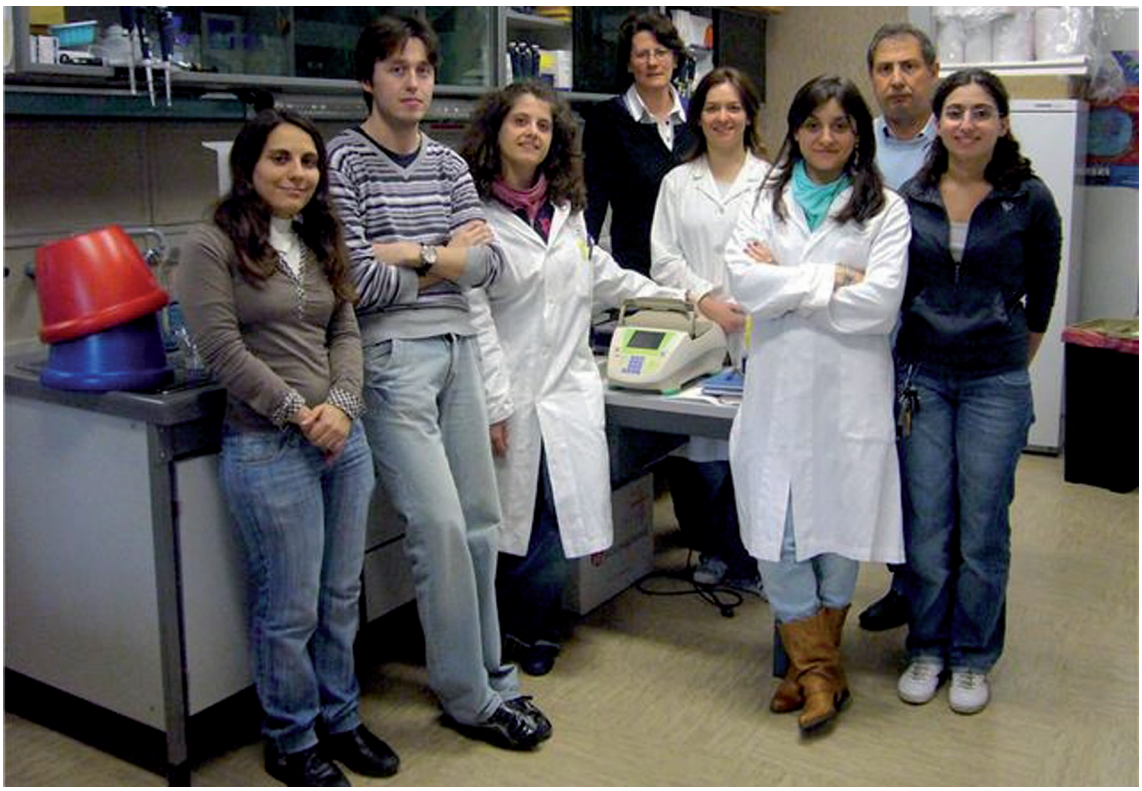
9. Sgambato A, Camerini A, Amoroso A, et al: *Expression of dystroglycan correlates with tumor grade and predicts survival in renal cell carcinoma*. Cancer Biol Ther 2007; 6:1840-46.
10. Camerini A, Tartarelli G, Martini L, et al: *Testicular metastasis from renal cell carcinoma in a responder patient to interleukine-2 treatment: a case report*. Int J Urol 2007; 14: 259-60.

THE CORE RESEARCH LABORATORY OF ISTITUTO TOSCANO TUMORI

GENETICS AND GENE TRANSFER IN ONCOLOGY



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Introduction

The Laboratory of Genetics and Gene Transfer in Oncology was established in 2007 and it became fully functional in 2008. The laboratory investigates various genetic aspects of clonal diseases with a specific focus on the investigation of individual genetic variability and on the role of somatic mutations.

The main research topics of the laboratory include:

1. Pharmacogenetics and chemotherapy.
2. Study of factors favoring the development and the selective growth of clonal populations of somatic cells.
3. The role of chromosomal rearrangement involving ETS proteins in development and progression of prostate cancer.

Main Research Themes

1. Pharmacogenetics and chemotherapy

The response to treatment of individual patients varies considerably both in terms of efficacy and toxicity. A number of factors affect the inter-individual variability of response to treatment. However, it is likely that much of this variability is associated with genetically determined individual differences in how drugs are metabolized. We are exploring the possible role of polymorphisms of a variety of genes (uridine diphosphate glucuronosyl transferase, cytochrome P450, CTLA-4, etc.) in affecting the response to treatments.

a) *Irinotecan toxicity and uridine diphosphate glucuronosyltransferase 1A gene family*. Irinotecan, a topoisomerase-I inhibitor used in cancer therapy, may have unpredictable and severe gastro-intestinal and hematological toxicity. The active metabolite of irinotecan, SN38, is inactivated through glucuronidation by Uridine diphosphate Glucuronosyltransferase (UGT) 1A1. The association of the *UGT1A1* –53$(TA)_7$ allele with an increased risk of irinotecan toxicity is still controversial. Since other UGT1A family isoforms, the extra hepatic UGT1A7 and hepatic UGT1A9, are involved in SN38 glucuronidation, it is possible that polymorphisms in other members of the *UGT1A* gene family could help in predicting the risk of irinotecan toxicity. Thus, we are investigating whether the polymorphic variants of these genes may contribute to irinotecan toxicity. We have found that in patients with advanced colorectal cancer treated with irinotecan, both the low activity alleles of UGT1A1 [$(TA)_7$] and UGT1A7 [622C] are associated with severe toxicity. The identification of genetic markers highly associated with a high risk of toxicity is a good example of how chemotherapy regimens could be more effectively tailored to individual patients.

b) *The role of CTLA-4 polymorphisms in the outcome of allogeneic stem cell transplantation*. Cytotoxic T-lymphocyte antigen-4 (*CTLA-4*) gene behaves as a negative regulator of T-cell activation. *CTLA-4* gene polymorphisms have been found to be associated with susceptibility to autoimmune diseases. Recently, conflicting observations have been reported about the role of *CTLA-4* gene polymorphisms in the outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT). We have investigated three polymorphisms of the *CTLA-4* gene (-318C > T, +49A>G, CT60G>A) in 133 donor/recipient pairs who underwent HLA-matched sibling donor HSCT for hematological malignancies. We found no association of the clinical outcome of the HSCT with either recipient or donor –318C>T and CT60G>A polymorphisms. At variance, we found a significant association of the donor +49A>G G/G genotype with longer overall survival (OS; $P = 0.04$), and the number of +49A>G G-alleles in the recipient with longer OS ($P < 0.03$), longer disease-free survival (DFS; $P < 0.04$) and reduced relapse rate ($P < 0.04$). However, multivariate analysis confirmed the independent prognostic significance of only recipient +49A/G genotypes for both OS and DFS. Our data show that the recipient *CTLA-4* +49A/G polymorphism, thus far not investigated, appears to be relevant to the clinical outcome of allo-HSCT. This suggests that CTLA-4 expression on leukemic cells and on recipient micro-environment cells might play a role in the post-transplant control of disease (a).

2. Study of inherited and acquired factors favoring the selective growth of abnormal clonal populations of somatic cells

a) *Disease model: paroxysmal nocturnal hemoglobinuria*. Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare acquired blood disorder characterized by intravascular hemolysis, a tendency for thrombosis, and a variable degrees of bone marrow failure (b). PNH is due to clonal proliferation of a Hematopoietic Stem Cell (HSC) in which a somatic mutation has inactivated the X-linked gene, *PIG-A* (Figure 1). The resulting deficiency of Glycosylphosphatidylinositol (GPI)-linked proteins on the surface of the progeny of the mutated HSC explains hemolysis and thrombophilia (b). Recently, treatment with a monoclonal antibody that blocks complement protein C5 (eculizumab) almost completely controlled intravascular hemolysis in PNH patients. However, the deficiency of GPI-anchored molecules on blood cells does not explain the bone marrow failure and the expansion of the PNH (GPI-negative) clone. Clinical observations, *in vitro* studies, and data from PNH mouse models indicate that GPI-negative HSC do not have an absolute growth advantage. In addition, very small GPI-negative clones exist in healthy subjects, but only in PNH patients do GPI-negative cells expand and contribute substantially to hematopoiesis. A plausible model that could explain both bone marrow failure and the PNH clone expansion is that normal (GPI-positive) HSC are the target of a selective auto-immune attack to which PNH HSC are resistant. Auto-reactive T-cells against HSCs are responsible for Idiopathic Aplastic Anemia (IAA); the close relationship of PNH with IAA has suggested that they may be present in PNH as well. Indeed, analysis of the TCR repertoire has revealed an increased frequency of expanded T-cell clones in PNH, similar to that observed in IAA. The identity of the putative auto-reactive T-cells and of their targets remains unknown. However, we have found that in PNH patients the expansion of CD8+CD57+ T-cells is relatively common. In addition, by a systematic sequence analysis of the TCR-beta molecules, we have shown that in PNH patients CD8+CD57+ T-cells are oligoclonal and that more than two-thirds of patients share, on this T-cell population, a set of highly homologous TCR-beta molecules (clonotypes) (7). These findings are consistent with the presence in PNH patients of an immune process driven by the same (or similar) antigen(s): probably a non-peptide antigen because patients sharing clonotypes do not all share identical HLA alleles (7). These findings provide strong support to our hypothesis that expansion of the GPI-negative blood-cell population in PNH is due to selective damage to

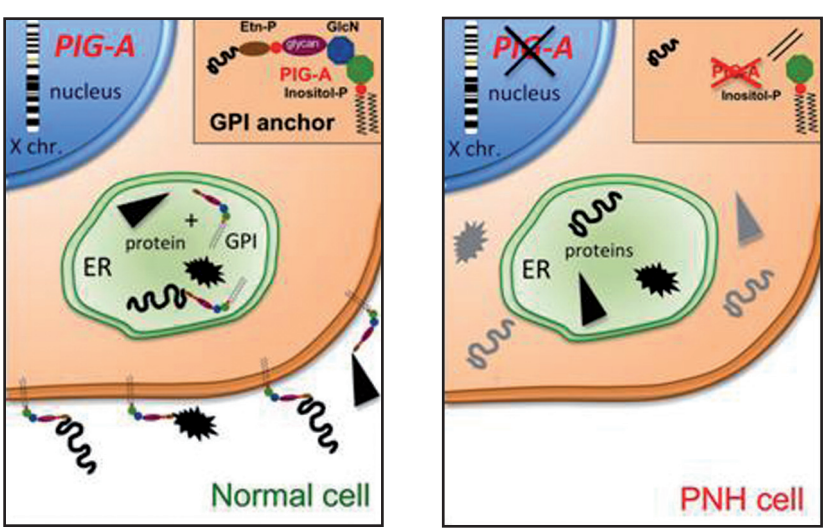

Figure 1 - The molecular basis of PNH. Complex biosynthetic machinery produces the GPI molecule (see inset) in the Endoplasmic Reticulum (ER) of a normal cell (left). An early step in this pathway is catalyzed by an acetylglucosaminyl transferase: one of the subunits of this enzyme is encoded by the gene *PIG-A*, located on the short arm of the X chromosome. A number of cellular proteins become covalently linked to the GPI molecule that serves for conveying and anchoring them on the surface of the cell membrane. The PNH cell (right) has a mutation in the *PIG-A* gene that impairs acetylglucosaminyl transferase activity and causes a total (or partial) block in the synthesis of the GPI molecule. As a result, the proteins requiring a GPI anchor are unable to bind to the membrane and will be lacking on the cell surface

normal hematopoiesis, mediated by an autoimmune attack of CD8+CD57+ T-cells against a non-peptide antigen(s) that could be the GPI anchor itself. In order to directly test the hypothesis that the GPI anchor itself is the auto-antigen targeted in PNH, the availability of the human GPI anchor is crucial. Mammalian GPI has never been synthesized; however, recently, Cristina Nativi (University of Florence) has succeeded in synthesizing the core structure of the mammalian (human) GPI anchor. The availability of the GPI molecules provides us with a powerful tool to directly investigate the characteristic autoimmune pathogenesis of the clonal expansion observed in PNH. In addition, it is possible that similar autoimmune mechanisms could be responsible for clonal expansion in others acquired clonal cytopenias related to IAA and PNH, such as Myelodysplastic Syndromes (MDS) that are much more frequent than PNH and far more prone to evolve to acute leukemia.

b) *The role of somatic mutation rate in human cancer*. Mutations are an inherent risk of cell duplication since they develop even in the absence of any exogenous agent. Thus, the frequency of mutants (*f*: the fraction of cells harboring a mutation in a given gene) and the mutation rate (μ: the probability of a new mutation occurring in a gene per cell division) are key biological features of any cell population. The accumulation of somatic cells plays a key role in the development of cancer. Since the final common pathway for cancer development is a sequence of somatic mutations, one common risk factor for the development of any tumor must be the rate of somatic mutation (μ). To measure *f* and μ, a potential sentinel gene is the *PIG-A* gene that encodes one of the subunits of an enzyme essential in the biosynthesis of GPI (see above, paragraph 2a). Since *PIG-A* is X-linked, mutational inactivation of the one single copy active in somatic cells results in the absence from the cell surface of all the proteins that require GPI for attachment to the membrane; thus, mutant cells display a GPI-negative surface phenotype (see above, Figure 1) that can be easily detected by flow cytometry (Figure 2).

The measurement of *PIG-A* mutants by counting cells with the GPI-negative phenotype has proved to be effective to measure mutant frequency in peripheral blood cells of humans and of others animals (1). Up to now, μ has been exceedingly difficult to measure in human cells (1); however, by using the *PIG-A* gene as a sentinel in long term culture of B Lymphoblastoid Cell Lines (BLCL), we

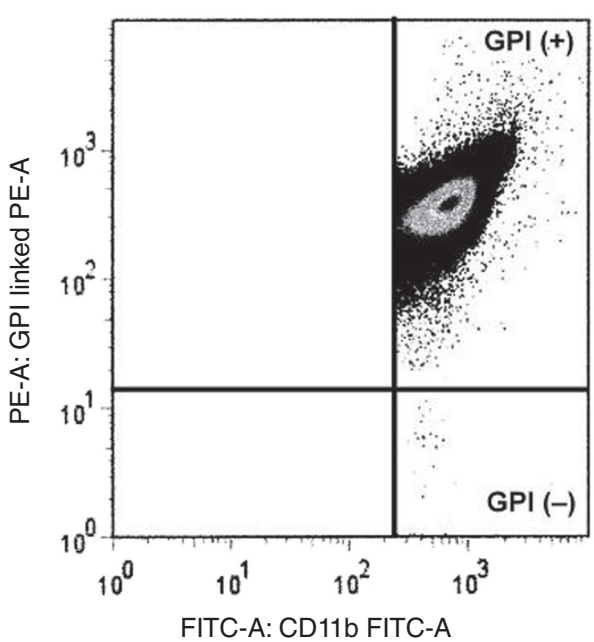

Figure 2 - Flow cytometry pattern displaying rare GPI-negative granulocytes from peripheral blood of a normal donor. The mutant (GPI-) cells are clearly resolved from the bulk (GPI+) cells: calculation gives 34 per million

have a test that makes it possible to measure μ in human cells. By using this approach, we have shown that μ is increased in patients with inherited cancer-prone syndromes, such as Fanconi anemia and ataxia-telangiectasia.

Now, we are addressing two important aspects: *a*) to determine to what extent μ is genetically determined; *b*) to explore the relationship between the value of μ (and *f*) and the risk of cancer.

i) In order to understand whether the normal variation of μ depends more on environmental factors or more on inherited factors, we have measured μ in a small set of clinically normal twins. Intriguingly, the μ variation in the same twin pair was smaller in mono- than in dizygotic twins, supporting the notion that, even within the normal range, μ may have a substantial inherited component. We plan to confirm this notion by testing a large group of pairs of twins.

ii) The determination of μ on BLCLs is still relatively laborious and since it is likely that in terminally differentiated cells the *PIG-A* mutant frequency (*f*) will be roughly proportional to the μ value of their progenitor cells, we have resorted to exploring the measurement of the *in vivo* frequency of mutant cells (*f*) in peripheral blood granulocytes as a surrogate of μ. We have determined the normal range of *f* in granulocytes studying a small group of healthy subjects. Now, we intend to compare the *f* values in granulocytes from a population exposed to environmental carcinogen, namely heavy smokers, and from cancer patients with the distribution of the *f* values in healthy subjects.

Our future plans include the identification of the genetic determinants of μ, the investigation of how μ is affected by different environmental exposures (*i.e.* smoking, etc.) and of how far it correlates with the risk to develop cancer.

3. The role of chromosomal rearrangement involving ETS proteins in development and progression of prostate cancer

Prostate cancer is the most commonly diagnosed cancer in elderly men in Western countries and it is the second leading cause of death in the male population. Recently, chromosomal translocations that juxtaposes the promoter of a gene highly expressed in the prostate to the coding sequence of one member of the ETS gene family (*ERG*, *ETV1*, *ETV4*, *ETV5*) have been found (c). Since then, a variety of translocations that juxtapose an ETS gene to the promoter of a series of partners that drive its prostate aberrant expression have been identified in prostate cancer. Since these translocations are recurrent in prostate cancer, it is reasonable to hypothesize that the resulting overexpression of an ETS transcription factor plays a direct role in prostate cancer pathogenesis. Evidence for this direct pathogenic role of *ERG* and *ETV1* overexpression in prostate cancer have been reported. We are investigating the pathogenic role of the overexpression of the *ETV4* gene that has been documented in a proportion of prostate cancer cases: in some cases it is associated with translocations that juxtapose the ETV4 gene to the promoter of genes highly expressed in the prostate (TMPRSS2, KLK2, DDX5, CANT1), and it is has been found in others without any detectable translocation. We have tested the expression of ETV4 in few human prostate cell lines: ETV4 expression was not detectable in LnCap and V-Cap whereas it was present in PC3 and Du145 cell lines. In order to test the role of ETV4 expression, we have used a doxycycline-inducible expression of short hairpin (sh) RNAs against ETV4: after induction, the growth of DU145 cell line transduced with the shRNA-containing vector was about 50% of that of DU145 transduced with an empty vector. In addition, we have observed that the reduction in ETV4 expression strongly impairs the ability to form colonies in soft agar (≥ 65% of reduction). ETV4 shRNA interference has produced a similar reduction in cell growth and in the number soft agar colonies in PC3 cell lines. Furthermore, the growth of DU145 xenografts is strongly reduced by the induction of ETV4 shRNA interference. These preliminary experiments show that the expression of ETV4 is functionally important in a cellular model of prostate cancer; thus, they suggest that ETV4 expression may play a direct role in the development and progression of a subset of prostate cancers.

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Research Grants

Year	Funding Agency	Amount
2009-2010	Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR)	€ 60,000
2008-2009	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 60,000
2008	Alexion Pharma Italia	€ 25,000

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» Flow Cytometry Unit, AOU Careggi, Firenze
» Pathology Unit, AOU Careggi, Firenze
» Laboratory of Mice and Animal Experimentation (L.I.Ge.M.A.), University of Florence
» Department of Pharmacology, University of Florence
» Department of Chemistry, University of Florence

With other Italian and Foreign Institutions/Organizations
» Istituto Nazionale per la Ricerca sul Cancro (IST), Genova
» "Federico II" University, Napoli
» University of Turin

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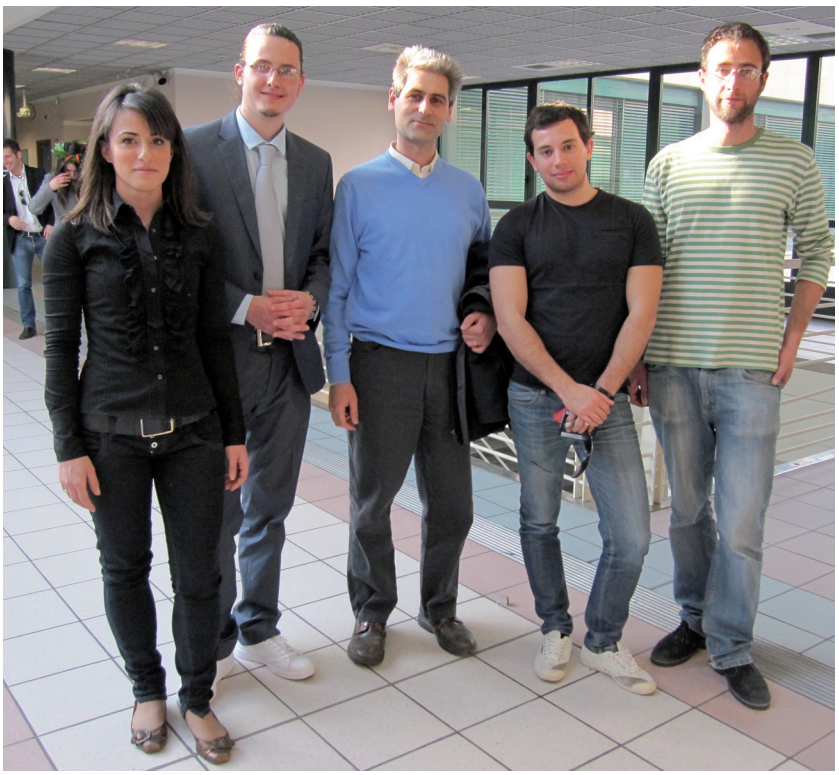
MOLECULAR MECHANISMS OF ONCOGENESIS



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Introduction

The Molecular Mechanisms of Oncogenesis Unit started its research activities in January 2008. Our research is focused on the identification and characterization of factors and pathways involved in the onset of genetic alterations in cancer.

Main Research Theme

Role of activation-induced deaminase in oncogenesis

Cancer is the outcome of a multistep path that progressively leads to uncontrolled cellular growth. Underlying this progression of events is the presence of genetic alterations or mutations that provide the framework, characterizing the type and evolution of the tumor. Mostly, we have access only to the ending point of this path – with the diagnosis of cancer – and it is not easy to intervene in this succession of events. However, understanding the factors and processes leading to the genetic alterations can improve our diagnostic and therapeutic options by a finer characterization of the tumorigenesis – the final aim being a more targeted treatment.

Surprisingly there is a class of enzymes, the AID/APOBECs, whose only role is to introduce mutations in DNA molecules (a). The founder member, Activation Induced Deaminase (AID), is a DNA mutator that, after recruitment to the transcribed immunoglobulin gene, deaminates cytosine residues to uracil. The deamination, followed by the recruitment of the DNA repair machinery, results in the mutation and recombination of the antibody genes, thus initiating the antigen-driven antibody diversification processes (b).

AID is a powerful tool to improve the immune response, but its ability to mutate DNA represents a double edged sword: transgenic mice constitutively expressing AID develop cancer (c) and there is increasing evidence linking AID to the onset of mature B-cell lymphomas (d). These tumors arise during the antigen dependent stages of the antibody gene diversification, and are often characterized by chromosomal translocations involving the IgH gene. While such chromosomal aberrations have been long hypothesized to be linked to the antibody gene diversification processes, only recently it has been proven that AID triggers c-myc/IgH translocations (a common trait in Burkitt's lymphomas) in Balb/c mice by targeting both the IgH locus and the c-myc locus. Moreover, expression of AID is needed in order to develop germinal center derived lymphomas in cancer-prone mice.

In addition to the B-cell lineage, AID is able to induce cancer in other experimental settings: ectopic expression of AID in transgenic mice induces cancer, and AID could be involved in the onset of chromosomal translocations specific for prostatic cancer in a prostate cell line.

At the current state of research, the specific processes by which AID can ultimately trigger cancer are not known. AID could induce DNA lesions somehow mimicking those occuring during B-cell development; these lesions could originate from a genome-wide mistargeting; or cells could be affected by changes in the methylation status of mistargeted genes. Indeed, much of the pathological effects of AID might depend on factors and pathways involved in its regulation: inflammation and inflammation-related signaling pathways can trigger expression of AID in B-cells and in other cell types. Unfortunately, very little is known about the crossroad between regulation of AID and its targeting of the DNA.

The main objective of our research is to investigate the role of AID in the onset and progression of human cancer through two converging approaches:

a) Identification and characterization of molecules and pathways controlling the function of AID: while overexpression of AID induces a generalized increase in mutations, under physiological conditions the primary target for AID is a specific region in the immunoglobulin locus. This suggests the existence of a targeting machinery and the possibility that AID misregulation could lead to DNA lesions and tumorigenesis. During my postdoctoral period at the MRC-LMB, I identified CTNNBL1, a spliceosome-associated molecule, as a specific interactor of AID involved in its targeting (e). Mutations in AID that interfere with the interaction lead to defects both in somatic hypermutation and class switch recombination. While the role of CTNNBL1 has not yet been clarified, its association with the spliceosome is intriguing: this is the first molecule specifically linking the targeting of AID to the mRNA processing machinery.

To this aim, we use a number of tools, ranging from biochemical and bacterial assays to cellular assays. In particular, DT40 cells – a chicken lymphoma cell line – which allow efficient gene targeting and provide a reliable assay system for AID function, and CH12F3 cells, a murine lymphoma clone in which AID can be induced to trigger IgM to IgA class switch recombination. In these models, we can analyze both the ability of AID to initiate the antibody diversification processes and its role in the mistargeting of somatic mutations and induction of cancer-like c-myc/IgH chromosomal translocations, analogous to those found in Burkitt's lymphoma. The information gathered through this research will be then used in transgenic mice to investigate whether forced expression of AID mutants and AID regulators makes B-cells more/less susceptible to tumorigenesis.

b) Analysis of human hematopoietic tumors: to assess whether distinct patterns of expression are associated with specific types of cancer and their prognosis, and to test our findings on the regulatory pathways of AID directly on the cancer tissues and thus evaluate their relevance to the biology of cancer. Constitutive expression of AID has been reported in a number of lymphoproliferative malignancies. In some cases, such as in chronic lymphocytic leukemia, AID is, paradoxically, expressed tumors with unmutated immunoglobulin V genes, while it seems absent from the ones with mutated immunoglobulin genes, which have a better prognosis. With AID expression being linked to the prognosis in B-cell lineage tumors, we are investigating whether AID is merely a relic of the developmental stage at the origin of the tumor or it plays an active role in the progression of the cancer. Whichever the answer, we will try to correlate the presence of AID to specific neoplastic subpopulations and to the relative prognosis. This approach will naturally complement the molecular dissection of AID regulatory pathways, as we will be able to test our findings directly on the neoplastic samples and thus evaluate their relevance to the biology of cancer.

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Main Collaborations

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» Hematology and Transplant Unit, University of Siena

With other Italian and Foreign Institutions/Organizations

» Medical Research Council, Laboratory of Molecular Biology, Cambridge (UK)

Publications

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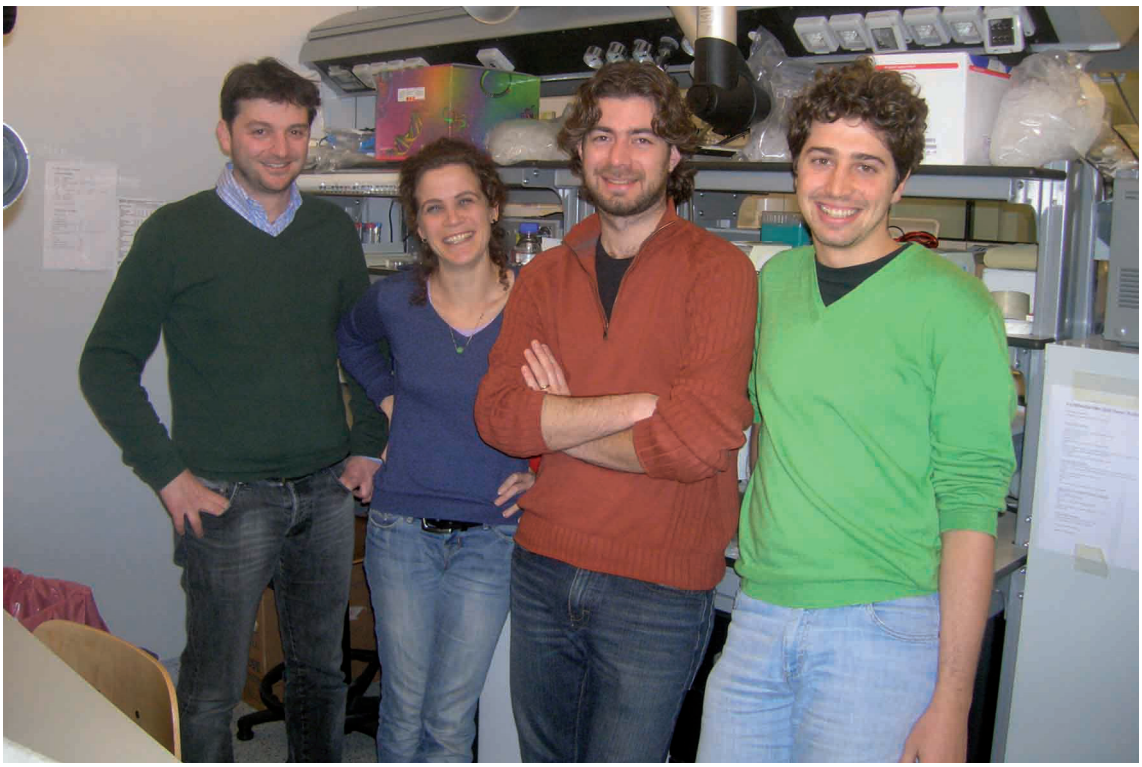
SIGNAL TRANSDUCTION



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Main Research Themes

MAP kinases are a family of proline-directed serine/threonine kinases that play a central role in signal transduction in all eukaryotic cells, from yeast to humans (a). They coordinate signaling from a variety of extracellular and intracellular stimuli controlling the activity of a vast array of cellular regulatory proteins, including protein kinases, transcription factors, cytoskeletal proteins and other enzymes (a) (Figure 1). Erk8 is the last identified member of the MAP kinase family of proteins. Expressed in several human tissues, its activity can be modulated by DNA-damaging agents and different activated oncogenes (b,c). Indeed, we have already shown that three human oncogenes, RET/PTC3, RET/MEN2B and bcr/abl, are able to activate Erk8 (b) while, in turn, this MAP kinase controls the activity of different nuclear receptors (d,e) (Figure 2). Interestingly, Erk8 has been recently involved in transformation in human

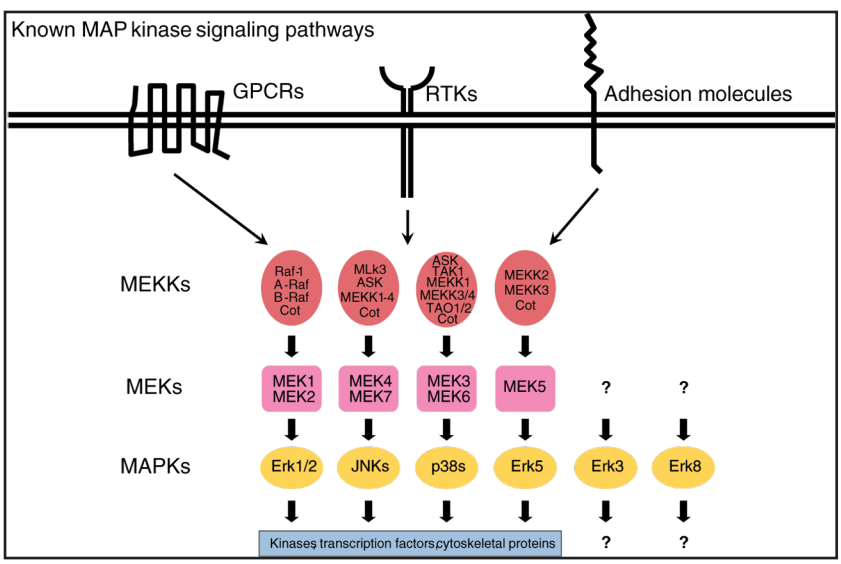

Figure 1 - Schematic illustration of the three-modular organization of MAP kinase (MAPK) cascades in which a MAPK kinase kinase (MEKK) modulates the activity of a MAPK kinase (MEK), which subsequently activates a MAP kinase, resulting in phosphorylation of downstream substrates

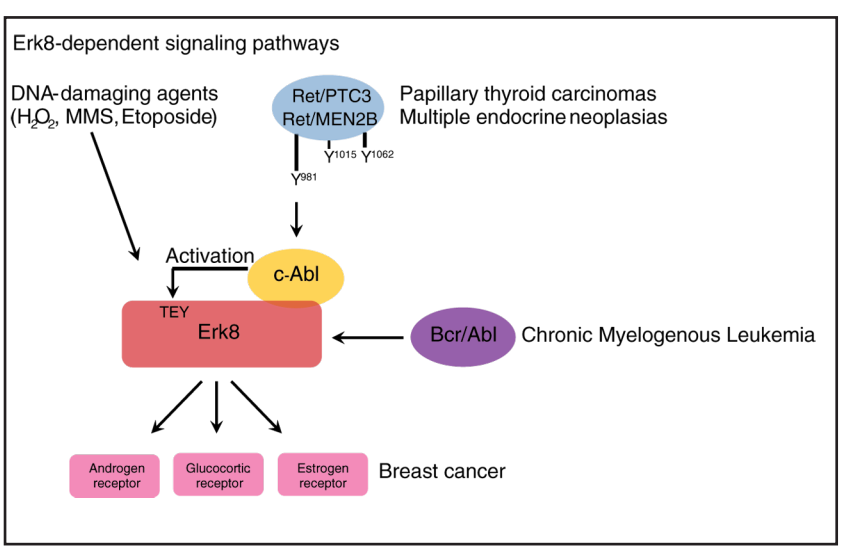

Figure 2 - Schematic representation of the currently known oncogenes and signaling pathways impinging on the regulation of Erk8. In turn, this MAP kinase controls the expression and activity of different nuclear receptors. Human tumors, currently studied because correlated to Erk8 functions, are also indicated

colon cancer cells. Still, the identity of its upstream activators and downstream effectors is almost completely unknown. Likewise, the mechanisms controlling the activity of this MAP kinase and its biological functions have yet to be defined.

The goal of this Unit is to achieve a comprehensive characterization of the mechanisms contributing to the regulation of cell growth and transformation by MAP kinases and, in particular, by Erk8. Therefore, we expect that full understanding of the signal transduction mechanisms governing the activity of Erk8 and its downstream targets will, in the near future, help in generating a rational approach to the management of tumors whose pathogenesis depends on the production of molecular information through this molecule.

To accomplish the goal of the Unit, we are pursuing the following research themes.

1. Unraveling basic signal transduction mechanisms involving the Erk8 protein

MAP kinases are able to control different signal transduction pathways based on their enzymatic activity and their ability to physically interact with other enzymes and scaffolding proteins (a). Erk8 is therefore expected to function as a key integration point, receiving signals from several independent transduction pathways and controlling different processes, such as cell proliferation and transformation. In our effort to identify Erk8-specific substrates and interacting proteins, using a two-hybrid approach, we have already discovered and validated several clones, among which transcription factors, trans-membrane proteins and nuclear receptors. In general, several of these proteins have already been involved in the control of cell proliferation, metabolism and neurodegenerative diseases and represent interesting potential targets for Erk8 functions, particularly in cell transformation.

2. Unraveling the role of Erk8 in Abl-dependent signaling pathways and in human Hematological malignancies

Based on our data showing the ability of the Bcr/Abl oncogene to interact and modulate Erk8 activity (b), we are addressing the role of Erk8 during the tumorigenic process sustained by this human oncogene and how it would be possible for us to interfere, generating a rational approach to the management of these tumors.

3. Studying the toll of Erk8 in mammary gland physiology and cancer

Erk8 has been recently correlated to the control of the protein levels and functions of different nuclear receptors, among which androgen, glucocorticoid and estrogen (d,e). Erk8 also strongly enhances degradation of the estrogen receptor-alpha, and loss of the MAP kinase has been correlated to breast cancer progression (e). Based on this information and on our preliminary results, we are addressing the role of Erk8 in breast cancer and characterizing novel Erk8-dependent signaling pathways contributing to this disease.

4. Using ERK8 as molecular target to select specific pharmacological inhibitors

We are building a homology model structure of the catalytic domain of Erk8 (Figure 3) to be used to screen libraries of kinase inhibitors, to select drugs showing *in silico* selectivity for this MAP kinase. Once obtained, we will first test them on Erk8 and then examine the capacity of such inhibitors to affect proliferation and resistance of human cancer cells to chemotherapy, to develop novel therapeutic approaches to human malignancies.

Through these studies, we expect to establish novel Erk8-dependent signaling pathways involved in controlling different aspects of both normal and cancer cell growth.

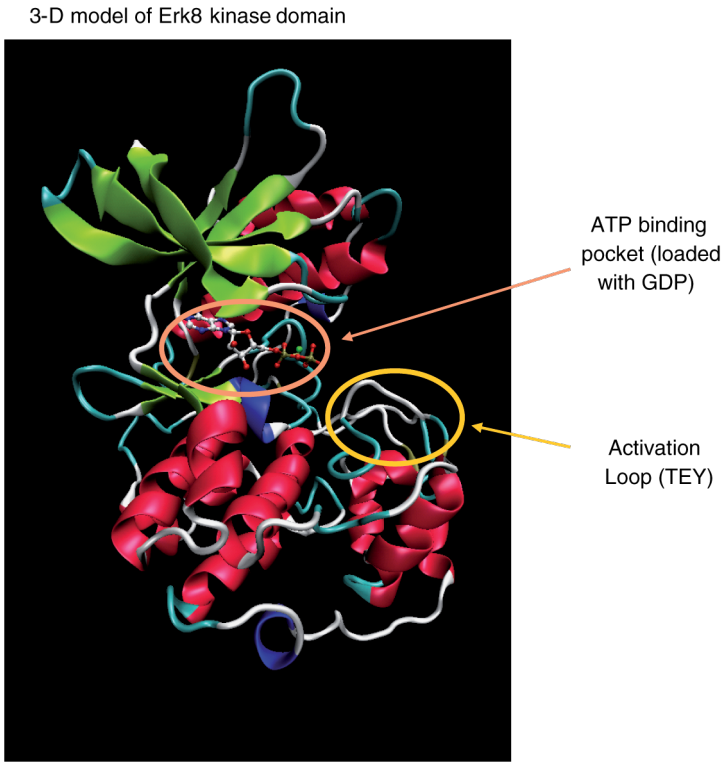

Figure 3 - Structure of the Erk8 catalytic domain obtained by Homology Modeling and subsequent Molecular Dynamics refinement. The N-term lobe is dominated by beta strands (yellow), the C-term lobe is mostly helical (orange) and contains the "Activation Loop" with the two phosphorylation sites (Thr-175 and Tyr-177) controlling kinase activity. The ATP-binding pocket is located at the interface between the two lobes

Understanding the mechanism involved in the regulation of Erk8 activity and the biological processes they participate in will therefore provide us a rationale for the development of new pharmacological strategies that are designed to target tumors whose pathogenesis and/or survival depends fully or in part on signals modulated by Erk8. Supporting this approach, several pharmacological inhibitors have already been developed, targeting other MAP kinases, and some of these inhibitors have already been effective in animal models and have therefore advanced to clinical trials for the treatment of inflammatory diseases and cancer. At the same time, fundamental new knowledge will be obtained that is expected to significantly advance the general field regarding the biology of MAP kinases and their intracellular signaling intermediates.

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Research Grants

Year	Funding Agency	Amount
2007- 2010	Istituto Toscano Tumori	€ 945,000
2007	Associazione Italiana per la Ricerca sul Cancro (AIRC)	€ 40,000
2006	AIRC	€ 40,000
2005	AIRC	€ 40,000
2004	AIRC	€ 45,000

Main Collaborations

With Units within ITT

» Clinical Physiology Institute, Consiglio Nazionale delle Ricerche (CNR), Siena
» Department of Experimental Pathology and Oncology, University of Florence

With other Italian and Foreign Institutions/Organizations

» Endocrinology and Experimental Oncology Institute, CNR, Napoli
» Fondazione IFOM, Istituto Europeo di Oncologia (IEO), Milano
» Department of Biology and Molecular Pathology, "Federico II" University, Napoli
» National Institute of Dental and Craniofacial Research, National Institutes of Health (NIH) (USA)
» Laboratorio de Oncología Molecular, Universidad de Castilla-La Mancha, Albacete (Spain)

Publications

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TUMOR CELL BIOLOGY

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Introduction

The Tumor Cell Biology Unit started its research activity in May 2009. Our research aims to understand how signaling pathways and gene expression programs control normal development, and how disruptions of these processes lead to tumorigenesis and cancer metastasis. We are approaching these questions by the study of the Hedgehog-Gli (Hh-Gli) signaling pathway. The Hh-Gli pathway plays critical roles in development, homeostasis and cancer. Secreted Hh ligands (Sonic, Indian and Desert) initiate signaling

in receiving cells by binding and inactivating the 12-pass transmembrane receptor, Patched (Ptch), which relieves its catalytic inhibition of the 7-pass transmembrane protein, Smoothened (Smo). Consequently, active Smo triggers an intracellular signaling cascade that enables activation and inhibits the formation of repressors of the Gli zinc finger transcription factors. Thus, Hh signaling regulates the function of the three Gli proteins and their activation. Among the targets, there are regulators of proliferation and differentiation, survival, self-renewal, angiogenesis and invasiveness (Figure 1).
During embryonic development, the Hh pathway is critical for normal patterning of diverse structures, such as the digits of the limb or the neuronal types in the spinal cord. Later, during embryogenesis, Hh-Gli is required for proliferation of neural precursors in dorsal brain regions. Loss of Sonic Hh causes cyclopia and ventral forebrain-patterning defects. On the contrary, abnormal Hh-Gli pathway activation can induce tumors, such as basal cell carcinoma, medulloblastoma and rhabdomyosarcoma.

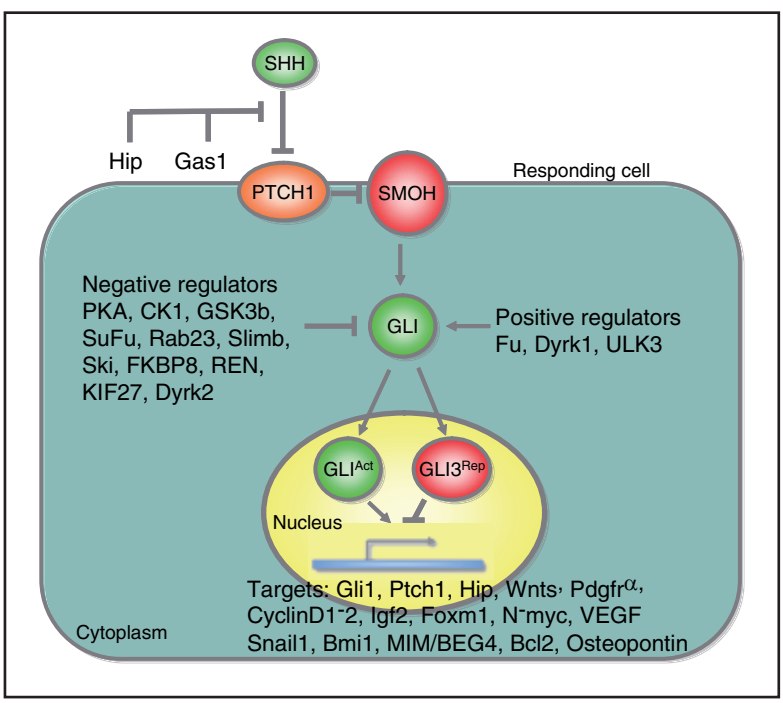

Figure 1 - Schematic representation of the Hh-Gli signaling pathway

Main Research Themes

1. Role of Hh-Gli signaling in the maintenance of Melanoma Stem Cells (MSC)

Main achievements: In the last few years, numerous studies have highlighted a critical role for continuous Hh-Gli signaling activity in driving the growth of an increasing number of human cancers. Our group has shown that the Hh-Gli pathway is required for melanoma growth. In fact, its blockade inhibits melanoma cell proliferation *in vitro* and leads to regression of melanoma xenografts and inhibition of lung metastases in mice (3). Recent studies point to a requirement for Hh pathway activity in maintenance and self-renewal of stem cells. In particular, we recently showed that Gli1 increases the number of brain stem cells in a conditional transgenic mouse model (1). These findings are relevant to cancer because of the possible derivation of Cancer Stem Cells (CSC), a sub-population within a cancer that is capable of its propagation, from adult tissue stem cells.

Current and future work: A recently described tumor cell subpopulation is under intense scrutiny due to its ability to self-renew and re-initiate tumor growth. These cells, called CSC or "tumor initiating cells", are thought to drive tumor initiation, development and metastasis, as well as to be responsible for their recurrence. CSC have been reported in primary melanomas and their derived metastases. However, although several studies have shown the existence of MSC, the identification of genes that are necessary for MSC generation and self-renewal is lacking. We plan to isolate and characterize MSC and investigate the requirement of Hh-Gli signaling for their proliferation and self-renewal *in vitro* and *in vivo*, by testing their tumorigenic and metastatic potential. These studies are carried out in collaboration with the Plastic Surgery Unit, coordinated by Dr. Borgognoni.

2. Modulation of Hh-Gli signaling in stem cells and cancer

Main achievements: Recent data suggest that the Hh-Gli pathway is positively regulated by multiple oncogenic signaling events, including peptide growth factors, receptor tyrosine kinases, RAS, MEK, phosphoinositide-3 kinase and AKT. We have previously shown that oncogenic H- or N-RAS and AKT1 potentiate Gli1 function, by enhancing its transcriptional activity and nuclear localization and counteracting its cytoplasmic retention by the negative regulator, Suppressor of Fused, in melanoma, gliomas and prostate cancer cells (3). Phenotypic interactions between RAS and Gli have been also described in various mouse models. Several lines of evidence also suggest that Gli proteins are regulated by tumor suppressors. Interestingly, we found that p53 negatively controls Gli1 by inhibiting its self-renewing and proliferation activities. Mechanistically, we showed that p53 inhibits the activity, nuclear localization and levels of Gli1 and that, in turn, Gli1 represses p53, establishing an inhibitory loop. We have proposed that the imbalance between Gli1 and p53 activities, and ultimately p53 loss, a common event during tumor progression, releases the normally restricted activities of Gli1, leading to an uncontrolled expansion of CSC and their derived progenitors (1).

Current and future work: It appears clear that a strict regulation of Hh-Gli signaling is critical not only for normal development, but also to maintain homeostasis, to control stem cell behavior and to prevent tumor formation. Also, Gli1 functions as a key integration point, where signals from several pathways converge. However, the identity of its upstream regulators is only partially known. Our goal is to understand the mechanisms contributing to the regulation of cell growth, transformation and tumor progression by Hh. In particular, we are testing the role of a novel putative oncogene in the modulation of Gli1 in stem cells and in cancer.

3. Identification of novel Hh-Gli targets by genome-wide mapping of Gli1 binding sites

Main achievements: The combinatorial function of the three Gli proteins modulates the cellular responses to Hh signaling. In particular, Gli1 plays a critical role in several human cancers, being frequently amplified in glioblastoma. Gli1 is a C_2-H_2 zinc finger transcription factor (a). Upon translocation into the nucleus, Gli1 elicits its activating function on target genes by recognizing the consensus sequence 5'-GACCACCCA-3' on the DNA (b). The molecular mechanisms that mediate cellular responses to Hh-Gli activation, however, have not been completely characterized and thus far only a few direct Gli target genes have been identified.

Current and future work: We will map Gli1 binding sites throughout the genome. Combining Chromatin Immunoprecipitation (ChIP) to the new sequencing technology developed by Illumina, we aim to identify genes or miRNAs whose expression is controlled by Gli1. We plan to validate a number of Gli1 binding sites by site-specific PCR on chromatin immunoprecipitated DNA and to correlate Gli1 binding to the regulatory region to Gli1-dependent target expression. Our attention will focus not only on genes that might be regulated by Gli1, but also on miRNAs whose expression might be induced by activation of the Hh-Gli pathway. Finally, we will perform functional studies on some of the newly identified targets to investigate whether they might be responsible for the increased proliferation, self-renewal and enhanced invasion ability observed in Hh-Gli1 activation.

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Main Collaborations

With Units within ITT
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» Department of Pathology and Experimental Oncology, University of Florence
» Department of Dermatology, University of Florence

With other Italian and Foreign Institutions/Organizations
» Centre Medical Universitaire (CMU), University of Geneva (Switzerland)

Publications

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CLINICAL TRIALS COORDINATING CENTER

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Daniela Baldari, Clinical Trials Administrator
Sandra Biancanelli, Clinical Trials Administrator

Introduction

The Clinical Trials Coordinating Center (CTCC), a Research Unit of the CRL of the Istituto Toscano Tumori (ITT), operates to promote multi-institutional independent research through the development, management and reporting of hypothesis-driven clinical trials.

For each trial, the CTCC provides the oncological network of Tuscany comprehensive services that include:

- developing protocols, forms and manuals of operations;
- designing web-based randomization;
- implementing trial management and web-based data gathering systems;
- providing training;
- recruiting investigators and centers;
- monitoring recruitment;
- conducting reviews and evaluations;
- monitoring adverse effects and events;
- maintaining data bases and analyzing data;
- assuring quality control;
- providing annual and interim reports;
- collecting final data and producing final data sets and final reports;
- overseeing regulatory compliance;
- monitoring vital status;
- disseminating study information.

Clinical Trials

Description	Year	Sponsor	Number of patients recruited to date
High-Intensity Focused Ultrasound (HIFU) in association with surgery and chemotherapy in patients with operable breast cancer	Planning phase	AUSL 7 Siena	0
Clinical activity of DNA damaging and non-damaging chemotherapy regimens in advanced breast cancer patients with different biological sub-types defined by biomarker evaluation on circulating tumor cells	Planning phase	AUSL 4 Prato	0
Multicenter phase III randomized study of cisplatin and etoposide with or without bevacizumab as first-line treatment in extensive stage small-cell lung cancer	2009	Gruppo Oncologico Italiano di Ricerca Clinica (GOIRC) and Agenzia Italiana del Farmaco (AIFA)	6
Phase II study of cetuximab in combination with cisplatin-docetaxel in the first-line treatment of patients with advanced NSCLC: a biomarker-based assessment of activity. Protocol GOIRC 03/2008	2009	GOIRC	3
Phase II randomized trial of an oral chemotherapy combination of capecitabine + vinorelbine and their sequential single agent use in metastatic breast cancer	2009	GOIRC	3

Description	Year	Sponsor	Number of patients recruited to date
Randomised placebo controlled phase III study with low doses of tamoxifen in women with intraepithelial neoplasia of the breast	2008	Ente Ospedaliero Ospedali “Galliera”	20
A phase III randomized trial of FOLFOXIRI + bevacizumab *versus* FOLFIRI + bevacizumab as first-line treatment for metastatic colorectal cancer	2008	Gruppo Oncologico del Nord Ovest (GONO)	240
An open-label, multicenter, randomized phase III study of second-line chemotherapy with or without bevacizumab in metastatic colorectal cancer patients who have received first-line chemotherapy plus bevacizumab	2008	GONO	70
Randomized phase II study of pemetrexed *versus* pemetrexed and carboplatin as second line chemotherapy in advanced Non-Small Cell Lung Cancer (NSCLC)	2007	GOIRC	241
A multicenter randomized phase III study: 5-fluorouracile *versus* 5-fluorouracile + oxaliplatin in combination with pelvic radiotherapy as preoperative treatment of resectable, locally-advanced rectal cancer	2003	University of Pisa	752
A randomized phase III multicenter study: prevention of chemotherapy-induced menopause by temporary ovarian suppression with triptorelin *versus* controls in young breast cancer patients	2003	Istituto Nazionale per la Ricerca sul Cancro (IST)	282

Research Grants

Year	Funding Agency	Amount
2009	GlaxoSmithKline	€ 15,000
2009	University of Modena	€ 25,000

Main Collaborations

With Units within ITT
» University of Florence
» Azienda Ospedaliero Universitaria Pisana

With other Italian and Foreign Institutions/Organizations
» Istituto Nazionale per la Ricerca sul Cancro (IST), Genova

- Ente Ospedaliero Ospedali "Galliera", Genova
- University of Genoa
- Istituto Giannina Gaslini, Ospedale Pediatrico, Genova
- GOIRC
- GONO

Publications

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LIST OF PIs AND AUTHORS

We have tried very hard to give in this Report a comprehensive account of the Research activities and related Service activities of the Istituto Toscano Tumori (ITT), the Comprehensive Cancer Network of our Region. We are aware of the fact that, despite our efforts, the picture is not complete, and for this we apologize. We are also confident that errors and omissions will be corrected in the next ITT Scientific Report.

The Directors

Midnight, May 22, 2010

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