IRE
ISTITUTO NAZIONALE TUMORI
REGINA ELENA

Istituti Fisioterapici Ospitalieri • Roma
Istituto Nazionale Tumori "Regina Elena" • Roma

Scientific Report 2007/2008

Ongoing biomedical, clinical and translational research

Istituti Fisioterapici Ospitalieri • Roma
Istituto Nazionale Tumori "Regina Elena" • Roma

Via Elio Chianesi, 53 - 00144 Roma
Tel. +39 06 52662728 - Fax +39 06 52665523
e-mail: dirscire@ifo.it - www.ifo.it

SCIENTIFIC REPORT 2007/2008
Ongoing biomedical, clinical and translational research

Publisher: IRE Regina Elena National Cancer Institute, Rome (Italy)
Number: 6
Month/Year: November 2008
Editor: Paola Muti
Editorial Assistant: Geraldine Williams
Editorial Secretary: Barbara Matrascia
Photographer: Ivana Zardin
Graphic design, layout and printing: L. G. Soc. Coop., Rome (Italy), +3906688444
Paper: Garda Pat

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Scientific Director's Report

Paola Muti
Scientific Director

In recent years the Regina Elena Cancer Institute has witnessed intense growth and progress. It is therefore timely and appropriate that we resume a long-standing tradition at the Institute – the publication of our scientific report.

This 2007-2008 report will focus primarily on our current scientific activity and in particular on our new molecular medicine program of *translational research*.

The program is based on a new collaboration between surgical, medical and pathology oncologists involved in clinical research and those involved in molecular investigation. The whole molecular medicine program has been organized around the opening of *new high technology laboratories* and a new program of *intramural funds*.

Research Facilities

Cancer is a disease characterized by numerous genetic aberrations and epigenetic modifications. It is our research mission to find out which molecular pathways or abnormalities contribute to cancer development and how we can act to *prevent* its initiation and its progression. Furthermore, in the last few years, there has been a substantial increase in the knowledge of cancer at a molecular level which has encouraged an improvement in *molecular diagnosis* and the subsequent application of the appropriate *targeted therapies* for cancer. However, there has been much less progress in the development of clinical tools for the *molecular characterization* of patients – those who would most likely benefit from particular targeted therapies and those who would not.

Therefore, it is our research mission to use all technologically advanced instruments to support the discovery and implementation of new targeted therapies and to identify the appropriate therapies for each patient and for each cancer through "*molecular signatures*" characterizing the individual and the disease.

The new Regina Elena Cancer Institute *molecular medicine facilities* area currently represents one of the most technologically advanced laboratories in Europe to support this new diagnostic, preventive, therapeutical, predictive and prognostic approach:

The *Pyrosequencer* will use pharmacogenetics together with pharmacokinetic aspects to predict response to chemo and radiotherapy on the basis of individual molecular characteristics.

The *Affimetrix* and *Agilent* platforms for microarray and the *Illumina* will facilitate the extremely advanced microarray DNA/RNA tests, genetic analysis sequencing and gene expression (single gene approach, genome-wide approach, candidate-pathway gene approach).

The *Maldi Tof/Tof* instrument will carry out serum and cellular protein analysis in general and proteomics analyses.

These analytical units are supported by specific international collaborations and memorandum of understanding drawn up with national and international institutions, each a leader in their particular field of interest.

Finally, the *Bioinformatic Unit* will support computer science activities which will analyze the complex and large dataset produced by the facilities in close cooperation with the Bioinformatic Unit at the Weizmann Institute for Science (Israel).

In supporting this important innovation in cancer research a major role has been played by the Regina Elena Cancer Institute Administrators: the present Director General Dr. Francesco Bevere, Dr. Giorgio Marianetti (Managing Director), Dr. Amalia Allocca (Chief Medical Officer) and Dr. Marino Nonis (former Director General 2006-2008). We also thank the Administration of the Lazio Region for the structural help in furnishing the new laboratories and the Minister of Health for their important support in the implementation of technology and, in general, for supporting research activity.

Intramural Funds

In the last two years, the Scientific Director's Office has recognized the need for, and subsequently approved, a standardized policy and procedure to provide intramural support to the Regina Elena Cancer Institute members and prospective members.
The subsequent policy and procedure can be found in this report and will be detailed on the Institute website. Intramural funds derived from funds provided by the Italian Minister of Health have been made available for providing grants to start up and to develop young researchers. The goal is to be flexible in providing such funding to qualified institutional principal investigators. The priorities in determining what to fund include an emphasis on start-up funds for new investigators, funds for novel ideas or pilot projects for new/junior investigators and the development of new or novel projects within an existing program.
Requests for intramural funds are made to the Scientific Director's Office which produces calls based on relevant subjects described by the Institutional Research topics approved by the Minister of Health. The Office works through an International Commission of evaluators to review requests. The Scientific Director's Office provides funds on the basis of the Commission ranking being blind to the review process.
Under this new program, thirty-two research projects have received intramural funds from the Scientific Director's Office. In all cases, the funds are being used by staff to perform requisite aspects of the investigators' research projects.

Conclusion

In the last twenty years, the Regina Elena Cancer Institute has grown as an organization, improving its grants management system and its clinical activities. In the last two years, the Institute has created a new intramural funds program, has worked to improve inter-institutional procedures, and has provided support for translational research through the establishment of a new, highly technological area of molecular medicine. Although the future holds further challenges, such as finding further research funds and support for young investigators, we are committed to meeting these challenges and thereby ensuring a robust and supportive environment for our research and clinical plans.

Our programs and results come from our history but also look forward to the future with the ambition to create a sound, comprehensive cancer center, able to carry out patient-oriented research.
A further growth in facilities and faculty recruitment continues to be a high priority for the Regina Elena Cancer Institute. ■

Board of Governors

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(2008)

Giorgio MARIANETTI Ad Interim
(August - December 2008)

Marino NONIS
(2006-2008)

Chief Medical Officer's Report

Amalia Allocca
Chief Medical Officer

In 2007 the main objective of the Institute has been to guarantee, in qualitative terms, the efficient use of all resources, in terms of productivity, levels of care, and the appropriateness of that care.

In accordance with regional directives, the Institute carried out a program of resource rationalization, including:

- more cost-effective distribution of resources, concentrating on day hospital and outpatients, through the implementation of Complete Day Hospital Services (PAC) and the Amalgamation of Day Hospitals (APA).
- cost savings on unproductive activities and a redistribution of surgical activity, in particular with regard to the use of operating theatres.
- more efficient management of health spending, strengthening management checks and introducing spending ceilings which take account of efficiency and productivity indicators (average cost of care, direct costs on proceeds etc, discharges per doctor/nurse, etc).

The mission of the Institute is to give the best responses to the health needs of the population, in terms of oncology, by consolidating scientific competence and technological resources, in terms of diagnostic and therapeutic processes, research and prevention. This also includes working with regional, national and international organizations and bodies, complementing the specific expertise they provide in their field, in order to provide complete welfare and health care.

The Institute's mission also recognizes the need to create the right conditions for the development of new technological knowledge and competence in oncology with other centers of excellence at a national and international level. This is being realized through strategic relationships with the Region (President, Department of Health) and the National Ministry of Health (National Institutes of Health and National Research Council).
The strategic vision of the Institute is aimed at consolidating a management system which is appropriate, timely and efficient and offers a quality of service which fulfills the population's expectations and needs.

The Institute has everything in place to fulfill the role of an Oncological Center for Regional Coordination "Center Hub", focused on creating conditions for better performance, through a more prudent use of resources.

The creation of a Center of Excellence and of satellite structures will favour more appropriate interventions and improve the relationship between cost, efficiency and effectiveness.

Scientific Advisory Board

Scientific Advisory Board visit - January 2008

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Secretary Ethics Committee

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Scientific Direction

Office of the Scientific Director

Office of the Scientific Director

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Pina Gioffrè
Barbara Matrascia
Carmela Matrascia
Geraldine Williams

The **Office of the Scientific Director** is made up of the following:

- **Secretarial Office**
- **Grants Office**
- **Research Administration Office (S.A.R.)**
- **Library**
- **Education, Training and Congress Center Management (C.M.E.)**
- **Rome Oncogenomic Center (R.O.C.)**
- **Fellowship and Intramural Grant Call**
- **The Journal of Experimental and Clinical Cancer Research**
- **Patent Office**
- **Office for Academic Affairs**

SECRETARIAL OFFICE

THE SECRETARIAL OFFICE IS STAFFED BY:

Carmela Matrascia
Pina Gioffrè
Geraldine Williams

■ The office acts as a liaison point between the Scientific Director and all the departments of the Institute, representing the Scientific Director in the work of the Institute. The office supervises the budget of the Scientific Director. All official communication from the Scientific Director is processed by this office and it is the first point of contact for the international scientific community wishing to contact the Director. It supports the research work of the Director, both international and national, including organizing and attending conferences, reviewing and submitting journal articles and formalizing official collaborations between institutes. The Institute's international Scientific Advisory Board is organized from the office. ■

GRANTS OFFICE

THE GRANTS OFFICE IS STAFFED BY:

John Fox
Barbara Matrascia

The Grants Office's main priorities are:

- to carry out regular searches for information on national and international grant opportunities available and which may be of interest to IRE researchers. These include calls from international bodies such as the European Commission, the National Cancer Institute in the United States and the Susan Komen Grants Program, as well as national opportunities such as AIRC and AIFA.
- to distribute information on grant calls to the Institute and identify specific researchers who may benefit from particular calls.
- to give administrative and technical support and advice to IRE researchers making grant applications.
- to give general information on how to make grant applications (according to the grant-making body).

In the past year, the grants office has supported applications to the NIH, DOD, the Susan Komen Grants Program and the European Community FP7 Grants Program, as well as national programmes such as AIRC and AIFA.

RESEARCH ADMINISTRATION OFFICE (S.A.R.)

THE DEPARTMENT IS STAFFED BY:

Enrico Del Baglivo Director
Piera Brugnoli
Giovanni Cavallotti
Maria La Rosa
Antonia Magnifico
Silvia Malvezzi
Doriana Salvati
Fortunato Varone

S.A.R. is dedicated to supporting the IRE Scientific Director and staff in their work in securing and monitoring research projects, academic and research activities and in assisting in post-award administration. The department manages research money, supervises project administration, acquisition and project personnel and human resource contracts. It is responsible for drawing up monetary reports on the internal and external (national and international) financing of projects. It provides assistance to all staff in their research work, through financing, recruitment and technical support.

THE LIBRARY IS STAFED BY:

Head Librarian: **Gaetana Cognetti**

Administrative Staff

Giuseppe Filardo
Domenico Verbicaro

Administrative and Research Assistant

Fabio D'Orsogna
Specialized Librarian (Patient Library)
Reasearcher (Azalea Project)

Katiuscia Dormi
Graduated Specialized Librarian Documentalist,
Security Information Systems and Privacy Expert

Amelia Mazzacuva
Digital Library / Patient Library
Researcher (Azalea Project)

Francesca Servoli
Specialized Librarian,
Periodicals management expert

Maura Tuveri
Specialized Librarian,
Periodicals management expert

■ The Scientific Library was created to guarantee the provision of up-to-date scientific research for researchers and doctors. The library's earliest collections date from the 1920s and 30s, the same period which witnessed the development of the Regina Elena National Cancer Institute. At the end of the 1980s, with the establishment of the Experimental Research Center (CRS) in via delle Messi d'Oro, basic science collection was moved to the new center. Currently the print collections are composed of approximately 8.000 books and 1.000 journals, warehoused at CRS. In 2005, together with the previous library's innovative center, a new innovative center was created: the Digital Library Knowledge Center "R. Maceratini" (BDCC-IRE), placed at the Institute's new location, opening on the 16 of June 2005. The Digital Library is situated within a historical rural home, near the hospital. There is a multimedia room with 15 computers and a special room for the Patient Library, which aims to guarantee access to biomedicals and updated information, especially online, as well as organizing training courses regarding information technologies. In recent years the library's collections have grown with the addition of journals, further electronic resources and databases.

The Regina Elena Cancer Institute library supports clinical research activities by offering scientific information and documentation. The objective of the Library is to guarantee easy access to up-to-date scientific material, as well as electronic support. Apart from acting as a library, it is also a knowledge center which facilitates access to important material in order to support clinical practices and the choices of patients. In other words, the library offers its services to both medical staff and patients. The Knowledge Center aims to contribute to the computerisation of the Institute, promoting the exchange of data between different professional areas and specialisations and more importantly between researchers within our structure and the scientific world in general. The center is easy to find, situated near the main entrance of the Institute and contains a multimedia room with 16 places for PCS. There is also in a Patient Library which offers information through the use of the most up-to-date health databases and websites. On January 31st 2006 the library in the old Institute was closed and the printed collections moved to the new site. In 2007 the Digital Library registered 488 members. Naturally, most services can be obtained on line through the purchase and organisation of electronic resources (eg. scientific periodicals), which are directly accessible from the workplace of each single researcher. The main activities of the library consist of the management of monographs, periodicals and databases (inventory, cataloguing, control of collections) following international standards and guidelines; updating collective catalogues (monographs and periodicals); reference service; personalised bibliographical research; consultation of the main biomedical data bases; supplying documents through inter-library exchange (Nilde); organisation of training courses; study and research regarding the problems of biomedical information and processes of integrated information technology; production of an electronic newsletter via e-mail for institutional users.

LIBRARY HOLDINGS

The library contains about 8,000 monographs and approximately 1,000 titles of various periodicals. In 2006-2007, we purchased electronic packages for approximately 3,000 periodicals and some important databases, including the Bibliosan project.
All library activities have been automized thanks to the use of auxiliary electronic systems, which are freely available on line through the institutional cooperation of the libraries present throughout Italy. The library also runs vocational training courses and is involved in numerous scientific research activities in the use of new technology. ■

ROME ONCOGENOMIC CENTER (R.O.C.)

SCIENTIFIC COORDINATOR

Giovanni Blandino

■ The Rome Oncogenomic Center (R.O.C.) is one of the four Oncogenomic Technological Platforms founded by the Italian Association for Cancer Research (AIRC) in 2004.
The R.O.C. Consortium is hosted at the Regina Elena Cancer Institute, one of the most prominent cancer institutes in Italy founded in 1932.
Since then, the Regina Elena Cancer Institute has mainly devoted its clinical and research activities to pursue new cancer therapeutic approaches.
The R.O.C. Consortium also includes, in addition to the Regina Elena Cancer Institute, founding Institutions in order to provide the required critical mass of high quality investigators.
The University of Rome "La Sapienza" joins R.O.C. mainly as an initiative of the 1st School of Medicine, with the partecipation of the Departments of Experimental Medicine and Pathology, Genetics and Molecular Biology, Cellular Biotechnology and Hematology.
The School of Medicine of the University of Rome "Tor Vergata" participates with the Departments of Experimental Medicine, Laboratory Medicine, Biopathology, Virology, Internal Medicine-Dermatology.
The Italian Ministry of Health participates with the following Departments: "Environment and Primary Prevention", "Cellular Biology and Neuroscience", "Epidemiology and Health Prevention".
Additional institutions in the Rome area are the National Council for Research (CNR), the Istituto Mendel-Casa Sollievo della Sofferenza, the Catholic University and the Fondazione A. Cesalpino (Laboratory of Gene Expression).
External to the Rome area, but also participating in the activities of R.O.C., are the University of Milan, the University of Chieti and the University of Firenze.
The development of new "genome-wide" approaches and the completion of the human genome sequencing now offer powerful opportunities to increase knowledge and progress in cancer research and to tailor cancer diagnosis and treatment to individual patients. However, to gather a deeper understanding of the basic mechanisms that control and determine cancer transcriptome, descriptive (phenotypic) oncogenomics must evolve into a more functional genome-wide approach. To this end we need to implement innovative technological approaches to identify new target molecules and define protein/DNA/RNA modifications that alter gene expression control in cancer cell progenitors during transformation and tumor progression, as well as in cancer cells response to therapy.

The ***R.O.C. Consortium*** will put together the efforts of leading scientists in the Rome/Central Italy area to:

1. set up a "**Central Core Facility**" to serve the purposes of the three research WorkPackages that constitute the R.O.C. "Core Research Program". This includes the production and validation (design, spotting and analysis) of biochips for immunopurified chromatin based techniques and for micro-RNA profiling; high-throughput ChIP analysis and real-time PCR; laser capture microdissection; cancer stem cell technologies; siRNA - and sncRNA-based innovative approaches to cancer cells gene expression.
2. foster an integrated functional genomics research effort aimed at building up a highly innovative "**Basic Research Core Program**" organized into 3 Research Workpackages:
   a. ***Integrated approaches for target gene identification in cancer***: Systematic ChIP based target gene identification applied to a number of TFs including E2Fs, p53 and the p53 paralogs p63 and p73, NF-Y, NFkB, beta catenin, PML/RARa, myc, Notch, Gli, HIF-1a, ER, their co-activators and co-repressors and, whenever identified and functionally characterized, their post-translational modified versions.
   b. ***Snc-RNAs (siRNAs and miRNAs) as diagnostic and therapeutic tools to fight cancer***: From comprehension of the basic mechanisms of mi-RNA functioning to mi-RNA profiles in cancer cells and the design of innovative approaches to modify altered gene expression based on RNA interference and selective modulation of RNA processing, the use of "cell based" assays transgenic mouse models.
   c. ***Stem cells, cancer and cancer stem cells: from development, through plasticity to cancer***: Hunting for "stem/progenitor cells signatures" (mRNAs expression profiles, post-translational modifications affecting the function of relevant proteins; micro-RNAs; RNA processing) and the development of new reagents for the identification of tissue specific cancer stem/progenitor cells;
3. integrate the R.O.C. "Central Core Facility" with a number of pre-existing and qualified "**External Technological Platforms**" for genome wide approaches to assist the R.O.C. "Core Research Program" and to implement, as a medium/long term goal, "individual patient-oriented" molecular approaches for the diagnosis, management and cure of cancer.
4. develop at the **R.O.C. Central Core Facility** new "low cost", "second generation" expression profiling chips oriented to study specific "cancer type" restricted signatures and relevant signaling/transcription pathways.
5. provide **Integrated Educational Activities** for the wider community of clinical oncologists, graduate and post-graduate students as well as for cancer patients.

If successful, these goals will help overcome the major existing limitations for a rapid translation from basic oncogenomics to patient-oriented therapy by implementing:

- communication between clinical centers and basic research groups;
- standardized experimental protocols, data management and interpretation;
- accessibility of cost-intensive technologies for basic, translational and clinical researchers.

www.romeoncogenomiccenter.eu/ROC_home.php

Education, Training and Congress Center Management Office

ADMINISTRATIVE BOARD

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Sabrina Soresi

SCIENTIFIC BOARD

Gabriele Alessandrini
Emilio Bria
Roberta Merola

■ The Education, Training and Congress Center Management Office (C.M.E.) is made up of an administrative and scientific board.

The priorities of the office are to organize and improve the educational program of the Institute. According to the Ministry of Health (MoH) education program, the establishment of both intra- and extra-institutional events is mandatory in order to obtain the Continuing Medical Education (CME) certification.

In this context, the office is committed to following the online credit-gaining process from the MoH, by supporting all the rules for any planned event.

According to MoH regulation, the office is also involved in the economic aspects of such planning, together with the process of scheduling reports.

With regard to the scientific board of the office, the aim is to investigate the educational needs of all professional figures working in the Institute. Cooperation between the administrative and scientific boards of the office enables the yearly educational program to be scheduled on the basis of personnel requirements, following proposals coming from the Institute itself. The establishment of an educational program is a dynamic process, in which personnel are required to submit their suggestions regarding topics as well as the pros and cons of all events.

Given the mission and the specific mandate of the Institution (i.e. oncology), the education program is dedicated mainly to basic and clinical research advances, by matching both the literature and news regarding future international meetings together with the research performed within the Institute. With this intent, the program is focused mainly on translational research, which constitutes the backbone of the seminars, thanks to the collaboration between the Institute's clinical and experimental research (CRS) centers, and by also involving international opinion leaders. The education program is based on two formats: 1) breakfast meetings, which are scheduled every Thursday morning on a weekly basis, and 2) research seminars, held monthly. ■

Computer-assisted molecular design for the development of intelligent anticancer drugs and for the engineering of peptide inhibitors of pathologically relevant protein-protein interactions

PRINCIPAL INVESTIGATOR
Emanuele Bellacchio
Laboratory "C"
Department for the Development
of Therapeutic Research Programs

Abstract

■ In the last few years the amount of information on tumor biology has expanded enormously, essentially due to the completion of human genome sequencing and to the application of new technologies that represent an exciting breakthrough in molecular analysis. High-throughput technologies recently applied to human cancer gene expression are powerful tools for understanding tumor biology and identifying novel cell transformation or tumor progression-related markers. The possibility of quickly identifying genes, whose abnormal expression is associated with specific human neoplastic diseases, facilitates diagnosis and prognosis. Growing expectations in the therapeutic field are the result of the integration of such knowledge with the information produced by experiments (enzymology, inhibition mechanisms and crystallography) and theoretical predictions (molecular docking and computer-assisted drug discovery). Such information makes it possible to achieve progress in drug discovery without the need to rely exclusively on empirical approaches. Two important aims of the project are the design and development of two different classes of pro-drugs with anticancer or antiinfective action, that are based on distinct mechanisms of activation and have the objective to selectively kill cancer or infected cells. For simplicity, the pro-drugs will be given the terms drug-A and drug-B. Details will be disclosed in a later phase (pending Intellectual Property issues): 1) Drug-A is a modified peptide that can be locally activated to cytotoxicity by specific proteases. Targets for this drug are cancer cells characterized by hyperactivity of endogenous proteases, or cells hosting infections that display a high activity of exogenous proteases. Specificity of action of the drug is obtained by the insertion of a peptide sequence cleavable by the desired protease; 2) Drug-B is a modified oligonucleotide that can be locally activated to cytotoxicity by the molecular recognition of complementary sequences of nucleic acids. Targets for this drug are cancer cells marked by abnormally high levels of endogenous nucleic acids, or cells infected and marked by the presence of exogenous nucleic acids.

Specificity of action towards targets is achieved by the insertion of the proper nucleotide sequence into the drug. Another aim is the design of peptides that interfere with the protein-protein interactions established between the oncoprotein E7 from Human Papillomavirus type 16 with endogenous cellular factors. ■

RNA interference *in vivo* to define the role of genes mainly involved in malignancy

PRINCIPAL INVESTIGATOR
Gianluca Bossi
Molecular Oncogenomic Laboratory
Department of Experimental Oncology

Abstract

RNA interference has emerged as a powerful tool to study biological effects generated by modulated expression of specific protein-coding genes. RNA interference can be triggered delivering into the target cells synthetic double-stranded RNA (siRNA), or vectors (plasmid, viruses) carrying short hairpin RNA (shRNA) or microRNA (miRNA). At present, the identification of efficient inducible systems for RNA interference in cells and tissues represents an objective of great interest in many areas of basic and applied research. Indeed, the identification of efficient conditional systems of RNA interference would allow a more profound analysis of the roles played by individual protein in cellular regulatory processes both in "*in vitro*" and "*in vivo*" approaches. With this aim, in the last two years, efforts were devoted to the identification of an efficient system for conditional RNA interference. As target gene of RNA interference, to define the accuracy of different experimental approaches, mutant p53 was selected, which has been for several years of central interest in cancer and is extensively studied in the Molecular Oncogenesis Laboratory. After several different attempts, a lentiviral-based conditional system was selected as a putative RNA interference model system, because of its accuracy in modulating gene expression, particularly in tumors. The model system generated proved valuable, not only for its ability in modulating *in vivo* the expression of target genes, but also because of the target gene relevance. Indeed, the p53 tumor suppressor gene is mutated in almost 50% of human tumors. Mutant p53-carrying tumors often show poor response to conventional anti-cancer therapy such as radiotherapy and chemotherapy. It is well known that TP53 mutations not only provide the inactivation of wild type p53 protein functions, but, also, may endow the mutant protein with novel properties (gain-of-function activities GOF) that contribute to tumor malignancy. Recently, we reported that the depletion of mutant p53 protein, by means of RNA interference, reduced tumor malignancy impacting on chemo-resistance and tumorigenicity *in vivo*. The submitted research proposal aims to use the lentiviral-based conditional model of RNA interference for a more detailed understanding of the gain of function activity of mutant p53 proteins *in vivo*. To this end, human tumor derived cell lines, endogenously expressing mutant p53 proteins, will be engineered with the lentiviral conditional system of RNA interference. Using this experimental approach in *in vitro* and *in vivo* systems we will be able to identify mutant-p53 target genes; microarray data generated in the laboratory will be validated by real-time PCR approach. Using this experimental approach we will also be able to compare whether the expression of target genes is differently modulated when different mutated p53 proteins are depleted. Furthermore, by using this model system a better evaluation of mutant p53 knockdown relevance will be achieved concerning tumor growth, angiogenesis and metastatic potential. Finally the lentiviral-based conditional RNA interference model, upon shRNA and/or microRNA engineering, will be employed to underscore the biological effects generated through the modulation of gene expression.

Investigating mutant p53 gain of function through oncogenomic approaches

PRINCIPAL INVESTIGATOR
Giulia Fontemaggi
R.O.C.

Abstract

P53 is considered a key tumor suppressor gene and is inactivated mainly by missense mutations in half of human cancers. It has becoming increasingly clear that mutant p53 proteins do not represent only the mere loss of wt-p53 tumor suppressor activity, but gain new oncogenic properties favoring the insurgence, the maintenance, the spreading and the chemoresistance of malignant

tumors. The recent generation of knock-in mice with common missense mutations at the p53 locus by Lozano's and Jacks's laboratories has convincingly and elegantly provided the formal proof that mutant p53 proteins selected in tumors exert pro-tumorigenic activities. The actual challenge is the fine deciphering of the molecular mechanisms underlying gain of function of mutant p53 proteins. The two following molecular scenarios can be proposed to explain gain of function of mutant p53 proteins: **(a)** mutant p53 can bind to DNA through the association with DNA binding proteins and transcriptionally activate specific target genes using its functional transactivation domain (TAD); **(b)** mutant p53 binds to and sequesters proteins whose function is required for anti-tumor functions such as apoptosis or growth inhibition. Interestingly, it has been reported that human tumor-derived p53 mutants can associate with p73 and interfere with its ability to induce apoptosis. Scenario **(a)** is the main subject of the studies that the proponent is currently leading. We performed an Affymetrix gene expression profile on H1299 cells overexpressing an inducible mutant p53His175 and found that mutant p53 clearly modulates gene expression. Moreover, using some of the modulated genes as models to study mp53 transcriptional activity we could assess that mutant p53 can also be recruited to promoter regions, probably through the participation of other transcription factors.

Specific aim: We aim to investigate the transcriptional signature determined by the binding of mutant p53 to a specific set of its target promoters in cancer. Moreover, to shed light on a novel/potential molecular mechanism underlying gain of function of mutant p53 proteins we propose to assess whether mutant p53 expression is capable of modulating the expression of miRNAs in tumor cells. **Experimental plan: 1)** ***ChIP on chip approach to identify in vivo transcriptional targets of gain of function mutant p53.*** The ChIP-chip technique is a very powerful technology that combines Chromatin Immunoprecipitation (ChIP) and microarray analysis allowing genome-wide evaluation of *in vivo* promoter occupancy of a certain transcription factor. At the Rome Oncogenomic Center platform we have generated the first dedicated ChIP-chip slide for mutant p53. The slide is a low-density array enclosing 50-mer oligonucleotides complementary to the promoters of all the known mutant p53 target genes and will be challenged with the following chromatin samples: **I)** p53-bound

chromatin from mutant p53 carrying cell lines; **II)** chromatin bound to the transcription factors that mediate mutant p53 binding to DNA (NF-Y, E2F-1, p65NFkB, etc.). **III)** chromatin bound to proteins that are usually associated with an active transcriptional status (histone acetyltransferases, acetylated histones and polymerase II). **IV)** p53-bound chromatin from tissue samples derived from head/neck and breast cancers. **2) *High-throughput approach to study the microRNAs profiling in normal vs tumor samples with reference to p53 status*:** to verify whether mutant p53 exerts gain of function through the modulation of miRNAs, whose gene targets silencing favors the insurgence, maintaining and chemoresistance of mutant p53 tumor cells, we will perform: **I)** a microarray analysis of 460 mature miRNAs using RNA samples from head/neck and breast cancers. To this end the LNA technology will be exploited to generate a low density microarray slide at the ROC platform; **II)** the validation of the miRNAs modulated in cancer through qRT-PCR Taq-Man method; **III)** the sequencing of the region of P53 gene (exons 5 to 8) holding the hot spot nucleotides for mutations; **IV)** the generation of low-density ChIP-chip dedicated arrays for the promoter analysis on the modulated miRNAs to identify possible direct interactions between mutant p53 and miR promoters. **Expected results:** The proposed genome-wide studies will lead to the identification of novel molecular pathways that could facilitate the development of both diagnostic markers and therapeutic targets for mutant p53 tumors. ■

Molecular mechanisms in endothelin A receptor-driven epithelial-to-mesenchymal transition and metastasis in ovarian cancer: implication for an effective targeted-therapy

PRINCIPAL INVESTIGATOR
Laura Rosanò Laboratory "A"
Associated to the Surgical Oncology Department

Abstract

■ Despite the combination of tumor debulking and chemotherapy that has led to improved treatment results in recent years, in most patients with ovarian cancer relapse rates are high, rendering treatment of this tumor with molecular targeted therapy of great clinical interest. The selective activation of the ETA receptor (ET_AR) by endothelin-1 (ET-1) promotes ovarian tumor progression by regulating cell proliferation, survival, angiogenesis, and epithelial to mesenchymal transition (EMT), a critical process in the invasive and metastatic competence of tumor cells. In order to better understand the molecular interactions that occur in EMT after ET_AR activation, in this project we propose to evaluate the role of ß-arrestin, that functions as regulator and adapter of G-protein-coupled receptor (GPCR), as cytoplasmic signal transducer to connect and expand the cross-talk between the GPCR ET_AR and the tyrosine kinase receptor, epidermal growth factor receptor (EGFR) in driving ß-catenin-mediated signalling and cell invasion. Moreover, we will explore whether ß-arrestin could function as nuclear messenger of ET_AR to mediate epigenetic signaling that control Snail and ß-catenin transcriptional programs leading to E-cadherin suppression and EMT. The findings that ET_AR is a molecular target that regulates structural and functional changes in EMT determinants, may allow the expansion of the therapeutic repertoire of ET_AR blockade by exploring the tumor response end points of ZD4054, a novel highly specific ET_AR antagonist, as a fruitful approach to control ovarian cancer cell progression, tumor growth and metastasis. Beside ET_AR, EGFR is a critical therapeutic target in ovarian cancer. However, the majority of ovarian cancer patients display acquired resistance to the treatment with EGFR inhibitors, such as gefitinib, suggesting that activation of alternative escaping signallings can counteract EGFR blockade. In this context, ET_AR-mediated EGFR transactivation could represent a possible mechanism of resistance to anti-EGFR therapy. Recently, as has been demonstrated, an inverse functional correlation between EMT-related gene expression and EGFR inhibitor resistance suggesting that E-cadherin and other EMT-related genes may represent potential markers of response to EGFR inhibitors and may also play a role in the mechanism underlying sensitivity to these drugs. In order to identify such mechanisms, we will evaluate whether pharmacologic blockade of ET_AR with specific ET_AR antagonist ZD4054, by restoring E-cadherin expression, can predict and influence response to gefitinib. The identification of the molecular changes that occur during ovarian tumor progression and EGFR inhibitor resistance will allow the design of integrated therapies in which combination with ZD4054 may overcome gefitinib resistance. Considering that new ET_AR targeting strategies are in clinical development, the combination therapy with ET_AR antagonist that concomitantly may block EGFR transactivation, revert EMT and restore E-cadherin, will offer an exciting opportunity to increase sensitivity to EGFR inhibitors, and provide a novel, potentially effective therapy for the treatment of metastatic ovarian cancer. The future design and implementation of clinical trials ultimately will determine the validity of these approaches. ■

Interplay between the endothelin axis and hypoxic microenvironment in melanoma progression: therapeutic implication

PRINCIPAL INVESTIGATOR
Francesca Spinella Laboratory "A"
Associated to the Surgical Oncology Department

Abstract

■ The steady increase of melanoma incidence in recent years, the early metastatization of the tumor, and the resistance of advanced melanoma to current treatment regimens underscore the importance of acquiring a better understanding of the genetic and environmental factors driving the natural history of this malignancy. Gene expression profiling and phenotypic analysis of human cutaneous melanoma identified endothelin B receptor (ET_BR) a tumor progression marker thus representing a potential therapeutic target. Upon activation by endothelin (ET)-1 or ET-3, ET_BR promotes tumorigenesis and melanoma progression by increasing the expression of angiogenenic and invasiveness markers. Moreover, these effects increase under hypoxic conditions suggesting that ETs may cooperate with hypoxia to potentiate the aggressiveness and invasion in melanoma cells. In order to define

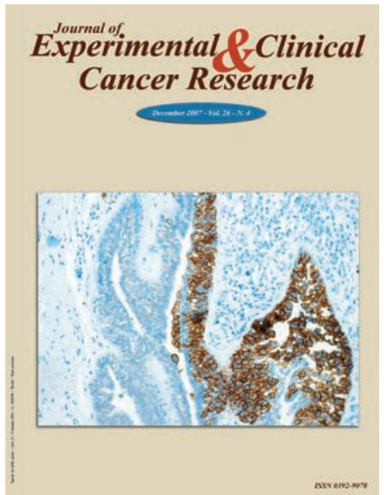
the interplay between ET_BR and hypoxia in modulating melanoma development and progression, this project aims to investigate the hypoxia-inducible factor (HIF)-1∂ driven cell fate influence in response to ETs under both normoxia and hypoxia setting. We will investigate in melanocytes and in primary and metastatic melanoma cells, the molecular mechanism by which ETs regulate HIF-1∂, the main transcriptional factor that allows cellular adaptation to hypoxia, leading to transcriptional programs that control the early steps of melanomagenesis and development of more aggressive tumors. Previous studies reported that a hypoxic microenvironment is necessary to allow melanocyte transformation initiated by Akt, that is reduced by disruption of the PI3K/Akt/mTOR pathway by inhibiting HIF-1∂ activity. Therefore, we will analyze the pathway involved for the formation of ET_BR-driven melanoma, potentially through regulation of HIF-1∂ activation. Another question that deserves additional study is whether hypoxic context may regulate ET axis in melanoma cells. Thus, tumor hypoxic microenvironment can activate HIF-1∂ enhancing the transcriptional activity of target genes, such as ET-1, ET-2 and ET-3 that through the binding of ET_BR, may, in turn, increase HIF-1∂ and related genes triggering a positive autocrine loop between HIF-1∂ and ET axis. This study may allow the disclousure of a regulatory mechanism which relies on the convergence of microenvironmental hypoxia and ET axis influencing melanoma development and progression through HIF-1∂ and related signaling cascade. The identification of regulatory mechanisms which are at the basis of the interplay between microenvironmental hypoxia and ET-1 axis may have significant implications on expanding the therapeutic repertoire of HIF-targeting agents, by developing novel protocols in the treatment of melanoma. In this regard, the confirmation of the role of ET_BR as a new molecular target that regulates melanoma development and progression, may allow the expansion of the therapeutic repertoire of ET_BR blockade by exploring the efficacy of A192621, a novel highly selective ET_BR antagonist, in the control of melanoma growth, invasion and metastasis formation. ■

Definition of molecular mechanisms involved in the angiogenic phenotype of bcl-2 and bcl-xl oncogenes: therapeutical implications

PRINCIPAL INVESTIGATOR
Daniela Trisciuoglio
Experimental Chemotherapy Laboratory
Department of Experimental Oncology

Abstract

■ Sustained angiogenesis is one of the essential alterations in cell physiology, which characterises cancer cells. How the interplay between environmental and genetic mechanisms influences tumor angiogenesis is at present a complex and unresolved matter. An understanding of the temporal and magnitude sequences of the generation of signals that can induce or block angiogenesis should help towards the development of effective therapeutic strategies. Since VEGF, CXCL8 and HIF-1 are the most important factors inducing angiogenesis, the definition of genes, such as bcl-2 or bcl-xL, able to modulate their expression, might have particular relevance. It could be useful in clarifying the basis of pathological angiogenesis and for early diagnosis. Similarly, since angiogenesis can be discerned in premalignant lesions, defining the relationship between bcl-2 or bcl-xL and angiogenesis will be relevant for prognosis prediction. The results of this project should have a significant impact in clinical cancer management. The definition of molecular mechanisms involved in the angiogenic phenotype will permit the design of new therapeutic approaches able to counteract tumor growth, metastatization and angiogenesis. Also, the study of bcl-2 overexpressing tumors sensitivity to multitargeted therapy could add important information for cancer treatment. Therefore, on the basis of previous results obtained in the Experimental Chemotherapy Laboratory, with the aim of improving cancer treatment, the objectives of this project are 1) to clarify the molecular mechanism by which bcl-2/bcl-xL increase angiogenesis, investigating the different pathways involved in this process; 2) to identify combination of agents which could improve the efficacy of anti-angiogenesis therapies (i.e. bcl-2/bcl-xL antisense oligonucleotides), targeting those pathways involved in tumor angiogenesis and, in general, in the metastatization process. ■



■ The Journal of Experimental & Clinical Cancer Research (JECCR), the official journal of the National Cancer Institute "Regina Elena", publishes quarterly original contributions dealing with basic and applied research in the field of experimental and clinical oncology; in particular it publishes scientific studies on the biological, epidemiological, immunological, pathological, radiobiological, and clinical aspects of oncology. Topics range from molecular genetics via infectious agents to surgery and therapeutic approaches and outcomes. The broad oncological focus, combined with the Editors' experience in evaluating and conducting systematic reviews and developing clinical practice guidelines, makes JECCR an attractive forum for publishing reviews and guidelines.
In 2007 117 manuscripts were submitted for publication, with 84 accepted by referees. Their place of origin is as follows:
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We believe that new editorial changes combined with the advantages of BioMed Central's open access publishing platform will greatly improve JECCR's dissemination and visibility. The Journal's online archive dating back to 1999 remains available from its former homepage.

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Scientific Activity

Laboratory “A” of Preclinical Experimental Chemotherapy

Director: **Gabriella Zupi**

STAFF
Annamaria Biroccio
Antonio Candiloro
Carmen D'Angelo
Donatella Del Bufalo
Francesca Di Modugno
Carlo Leonetti

Scientific Activity*

■ The work of the Laboratory centers on the issues listed below.

ROLE OF GENES (BCL-XL) AND MOLECULES (MEK INHIBITOR) ON TUMOR ANGIOGENESIS

Based on our previous studies, which demonstrated that i) bcl-2 overexpression in tumor cells synergistically interacts with hypoxia to modulate the vascular endothelial growth factor (VEGF) and angiogenesis, ii) bcl-xL upregulates the proangiogenic chemokine interleukin-8 (CXCL8) through AP-1 and NF-kB-dependent mechanism, in 2006 we performed experiments to evaluate in more detail the molecular mechanism by which bcl-xL modulates NF-kB pathway, and to analyse whether bcl-xL can also be involved in the modulation of the angiogenic phenotype of other tumor hystotypes. Using the ADF human glioblastoma cell line and two bcl-xL overexpressing clones, we demonstrated that the overexpression of bcl-xL in the ADF line induced NF-kB activation through phosphorylation of IKKa/B and subsequent IkBa phosphorylation and degradation. Moreover, the relevance of NF-kB in bcl-xL-induced increase of CXCL8 was confirmed by the use of a mutant form of IkB-a.
Transient overexpression of bcl-xL extended the ability of bcl-xL to increase CXCL8 expression to other tumor cell lines of different origin, such as colon and prostate carcinomas and melanoma. In particular, using M14 melanoma cells, the role of CXCL8 on bcl-xL-induced angiogenesis was validated through CXCL8 neutralizing antibody, while bcl-xL antisense oligonucleotides and RNA interference confirmed the involvement of bcl-xL on CXCL8 expression. In conclusion, our results demonstrated that NF-kB-mediated CXCL8 upregulation by bcl-xL increases tumor angiogenesis, and they point to elucidate an additional function of bcl-xL protein.
In collaboration with Dr Milella, using preclinical models of melanoma we also investigated the anti-tumor activity (in terms of effects on cell growth, cell cycle progression, and induction of apoptosis) and the anti-angiogenic potential of the novel MEK inhibitor PD0325901. Our results indicate that PD0325901 inhibited VEGF and CXCL8 production *in vitro* under both normoxic and hypoxic conditions, through inhibition of hypoxia-stimulated HIF-la expression and transcriptional activation.

CHEMOKINE RECEPTORS INHIBITORS FOR MELANOMA TREATMENT

The chemokine receptors CXCRl and CXCR2 and their ligand CXCL8 have been found to regulate the tumor progression and the invasion of melanoma cells to sites of metastases. In 2006, in the search for the development of new anticancer drugs, the effect on tumor growth and metastasis of Meraxin, a novel noncompetitive allosteric inhibitor of the chemokine receptors, CXCRl and

CXCR2, was evaluated. Two different human melanoma cell lines (A375SM, M20) expressing CXCRl and CXCR2 and a human melanoma cell line (SbCll) negative for both receptors were employed for *in vitro* and *in vivo* studies. Our results demonstrated that treatment with CXCL8 resulted in ERK1,2 and AKT phosphorylation an increase of *in vitro* cell proliferation, invasion and migration of melanoma cells expressing CXCRl and CXCR2, but not in melanoma cells negative for both receptors. Meraxin significantly suppressed *in vitro* CXCL8-induced signaling and functional activation of melanoma cell lines expressing CXCRl and CXCR2, including invasion and migration. *In vivo*, Meraxin efficiently inhibited artificial metastasis of A375SM and M20 cell lines while not affecting the number of artificial metastases of SbCll cells. Finally, the strong antimetastatic effect was further confirmed on M20 spontaneous metastases. In contrast, local growth of melanoma tumors was not affected by the compound treatment. In conclusion this is the first evidence indicating that a small molecular weight inhibitor of CXCRl and CXCR2 shows a relevant antimetastatic effect *in vivo* and, taken together, these results support the clinical development of Meraxin in the therapy of metastatic melanoma.

COMBINATION TARGETED THERAPY FOR HUMAN SOLID TUMORS

A number of molecular genetic changes have been associated with the development of hormone-refractory prostate cancer (HPRC), including the overexpression of Bcl-2, a proto-oncogene that belongs to a family of related genes whose proteins regulate apoptosis, and of c-myc oncogene which plays a significant role in the regulation of cellular proliferation, differentiation and apoptosis. Based on this background and with the aim of improving the response to HPRC chemotherapy, we investigated the antitumor efficacy of docetaxel in combination with antisense oligonucleotides (ASOs) against bcl-2 (G3139) and c-myc (INX-6295) in an experimental model of HRPC *in vitro* in culture cells and in xenografts. To this end, PC-3 androgen-independent human prostate cancer cells exhibiting over expression of c-Myc and Bcl-2 protein, were treated *in vitro* with different scheduling of administration of the three agents. Our results showed that the triple combination of drugs given in the sequence G3139/docetaxel/INX-6295 was the most active, compared to the other scheduling of treatments, in reducing the survival of PC3 cells with colony formation around 2%. This effect was consistent with the ability of G3139/docetaxel/INX-6295 combination to trigger apoptosis as more than 80% TUNEL-positive cells were observed. The *in vivo* experiment confirmed that this schedule was the most active as the treatment of PC3 tumor-bearing inhibited about 80% of the tumor weight and this effect persisted for more than 40 days; moreover a complete regression of tumors was observed in 50% of mice. The treatment produced an overall increase of mice survival of 111% and a complete cure in 2 out of 8 mice. This treatment exhibited efficacy also when started at a very late stage of tumor growth (about 500 mg of tumor mass) producing about 80% of tumor weight inhibition with the regression of tumors maintained for 1 month. This antitumoral effect resulted in a significant increase (70%) of mice survival. These results indicate that a combination strategy based on the use of antisense ODNs targeting different pathways is able to sensitize human prostate cancer to therapy and that the success of this therapy seems to be related to the rationale sequence of administration of the agents employed.

In the course of the collaboration with Tor Vergata University, Rome, we have demonstrated the ability of poly (ADP-ribose) polymerase-1 (PARP-1) inhibitors to enhance the antitumor efficacy of chemotherapy. In particular, we observed that the novel PARP-1 inhibitor GPI 15427 administered orally, was able to sensitize melanoma and primary and secondary brain tumors to the methylating agent temozolomide by inhibiting the activity of DNA repair enzyme 06 -alkylguanine DNA alkyltransferase (AGT) and the mismatch repair system (MR), two mechanisms involved in the resistance to methylating agents. Clinical trials are now evaluating the therapeutic efficacy of temozolomide in combination with

irinotecan, a topoisomerase I inhibitor, in colorectal cancer, one of the most common gastrointestinal tract malignancies, due to the synergistic effects observed in experimental models. Since a novel molecular approach to enhance the antitumor activity of temolozomide and irinotecan relies on the use of chemical inhibitors of PARP, we evaluated whether the PARP inhibitor GPI 15427 increases the antitumor activity of temozolomide and irinotecan combination against colon cancer. Results of colony forming assay demonstrated that GPI 15427 combined with temozolomide and irinotecan showed a synergistic activity (combination index < 1) in reducing the proliferation of colon cancer cells. Moreover, this combination was highly active *in vivo* reducing significantly the growth of colon cancer xenografts resistant to the chemotherapy. A major concern in the use of biomodulators of resistance is that they can potentially increase the toxicity exerted by chemotherapy towards normal tissue. To this purpose, we demonstrated that the intestinal damage provoked by irinotecan is due to induction of PARP-1 activation with ADP-ribose polymer accumulation which is known to cause cell death. The administration of GPI 15427 prevents ADP-ribose polymer accumulation and protects normal epithelial cells from cell death. Moreover, we observed that GPI 15427 did not exarcerbate myelotoxicity induced by the treatment with temozolomide. In conclusion, GPI 15427 sensitizes colon cancer to the methylating agents and topoisomerase I inhibitors and reduces the toxicity of chemotherapy thus representing a novel and promising strategy for the therapy of colon cancer.

ROLE OF C-MYC IN CELLULAR RESPONSE TO STRESS

In addition to the well-established role of c-Myc in regulating cell growth, a novel picture is beginning to emerge identifying c-Myc new functions in multiple metabolic pathways. We defined a new function for c-Myc in determining cellular redox balance, identifying glutathione (GSH) as the leading molecule mediating this process. The link between c-Myc and GSH is g-glutamyl-cysteine synthetase (g-GCS), the rate-limiting enzyme catalyzing GSH biosynthesis. We found that c-Myc transcriptionally regulates the y-GCS genes by binding and activating the promoters of g-GCS heavy and light subunits. The transcriptional control of g-GCS gene expression by c-Myc occurs through its binding to the non-canonical c-Myc consensus sites identified on g-GCSH and g-GCSL gene promoters. We also demonstrated that the increased transcriptional rate and mRNA expression of g-GCS genes following oxidative stress is attributable to a higher recruitment of c-Myc to both y-

GCS promoters. Moreover, the stress-dependent regulation of c-Myc recruitment to target genes occurs quickly and strongly depending on the higher GSH synthesis requirement displayed by the stress-exposed cells.
The fast c-Myc-mediated response to stress raised the question as to whether it could be tightly regulated by post-translational modification. Indeed, exposure to H202 did not change the expression levels of c-Myc protein, but instead triggered ERK-dependent Thr-58/Ser-62 phosphorylation. The findings reported in this paper add mechanistic insights of this c-Myc phosphorylation function. Indeed, Ser-62 phosphorylation not only regulates protein stability, but can also dictate the choice of target genes. Specificala, c-Myc phosphorylation at Ser-62 is required for activation of y-GCS genes. Moreover, microarray analysis revealed that, beside both g-GCS genes, other genes resulted differentially regulated in the c-MycS62A compared to Mycwt cells, exclusively upon H202 treatment, supporting the key role of this c-Myc phosphorylation site during oxidative stress response. Finally, in this paper we attribute a biological function to c-Myc phosphorylation at Ser-62 in determining cellular response ROS-triggering agents, including H202 and anticancer drugs.
In summary, we identified an oxidative stress-induced survival signal pathway, depending on c-Myc phosphorylation, which can contribute to resistance to oxidative damage in tumor cells, which generally exhibit deregulated c-Myc expression.
TELOMERE MAINTENANCE MECHANISMS IN CANCER THERAPY TRF2, an ubiquitously expressed protein binding the tandem array of duplex telomeric repeats, is involved in telomere structure and chromosome end protection. The multiple roles of TRF2 in telomere structure and functions render it an interesting target for anti-telomere pharmacological interventions. TRF2 inhibition, by expressing the dominant negative form TRF2ABAC, has been used as a model of anti-telomere strategy to induce a reversion of the malignant pheno-type of M14 and JR5, two telomerase-positive human melanoma lines. We found that over-expression of TRF2ABAC induced apoptosis and reduced tumor genicity exclusively in JR5 cells. The inability of M14 line to respond to TRF2ABAC over-expression is not due to a lack of an apoptotic response to DNA damage because apoptosis is efficiently activated in M14 cells following treatment with the DNA-damaging agents cisplatin and adriamycin. Analysis of p53 and Rb status demonstrated that both melanoma lines are defective for p53 induction but functional for the cell cycle related functions of pRb. Thus, the different effect of TRF2ABAC in M14 and JR5 cells is unlikely to be explained by a differential functioning of the p53 or Rb pathways.
We also found a correlation between the sensitivity to TRF2 inhibition and the capping capacities of the tumor cells. Analysis of telomere status demonstrated that JR5 cells have shorter and more dysfunctional telomeres compared to M14 cells. Moreover, the gene expression profile of multiple telomere-related proteins revealed that the better capping in M14 than in JR5 was associated with a higher expression of the mRNA corresponding to the genes encoding the Mrell-Rad50-Nbsl (MRN) complex. Furthermore, we demonstrated that treatment with the G quadruplex ligand RHPS4 sensitises M14 resistant cells to TRF2 inhibition through activation of apoptosis. On the contrary, over-expression of either hTERT or TRF2 renders cells resistant to the drug. In conclusion, our results show that TRF2 inhibition can limit the proliferation of human cancer cells both *in vitro* and *in vivo*, thus strengthening TRF2 as an important target for the development of new anti-cancer therapies. The sensitivity to this inhibition depends, at least in part, upon the capping functions of the tumor cells, suggesting that the level of telomere instability in cancers can predict the efficacy of anti-TRF2 or anti-telomere therapeutics. Moreover, the combined action of both G4-ligand and anti-TRF2 molecules would represent an efficient strategy for eliminating tumor cells exhibiting a robust telomere capping. ■

* 2006 Scientific Activity because the 2007 Scientific Activity report was not submitted

DEPARTMENT OF EXPERIMENTAL ONCOLOGY

Laboratory "B" of Immunology

Director: **Pier Giorgio Natali**

STAFF

Sergio Anastasi
Francesca Baietti
Andrea Conidi
Duilia Del Bello
Giuliana Falasca
Valentina Federici
Rocco Fraioli
Patrizio Giacomini
Rossana La Sorda
Elisa Lo Monaco
Paola Nisticò
Belinda Palermo
Maria Vincenza Sarcone
Oreste Segatto
Agnese Serafini
Leonardo Sibilio
Alessandra Ranieri
Frosi Yuri

Scientific Activity*

MORPHOLOGICAL AND MOLECULAR ASSESSMENT OF APOPTOTIC MECHANISMS IN PERIPHERAL NEUROBLASTIC TUMORS

Multiple defects in apoptotic pathways have been described in peripheral neuroblastic tumors (NTs). Mitosis–karyorr-hexis index (MKI) is a reliable morphological marker identifying favourable and unfavourable NTs. The extent to which apoptotic processes contribute to determining the clinical significance of MKI is still undefined. Apoptosis was investigated in a series of 110 peripheral NTs by comparing MKI to immunohistochemical and molecular apoptotic features. High MKI was found in 55 out of 110 NTs (50%) and was associated with advanced stage ($P<0.007$), neuroblastoma (NB) histological category ($P<0.024$), MYCN amplification ($P<0.001$), and poor outcome ($P<0.011$). Overall survival probability was 45% in patients with high MKI compared to 73% in patients with low MKI. In the same 110 NTs, the expression of Bcl-2, Bcl-XL, Bax and Mcl-1 was studied by immunohistochemistry, but no significant associations were found with clinicohistological features. Microarray analysis of apoptotic genes was performed in 40 out of 110 representative tumors. No significant association was found between the expression of apoptotic genes and MKI or clinicohistological features. Proliferative activity was assessed in 60 out of 110 representative tumors using Ki67 immunostaining, but no significant correlations with MKI or clinicobiological features were found. In NTs, the combination of apoptosis and proliferation as expressed by MKI is a significant prognostic parameter, although neither of them is per se indicative of the clinicobiological behaviour and outcome.

FLAVONOIDS INHIBIT MELANOMA LUNG METASTASIS BY IMPAIRING TUMOR CELLS ENDOTHELIUM INTERACTIONS

Flavonoids comprise a class of low molecular weight compounds displaying a variety of biological activities including inhibition of tumor growth and metastasis. To gain insight into the mechanisms underlying metastasis inhibition, we have employed the B16-BL6 murine melanoma metastasis model. B57BL/6N mice were injected I.V. with tumor cells and Apigenin, Quercetin, or Tamoxifen, each at 50 mg/kg given i.p., and lung tumor cell colonies counted 6–14 days thereafter. Three different injection schedules were used for each drug: (a) daily injection, starting 24 h before injection of the tumor cells; (b) single dose, 24 h preceding tumor challenge; (c) daily injection, starting 24 h after the injection

of the tumor cells. All three compounds significantly reduced tumor lung deposits (Apigenin1/4Quercetin>Tamoxifen).
However, when treatment was delayed by 24 h after tumor cells, multiple daily doses of Apigenin or Quercetin were less effective than a single dose of the same compound given 24 h before tumor challenge. Apigenin and Quercetin, but not Tamoxifen, were found to inhibit VCAM-1 expression in a dose-dependent manner in HUVEC and in murine pulmonary endothelial cells. In *ex vivo* experiments, the number of tumor cells adhering to lung vessels was significantly diminished in animals treated with a single dose of Apigenin and Quercetin. These findings indicate that the inhibition of tumor cell metastasis by Apigenin or Quercetin may significantly depend on the ability of these compounds to alter the host's microenvironment, further substantiating the role of the intravascular processes in the metastatic cascade.

LAMININ ∂2CHAIN-POSITIVE VESSELS AND EPIDERMAL GROWTH FACTOR IN LUNG NEUROENDOCRINE CARCINOMA. A MODEL OF A NOVEL COOPERATIVE ROLE OF LAMININ-2 AND EPIDERMAL GROWTH FACTOR IN VESSEL NEOPLASTIC INVASION AND METASTASIS

Capillaries expressing the laminin ∂2 chain in basement membranes may be considered early developing vessels in normal and neoplastic human tissues. Therefore, we investigated whether up-regulation of this extracellular matrix protein favors transendothelial migration of neoplastic cells and then metastasis. In lung small and large cell neuroendocrine carcinomas, which exhibit a stronger metastatic tendency among carcinomas, laminin ∂2 chain-positive vessels were more numerous than in carcinoid tumors and supraglottis, breast, and lung non-small cell carcinomas, suggesting a direct relationship between these vessels and metastasis. *In vitro* studies showed that epidermal growth factor (EGF) induced a more efficient migration of the AE-2 lung neuroendocrine carcinoma cell line through the purified laminin ∂2 chain rather than through the laminin fl1 chain and fibronectin. AE-2 cells constitutively expressed all EGF receptors and the a6fl1 integrin, which is one of the laminin ∂2chain receptors. EGF up-regulated a6fl1 expression in several tumors. In this regard, we show that EGF increased the chemokinetic migration of AE-2 cells through EAHY endothelial monolayers, which was inhibited by the anti- a6 integrin chain monoclonal antibody. This data indicates that the laminin ∂2 chain and a6fl1 may be equally t involved in EGF-dependent migration of AE-2 cells and that laminin ∂2 chain-positive vessels may favour metastasis of EGF-dependent tumors.

HUMAN GLIOBLASTOMA ADF CELLS EXPRESS TYROSINASE, L-TYROSINE HYDROXYLASE AND MELANOSOMES AND ARE SENSITIVE TO L-TYROSINE AND PHENYLTHIOUREA

Melanocytes and neuroblasts share the ability to transform L-tyrosine through two distinct metabolic pathways leading to melanogenesis and catecholamine synthesis, respectively. While tyrosinase (TYR) activity has been shown to be expressed by neuroblastoma it remains to be established as to whether glioblastomas cells are also endowed with this property. We have addressed this issue using the human continuous glioblastoma cell line ADF. We demonstrated that these cells possess tyrosinase as well as L-tyrosine hydroxylase activity and synthesize melanosomes. Because the two pathways are potentially cyto-genotoxic due to the production of quinones, semiquinones, and reactive oxygen species (ROS), we also investigated the expression of the peroxisomal proliferators activated receptor a (PPAR) and nuclear factor-kB (NFkB) transcription factor as well the effect of Ltyrosine concentration on cell survival. We report that L-tyrosine down-regulates PPARa expression in ADF cells but not neuroblastoma and that this aminoacid and phenylthiourea induces apoptosis in glioblastoma and neuroblastoma.

PHENOTYPIC AND FUNCTIONAL CHANGES OF HUMAN MELANOMA XENOGRAFTS INDUCED BY DNA HYPOMETHYLATION: IMMUNOTHERAPEUTIC IMPLICATIONS

Emerging *in vitro* evidence points to an immunomodulatory activity of DNA hypomethylating drugs in human malignancies. We investigated the potential of 5-aza-20-deoxycytidine (5-AZA-CdR) to modulate the expression of cancer testis antigens (CTA) and of HLA class I antigens melanoma xenografts, and the resulting modifications in immunogenicity of neoplastic cells. Three primary cultures of melanoma cells, selected for immune phenotype and growth rate, were grafted into BALB/c nu/nu mice that were injected intraperitoneally with different dose- and time-schedules of 5-AZA-CdR. Molecular analyses demonstrated a de novo longlasting expression of the CTA MAGE-1, -2, -3, -4, -10, GAGE 1–6, NY-ESO-1, and the upregulation of MAGE-1, MAGE-3, and NYESO-1 levels in melanoma xenografts from 5-AZA-CdR-treated mice. Serological and biochemical analyses identified a de novo expression of NY-ESO-1 protein and a concomitant and persistent upregulation of HLA class I antigens and of HLA-A1 and -A2 alleles. Immunization of BALB/c mice with 5-AZA-CdR-treated melanoma cells generated high titer circulating anti-NY-ESO-1 antibodies. Altogether, the data obtained identifies an immunomodulatory activity of 5-AZA-CdR *in vivo* and strongly suggests the development of novel strategies of CTA-based chemoimmunotherapy for melanoma patients.

CDNA-ARRAY PROFILING OF MELANOMAS AND PAIRED MELANOCYTE CULTURES

Through a non-conventional 'paired' approach of gene expression profiling, we have compared malignant and non-transformed 'autologous' counterparts (paired cultures of melanoma cells and melanocytes from the skin surrounding tumor lesions). Signatures were identified (and validated) for early melanocyte transformation, with no need to implement formal statistical criteria. We found that CD9, Collagen type VI, and STAT2 are turned off as Breslow thickness increases, but with distinct immunohistochemical patterns. Experiments are in progress to develop a 'melanochip' for the complementation of conventional melanoma staging.

EXPRESSION OF ENDOPLASMIC RETICULUM AMINOPEPTIDASES IN EBV-B CELL LINES FROM HEALTHY DONORS AND IN LEUKEMIA/LYMPHOMA, CARCINOMA, AND MELANOMA CELL LINES

ERAP1 and ERAP2 are two Endoplasmic Reticulum Aminopeptidases recently involved in antigen processing and presentation. To determine whether they play a role in tumor immune escape, their expression and function were studied in a large panel of normal and neoplastic cell lines. Interestingly, ERAP1 but not ERAP2 was coordinately expressed with HLA class I molecules. A novel phenotype characterized by low and/or imbalanced ERAP1/ERAP2 expression was detected. Transfection with ERAP1 or ERAP2 of selected, ERAP-low tumor cell lines restored class I expression. Thus, ERAP imbalance is a novel T-cell tumor escape phenotype.

FUNCTIONAL EXPRESSION OF A SINGLE-CHAIN ANTIBODY TO ERBB-2 IN PLANTS AND CELL-FREE SYSTEMS

Antibodies targeting the ErbB-2 pathway are a preferred therapeutic option for patients with advanced breast cancer. We have developed a multi-platform approach for the production of recombinant Single chain Fragments of antibody variable regions (ScFvs) to ErbB-2 in (a) bacteria, (b) transient as well as stable transgenic tobacco plants, and (c) a newly developed, disulphide-capable, high-yield, cell-free transcription-translation system. The ultimate goal is to manufacture recombinant antibodies with biological properties comparable to those of Trastuzumab/Herceptin®, but without the inherent disadvantages of whole antibody production in mammalian CHO cells (large size, high cost, zoonotic infection carryover).

THE CYTOSKELETON REGULATORY PROTEIN HMENA (ENAH) IS OVEREXPRESSED IN HUMAN BENIGN BREAST LESIONS WITH HIGH RISK OF TRANSFORMATION AND HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR-2-POSITIVE/HORMONAL RECEPTOR-NEGATIVE TUMORS.

hMena (ENAH), a cytoskeleton regulatory protein involved in the regulation of cell motility and adhesion, is overexpressed in breast cancer. The aim of this study was to define at what stage of breast carcinogenesis hMena is overexpressed and to correlate hMena overexpression with established prognostic factors in breast cancer, focusing on human

epidermal growth factor receptor-2 (HER-2).
hMena expression was assessed immunohistochemically in a prospective cohort of cases (n = 360) encompassing a highly representative spectrum of benign breast diseases associated with different risks of transformation, in situ invasive, and metastatic tumors. Correlations with conventional pathologic and prognostic variables, such as proliferation index, hormonal receptor status, and HER-2 overexpression, were also evaluated. *in vitro* experiments were done to study the effect of neuregulin-1 and Herceptin treatments on hMena expression.
hMena protein is undetectable in normal breast and is weakly expressed in a small percentage of low-risk benign diseases (9%), but displays a progressive and significant increase of positivity in benign lesions at higher risk of transformation (slightly increased risk 43%; moderate increased risk 67%), in in situ (72%), invasive (93%), and metastatic breast cancer (91%). A significant direct correlation with tumor size (P = 0.04), proliferation index (P < 0.0001), and HER-2 overexpression (P < 0.0001) and an inverse relationship with estrogen (P = 0.036) and progesterone receptors (P = 0.001) are found in invasive carcinomas. *in vitro* experiments show that neuregulin-1up-regulates, whereas Herceptin down-regulates, hMena expression.
Our data provides new insights into the relevance of acting-binding proteins in human breast carcinogenesis and indicates hMena overexpression as a surrogate indicator in breast disease management.

THE EVOLUTIONARILY CONSERVED EBR MODULE OF RALT/MIG6 MEDIATES SUPPRESSION OF THE EGFR CATALYTIC ACTIVITY

Physiological signalling by the EGF receptor controls developmental processes and tissue homeostasis, whereas aberrant EGFR activity drives oncogenic cell transformation. Under normal conditions the EGFR must therefore generate outputs of defined strength and duration. With this aim, cells balance EGFR activity via different modalities of negative signalling. Increasing attention is being given to transcriptionally controlled feedback inhibitors of EGFR, namely RALT/MIG6, LRIG1, SOCS4 and SOCS5. Genetic studies in mice have revealed the essential role of Ralt/Mig6 in regulating Egfr-driven skin morphogenesis and tumor formation, yet the mechanisms through which RALT abrogates EGFR activity are still undefined. We report that RALT suppresses EGFR function by inhibiting its catalytic activity. The evolutionarily conserved ErbB binding region (EBR) is necessary and sufficient to carry out RALT-dependent suppression of EGFR kinase activity *in vitro* and in intact cells. The mechanism involves the binding of the EBR to residues of the EGFR kinase that participate in allosteric control of EGFR catalytic activity. Our results uncovered a novel mechanism of temporal regulation of EGFR activity in vertebrate organisms. ■

* 2006 Scientific Activity because the 2007 Scientific Activity report was not submitted

DEPARTMENT OF EXPERIMENTAL ONCOLOGY

Laboratory "C" of Molecular Oncogenesis

Director: **Ada Sacchi**

STAFF
Rita Falcioni
Maria Pia Gentileschi
Giulia Piaggio
Maria Giulia Rizzo
Silvia Soddu

Scientific Activity*

■ The work of our laboratory has traditionally concentrated on four major issues:
1. p53 and p53 family members: tumor response and tumor progression
2. Signal control of cell cycle progression
3. Integrin signaling in growth control
4. Hormone regulation of telomere dysfunction in cancer

1. P53 AND P53 FAMILY MEMBERS:TUMOR RESPONSE AND TUMOR PROGRESSION

The TP53 gene is the most frequent target for genetic alterations in tumors mutating in over 50% of human cancers. The primary outcome of these mutations is the loss of tumor suppressing functions of the wild type-p53 (wtp53) protein. However, the high frequency of missense mutations and the high expression levels of mutant-p53 (mp53) proteins in cancer cells would suggest that mp53 proteins have possibly acquired new functions ("gain of function" GOF), that actively contribute to cancer development and progression. In recent years, a growing number of studies have provided compelling evidence which indicates mp53 GOF activity in tumor cells. These activities range from enhanced proliferation in culture, to increased tumorigenicity *in vivo*, and enhanced resistance to a variety of anti-cancer drugs commonly used in clinical practice. The molecular mechanisms underlying gain-of-function activity of human tumor-derived p53 mutants are yet to be established. According to several *in vitro* and *in vivo* studies, two potential models can be depicted. The first relies on the fact that almost 90% of p53 mutations reside in the core domain of p53 protein. Thus, mutant p53 proteins are unable to recognize wt-p53 DNA binding sites, while their N-terminal transactivation domains are still intact and functional. The second one relies on the large amount of mutant p53 protein that is present in tumor cells and that could serve as a protein-protein interaction platform for sequestering and inactivating the proteins (for instance p73 and p63) required for anti-tumoral effects. Growing evidence suggests that the integration of both models might explain gain-of-function activity of mutant p53.
In view of the fact that most of the studies reported so far regarding GOF activity of mp53 proteins have been performed upon overexpression of exogenous mutant proteins, we evaluated whether depletion of mp53 by RNA interference (RNAi) compromises mp53 GOF activity in more physiological systems such as human cancer cell lines endogenously expressing different forms of mp53 proteins. In these studies three human cancer cell lines (i.e. SKBR3 breast cancer cells, HT29 and SW480 colon cancer cells) were employed, and RNA interferences were introduced by a pRetroSuper vector carrying oligonucleotide sequences specific to either the human p53 mRNA (p53i) or the bacteri-

al b-galattosidase mRNA (LAcZi, RNAi control). Even if to different degrees, the knocking down of mp53 proteins was efficient and specific in all three cell lines tested. Thus, we evaluated whether the mp53 knockdown modifies the cell growth, the tumorigenicity and the resistance to anticancer drugs of cancer cell lines. Interestingly, the results showed that mp53 depletion reduces:
1) the growth rate and the replication rate, such as the number of BrdU positive cells, in all three cell lines; 2) the *in vivo* tumorigenicity of HT29 and SW480 cells as in xenograft experiments, and the *in vitro* tumorigenicity of SKBR3 cells as in soft-agar assay; 3) the chemo-resistance of SKBR3, HT29, or SW480 cells to the treatment with different drugs (i.e., cisplatin, adriamycin, or etoposide).
P73, the homologue of p53, is a nuclear protein whose ectopic expression, in p53+/+ and p53 -/- cells, recapitulates the best-characterized p53 effects, such as growth arrest, apoptosis and differentiation. Altered expression of the p73 gene has been reported in neuroblastoma, lung cancer, prostate cancer and renal cell carcinoma as well as in breast cancer, ovarian tumor, melanoma and hematopoietic neoplasia. P73 has a complex genomic organization that largely results from an alternative internal promoter in intron 3 generating NH2-terminally deleted dominant negative proteins (DN-p73) and differential splicing of the COOH-terminal exons (a, b, d, e, x, Ë, Ë1 isoforms), of which the two major forms are p73a and p73b. These different splicing variants at the COOH-terminus were shown to have variable homo- and heterotypic interactions between each other and with p53 whereas the DN-p73, that lacks the transactivation domain, exerts a dominant negative function towards p53 and p73 activity. Therefore, it is likely that the various products of this gene participate, in different ways, in a complex network that regulates cell growth, death, and differentiation, giving rise to a family of proteins that adds a new level of complexity to understanding p73 signaling in cancer cells. Indeed, several studies have demonstrated that expression of p73 is markedly enhanced during differentiation of myeloid leukemic cells. We have shown that leukemic blasts from acute myeloid leukemia (AML) patients exhibit an increased expression of shorter p73 isoforms (g, d, e,). In addition, we described a distinct expression pattern of the DN-p73 isoform in the peculiar acute promyelocytic leukemia (APL) subset as compared to other AMLs, thus indicating a potential role of p73 isoforms in acute myeloid leukemias (AMLs). We speculate that a complex p73 isoform profile with an alternative expression pattern of a particular p73 variant (DN-p73 in APL) might represent a non-mutational mechanism of leukemogenesis of which the study can shed light on the pathogenesis of AMLs.
The transcriptional coactivator Yes-associated protein (YAP) has been shown to interact with and to enhance p73-dependent apoptosis in response to DNA damage. Here, we show that YAP requires the promyelocytic leukemia gene (PML) and nuclear body localization to co-activate p73. YAP imparts selectivity to p73 by promoting the activation of a subset of p53 and/or p73 target promoters. Endogenous p73, YAP, and p300 proteins are concomitantly recruited onto the regulatory regions of the apoptotic target gene p53AIP1 only when cells are exposed to apoptotic conditions. Silencing of YAP by specific siRNA impairs p300 recruitment and reduces histone acetylation on the p53AIP1 target gene, resulting in delayed or reduced apoptosis mediated by p73. We also found that YAP contributes to the DNA damage-induced accumulation of p73 and potentiates the p300-mediated acetylation of p73. Altogether, our findings identify YAP as a key determinant of p73 gene targeting in response to DNA damage.
To define the modulation of p53 tumor suppressor activity in tumor cells, we studied HDMX expression and activity in collaboration with Dr. Fabiola Moretti (CNR - Rome). The HDMX protein is closely related to HDM2 with which it shares different structural domains, particularly the p53 binding domain and the ring finger domain, where the two HDM proteins interact. Several oncogenic forms derived from the splicing of HDM2 have been described in cancer.

This work investigated whether analogous forms of HDMX exist in human tumors. Here, we report the characterization of an aberrantly spliced form of HDMX, HDMX211, isolated from the thyroid tumor cell line, ARO.
HDMX211 in samples that over-express HDM2 protein, supporting a pathologic role for this new protein. This is the first evidence of a variant form of HDMX that has oncogenic potential independently of p53. HDMX211 reveals a new mechanism for overexpression of the oncoprotein HDM2. Most interestingly, it outlines a possible molecular explanation for a yet unclarified tumor phenotype, characterized by simultaneous overexpression of HDM2 and wild-type p53.
The p53 tumor suppressor gene is activated in response to DNA damage resulting in either growth arrest or apoptosis. We previously demonstrated the specific involvement of homeodomain interacting protein-kinase 2 (HIPK2), a nuclear serine / threonine kinase, in inducing p53-dependent apoptosis through selective p53 phosphorylation at serine 46 after severe genotoxic damage. Continuing the collaboration with Dr. Gabriella D'Orazi (Chieti University), we can now show that HIPK2 contributes to p53 regulation, independently from serine 46 phosphorylation upon non-apoptotic DNA damage such as that induced by cytostatic doses of cisplatin. We show that HIPK2 depletion by RNA interference inhibits p53 binding to the p21Waf1 promoter affecting its p53-induced transactivation, thereby allowing cell proliferation. We found that non-apoptotic DNA damage induces p53 acetylation mediated by the HAT protein PCAF and this p53 post-translational modification is abolished by HIPK2 depletion. We found that HIPK2 cooperates with PCAF to induce selectively p53 transcriptional activity towards the p21Waf1 promoter, while depletion of either HIPK2 or PCAF abolished this function. This data shows that HIPK2 regulates the p53 growth arrest function through its PCAF-mediated acetylation. The involvement of the homeodomain-interacting protein kinase 2 (HIPK2)/p53 complex on MDM2 regulation, following apoptotic DNA damage, was also studied. Interestingly, our results provide a plausible transcriptional (p53-dependent) and post-transcriptional (p53-independent) double mechanism by which HIPK2 accomplishes MDM2 down-modulation. In wtp53-carrying cells, HIPK2-dependent p53Ser46 phosphorylation selectively inhibits MDM2 at transcriptional level. HIPK2 interacts with MDM2 *in vitro* and *in vivo* and promotes MDM2 nuclear export and proteasome degradation, in p53-null cells. This p53-independent effect is likely to be mediated by HIPK2 catalytic activity and we found that HIPK2 phosphorylates MDM2 *in vitro*. In response to DNA damage, depletion of HIPK2 by RNA-interference abolishes MDM2 protein degradation. We propose that HIPK2 contributes to drug- induced modulation of MDM2 activity at transcriptional (through p53Ser46 phosphorylation) and post-transcriptional (through p53-independent subcellular re-localization and proteasome degradation) levels.
By characterizing the HIPK2 knock out mice (kindly provided by A. Fusco and F. Trapasso) together with a series of cells in which HIPK2 was either overexpressed by recombinant adenovirus infection or depleted by RNA interfering strategies, we found that HIPK2 is dispensable for cell proliferation and its depletion contributes to the generation of genomic instability. Thus, we are currently studying the molecular mechanisms underlying the HIPK2-dependent proliferation defects and directly testing the possible interrelationship between HIPK2 and p53 in this activity.

2. SIGNAL CONTROL OF CELL CYCLE PROGRESSION

Studies of the transcription control of cell cycle progression and differentiation have particularly focused on understanding how the NF-Y transcription factor regulates cellular functions such as cell fate, cell proliferation and/or transformation. The rationale is based on the consideration that the majority of genes essential for the progression of the cells throughout the cell cycle phases are regulated, at transcription level, by the NF-Y complex. NF-Y is a highly conserved transcription factor that binds to CCAAT motifs in the promoter regions in a variety of genes involved in cell cycle progression. It is

composed of three subunits, NF-YA, NF-YB, and NF-YC, all required for DNA binding. Expression of NF-YA fluctuates in a cell cycle-dependent manner and is down-regulated in post-mitotic cells, indicating its role as the regulatory sub-unit of the complex. We have demonstrated that the levels of NF-YA protein are regulated at post-translational level by proteasome degradation both in proliferating and post-mitotic cells. We have shown that endogenous NF-YA protein is ubiquitinated. Interestingly, the lysines that are the target for ubiquitination are also a target for acetylation by p300. NF-Y activates transcription through mechanisms that are still unclear. In the last year, we demonstrated that the binding of NF-Y to its target promoters is essential for post-translational modifications of histones, resulting in the modulation of cell cycle gene transcription.We have shown that in proliferating cells, when NF-Y is recruited onto target promoters, histones H3 and H4 became locally hyperacetylated, p300 is recruited and transcription of cell cycle genes is induced. We have also shown that in post-mitotic tissues, transcription of NF-Y target genes responsible for cell cycle progression is switched off and this is accompanied by a hypermethylation of histones H3 and H4 implying a crucial role for the NF-Y transcription factor in organizing chromatin conformation. Moreover, by studying the role of NF-Y in cell growth, we observed that an ectopic NF-Y activity caused a strong inhibition of colony formation in mouse embryo fibroblasts and that the growth inhibition was dependent upon the presence of a wild-type p53. This date indicates a new role for the ubiquitously expressed transcription factor NF-Y in the transduction pathway of the apoptotic signal.

3. INTEGRIN SIGNALING IN GROWTH CONTROL

Many studies have indicated that the a6b4 integrin, a laminin receptor, may be involved in tumor progression and invasion. We demonstrated that b4 integrin sub-unit associates with ErbB-2 tyrosine kinase in human mammary carcinoma cell lines and that its overexpression in NIH3T3/ErbB-2 transformed cells, causes a constitutive activation of PI3K and inducing a strong increase in their invasive capacity. We are now investigating the biological consequences of the interference with the endogenous b4 integrin sub-unit expression in mammary tumor cells. To this end, the shRNA approach in mammary tumor cells has been employed. The results indicate that the loss of b4 integrin does not affect the proliferation of interfered cells and induces minimal effects on the ability of the cells to adhere to laminin. More interestingly, b4 depletion caused a strong reduction of the colony forming ability confirming the role of b4 integrin in tumorigenicity. Based on previous findings which demonstrate that the activation of PI3K by the a6b4 integrin promotes the invasion and survival of transformed cells, we verified the consequences of b4 depletion on PI3K activity. The results showed a strong down regulation of PI3K activity in b4-shRNA cells, which was independent of mutations in the catalytic sub-unit (PIK3CA), and a reduced downstream signaling of PI3K (reduced AKT and mTOR phosphorylation). Moreover, since it is well known that mammary cells proliferate and survive under hormone stimuli and that the hormones activate PI3K activity, we decided to investigate whether the interference with b4 integrin sub-unit could be relevant for cell growth in the absence of hormones. Interestingly, we found that b4-shRNA cells in the absence of hormones show reduced survival and that treatment with tamoxifen further increases b4-shRNA cell death. To conclude, our data indicates that b4 expression is relevant for 1) anchorage-independent growth of mammary tumor cells, 2) PI3K activity and signaling, and 3) survival upon hormone deprivation. Overall, these results confirm the relevance of b4 expression in mammary tumors and indicate this integrin as a relevant target for tumor therapy.

4. HORMONE REGULATION OF TELOMERE DYSFUNCTION IN CANCER

The following studies were carried out in close collaboration with the Molecular Oncogenesis Laboratory and Dr. Antonella Farsetti (CNR, Rome): 1) signaling through estrogen receptors modulates angiogenesis via human telomerase; and 2) gene profiling and sex hormone receptors status: towards a predictive molecular signature for prostate cancer.

1. Angiogenesis is a hallmark in the pathology of many diseases, including cancer, ischemia, atherosclerosis, and inflammatory diseases. A considerable amount of evidence suggests that estrogens directly modulate angiogenesis and cardiovascular aging, acting primarily at the level of endothelial cells, which constitutively express estrogen receptors (ERs). Estrogen has a dramatic impact on response to vascular injury and the development of atherosclerosis. To address, at molecular level, the role of estrogens as regulators of the endothelial function and the mechanism underlying the modulation of eNOS action, we evaluated the effects of 17b-estradiol (E2) or 4-hydroxytamoxifen (OHT) on a non-traditional target of estrogen receptor signaling, the catalytic sub-unit of human telomerase, hTERT. It was demonstrated that overexpression of telomerase allows human umbilical endothelial cells (HUVEC) to bypass senescence, although the molecular mechanisms underlying this phenomenon and their relationship to estrogen action are largely unknown.

2. The histopathological and molecular heterogeneity of prostate cancer and the limited availability of human tumor tissue makes it difficult to establish the mechanisms of prostate carcinogenesis.

Our goal was to develop an *ex vivo* model that could be reliably utilized to define a prognostic signature based on the gene expression profiling of cell cultures that maintained the tumor phenotype. To this end, we derived epithelial cultures from tissue explanted from 59 patients undergoing radical prostatectomy or cistoprostatectomy because of prostate benign hyperplasia prostate cancer or bladder carcinoma. Patient selection criteria was based on those without hormonal neo-adjuvant treatment before surgery and a diagnosis of clinically localized disease. Using this unique experimental material, we analyzed the expression of 22,500 transcripts on the Affymetrix Human U133A Gene Chips platform. Cultures from normal/hyperplastictissues with a prevalent luminal phenotype, and from normal prostate epithelial tissue with basal phenotype (PrEC), served as controls. We established a large number of prostate primary cultures which were highly enriched in the secretory phenotype. From these, we derived an epithelial-restricted transcriptional signature that: 1) differentiated normal cells from tumor cells; and 2) clearly separated cancer-derived lines into two distinct groups which correlated with indolent and aggressive clinical behaviors of the disease.

Our findings provide: 1) a method to expand human primary prostate carcinoma cells with a luminal phenotype; 2) a powerful experimental model to study primary prostate cancer biology; and 3) a novel means to characterize these tumors from a molecular genetic standpoint for prognostic and/or predictive purposes. ■

* 2005 Scientific Activity because the 2007 Scientific Activity report was not submitted

DEPARTMENT OF EXPERIMENTAL ONCOLOGY

Laboratory "D" of Virology

Director: **Aldo Venuti**

STAFF

Researchers
Gianna Badaracco
Federico De Marco

Technicians
Silvio Flamini
Antonio Muller
Sabatino Petrelli

Post Doc
Rossella Brandi
Fabio Isidi
Francesca Paolini
Consuelo Rizzo

Collaborators
Giuseppe Borzacchiello Federico II University, Naples
Saveria Campo Glasgow University, UK
Chiara Cini Sapienza University, Rome
Raffaella Coccia Sapienza University, Rome
Fabio Di Domenico Sapienza University, Rome
G. Di Guardo Teramo University
Rosella Franconi ENEA, Rome
Elena Illiano ENEA, Rome
Fatma Houissa Habib Tameur Hospital, Tunis
Eduard Kakarriqy Inst. of Public Health, Tirana
Maijlinda Kota Inst. of Public Health, Tirana
Kozeta Kule Inst. of Public Health, Tirana
Silvia Massa ENEA, Rome
Marzia Perluigi Sapienza University, Rome
Franco Roperto Federico II University, Naples
Sante Roperto Federico II University, Naples
Della Salde Teramo University
Cristiano Spadaccio Tor Vergata University, Rome

Scientific Activity

■ The activity of the Virology department focused on translational and basic research in HPV–associated cancers and on aspects of the melanocyte transformation. The main achievements of these studies are reported below.

A) TRANSLATIONAL RESEARCH

The viral characteristics of HPV-induced pathogenesis, like genotype and variant, oncogene expression, physical status and viral load, were analysed by PCR-based methods to ascertain their biological relevance on population screenings and patient follow-up.

Genital tumors

Studies of the circulation of HPV types in the Mediterranean have confirmed that HPV16 is the most prevalent and that neither the European nor African variants appear to be associated with any additional risk of cervical cancer.

In a group of women treated with neo-adjuvant chemo- and/or radio-therapy for cervical cancer, the molecular analyses carried out on multiple specimens collected before and after treatment indicated that the presence of HPV18 was related to a major persistence of the infection. The global HPV clearance rate was about 38%, independent of the initial tumor stage. This study is still ongoing but there is a clear indication that HPV persistence is linked to a lower overall survival rate. Typing is expected to be increasingly utilized in order to monitor the efficacy of preventive vaccines. Comparative analysis of several commercial tests, namely: high risk Hybrid Capture II assay (Digene, Italy); PreTect-HPV-Proofer (Norchip, Norway); and Reverse Linear Array (Roche, Italy) performed in our laboratory, indicated that typing gave discordant results, suggesting that the existing assays still failed to identify all of the HPV types in the sample. In order to improve the typing performance in our clinical samples we utilized a new technique based on nucleic acid hybridation on polymer chip. To check the specificity and sensitivity of the methods, the same samples were also analyzed by homemade PCR with MY or GP 5+/6+ primers and with the mentioned previously commercial kits.
Considering a K over 0.60 as a good concordance value, all the methods, excluding Norchip, appeared to give the same performance. The typing analysis showed that all the various methods can detect easily the HPV 16,18 and 33, whereas for other types, different methods detected different HPV types. Therefore typing on polymer chip may be a valid tool in the typing of HPV infection and is less time consuming (the test can be performed in just 6 hours) even if new and improved HPV typing tests are still required.

Extra-genital tumors

In a group of 115 patients affected by head and neck cancer we identified a subset of tumors (18%) significantly associated to HPV, mostly to the high risk HPV16. Notably tonsil cancer is particularly associated with HPV (75%). In the tumors the viral DNA is present in episomal and integrated forms, both concurring to viral oncogene transcription. Conversely, HPV viral load was largely variable. By multivariate analysis, the HPV bearing tumors presented a more differentiated phenotype and were associated with better survival, both as DFS and OS at two year intervals. Overall, the study has shown that different mechanisms are involved in malignant transformation for each single HN sub-site and that the presence of HPV is a useful tool for the prognostic assessment of HN carcinomas.
In HPV associated cutaneous tumors we have further improved our diagnostic tools in order to detect and type the enormous number of HPV infecting the epidermis as saprophytes. A special micro-array was generated on glass-slides in order to detect, by laser scanning, the presence and typing of 24 beta and gamma HPVs (HPV types 4, 5, 8,9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 48, 49, 50, 60, and 65). Because the HPV-specific PCR products are only 72 bp in size, the system is suitable for formalin-fixed, paraffin-embedded specimens and other samples in which the DNA is of suboptimal quality. This technique, together with dedicated PCR, was utilised in order to identify a broad spectrum (including both alfa and beta genus) of HPV DNA sequences in three different groups of patients: i) patients with pre-neoplastic lesions (e.g. leukoplakia, erythroplakia), ii) patients with carcinomas of the oral cavity and oropharynx, and iii) patients attending the outpatients' department for dental diseases. Surprisingly cutaneous HPVs (genus beta) are present to the same extent in normal and pre-neoplastic samples, whereas their presence dramatically decreases in tumor specimens. The prevalence of mucosal HPVs shows an opposite tendency with the highest value in the tumor samples, resembling the mucosal HPV trend in cervix cancer. HPV mRNA analysis was performed to ascertain the role of these cutaneous HPV in the oro-pharynx carcinogenesis.

B) THERAPY OF HPV AND ASSOCIATED CANCER

In order to increase the efficacy of therapeutic vaccines against the E7 antigen based on recombinant plant and plant virus sequences, genetic immunization and plant-derived antigens were studied.

Genetic immunization

Two DNA constructs, already produced by our group, and expressing the E7 antigen fused to the signal sequence of plant protein (PGIPss) or to the coat protein (CP) of Patatovirus X (PVX), were used. In particular, studies were focused on the CP construct. Western blot analysis and immunoprecipitation showed that fusion proteins were produced at low levels. The addition of proteasome inhibitors caused a dramatic increase in fusion protein content in cellular lysates, suggesting that the fusion of CP to the C-terminus of E7 leads to an enhanced processing through the proteasome-ubiquitin pathway and, in turn, a better antigen presentation. Immunofluorescence of transfected cells confirmed expression of the fused oncoproteins. This data gives a biological explanation

for the already reported efficacy of the fusion HPV16 E7-CP gene in curing animals from experimental induced cancer. The fusion of CP to E7 antigen seems to be a valid strategy to improve the efficacy of therapeutic genetic vaccines targeting cervical carcinoma.

Plant derived antigens for immunotherapy

A totally new construct and new technology were employed to produce a mutated form of the HPV16 E7 oncoprotein devoid of oncogenixc potentials and toxic effect (E7GGG). The E7GGG was fused to the Clostridium thermocellum b-1,3-1,4- glucanase (LicKM), already utilised for expressing target antigens in plants. The construct was introduced into agrobacterium rhizogenes strain A4 and the resulting bacteria were inoculated into nicotiana benthamiana. Five to seven days post infiltration, target antigens were produced and their estimated yields were approximately 400 mg per kg of fresh leaf tissue. The antigenicity of the plant-expressed proteins was verified by immunoblotting, using antibodies specific to LicKM and E7, in mice for their potential as prophylactic and therapeutic vaccine candidates. The fusion proteins induced HPV16 E7-specific IgG and cytotoxic T-cell responses and protected mice against challenges with E7-expressing tumor cells. Furthermore, when administered after challenge, these plant-produced antigens prevented tumor development. The reported results support the possibility of producing an anti-tumor vaccine with both therapeutic and prophylactic potential in plants. Further studies are being conducted to characterize the potential role of LicKM in the enhanced immunogenicity and protective efficacy of fusion vaccine candidates for other HPV (I.E. Types 18, 31, 45).

C) MOLECULAR CARCINOGENESIS

Animal models

Animal studies provide precious tests for the development of new anti-viral therapies and vaccines. A collaborative research with the Veterinary Faculty of the Federico II University of Naples, has revealed unsuspected activities of the BPV2 E5 and E7 oncogenes that are consistently expressed both in epithelial and in the mesenchymal component of the bovine urinary haemangiosarcomas. Moreover the E5 PDGFb receptor was also shown to be expressed and co-localized with E5 in neoplastic blood vessels. These achievements underline the importance of this animal model for target therapy studies. In collaboration with the Veterinary Faculty of the University of Teramo another animal model in caprine species was analysed; goat epithelial tumors were infected by a putative new caprine hircus papillomavirus (ChPV). DNA sequence analyses will be performed on amplified products to verify this hypothesis.

HPV oncogenes

The HPV-16 E5 inhibition of programed cell death was studied in human keratinocytes treated with paclitaxel. Extending our previous studies on paclitaxel induced apoptosis we showed that the HPV16 E5 protein dramatically inhibits the paclitaxel-induced apoptosis by a direct block of the caspase-3/caspase-8 loop without affecting the cytocrhome–c release. The hypothesis that E5 could account for the poor response of cervical tumors to taxanes chemotherapy is currently being evaluated in a follow-up study of cervical cancer patients.

Oxidative stress and malignant transformation

The effect of Reactive Oxygen Species (ROS) generated under sub-lethal UV-B irradiation on HPV-16 mRNA expression had already been studied by our research group in human keratinocytes transfected with the whole HPV-16 genome (HK-168). This new cell line has now been extensively characterised. HK-168 cells showed a basal/para-basal keratinocyte phenotype, requiring the use of serum-free chemically defined media and maintaining the ability to differentiate towards pluri-stratified epithelia. Although immortalised they were unable to anchor independent growth and were not tumorigenic. HK-168 showed a distinctive kariotype, with a complete, transcriptionally active HPV-16 genome integrated at an almost 1:1 ratio into the host haploid genome thus providing a convenient experimental model for viral transformed pre-neoplastic cell phenotype. The oxidative stress, induced by mild UVB irradiation, caused a general suppression of viral transcription in HK-168, accompanied by a moderate growth arrest, an appropriated response of cellular antioxidant enzymes, the activation of cell repair mechanisms and a mild induction of apoptosis. This response was similar to the one observed in the highly resistant diploid keratinocytes. Conversely, transformed cells devoid of HPV sequences (HaCaT), appeared extremely susceptible to apoptosis. We propose that reported suppression of viral oncogenes, possibly orchestrated by the UVB responsive E2 gene, restoring the cell control on growth and repair mechanisms, allows the damage to be repaired, ultimately resulting in a surviving response.

In the protection from ROS-generating stresses, a fundamental role is played by the NADH-Quinone-Oxidoreduttase1 (NQO1). In melanocyte/melanoma cells this enzyme, through the two electron reductions of quinones to hydroquinones, prevents the generation of the highly toxic semiquinone intermediates and provides a precursor for the generation of melanin. This scavenging mechanism is also present in dopaminergic neuronal cells, as well as in keratinocytes where it detoxifies aldehydes and quinones through their incorporation into senile pigments. Preliminary proteomic data on melanocytes and keratinocytes indicates that at least four proteins were involved in oxidative stress (OS) response, namely: Alpha Enolase; HSP 75, Elongation factor Tu and Annexin II. Further studies are underway to ascertain the actual role of these proteins in OS response during neoplastic progression.

MAJOR TRANSLATIONAL ACHIEVEMENTS

- New profiling for high-risk HPV infection.
- HPV as prognostic marker in genital and extragenital tumors.
- New fusion antigens from the plant production of therapeutic vaccines.
- Immunotherapy by genetic immunization.

DEPARTMENT FOR THE DEVELOPMENT OF THERAPEUTIC PROGRAMS

Laboratory "A" associated to the Surgical Oncological Department

Director: **PIER GIORGIO NATALI** ad interim

STAFF
Anna Bagnato
Valeriana Di Castro
Aldo Lupo
Stefania Miccadei

Scientific Activity*

ZD4054, A POTENT ENDOTHELIN RECEPTOR ANTAGONIST, INHIBITS OVARIAN CARCINOMA CELL PROLIFERATION

■ Endothelin-1 (ET-1) is present in high concentrations in ovarian cancer ascites and is overexpressed in primary and metastatic ovarian carcinomas. In these tumors, the presence of ET-1 correlates with tumor grade, enhanced neovascularization, and with vascular endothelial growth factor (VEGF) expression. ET-1 acts as an autocrine factor selectively through ETA receptor (ET_AR), predominantly expressed in ovarian carcinoma cells resulting in increased VEGF production and VEGF-mediated angiogenic effects. Previous results demonstrated that in ovarian carcinoma cells, activation of the ET-1/ ET_AR axis promotes cell proliferation, neovascularization, and invasion, which are the principal hallmarks of tumor progression. The present study was designed to investigate the *in vitro* effects of trans, N-(3-methoxy-5methylpyrazin-2-yl)-2-(4-1,3,4-oxadiazol-2-ylphenyl) pyridine-3-sulfonamide, an orally active specific ET_AR antagonist, on the ET-1–induced mitogenic effect in OVCA 433 and HEY ovarian carcinoma cell lines secreting ET-1 and expressing ET_AR and ETBR mRNA. We show that ET_AR blockade by ZD4054 inhibits ET-1–induced mitogenic effects, while the ETBR antagonist, BQ 788, is ineffective. In conclusion, our data demonstrates that ZD4054 is capable of inhibiting the proliferative activity of ET-1, indicating that this specific ET_AR antagonist may be a potential candidate in developing novel treatments of ovarian carcinoma.

ENDOTHELIN-1 IS REQUIRED DURING EPITHELIAL TO MESENCHYMAL TRANSITION IN OVARIAN CANCER PROGRESSION

In a range of human cancers, tumorigenesis is promoted by activation of the endothelin A receptor (ET_AR)/endothelin-1 (ET-1) axis. ET-1 and ET_AR are overexpressed in primary and metastatic ovarian carcinomas, and high levels of ET-1 are detectable in patient ascites, suggesting that ET-1 may promote tumor dissemination. Moreover, in these tumors, engagement of ETA receptor by ET-1 triggers tumor growth, survival, angiogenesis, and invasiveness. Thus, ET-1 enhances the secretion of matrix metalloproteinases, disrupts intercellular communication, and stimulates cell migration and invasion. Therefore, we investigated the role of the ET-1/ ET_AR autocrine axis in promoting epithelial to mesenchymal transition (EMT) in ovarian tumor cells, a key event in cancer metastasis, in which epithelial cells depolarize, disassemble cell-cell contacts, and adopt an invasive phenotype. Here, we examine the potential role of ET-1 in regulating cell morphology and behavior and epithelial and mesenchymal proteins employing an *in vitro* 3-D culture system. We found that in 3-D serum-free collagen I gel cultures, HEY and OVCA 433 ovarian carcinoma cells undergo fibroblast-like morphologic changes between 3 and 5 days of ET-1 treatment. In these cells, ET-1 induces loss of adherens and tight-junction protein expression, E-cadherin, catenin, and zonula occludens-1, and gain of N-cadherin and vimentin expression. These results confirm the ability of ET-1 to promote EMT, a metastable process involving sustained loss of epithelial markers and gain of mesenchymal markers. Collectively, these findings provide evidence of a critical role for the ET-1/ ET_AR axis during distinct steps of ovarian carcinoma progression, thus underlining this axis as a potential target in the treatment of ovarian cancer.

INTEGRIN-LINKED KINASE FUNCTIONS AS A DOWNSTREAM MEDIATOR OF ENDOTHELIN-1 TO PROMOTE INVASIVE BEHAVIOR IN OVARIAN CARCINOMA

The endothelin-1 (ET-1) axis represents a novel target in several malignancies, including ovarian carcinoma. Upon being activated, the endothelin A receptor (ET_AR) mediates multiple tumor-promoting activities, including mitogenesis, escape from apoptosis, angiogenesis, metastasis-related protease activation, epithelial-mesenchymal transition, and invasion. Integrin-linked kinase (ILK) is a multi domain focal adhesion protein that conveys intracellular signaling elicited by 1-integrin and growth factor receptors. In this study, we investigate whether the signaling triggered by ET_AR leading to an aggressive phenotype is mediated by an ILK-dependent mechanism. In HEY and OVCA 433 ovarian carcinoma cell lines, activation of ET_AR by ET-1 enhances the expression of ∂2ß1 and ∂3ß1 integrins. ILK activity increases as ovarian cancer cells adhere to type I collagen through ß1 integrin signaling, and do so to a greater extent on ET-1 stimulation. ET-1 increases ILK mRNA and protein expression and activity in a time and concentration-dependent manner. An ILK small-molecule inhibitor (KP-392) or transfection with a dominant-negative ILK mutant effectively blocks the phosphorylation of downstream signals, Akt and glycogen synthase kinase-3ß. The blockade of ET-1/ET_AR-induced ILK activity results in an inhibition of matrix metalloproteinase activation as well as of cell motility and invasiveness in a phosphoinositide 3 kinase–dependent manner. In ovarian carcinoma xenografts, ABT-627, a specific ET_AR antagonist, suppresses ILK expression, Akt and glycogen synthase kinase-3ß phosphorylation, and tumor growth. This data shows that ILK functions as a downstream mediator of the ET-1/ ET_AR axis to potentiate aggressive cellular behavior. Thus, the ILK-related signaling cascade can be efficiently targeted by pharmacologic blockage of ET_AR.

ANTITUMOR EFFECT OF GREEN TEA POLYPHENOL EPIGALLOCATECHIN-3-GALLATE IN OVARIAN CARCINOMA CELLS: EVIDENCE FOR THE ENDOTHELIN-1 AS A POTENTIAL TARGET

The green tea polyphenol, epigallocatechin-3-gallate (EGCG), has been shown to prevent cancer; however, a precise mechanism responsible for tumor growth inhibition has not yet been clearly described. The endothelin (ET) A receptor (ET_AR)/ET-1 autocrine pathway is overexpressed in ovarian carcinoma and triggers tumor

growth, neoangiogenesis, and invasion. These latter tumor-promoting effects are mediated through the activation of cyclooxygenase (COX)-1– and COX-2–dependent pathways by ET-1. In the present study, pre-treatment of HEY and OVCA 433 ovarian carcinoma cell lines with green tea and EGCG inhibited ET-1/ET_AR expression, ET_AR-mediated COX-1/2 mRNA expression, and COX-2 promoter activity. These effects were associated with a significant reduction in the COX-1/2–derived prostaglandin E2 (PGE2) production. These results provide a novel insight into the mechanism by which EGCG, affecting ET_AR-dependent COX-1/2 pathways, may inhibit ovarian tumors. This suggests that EGCG may be useful in preventing and treating ovarian carcinoma in which the activation of ET_AR by ET-1 plays a critical role in tumor growth and progression.

GREEN TEA POLYPHENOL EPIGALLOCATECHIN-3-GALLATE INHIBITS THE ENDOTHELIN AXIS AND DOWNSTREAM SIGNALLING PATHWAYS IN OVARIAN CARCINOMA

The polyphenol epigallocatechin-3-gallate (EGCG), the principal mediator of green tea, has been known to possess antitumor effects. The endothelin A receptor (ET_AR)/endothelin-1 (ET-1) axis is overexpressed in ovarian carcinoma, representing a novel therapeutic target. In this study, we examined green tea and EGCG effects on two ovarian carcinoma cell lines, HEY and OVCA 433. EGCG inhibited ovarian cancer cell growth and induced apoptosis that was associated with a decrease in Bcl-XL expression and activation of caspase-3. Treatment with green tea or EGCG inhibited ET_AR and ET-1 expression and reduced the basal and ET-1-induced cell proliferation and invasion. The EGCG-induced inhibitory effects were associated with a decrease of ET_AR-dependent activation of the p42/p44 and p38 mitogen-activated protein kinases and phosphatidylinositol 3-kinase pathway. Remarkably, EGCG treatment resulted in a lowering of basal and ET-1-induced angiogenesis and invasiveness mediators, such as vascular endothelial growth factor and tumor proteinase activation. Finally, in HEY ovarian carcinoma xenografts, tumor growth was significantly inhibited by oral administration of green tea. This effect was associated with a reduction in ET-1, ET_AR, and vascular endothelial growth factor expression, microvessel density, and proliferation index. These results provide a novel insight into the mechanism by which EGCG, affecting multiple ET_AR dependent pathways, may inhibit ovarian carcinoma growth, suggesting that EGCG may be useful in preventing and treating ovarian carcinoma in which ET_AR activation by ET-1 plays a critical role in tumor growth and progression.

IDENTIFICATION OF THE MELANOCYTE-SPECIFIC ELEMENT IN THE MELANOCORTIN RECEPTOR 1 GENE PROMOTER.

∂-melacortin and its specific receptor play a crucial role in cutaneous pigmentation, since their binding on the plasma membrane of the melanocytes regulates the biosynthesis of the melanins. Therefore, it is important to study the regulation of the ∂-melacortin receptor gene expression because their interaction on the melanocyte cell membrane modulates melanins biosynthesis. The understanding of pigmentation biology is relevant from the biological and clinical point of view. The minimal ∂-melacortin receptor gene promoter has been characterised and as with several gene promoters of the G-protein coupled family of receptors, it is G-C reach and TATA-less. However, the *cis* acting elements responsible for tissue-specific activity of the promoter have not been identified. We show that the first 150 base pairs upstream of the initiation codon are able to drive the melanocyte-specific promoter activity. Furthermore, we provide some experimental evidence suggesting that positive and negative complexes can assemble on such a minimal melanocyte specific gene promoter. We are performing these experiments in collaboration with Dr.D.Civitareale, Institute of Neurobiology and Molecular Medicine CNR, Rome.

THE SYNERGISTIC ACTIVITY OF THYROID TRANSCRIPTION FACTOR 2 AND PAX8 IN THE TRANSCRIPTION REGULATION OF THYROPEROXIDASE GENE

We are studying the regulation of the tissue-specific gene expression in thyrocytes. We have focused on the role of the transcription factor Pax8 in the control of the thyroperoxidase (TPO) gene promoter activity, and we are interested in its relationship with the thyroid transcriptional factor 2 (TTF2). TTF2 is a forkhead-containing protein highly enriched in thyroid follicular cells. TTF2 is a thyroid-specific DNA binding activity recognizing the promoter of TPO and thyroglobulin (Tg) genes and is necessary for thyroid morphogenesis. Previous studies have demonstrated that TTF2 is able to inhibit the activity of the thyroid-specific transcription factor 1 (TTF1) and Pax8 only on certain promoters and have shown that repression by TTF2 is DNA-binding independent. However, the data does not produce a conclusive result with respect to the role of TTF2 in the thyroid transcription. Since we have previously demonstrated that the combined activity of both the TPO gene promoter and the TPO enhancer drives a strong synergistic activity between TTF1 and Pax8, we considered again whether TTF2, to initiate the transcription machinery, requires cooperation with TTF1 and PAX8. Preliminary data indicates the synergistic activity of TTF2 and Pax8 in the transcriptional regulation of the thyroperoxidase gene.

We are performing these experiments in collaboration with Dr. D. Civitareale, Institute of Neurobiology and Molecular Medicine CNR, Rome.

ANTIOXIDATIVE AND APOPTOTIC PROPERTIES OF POLYPHENOLIC EXTRACTS FROM THE EDIBLE PART OF ARTICHOKES

Epidemiological studies have shown that the consumption of fruit and vegetables is associated with a reduced risk of chronic disease. Diets rich in grain, fruit and vegetables are known to reduce cancer risk, implicating edible plants as a potential source of anticancer agents, many of which belong to the flavonoid family. Extracts from artichokes, Cynara scolimus, have been claimed to exert a beneficial action against hepato-biliary disease. Some of which is due to the antioxidant potential of artichoke extracts. The therapeutic activity of the extract is probably due to the phenolic structure of these substances which is responsible for the free radical mediated process inhibition.

The major constituents of artichoke extracts are hydroxycinnamic acids such as chlorogenic acid, dicaffeoylquinic acids, caffeic acid, ferulic acid and flavonoid such as luteolin and apigenin glycosides. Cultured rat hepatocytes and human hepatoma HepG2 cells were used to evaluate the hepatoprotective properties of polyphenolic extracts from the edible part of artichokes (AE). The hepatocytes were exposed to H2O2 generated in situ by glucose oxidase, to cause an oxidative stress, and treated with AE. Depletion of glutathione (GSH), accumulation of malondialehyde (MDA) in the cultures, as lipid peroxidation indicator, and cell death due to the presence of H2O2, were prevented either by the presence of AE or by the addition of a well known antioxidant, N,N'-diphenyl-p-phenilenediamine (DPPD). These results demonstrated that AE protected cells from the oxidative stress caused by glucose oxidase, comparable to the antioxidant DPPD. Treatment of HepG2 cells for 24 h with AE induced apoptosis in a dose-dependent manner. Our findings indicate that AE had a marked antioxidative potential that protects hepatocytes from oxidative stress and had apoptotic activity on human liver cancer cell line HepG2 cells.

Furthermore, we studied the human bioavailability and metabolism of hydroxycinnamates derivates. Different concentrations of chlorogenic acid, caffeic acid and ferulic acid were determined in human plasma after ingestion of cooked Cynara scolimus L. These results confirmed bioavailability of metabolites of hydroxycinnamic acids.

We are performing these experiments in collaboration with Dr. G. Maiani, National Institute for Research and Nutrition (INRAN) Rome and Dr. D. Di Venere, CNR-Institute of the Science of Food, Bari. ■

* 2006 Scientific Activity because the 2007 Scientific Activity report was not submitted

DEPARTMENT FOR THE DEVELOPMENT OF THERAPEUTIC PROGRAMS

Laboratory "B" associated to the Medical Oncology Department

Director: **Alfonso Bellacosa**

STAFF
Tiziana Bruno
Serena Buontempo
Valeria Catena
Nicoletta Corbi
Francesca De Nicola
Agata Desantis

Monica Di Padova L' Aquila University
Maurizio Fanciulli
Aristide Floridi L' Aquila University
Simona Iezzi
Annalisa Onori
Claudio Passananti
Institute of Molecular Biology and Pathology,
National Research Council (CNR)

Scientific Activity

■ The work of this laboratory focused on the following topics:

1. Functional characterization of the Che-1 protein
2. Artificial transcription factors
3. Maintenance of genomic stability at CpG sites
4. Identification of early markers of transformation

1. FUNCTIONAL CHARACTERIZATION of the Che-1 protein

We have previously demonstrated that DNA damage leads to stabilization and accumulation of Che-1, a RNA polymerase II binding protein that plays an important role in transcriptional activation of p53 and in maintenance of the G2/M checkpoint. We found that Che-1 is downregulated during the apoptotic process. The E3 ligase HMD2 physically and functionally interacts with Che-1 and promotes its degradation via the ubiquitin-dependent proteasomal system. Furthermore, we found that in response to apoptotic stimuli Che-1 interacts with the peptidyl-prolyl isomerase Pin1, and conformational changes generated by Pin1 are required for Che-1/HDM2 interaction. A Che-1 mutant lacking the capacity to bind Pin1 exhibits an increased half-life and this correlates with diminished apoptosis in response to genotoxic stress. We also found that Che-1 interacts with acetyl-transferase protein p300 and that in response to DNA damage Che-1 is acetylated by p300. A mutant lacking acetylable residue resulted in much less stability and was ubiquitinated when compared to wild-type. From a microarray analysis, we also found that Che-1 activates XIAP expression in response to DNA damage. This effect is mediated by Che-1 phosphorylation and requires NF-kB. Notably, we found that XIAP expression is necessary for antiapoptotic activity of Che-1 and that *in vivo* down-regulation of Che-1 by siRNA strongly enhances the cytotoxicity of anticancer drugs.

Increasing evidence suggests that Che-1 may be involved in apoptotic signalling in neural tissues. Indeed, in cortical neurons Che-1 exhibits an anti-apoptotic activity, protecting cells from neuronal damage induced by amyloid b-peptide.

We demonstrated that Che-1 interacts with NRAGE, a novel cell-death inducer, and that EGFP-NRAGE inhibits nuclear localization of Che-1, by sequestering it within the cytoplasm. Furthermore, NRAGE overexpression down-regulates endogenous Che-1 by targeting it for proteasome-dependent degradation. Finally, we produced evidence that Che-1 may be a functional antagonist of NRAGE, since its overexpression completely reverts NRAGE-induced cell-death.

Che-1 remains intact during evolution, in particular the carboxyl terminal portion is particularly conserved from yeast to human. Using this peculiar portion of human Che-1 in yeast two-hybrid experiments, we identified, from a human brain cDNA-library, several interacting proteins, all involved in some way in the control of cell-death programs. One of these proteins was the mitochondrial protein Hax1, mutated in the autosomal recessive severe congenital neutropenia, or Kostmann disease. This disease constitutes a primary immunodeficiency syndrome associated with increased apoptosis in myeloid cells. The preliminary data produced on Che-1-Hax1 interaction are: 1) both full length and C-terminal portion of Che-1 are able to interact with Hax1 2) The Hax1 Kostmann mutant binds Che-1 with very low efficiency (almost undetectable) 3) In HCT-116 cells Che-1 and Hax1 share the compartmentalization in the mitochondrial district.

2 ARTIFICIAL TRANSCRIPTION FACTORS

Our main aim is to up-regulate the expression level of the dystrophin-related gene utrophin in Duchenne Muscular Dystrophy (DMD), complementing in this way the lack of dystrophin functions. Indeed, it is now well established that utrophin up-regulation is a possible strategy to cure dystrophy. To reach utrophin up-regulation, we have engineered artificial zinc finger based transcription factors (ZF ATFs), capable of binding and activating transcription from the promoter A of both human and mouse utrophin genes. In particular, we generated transgenic mice that specifically over-express an artificial three-zinc finger protein at the muscular level, that we named Vp16-Jazz, which is able to specifically up-regulate the utrophin gene. The achievement of Vp16-Jazz transgenic mice validates the strategy of transcriptional targeting of endogenous genes and represents a unique animal model for drug discovery and therapeutics. Moreover, we engineered a novel artificial four-zinc finger protein, named Bagly, by adding an extra-fourth zinc finger to Jazz protein, derived from the transcription factor YY1. Bagly is able to bind with optimised affinity/specificity a 12-base pair DNA target sequence, internal to the human utrophin promoter A at the endogenous chromosomal site. Importantly, Bagly DNA target sequence is statistically present in the human genome only 210 times, about 60 times less than the 9 base pair Jazz DNA target sequence.

3. MAINTENANCE OF GENOMIC STABILITY AT CPG SITES

The long-term goal of this project is to determine the function of the base excision repair (BER) glycosylase MED1, originally identified by us as a protein interacting with the mismatch repair (MMR) enzyme MLH1. Ourseleves and others have previously shown that MED1 (also named MBD4) acts as a G:T and G:U mismatch-specific thymine and uracil DNA *N*-glycosylase. The thymine and uracil glycosylase activity of MED1 prefers G:T and G:U mismatches located in the context of methylated and unmethylated CpG sites. Since G:T and G:U mismatches can originate via spontaneous hydrolytic deamination of 5-methylcytosine and cytosine to thymine and uracil, respectively, MED1 is likely involved in the repair of deaminated 5-methylcytosine and cytosine at CpG sites, preventing transition mutations that would turn CpG sites into CpA sequences. In parallel studies, we found that MED1 is required for the cellular response to alkylating agents and other chemotherapeutic drugs. Also, absence of MED1 leads to a reduction in the levels of several MMR proteins, including MLH1, MSH2, PMS2 and MSH6.

Recently, we showed that MED1 has a remarkable preference for mismatches containing halogenated pyrimidines: the k_{st} values obtained with 5-fluorouracil (5FU) and 5-iodouracil were 20-30-fold higher than those obtained with uracil and thymine. We also found that MED1-null mouse embryo fibroblasts (MEFs),

obtained from our mice with targeted inactivation at the Med1 locus, are dramatically sensitive to cell killing by 5-iododeoxyuridine (5IdU), a precursor to 5FU used in the oncology clinic as a radiosensitizing agent. These findings establish MED1 as a *bona fide* repair activity for the removal of halogenated bases and indicate that MED1 may play a significant role in 5IdU cytotoxicity.
In the course of the studies on Med1-mutant mice, we made an unexpected discovery. We previously engineered mice bearing two different targeted alleles at the *Med1* locus, lacking exons 1 to 3 (D1-3 allele) and exons 2 to 5 (D2-5 allele), respectively. While mice homozygous for the D2-5 allele are viable, embryos homozygous for the D1-3 allele show multiple developmental defects that result in lethality. In the past year, we found that this is due to concurrent inactivation, in the D1-3 allele, of the overlapping gene *Ift122/Wdr10* that shares a portion of exon 1 with *Med1* in the opposite orientation. Since orthologs of *Ift122* in invertebrates are involved in ciliogenesis, we examined the phenotype of primary cilia and found them to be absent in the node of mutant embryos, and obtained evidence linking the primary cilia defect to defective Sonic hedgehog (Shh) signaling and lethality. These findings indicate that *Ift122* is required for ciliogenesis and Shh signaling. The significance of the unusual arrangement of the *Med1* and *Wdr10* genes will require additional investigation.

4. IDENTIFICATION OF EARLY MARKERS OF TRANSFORMATION

By combining Knudson's "two-hit" and the multistep tumorigenesis theories, we hypothesized that while biallelic inactivation of the gatekeeper tumor suppressor gene is necessary to initiate tumorigenesis of a given target epithelium, single-hit mutations of this gene might be associated with initial molecular alterations (pre-initiation) present in the morphologically "normal" mucosa. These early changes might represent molecular targets for strategies of intervention based on novel chemopreventive agents. In order to detect these changes, we conducted microarray studies by comparing the gene expression profile of primary breast and ovary epithelial cultures from patients predisposed to cancer (carriers of BRCA1 or BRCA2 mutations), with those from control individuals with intact copies of the tumor suppressor genes. By using class-comparison bioinformatic analyses, we identified a set of extremely promising markers for breast and ovarian cancer risk, including genes involved in cell cycle and growth control, cell-cell and cell-matrix adhesion. Interestingly, several of these genes/gene products had been previously proposed as markers of breast and ovarian cancer, thus providing an initial indirect confirmation to our hypothesis. ■

DEPARTMENT FOR THE DEVELOPMENT OF THERAPEUTIC PROGRAMS

Laboratory "C" associated to the Prevention and Diagnosis Department

Director: **Marco Giorgio Paggi**

STAFF
Claudia Abbruzzese
Emanuele Bellacchio
Maria Canale
Mauro Castelli
Antonio Federico
Stefano Mattarocci
Anna Maria Mileo
Fulvia Rocchi

Scientific Activity

■ Our laboratory has a long history in cell cycle-related studies. Currently we are also involved in studies regarding the molecular bases of cellular transformation (our model is HPV oncogenesis) and tumor progression (our model is melanoma progression), in order to identify sets of diagnostic, prognostic and therapeutic targets of potential interest in cancer clinical practice. Recently, the department has been involved in studies concerning tridimensional molecular modeling for protein-protein or drug-protein interactions.

CELL CYCLE

Our interest in this topic led us to identify the factor Pkn as a partner of cyclin T2a and to investigate the role of this interaction in muscle differentiation.
We then delimited the minimal portion of the RB-related protein pRb2/p130 able to inhibit Cdk2 activity and validated the effect of this short amino acid sequence in reducing tumor growth *in vitro* and *in vivo*. Subsequently, via in silico molecular modeling, we developed the tridimensional model of the interaction between Cdk2/Cyclin A complex and the pRb2/p130-derived small molecule described above.

CELLULAR TARGETS OF THE VIRAL ONCOPROTEINS FROM SMALL DNA TUMOR VIRUSES

In this field, we identified the physical interaction between E7 and the antimetastatic protein Nm23, demonstrating that such an interaction is able to drastically modify specific functions of the Nm23 cellular factor. More recently we illustrated the functional significance of the interaction of HPV-16 E7 with the pro-apoptotic factor Siva-1, showing that its inactivation is one of the mechanisms used by the oncoprotein to inhibit the apoptotic processes. We also published a review article concerning the up-to-date functional implications of the interaction between the small DNA tumor virus oncoproteins and the RB family of tumor and growth suppressor factors.

IDENTIFICATION OF GENES INVOLVED IN MELANOMA PROGRESSION: FERRITIN DOWN-MODULATION AND MELANOMA PIGMENTATION AND TYROSINASE ACTIVITY IN CULTURED MELANOCYTES

We started a new investigation from the observation that L-ferritin down-regulat-

ed LM cells displayed a less pigmented phenotype, confirmed by a major decrease of total melanin content. This finding was alongside a dramatic decrease in tyrosinase activity not matched by a reduction of tyrosinase specific mRNA. Indeed, we detected in these cells an improper tyrosinase post-translational maturation and a consequent reduction of its activity. Consequently, we demonstrated a correlation between melanogenic and catalase activity in human melanocytes, a synergic strategy against oxidative stress. We also wrote a review article on the use of the new high-throughput technologies for the study of the malignant melanoma.

MOLECULAR MODELING

Besides the molecular model of the interaction between Cdk2/Cyclin A complex and a pRb2/p130- derived small molecule described above, we developed a protease-mediated arsenic pro-drug strategy based on the design of polythiol peptides able to neutralize the toxicity of As(III) through chelation, and at the same time recognized as substrates of cancer-linked specific proteases.

DEPARTMENT FOR THE DEVELOPMENT OF THERAPEUTIC PROGRAMS

Laboratory “D” associated to the Neurosciences, Head, Neck and Facial Pathologies Department

Director: **Ada Sacchi** ad interim

STAFF
Rossella Galati
Manlio Mattioni
Alessandra Verdina

Scientific Activity*

1. PEPTIDE SYNTHESIS AND ANTIBODY PRODUCTION

The laboratory served as a facility to synthesize peptides when requested by internal and external institutional researchers. These peptides have been used as inhibitors of target protein activities or to generate specific antibodies. The production of specific antibodies against a protein requires an in-depth study to underscore the structure / function relationship (i.e. conformational, chemical, etc,) that leads to the identification of the immunogenical regions and the necessary amino acid sequence (peptide). The preparation of the antigen can be started only after peptide characterization (i.e. length, hydrophilicity, side residues). This investigation makes it possible to choose the proper carrier together with the amino acidic residue to be used in the carrier-peptide conjugation reaction. After the completion of these theoretical studies, the peptide synthesis can begin.The characterization of the protein is necessary in order to use the peptides as specific protein inhibitors.
Furthermore, it is important to decide at which level the inhibition of the proteins needs to take place. It is possible to use peptides that impair the protein-receptor binding or peptides that prevent conformational change (i.e. the passage from monomer to dimmer).This structural study makes it possible to identify groups of peptides that are synthesized and tested. In the last year, a peptide has been synthesized which corresponds to an extracellular domain of a protein called “prostein” and an antibody recognizing this protein has been developed.This antibody might be useful for diagnostic tests and *in vivo* imaging. Indeed, prostein has been identified as a molecule which is specifically expressed in prostate tissues that are responsive to hormone treatments. It is important to develop new tools for prostate cancer diagnosis because PSA detection in serum is not completely adequate for the identification of benign versus malignant tissues.

2. ROLE OF DNA-REPAIR POLYMORPHISMS IN INDIVIDUAL SUSCEPTIBILITY OF OCCUPATIONALLY EXPOSED POPULATIONS

The association between biomarkers of DNA damage and polymorphisms for DNA repair genes and NAT2 was investigated in a population of healthy Italian traffic wardens who had previously been enrolled to evaluate the effects of air pollution.
Due to the potential role played by genetic factors in modulating individual DNA damage through either endogenous or exogenous agents, in this and previous studies,we evaluated the relationships between polymorphisms for xenobiotics-metabolising enzymes, folic acid metabolism and DNA repair genes and three biomarkers of DNA damage.These were SCE, tail moment length after single cell gel electrophoresis and micronuclei in binucleated cells.These were all detected in peripheral blood lymphocytes.

In 2005, as part of the joint project with the Italian Health Ministry and Ministry of Environment, different DNA-repair polymorphisms were analyzed in a human cohort of Rome traffic officers. In 2005 this study was completed and the NAT2 polymorphisms analyzed, in order to extend our panel of xenobiotict metabolizing polymorphisms. The NAT2 enzyme is involved in the N-acetylation of aromatic amines and o-acetylation of heterocyclic amines. Several NAT2 polymorphisms have been identified in human populations and the subjects can be classified on the basis of the number of variant alleles in slow and rapid acetylator with good concordance with phenotypical determination. Despite discordant evidence, slow acetylator and rapid acetylator have been shown at risk for bladder and colon cancer, respectively, and high levels of baseline chromosome aberrations and DNA adducts in smokers has been associated to NAT2 slow acetylators. However, the few studies that have analysed SCE and NAT2 polymorphisms have found smoking effects this biomarker, but have failed to observe any association with this polymorphism.This is consistent with our results. In fact, no association was observed between the combined NAT2 polymorphism and any biomarker (SCE, tail moment, micronuclei), also when the interaction with polymorphisms for the other xenobiotic metabolizing enzymes (previously analyzed) was taken into account.

3. SERUM P53 ANTIBODY AS A USEFUL MARKER IN CANCER

Disfunctions in the TP53 tumor suppressor gene represent the most common genetic alterations in cancer. They can lead to the expression of a disfunctional p53 protein with a longer half-life than the functional one, resulting in an accumulation of the dysfunctional protein in cancer cells.The accumulated protein may act as an antigen and induce an immune response with the production of anti-p53 antibodies (p53-Abs), which are detectable in the sera of patients with various types of cancer. There is a close correlation between the presence of p53-Abs and an increased expression of p53 protein in the corresponding tumor. Therefore, the detection of p53-Abs can be used as a marker for the occurrence of p53 gene alterations.These antibodies show high specificity, since healthy controls rarely resulted positive, and sensitivity up to 30 % in most tumor types.
In lung cancer, p53 protein accumulation and the production of p53-Abs represent early events in neoplastic processes. In fact, p53-Abs were found in patients at high risk of lung cancer, such as heavy smokers with chronic obstructive pulmonary disease (COPD), months before any clinical evidence of cancer. However, any clinical implications of p53- Abs in lung cancer remain controversial. In non-small-cell lung cancer, p53-Abs were predominantly related to short survival, but they also predicted better survival, after radiotherapy, or showed an absence of prognostic relevance; in small cell lung cancer, studies were very divergent. The aim of our work, regarding pulmonary disease, was to evaluate the role of the detection of p53-Abs in the early diagnosis of patients at high risk of lung cancer and to investigate the actual prognostic significance of these antibodies in lung cancer patients. In collaboration with the clinical departments of our Institute, we have started a large prospective study to analyse, by a specific ELISA, p53-Abs in non-neoplastic people, including patients at high risk of lung cancer, and of patients with histological diagnosed lung cancer. Results in a large number of people indicate that approximately 10% of non-neoplastic people tested positive for p53 Abs, of which approximately 6% have a diagnosis of COPD. The percentage of people tested positive increases significantly in lung cancer patients who also show high levels of p53-Abs in the serum.

4. COX-2 AND PATHOGENESIS AND THERAPY OF HUMAN MESOTHELIOMA

Malignant mesothelioma (MM) is a rare, highly aggressive tumor, accounting for less than 1% of all cancer deaths in the world. Although the association between exposure to asbestos and the development of MM is commonly accepted, the exact mechanism whereby asbestos induces MM is unknown. Moreover, MM has proved resistant to classical chemotherapeutic and radiation therapies

and the natural history has yet to be influenced by standard therapy.
We evaluated the *in vitro* effects of piroxicam, a widely used non-steroidal anti-inflammatory drug (NSAID), alone or in combination with cisplatin (CDDP), on cell growth of two mesothelioma cells lines (MSTO-211H and NCI-H2452) and the anti-tumor potential of the drug *in vivo*, in a mesothelioma flank tumor model and in a mesothelioma orthotopic tumor model.
A significant, dose-dependent inhibition of proliferation level was demonstrated in the two mesothelioma cell lines. In particular, the MSTO-211H cell line showed a 50% reduction (IP50) with 760 mM of the drug, while NCI-H2452 reached an IP50 with 680 mM. In order to analyze the mechanisms by which piroxicam affects mesothelioma cells proliferation in greater detail, flow cytometry was used. Cell cycle analysis on MSTO-211H showed an increase of the sub-G1, that is an index of apoptosis. On the other hand, in NCI-H2452, a decrease in the S-phase accompanied by an increased G2/M fraction suggested there is a G2-block of the cell cycle and this was confirmed by the fact that there was only a slight increase of apoptosis in these cells. Regarding *in vivo* experiments, a marked tumor growth inhibition was observed in mice carrying subcutaneous mesothelioma when piroxicam was administered in combination with CDDP. Histopathological and immunohistochemical analyses of the explanted mesothelioma xenografts revealed that the combination of piroxicam plus CDDP was effective in blocking tumor growth, essentially through a pro-apoptotic activity with no or little effects on tumor angiogenesis.
In mice with orthotopic mesothelioma, piroxicam increases survival of CDDP treated mice, confirming that the addition of piroxicam could be instrumental in increasing mesothelioma control by platinum compounds. In MSTO cells COX-2 protein and PGE2 levels were assessed: the low expression of COX-2 was confirmed by the lack of detectable levels of PGE2 in the cell media analyzed. For this reason, we suggest that piroxicam in these cells exerts its anti-proliferative activity via COX/PGE2-independent mechanisms. ■

* 2005 Scientific Activity because the 2007 Scientific Activity report was not submitted

Training and Prevention in Psycho-Oncology

Director: **Anita Caruso**

STAFF
Laura Bongiorno
Simona Campagna
Sara Cattaneo
Bruna Marozzo
Wissya Santoni
Antonia Tramontana
Cristina Vigna

Scientific Activity

■ Training and Prevention in Psycho-Oncology is involved in these different areas:

BREAST CANCER

"Psycho-social impact of genetic counselling for hereditary breast and ovarian cancers".
The aim of the study is to assess the psycho-social variables of the individuals who seek genetic counselling, in order to modulate the intervention of the team based on patients' needs, to survey the psycho-social impact of genetic counselling processes and to survey the possible correlation between psycho-social variables, life stressful events and development of cancer.

COLON CANCER

"Psycho-social impact of genetic counselling for hereditary colon cancer".
The aim of the study is to assess the psycho-social variables of individuals who approach genetic counselling, in order to survey the psycho-social impact of the genetic counselling process, to survey the possible correlation between psycho-social variables and development of cancer and to modulate the intervention of the multidisciplinary team on patients' needs.

SCREENING OF PSYCHOLOGICAL DISTRESS

Multicohort Project "Distress screening among cancer patients in follow-up: A feasibility study".
The aim of the study is to validate a screening procedure which can facilitate the recognition of cancer-affected patients with a high level of distress, and who are in follow-up for two years or more.

EVALUATION OF PHYSICIANS' COMMUNICATION SKILLS

Multicohort Project "Improving the physician's communication skills and reducing the psychological distress of the patient: a randomized clinical study". The aim of this study is to evaluate the impact of training for physicians, by measuring the satisfaction and the level of anxiety of patients evaluated before and after a medical visit. There will be a control group composed of non-trained physicians. ■

DEPARTMENT OF CRITICAL AREA

Anaesthesiology

Director: **Edoardo Arcuri** ad interim

STAFF

Cecilia Cioccia
Luca Colantonio
Piera Di Angelo
Pietro Di Palma
Luana Fabrizi
Ester M.A. Forastiere
Claudia Frigieri
Maddalena Giovannetti
Paolo Moricca
Federico Pierconti
Francesca Principi
Maria Sofre

Scientific Activity*

■ The Department of Anaesthesiology and the Operating Theatre collaborates closely with the various Departments of Surgery in order to guarantee daily surgical activity for patients suffering from complex neoplastic pathologies who require highly destructive surgery.
With this major surgery, which involves the entire staff of the Department, from the nursing personnel to the operating theatre technicians, the Department actively collaborates with the Department of Surgery B in the field of liver transplants.
The Anaesthesiology Department also acts as consultant in various divisions in order to establish operating risks. The patients receive detailed information regarding these risks. Pre-hospitalization processes are organised by the Anaesthesiology Department, which makes it possible to plan procedures and processes, resulting in patient satisfaction. This has helped reduce the length of waiting lists and has almost completely eliminated unnecessary admissions.
Moreover, correct anaesthetic assistance is guaranteed for all out-patients and those who have to undergo various types of diagnostic and therapeutic exams including: CAT, MRI, digital angiographs, gastroenterology endoscopies.
A large part of the routine operations of the Operating Theatre contributed to the scientific research in the Anaesthesiology and Operating Theatre Department. Participation in DMTs, which today have become standard practice, has made it possible to outline anaesthesiology protocols in the various surgical fields. Furthermore, they have considerably stimulated collaboration between all the medical sections concerned.
The oncological pathologies of the patients which we deal with are particularly complex. This means it is of the utmost importance to continually improve knowledge, promote continuous professional training, and study further the most appropriate treatments in order to minimize risks and complications.
This useful collaboration made it possible to propose certain studies which are now ongoing and also establish new lines of research to pursue in the future.
We completed the study: "A multimodal approach to postoperative pain treatment in oncological surgery: evaluation of the efficacy of the ONQ Pain Management System". This study analysed the potential synergic effects of various analgesic drugs at differing pain levels. The research produced highly positive results, which were presented by Dr. E. Forastiere (in charge of research) at two important conferences. The first was held in Rome at the SICD (The Italian Society of Clinical Pain) National Congress. The second was held in Bari at a national congress organised by SIAARTI (The Italian Society of Analgesic Anaesthic Reanimation and Intensive Therapy).
Furthermore, these excellent results have helped us extend the system of postoperative pain treatment to all patients undergoing major surgery in each of the differing surgical areas.
As a result of these studies, the use of radiotherapy in the treatment of prostrate cancer has increased considerably. However, there are a number of short and long-term side effects, including rectal haemorrhages, cystitis and incontinence.
In order to resolve these complications arising from the use of cortisone-based therapies, a research project has been set up to evaluate the efficacy of the COX-2 inhibitor.
The study, "A comparison of conventional cortic-steroid treatment and treatments with the COX-2 inhibitor to resolve complications arising after radiotherapy for prostrate carcinoma", had already enrolled 25 patients at the beginning of 2005. However, due to well-known developments involving COX-2 inhibitors, late that year, the study was interrupted for many months.
These difficulties have now been resolved. The enrolment of patients has recommenced and the study is once again ongoing.
In the near future, we hope that Dr. Tessitore, the head of this research, together with her radiotherapy colleagues, will be able to produce the preliminary results and confirm the validity of the study.
The enrolment for the study, "Factors of renal protection in patients undergoing Nephrectomies", has been completed and the data collected is being statistically analysed. ■

* 2005 Scientific Activity because the 2007 Scientific Activity report was not submitted

Cardiology

Director: **Italo Sacchi**

STAFF

Anna Angelocola
Armando Carpino
Stefania Di Paolantonio
Mara Lotti
Fabio Maramao
Nicola Morace
Susanna Pampinella
Silvia Santacroce
Giuseppe Toglia

Scientific Activity

In 2007, 24,746 assisted and specialized consultations were carried out by the Department of Cardiology. These included: 17,496 clinical tests for patients of the Department and the day hospital, 2,604 for patients of the Department and day hospital of the San Gallicano Institute and 4,646 for outpatients.

Apart from the general pre-surgery cardiac evaluation of oncology patients aimed at defining individual cardiac risk as well as post surgery cardiac assistance for complications from surgery and intra-hospital emergencies, the primary institutional aim of the Department of Cardiology has been the prevention, early diagnosis and cure of cardio-toxic effects of anti-tumoral drugs. In particular, our work has concerned the derivatives of anthracyclines together with the damage due to oncological radiotherapy treatment.

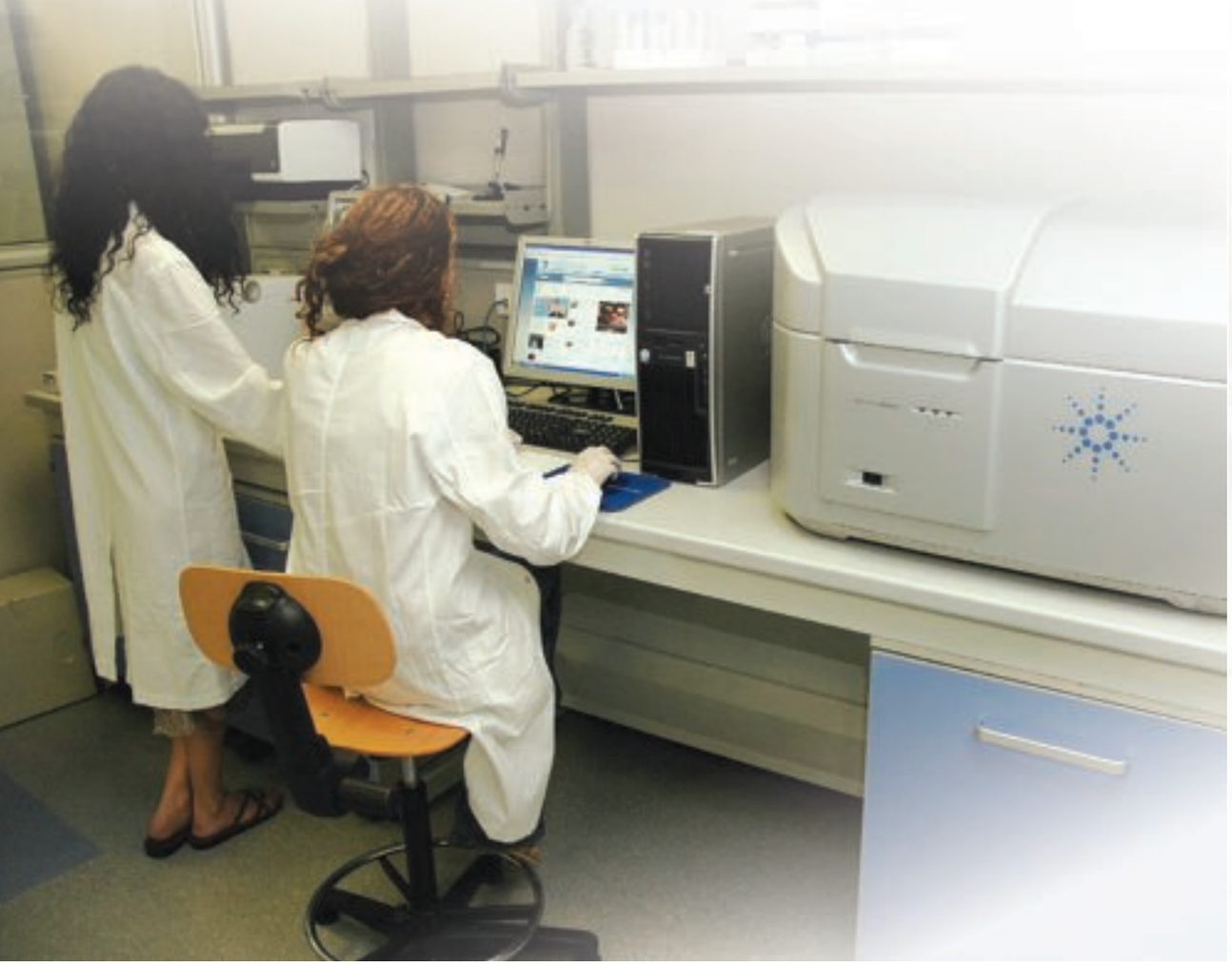
Apart from anthracyclines, current oncological therapy also uses chemotherapy drugs, already in common use, such as taxanes, trastuzumab and bevacizumab. These are often used in temporal or sequential association. This, however, has been widely shown to hold some risks, even chronic, after months or years of cardiotoxicity, which are enough to cause an unfavorable prognosis of cardiomyopathy at 5 years in nearly 50% of cases.

The outpatients division of our Department is dedicated to cardioprotection against cardio-toxicity of antiblastic drugs. There are two aims. The first is to gather information on preclinical manifestations and its signs in early, asymptomatic phase (subclinical cardiotoxicity). The second is, in cooperation with oncologists, to conduct monitoring procedures, in particular for oncology patients with associated cardiovascular pathology, aimed at defining compatible therapeutic strategies.

This involves variation of dose, type and modality of administration of chemotherapeutics and early chemioprotective cardiologic therapy, for those patients who may benefit in some way from favorable expectations and quality of life after chemotherapy for their oncologic disease.

Apart from a cardiac visit with ECG this service makes use of echocardiograph colour Doppler, which is the most common method of monitoring the study of the left ventricular systolic function (ejection fraction). In particular, it is useful in the study of diastolic relaxation through colour flow Doppler analysis.

The results obtained from some recent studies and their projected follow-up at one year have in fact demonstrated the early dysfunction of the ventricular myofibrilation. Above all, its diastolic relaxation properties (analyzed with precision with the tissular Doppler technique),



rather than systolic contractions, represent a more sensitive and accurate early marker of subclinical cardiotoxicity.
In these studies, along with the diastolic dysfunction, some biochemical markers such as troponine (TnT and TnI) and the cardiac natriuretic hormones, (in particular the cerebral BNP and NT- proBNP) have been suggested as being early and specific markers of left ventricular dysfunction in the subclinical stage. This can be seen possibly even before the pathological reduction of the ejection fraction (F.E.)
Our Department has also adopted, in selected patients, new and safer methods for procedures and monitoring of subclinical cardiotoxicity and associated preliminary results are in accordance with published studies.

The research activity of the Department on various current and finalized institutional clinical research protocols, which involves all the heads of department, is generally carried out in close cooperation with the three oncological divisions as well as the hematology division of the Regina Elena Institute.

In 2007 the Cardiology Department of IRE was also involved in the following studies:

- The monitoring of cardiotoxicity related to antiblastic chemotherapy: cardiac visits are carried out before, during and in the follow up to chemotherapy with ECG and echocardiogram intended to highlight subclinical cardiotoxicity signs and to prevent the onset of dilated cardiomiopathy:
  Anthracycline derivatives used alone or in cooperation with the A, B and C Divisions of Medical Oncology:
  In breast cancer (in the metastatic phase or not) with doxorubicin, epiribicin, trastuzumab, paclitaxel, docetaxel and gemcitabine in primary or adjuvant chemotherapy programs;

- Solid tumors treated with high dose chemotherapy schedules

  Clinical-instrument cardiac monitoring, in cooperation with the Hematology Division, for patients affected by hemopoietic tumors due to both therapeutic regimes (chemoradiotheraputic), which are often aggressive and intensive and which may cause acute or chronic cardiac events, or due to the heart affected by the tumors because of direct infiltration or compressive mechanisms or alteration of the hemorrheologic characteristics of the blood;

- echocardiograph monitoring of the clinical evolution of pericardiac effusion, of the paracardiac masses and of tumors of the heart

Since November 2006 the Cardiology Department of IRE has been involved, in cooperation with the oncological surgical divisions, in the international multicentre study VISION : Major Vascular events In noncardiac Surgery patIents cohOrt evaluatioN study, involving the screening of troponin measurements after surgery. ■

DEPARTMENT OF ONCOLOGICAL PREVENTION AND DIAGNOSES

Clinical Pathology

Director: **Gaetano Vitelli**

STAFF

Anna Antenucci
Roberta Ascani
Marianna Attanasio
Lidia Autullo
Anna Maria Cianciulli
Giovanni Cigliana
Roberta Cocco
Laura Conti
Iole Cordone
Michele Crestini
Gabriella D'Alessandro
Francesco De Bellis
Carlo Del Carlo
Giovanna Digiesi
Alessandria Drusco
Assunta Esposito
Renzo Fontinovo
Anna Maria Frasca
Barbara Frollano
Simone Giommi
Antonio Gugliotta
Claudia Lattanzio
Giulia Mancusi
Chiara Mandoj
Aline Martayan
Serena Masi
Roberta Merola
Giulia Orlandi
Carmine Parracino
Alessia Pasquale
Eliana Preziosi
Immacolata Anna Rosito
Patrizia Spinosa
Chiara Timarco
Giuseppe Vercillo
Erika Vico

Scientific Activity

The unit of Molecular Genetics (consisting of the SSO Cytogenetic and the Molecular Genetic Area), has reached an outstanding level based on the establishment of classic and molecular methods of cytogenetic, and based on results interpretation. The latter involved correlating genetic alterations to patient diagnosis and prognosis and also the analysis of genetic mutations through sequencing and the identification of new therapeutic targets in breast, ovary and colonic cancer.
The hematology unit (composed of basic haematology, oncohematology and the urine area) is working on the diagnostic, prognostic definition and monitoring of all incoming patients, particularly with hematologic malignancies (Acute and Chronic leukemias, lymphomas, myeloma, etc.) achieving a high level of activity. Hematopioetic malignancies morphologic characterization, both immunocytochemical and immonophenotipic, is accomplished by using cytogenetic and molecular biology tests.

The Hemostasis and Thrombosis Department studies the different coagulation parameters of the fibrinolytic system, and also identifies (through molecular biology techniques) genetic polymorphisms in order to study hereditary trombophylia, already known as a high risk condition, especially at a later age, for developing a venous and/or arterious trombotic event with frequent relapse. During recent years, these genetic tests have been included among the prescriptionable tests and the unit has been investigating some parameters of the hemostatic system activation, in order to predict the thrombotic event in neoplastic patients who represent a high risk category for TVP.

The study results of acquired and hereditary thrombophylia is of primary importance in female patients who present relapsed abortive events and also in women undergoing assisted fecundation. In this area the department is a regional referential center.

The Chemical Chemistry unit (made up of analytical chemistry and of immunoprotidology) has participated in the global project QoL (Quality of Life), collaborating in the selection and the study of patients in follow-up, chosen for the post-chemotherapy evaluation, and managing the biological samples and the related database. Moreover, the unit is studying and monitoring patients with Multiple Myeloma (MM) and with other B cell dyscrasias, through the evaluation of the Kappa and Lambda free light chains.

The Serology Unit will benefit from the recently approved contract for Service instrumentation which could facilitate the transfer of some RIA performances to non isotopic methods, thereby responding to an increase in requests.

INFORMATICS

Updating the Italab C/S system is currently in progress through the installation of the new release DNLab and re-engineering the operative flows of the two analysis labs (IRE and ISG). We are waiting for the divisions to be brought together before being able to experiment in the introduction of electronic procedure signature on lab test results.

Updating the staff structure continues to represent one of the priorities for the lab and with this in mind, internal and external courses for technicians, graduates and contracted personnel and also for attendants of the Service have been activated.

TECHNOLOGICAL AND INSTRUMENTAL RESOURCES ENGAGED

The just approved contract for the renovation of the Service Instrumentation should give us the possibility of much more reliable instruments. These new resources will allow us to better manage the operative flow and the economical resources.

The Division of Clinical Pathology research activity has had much success.

The activity of the Division of Clinical Pathology is organized around the most interesting tools used in the diagnosis and monitoring of human cancer. More than 1.040,000 analyses were carried out in 2007. More than 22,000 analyses concerned genetics and molecular biology. ■

DEPARTMENT OF ONCOLGICAL SURGERY

General Surgery “A” Breast-Melanoma-Soft Tissue Sarcomas

Director: **Franco Di Filippo**

STAFF
Michele Anzà
Claudio Botti
Pietro Bruno
Fabrizio Frezza
Rosa Garinei
Roberto Maialetti
Pasquale Perri
Loredana Piarulli
Angelo Psaila
Fabio Massimo Sega
Carlo Vitucci

Nursing staff
Agostino Castaldo
Gianni Chiarabini
Antonietta Di Ceglie
Giuseppina Gemma
Rossella Leonardi
Roberta Melis
Maria Antonietta Picano
Aurelio Tirimagni

Specialists
Andrea Felice Armenti
Federico Corrias
Simona Di Filippo
Davide Gagliardi
Samantha Palermo
Simona Rinaldi

Secretary
Tiziana Piccolo

Scientific Activity

■ The activity of our department focuses on 4 areas: breast cancer, melanoma, soft tissue sarcoma and peritoneal carcinomatosis.

1. BREAST CANCER

Nipple Skin Sparing Mastectomy (NSM)
By the end of December 2007, 304 patients had been treated with NSM. At a mean follow-up of 22 months the incidence of recurrence after NSM is similar to that of radical mastectomy. Moreover the incidence of NAC necrosis is 1%. All patients have now been submitted to psycological testing to verify compliance with this operation.

Multicentric randomized study: IORT vs. external radiotherapy
Our department is participating in a prospective randomized trial that compares the effectiveness of IORT to external radiotherapy. The aim of the study is to demonstrate an equivalence in the two techniques in terms of efficacy. So far, more than three hundred patients have been enrolled.

Evaluation of lymphangiogenesis as a potential predictor of sentinel node status
The identification of intratumoral and peri-tumoral lymphatics is carried out by immunohistochemistry using an antibody anti podoplanin. Twenty patients with

SN negative and twenty patients with SN positive were evaluated.
There is a clear correlation between LVD and sentinel node status with the mean and median LVD values higher in positive than in negative sentinel nodes.

Breast cancer surgery in the elderly
We have selected 814 patients with age >65 from our database of 5.000 patients. 336 were submitted to demolitive surgery and 478 were treated with conservative surgery. Our experience shows that age is not the conditioning factor for the type of surgery. The surgical strategy was mainly conditioned by T and N status.

Tissue microarray analysis of FAS, Bcl-2, Bcl-x, ER, PgR, Hsp60, p53 and Her2-neu
The aim of this study was to detect immunohistochemical markers in breast carcinoma by means of tissue microarray analysis (TMA). Statistical analysis revealed that tumor stage (p=0.003) and node status (p=0.001) were the only two prognostic markers of disease-free survival. Moreover, FAS and Bcl-x showed an independent effect on recurrence (p=0.005).

2. MELANOMA

The Impact of Lymphoscintigraphy Technique on the Outcome of Sentinel Node Biopsy in 1.313 Patients with Cutaneous Melanoma: An Italian Multicentric Study (SOLISM-IMI)
On multivariate analysis only the number of peritumoral injections was inversely associated with the number of excision of SNs (p=0.002), whereas none of the technical variables showed an independent impact of SN status when Breslow thickness was included as a control variable.

cDNA-Array Profiling of Melanomas and Paired Melanocyte Cultures
With cDNA hybridization arrays, it is now possible to simultaneously examine changes in the expression of thousands of genes. Gene expression profiles identify "signatures" of neoplastic transformation and progression.
The data obtained thus far provides proof that cDNA profiling of paired melanocyte/melanoma cultures highlights novel, early signatures of melanocyte transformation that could contribute to the clinical management of patients at high risk of metastatic disease.

Hyperthermic Isolation Limb Perfusion with TNF∂ in the Treatment of In-transit Melanoma Metastasis
A total of 113 patients were enrolled in this study. The complete and partial responses were 63% and 24.5%, with an OR of 87.5%. The tumor mass was the only factor influenced by therapy.

3. SOFT TISSUE SARCOMA

Liposomal Doxorubicin with and without TNF∂ in the Perfusional Treatment of Advanced Soft Tissue Sarcoma: Preliminary Results
A combination of doxorubicin and TNF∂ has been proven to be very effective, but in some patients a grade IV limb reaction was recorded. Twenty patients were treated with liposomal doxorubicin (Caelyx): 14 with Caelyx alone and 6 in combination with a low TNF∂ dose (1 mg).
The limb toxicity was always mild (I-II), in patients treated with Caelyx and TNF∂. There was a consistently high percentage of tumor necrosis and conservative surgery was always carried out.

4. PERITONEAL CARCINOMATOSIS

120 Peritoneal Carcinomatosis from Colorectal Cancer Treated with Peritonectomy and Intra-abdominal Chemohyperthermia: A S.I.T.I.L.O. Multicentric Study
A multicentric study was carried out on 120 patients affected with peritoneal carcinomatosis from colorectal cancer. Patients were treated with cytoreductive surgery and intra-operative hyperthermic chemoperfusion (HIPEC) with cisplatin (CDDP) and mitomycin-C (MMC).
Major morbidity and mortality rates were 22.5% and 3.3%, respectively. No G4 toxicity was registered. The three-year survival was 25.8%. The difference in survival evaluation in relation to complete cytoreduction (CC-0) vs. incomplete (CC1-2; residual tumor nodules greater than 2.5 mm) was statistically significant (p<0.0001). Evaluating only the patients that could be cytoreduced to CC-0, the 3-year survival rose to 33.5%. ■

Digestive Endoscopy

Director: **Giovanni Viceconte**

STAFF

Daniela Assisi
Paola Capra
Alessandra Cinti
Antonio Grassi
Guglielmo Irti
Rocco Lapenta
Luigi Malatesta
Stefano Podestà
Marina Santamaria
Vittoria Stigliano

Scientific Activity

For some time now, our structure has been actively involved in the definition and use of protocols for screening as well as innovative diagnostic and therapeutic methods.

The major effort in research activity during 2006/2007 has been focused on:

1) Screening for colorectal cancer
2) Nutritional assessment of oncological patients
3) Cooperation with other facilities
4) Census of the gastrointestinal Endoscopy centers in Italy – 2007
5) Palliation of advanced cancer of gastrointestinal tract
6) Endoscopic treatment of bilio-pancreatic disease
7) Endoscopic ultrasound in oncology
8) Capsule endoscopy for the small bowel diseases
9) Clinical services

In 2007 the first videocapsule endoscopic examination was performed. This allows the diagnosis of small bowel diseases. Its application in oncology is important in those patients with a high risk of small bowel neoplasia such as families affected with Lynch syndrome and Familial Polyposis and in all patients with obscure gastrointestinal bleeding with negative upper and lower endoscopic examination.

1) SCREENING FOR COLORECTAL CANCER

a) **Cooperation with A.S.P** (Regional Public Health Authority), for colorectal cancer screening in subjects with average risk. The screening started in February 2006 and ended June 2007. 7428 subjects were recruited, of which 1495 performed a Fecal occult blood test. The compliance to perform Fecal occult blood (first level screening) was about 20%, of which 6.3% resulted positive. About 84% of positive patients agreed to undergo colonoscopy (second level screening).
Colon neoplasia (including adenomas) was found in about 18.7% of second level patients. 3.3% of cases were adenocarcinomas.

b) A study was performed to identify and define the **risks of colon cancer in breast cancer patients,** in accordance with many international studies, acknowledging a class of greater risk for these patients and the necessity for protocols of careful surveillance.

In December 2007 approximately 150 patients and 150 controls were recruited, and of these, approximately 15% had 1 or more colon adenomas. The study has now finished and statistical analysis is on going.

c) The **identification of relatives at risk of colorectal cancer linked to heredity** is one of the most successful activities of our facility and has already been in use for 20 years. For some time, our center has been a reference point for the study of HNPCC and of familial polyposis with a high number of immediate family members observed in our dedicated outpatients unit both from the clinical and endoscopic as well as the bio-molecular point of view. Patients and their relatives undergo multidisciplinary (genetic, gastroenterological, psychological) counselling. The relative data on the whole family is stored on a data base, which also contains family tree information, acquired during genetic counselling. Should there be any indication, the patients undergo a biomolecular test and a subsequent clinical and endoscopic follow up. In 2007 we enrolled 23 patients with suspected HNPCC and 16 patients affected with FAP.

d) Patients with early onset colorectal cancer underwent genetic counselling and immunohistochemical screening for HNPCC to identify hereditary syndromes, whose prevalence is currently under -estimated, and to design, within the family, adequate surveillance programs. The project I is financed by "Italian League Against Cancer". In 2007 we enrolled 11 patients.

2) NUTRITIONAL ASSESSMENT OF ONCOLOGICAL PATIENTS

a) Nutritional assessment of oncological patients. The belief that nutritional status is the basis in planning surgical and/or chemoradiotherapy treatment, led us to concentrate our effort on nutritional support during recovery status or for a domiciliary treatment. Four years ago we created a multidisciplinary team (doctors, dietitians, pharmacists and nurses) to evaluate every patient with oncological disease that showed an alteration in nutritional status (weight loss or obesity). Evaluation of nutritional status consists in antropometric and biochemical assessment (weight, height, arm circumference etc).
Severity of dysphagia, regarding localization of neoplasm, versus what kind of nutritional device it is necessary to implement.

b) Early recognition of patients that are malnourished or are likely to be, can influence the access of enteral nutrition. Furthermore, a multidisciplinary approach in the management and nutritional intervention for malnourished patients (enteral vs parenteral nutrition) is very important.

c) Feeding through tubes placed in the stomach or duodenum (**nasogastric tubes, nasoduodenal tubes or nasojejunal tubes**) has been used successfully for short term feeding.

d) **Percutaneus endoscopic gastrostomy (PEG)** is frequently performed in our endoscopy unit. It is technically easy to carry out, and less expensive than surgical gastrostomy. In this regard our center has been the reference point for some years for the PEG positioning of many facilities in the Lazio region.

e) Nutritional assessment of patients who have undergone prothesis placement of gastroenteric tract.

3) CO-OPERATION WITH OTHER FACILITIES

a. In co-operation with some of the IRE and ISG facilities a **multidisciplinary clinical study is underway involving patients affected by Celiac disease** in order to identify the relationship between this pathology and the disease in dermatologic, endocrinologic, and oncologic conditions.

b. There is close cooperation with the **Radiotherapy** facility for the endoscopic evaluation before and after treatment of patients undergoing radiation therapy for prostate cancer.

c. An international **multicenter clinical study on** the efficiency and use of **Celecoxib** (inhibitor of Cox-2) in the prevention of sporadic adenomatosis polyposis was closed in December 2007.

d. An international **multicenter clinical study on** the efficiency and use of **Celecoxib** (inhibitor of Cox-2) in the prevention of familial adenomatosis polyposis in children has been approved.

e. There is a close cooperation with the Hematology Unit for the evaluation of gastric-wall infiltration of lymphoma, before and during chemotherapy. The evaluation is performed with periodical EUS examination, according to a well- defined algorithm.

5) NATIONAL CENSUS OF THE ENDOSCOPY UNITS IN ITALY PROMOTED BY SIED (Italian Society of Gastrointestinal Endoscopy)

The Italian Society of Gastrointestinal Endoscopy (SIED) set up a committee to perform a Census of Gastrointestinal Endoscopy (GIE) Centers in Italy; Dr. Antonio Grassi is part of the Organizing Committee. The Census is designed to collect data about the number, the state of and the clinical activities of the Italian GIE Centers. After preliminary meetings to organize the Census a questionnaire was prepared and in 2007 it was due to be sent to all Italian GIE Centers. Dr. A. Grassi is also responsible for the data collection in the Roman Province in the Region of Lazio.

6) PALLIATION OF ADVANCED CANCER OF GASTROINTESTINAL TRACT

With regard to the numerous palliative treatment used by our unit we focused particular attention on the new technique of prothesiation in the stenosis of intestinal tract and in the stenosis of biliary tree.

7) ENDOSCOPIC TREATMENT OF BILIO-PANCREATIC DISEASE

In the last year, therapeutic endoscopy has increased remarkably. The area in which application is most common is in obstructive neoplastic jaundice, but patients affected with non neoplastic disease of biliary tree are treated as well.

8) ENDOSCOPIC ULTRASOUND IN ONCOLOGY

In the last 3 years we have started to perform routine endoscopic ultrasound (EUS) of upper and lower gastrointestinal tract for the evaluation of mucosal and sub mucosal lesion, pancreatic cysts and cancer stadiation.
This highly specialized endoscopic technique is one of the most important activities of our Unit. Because of the poor diffusion of EUS within Italy the unit is the reference center for Central and Southern Italy.

a) Selected criteria to identify patients with colorectal cancer treated with neoadjuvant treatment (radiotherapy plus chemotherapy preoperative and postoperative) are important to determine the response rate to treatment. EUS has a high accuracy and specificity for definition of T stage (particularly for differentiation T2 from T3) and for the detection of perilesional lymphonode (N stage).
Furthermore, EUS preoperative staging provides an accurate evaluation of the response to therapy and a better definition of the treatment (transanal excision or traditional surgery), especially if the endoscopic feature shows a complete response.

b) Upper EUS is useful to stage mucosal lesion of esophagogastroduodenal tract, to diagnose submucosal lesion (lipomas, GIST, leiomiomas), to stage pancreas adenocarcinoma and to diagnose benign pancreatic lesion.

c) EUS is the most important diagnostic tool to stage gastric lymphoma.
Furthermore, its accuracy and ability to stage gastric lesions is of critical importance in the definition of the most successful therapies.

9) CLINICAL SERVICES

In 2007, 12305 services, 83.3 % outpatients and 16.7 % admitted patients, were carried out. Out of a total of 3914 endoscopic examinations carried out, 416 were therapeutic examinations, 148 consultations made through the dedicated outpatients department for nutritional evaluation and 63 for hereditary tumors of the colon.

Epidemiology

Director: **Holger Schünemann**

RESEARCHERS
Maddalena Barba
Jan Brozek
Maria Cecilia Cercato
Valerio Ramazzotti
Nancy Santesso
Francesca Sperati
Irene Terrenato

STAFF
Vitolo Armando
Marco Canfora
John Fox
Tania Merlino
Isabella Sperduti

Scientific Activity

The research activity mainly focused on Clinical Epidemiology and Molecular and Metabolic Epidemiology.

CLINICAL EPIDEMIOLOGY

The core activities of the SC Epidemiologia include the synthesis and knowledge transfer of evidence to clinicians and patients. To this end, staff have been involved in the production of many systematic reviews of health care interventions, such as those from the Cochrane Collaboration. To ensure that evidence is used to change clinical practice, this department has also led and been involved in the production of clinical guide-lines for organisations such as WHO and professional associations for chronic obstructive pulmonary disease. We devoted resources to developing the methods and processes behind the production of systematic reviews and clinical guidelines. Work was conducted and continues to perfect the GRADE approach to evaluating evidence. Work has also been conducted to produce a software program, called GRADEpro, which assists researchers and clinicians to use the GRADE approach to summarise the evidence from the literature and make recommendations for clinical guidelines.

This department also builds capacity in clinicians and researchers to synthesise and transfer evidence to clinicians. Members of this department have provided training at national and international workshops for evidence based medicine, GRADEing the evidence and producing clinical guidelines.
Staff also supported the production of primary research by providing statistical support to clinical studies at IFO.

MOLECULAR AND METABOLIC EPIDEMIOLOGY

"Endogenous hormones and pre-menopausal breast cancer risk"
Funded Grant: NIH/NCI, 2004-2007
The overall goal of this application is to evaluate the role of ovarian sex hormones, insulin and insulin-like-growth factor 1 (IGF-I) bioavailability, based on levels of IGF-I and IGF binding proteins 1,2 and 3, in pre-menopausal breast cancer etiology.
During the past decade, several prospective cohort studies have examined the relation between serum concentrations of ovarian steroid hormones and breast cancer risk. For postmenopausal women, the studies have shown a consistent positive association between levels of serum estrogens and androgens and risk of the disease. However, there is little data from

prospective studies for pre-menopausal women, and the results that do exist are inconclusive. There are a number of likely reasons for this inconsistency. These include: small sample size, inability to control fluctuations in levels of serum sex steroids related to the ovarian cycle, issues with the methods used for biological specimen collection and issues with hormone determination assays.

There is also evidence supporting an etiological role of insulin and IGF-I bioavailability with breast cancer in premenopausal women. Besides the direct effect on proliferation and apoptosis inhibition exerted by both insulin and IGF-I on tumor cells, the etiological role of these factors may be also explained by their effect on tumor promotion by up-regulation of ovarian steroid secretion.

Therefore, our hypotheses are that in premenopausal women: 1) increased serum estrogen, androgen, and progesterone levels are associated with breast cancer development; 2) insulin, and increased IGF-I bioavailability are directly associated with breast cancer risk; 3) the association of estrogens and androgens with breast cancer is explained, at least in part, by the insulin and IGF-I induced up-regulation of steroid secretion.

"Glucose Metabolism and Breast Density: a Pathway for Breast Cancer Etiology" Funded Grant: FP-6 European Community Marie Curie Reintegration Grant 2005-2008

Breast cancer is the most common cancer in women, and a leading cause of cancer death in Europe. We proposed to improve understanding of breast cancer etiology by investigating the metabolic effect of glucose and genetic variants implicated in glucose metabolism and breast mammographic density, one of the strongest known risk factors for breast cancer.

There is increasing evidence that obesity and diabetes mellitus are associated with increased risk of breast carcinomas. Biological and epidemiological data provide support for a role of glucose and other factors related to glucose metabolism, such as insulin and insulin-like growth-factors (IGFs), in breast cancer development. However, it is still not clear how impaired glucose metabolism exerts its influence on breast cancer risk.
Mammographic density (MD) refers to variations in the breast among women that reflect differences in tissue composition. Women who have >75% of their breast area as dense tissue have a risk of breast cancer 3-6 times greater than women of the same age with zero area representing one of the most powerful predictors of breast cancer development
The conducted study aims to investigate whether glucose metabolism influences risk of breast cancer through direct effects on proliferative activity and quantity of stromal and epithelial tissue in the breast, that is reflected in mammographic density. Thus, we postulate that genetic and metabolic factors related to glucose metabolism influence breast tissue composition through effects on the levels of exposure to growth factors such as IGF-1 that are mitogens in the breast resulting in greater quantities of stromal and epithelial tissue in the breast, increase susceptibility to carcinogens, and risk of breast cancer. The long term goal of the on-going investigation is to understand the etiology of breast cancer, and to use this knowledge to guide the development of preventive strategies for the disease.

Estrogen Metabolism & Prostate Cancer Risk: A Prospective Study, 2005 2007, Department Of Defense, USA
This study investigated prostate cancer (Pca) risk in relation to estrogen metabolism, expressed as urinary 2-hydroxyestrone (2-OHE1),16_- hydroxyestrone (16_-OHE1) and 2-OHE1 to16_-OHE1 ratio.
We conducted a case–control study within the Western New York Health Cohort Study (WNYHCS) from 1994 to 2001. From January 2003 through September 2004, we completed the WNYHCS recall and follow-up. Cases (n = 26) and controls (n = 110) were matched on age, race and recruitment period according to a 1:4 ratio.We used unconditional logistic regression to compute crude and adjusted odds ratios (OR) and 95% confident

interval (CI) of Pca in relation to 2-OHE1, 16_OHE1 and 2-OHE1 to16_-OHE1 by tertiles of urine concentrations (stored in a biorepository for an average of 4 years). We identified age, race, education, body mass index as possible covariates. After conducting an updated search of the literature which revealed no additional studies, we pooled the results from this study with those from a previously conducted case-control study using the DerSimonian-Laird random effects method.
We observed a non significant risk reduction in the highest tertile of 2-OHE1 (OR 0.72, 95% CI 0.25-2.10). Conversely, the odds in the highest tertile of 16_-OHE1 showed a non significant risk increase (OR 1.76 95% CI 0.62-4.98). There was a suggestion of reduced Pca risk for men in the highest tertile of 2-OHE1to16_-OHE1 ratio (OR 0.56, 95% CI 0.19–1.68). The pooled estimates confirmed the association between an increased Pca risk and higher urinary levels of 16_-OHE1 (third vs. first tertile: OR 1.82, 95%CI 1.09-3.05) and the protective effect of an higher 2-OHE 1to16_-OHE1 ratio (third vs. first tertile: OR 0.53, 95% CI 0.31-0.90).
Our study and meta-analysis provides evidence of a differential role of the dominating estrogen hydroxylation pathway in Pca development and encourage to the conduction of further studies.

Endogenous 6-Hydroxymelatonin Excretion & Subsequent Risk of Breast Cancer: A Prospective Study - 2005- 2007
Department Of Defense, USA

Several factors including light at night, age, and body mass index appear to influence melatonin production. Lower urinary melatonin levels have been associated with a higher risk of breast cancer in premenopausal women. The association between melatonin levels and breast cancer risk in postmenopausal women remains unclear.
In a prospective case–control study nested within the ORDET, we measured the concentration of melatonin's major metabolite, 6-sulphatoxymelatonin (aMT6s), in the 12-hour overnight urine of 178 postmenopausal women with incident breast cancer and 710 matched control subjects.
In conditional logistic regression models, the multivariate relative risk [reported as the odds ratio (OR)] of invasive breast cancer for women in the highest quartile of total overnight aMT6s output compared with the lowest was 0.56 [95% confidence interval (CI) = 0.33 to 0.97; Ptrend = 0.02]. This association was strongest among current non-smokers, excluding 28 cases who reported smoking cigarettes at the time of urine collection (OR, 0.38, 95% CI, 0.20-0.74; Ptrend = 0.001). Overnight urinary aMT6s level and breast cancer risk were more strongly associated in women who were diagnosed with invasive breast cancer more than 4 years after urine collection, compared to their controls (OR for highest versus lowest quartile of urinary aMT6s output = 0.34, 95% CI = 0.15 to 0.75). We did not observe important variations in relative risks by hormone receptor status of breast tumors.
These prospective data provide, to our knowledge for the first time, evidence for a significant, inverse association between melatonin levels, as measured in overnight morning urine, and breast cancer risk in postmenopausal women. ■

Haematology Oncology

Director: **Maria Concetta Petti**

STAFF

Mariella D'Andrea
Maria Laura Dessanti
Andrea Mengarelli
Francesca Palombi
Francesco Pisani
Atelda Romano
Antonio Spadea

Scientific Activity

■ In 2007 the Division of Haematology Oncology was involved in carrying out clinical trials of primary relevance in different hematological malignancies, working in cooperation with other hematological institutions. In particular, the Unit is a member of the following cooperative group:

- Italian Association of Haematology Illnesses (GIMEMA)
- European Organisation for Research and Treatment of Cancer (EORTC)
- Non-Hodgkin Lymphoma Cooperative Study Group (NHLCSG)
- Italian Lymphoma Group (ILL)
- Italian Multiple Myeloma Study Group (IMMSG)

THE UNIT WAS INVOLVED IN THE FOLLOWING STUDIES:

Modern combination chemotherapy in acute myeloid leukaemia (AML) can induce a complete remission in 70-80% of adult patients (age less than 60 years), with 25 to 35% long-term leukaemia-free survivors. Results in elderly patients are still consistently worse in terms of response rates, duration of response and survival time.

An increase in the CR rate after induction, improvement of the applicability of a stem cell transplantation, a reduction in relapse rate, and monitoring of minimal residual disease are the main aims of the current phase III randomized trial of the EORTC/GIMEMA (AML 12). The AML 12 randomized phase III trial of EORTC – LG assessed the efficacy and toxicity of HD – AraC (3 g/m^{2}q 12 hrs for 4 days) in combination with daunorubicin (50 mg/m^2 for 3 days) and etoposide (50 mg/m^2 for 5 days) vs SD – AraC (100 mg/m^2 for 10 days) combined with the same drugs. All patients (pts) who reached complete remission (CR) received one consolidation course consisting of ID – AraC (500 mg/m^{2}q 12 hrs for 6 days) and daunorubicin. Subsequently, an allogeneic (allo–SCT) or an autologus stem cell transplantation (auto–SCT) was planned according to donor availability and age. A second randomization was performed after consolidation in pts without a donor. Auto–SCT followed or not by maintenance therapy with low dose IL – 2 (4 – 8 x 10^6 IU s.c. for 5 days per month) during one year. From 1999 till July 2005, 1359 AML pts (APL excluded), age < 61 years, from 65 centers (23 EORTC – LG and 42 GIMEMA) entered the trial. To date, 1235 pts have been randomized for induction and 355 pts for post–consolidation. During the induction course toxicity profiles were similar in the 2 groups. However, in the HD – AraC group, the incidence of grade 3–4 liver transaminase abnormalities (9% vs 5%) and conjunctivities (16% vs 1%) was higher, and neutrophil and platelet recovery time was shorter. HD – AraC in the induction cycle had no impact on organ toxicity during the consolidation course, but the

platelet recovery time (> 50 x 10^9/l) was significantly longer (median 4.4 vs 3.1 weeks). The IL – 2 schedule was well tolerated in most pts with fatigue (20%), rigor/chills (6.5%), arthalgia/myalgia (4%) being the main grade 3–4 toxicities.

So far, our results indicate: (1) the toxicity of HD – Ara – C is acceptable in induction of de novo AML pts < 61 years old, but better prevention of conjunctivities should be a priority; (2) platelet recovery after consolidation is longer in those who received HD – Ara – C in induction; (3) transplantation rates are high after consolidation; (4) IL – 2 toxicity is acceptable; (5) pts with a donor have a better outcome, (6) those good/poor risk cytogenetics have an excellent/poor outcome, respectively.

The conventional treatment of chronic myeloid leukaemia (CML) in early chronic phase (ECP) is Imatinib mesylate (IM) 400 mg daily. The estimated rates of major (MCgR) and complete cytogenetic response (CCgR) at 42 months are 91% and 84%, respectively (IRIS Trial – F Guilhot, ASH 2004), with a survival free rate from accelerated and blastic phase of 84%. The rates of CCgR are significantly different according to Sokal score, being 92%, 84% and 69% for low, intermediate and high risk categories. Several biological and clinical observations suggest that increasing the dose may improve the results: therefore, high risk patients could benefit from a dose increase front-line. To compare the effects of 400 mg and 800 mg daily in previously untreated, early chronic phase, Sokal high risk patients, the GIMEMA (Italian Group of Haematology Illnesses) CML WP is conducting a phase III trial in a multicentric international study running in Italy, Sweden, Denmark, Finland, Norway, Turkey and Israel. The preliminary results of this trial were presented at the 2007 ASH meeting held in Atlanta, Georgia, U.S.A. Overall, 215 pts were enrolled over a 3-year period and randomized (1:1) to receive IM 400 or 800 mg daily. As of August 2007, 137 pts were evaluated for CCgR rate at 12 months and 78/137 (57%) at that time were in CCgR. Treatment failures during the study (no complete hematologic response or 100% Ph+ at 6 months, or loss of response) were 24/137 (17%); patients off-treatment for protocol violations or refusal were 10/137 (7%); patients off-treatment for toxicity were 7/137 (5%). The results of this preliminary analysis show that the CCgR rate at 12 months is 57% overall, in line with the results of the IRIS trial in the same risk category (69% all risks, 49% high Sokal risk). It is currently too early to analyze the results and a second analysis will be performed at the beginning of 2008.

Several trials have shown the superior impact of high-dose melphalan (usually 200 mg/sqm, MEL200) versus standard therapy in newly diagnosed multiple myeloma (MM) patients. Intermediate-dose melphalan (100 mg/sqm, MEL100) was superior to the standard dose, but MEL100 has not been clinically compared with MEL200 in a randomized study. In a prospective, randomized, multicenter, phase III trial the Italian Multiple Myeloma Study Group (IMMSG) compared the efficacy and toxicity of MEL200 and MEL100: the results of the analysis were presented at the 2007 ASH meeting held in Atlanta, Georgia, U.S.A. The primary end points were complete remission (CR) rate, event-free survival (EFS) and incidence of gastrointestinal toxicity, infections and treatment-related mortality (TRM). Inclusion criteria were previously untreated myeloma, age less than 65 and Durie and Salmon stage II or III. All patients received 2 cycles of 28 day dexamethasone-doxorubicin-vincristine and 2 cycles of high-dose cyclophosphamide followed by stem cell harvest. MEL200 patients were conditioned with 2 cycles of melphalan 200 mg/sqm and MEL100 patients with 2 cycles of melphalan 100 mg/sqm, both followed by stem cell reinfusion. 298 patients (median age 57) were randomized, 149 to MEL200 and 149 to MEL100: all patients were evaluated for response, EFS and overall survival (OS). Patient characteristics were similar in both groups: 96 patients completed tandem MEL200 and 103 tandem MEL100. In intention-to-treat analysis, the very good partial response (VGPR) rate was higher in MEL200 group (37% vs 21%, p.=0.003) but CR was 15% in MEL200 and 8% in MEL100 group (p.=0.07). After a median follow-up of 30.5 months, the 3-years EFS was 46% in the MEL200 group and 26% in the MEL100 group (p.=0.03). The 3-years OS was 81% in the MEL200 group and 73% in the MEL100 group (p.=0.14). Duration of grade 4 neutropenia and thrombocytopenia was comparable, but a higher proportion of MEL200 patients required platelet transfusions (p.=0.002). Grade 3 or 4 non-hematologic adverse events were more frequent in the MEL200 patients (38% vs 19%, p.<0.0001). The incidence of grade 3-4 mucositis was 16% after MEL200 and 3% after MEL100 (p.<0.0001). The incidence of severe gastrointestinal toxicity was 19% after MEL200 and 2% after MEL100 (p.<0.0001). The incidence of grade 3-4 infections and of TRM was similar in both groups. In conclusion, MEL200 resulted in a significantly higher VGPR rate that translated in superior EFS but not OS.

Radiolabeled antibodies may be particularly effective in treating Non-Hodgkin's lymphomas (NHL) for the following reasons: lymphocytes and lymphoma cells are inherently sensitive to radiotherapy; the local emission of ionizing radiation by radiolabeled antibodies can kill cells with or without the target antigen being in close proximity to the bound antibody; and penetrating radiation may avoid the problem of limited access in bulky or poorly vascularized tumors.

The immunoglobulin ibritumomab is the murine parent IgG, kappa monoclonal antibody of Rituximab which also target the CD20 antigen. Ibritumomab is covalently linked to the tiuxetan chelate and radiolabeled with 90Yttrium. To optimize biodistribution, Rituximab is given prior to the radiolabeled antibody in order to deplete all normal circulating B-cells and thereby avoid non-targeted radiation.

We are performing a pilot study in relapsed Follicular Lymphoma (FL) to evaluate the efficacy and safety of Fludarabine, Cyclophosphamide, Rituximab (FCR) regimen followed by 90Y-ibritumomab tiuxetan (Zevalin) as consolidation. The preliminary results of this study were presented at the 2007 Italian Society of Hematology meeting held in Bologna, Italy, on October 14-17, 2007. At the time of the presentation, 4 patients were enrolled: FCR regimen produced a Complete Remission (CR) rate of 100%, was well tolerated and neutropenia was the only grade 3-4 toxicity. After Zevalin grade 3 thrombocytopenia and neutropenia occurred in 2 cases and extra-hematologic toxicity was absent. Three patients in CR after FCR were still in CR after Zevalin at 5, 6 and 11 months respectively; the fourth patient is "too early" for Zevalin evaluation. These preliminary data indicate feasibility, tolerability and efficacy of FCR regimen followed by Zevalin in patients with relapsed FL. Hematologic toxicity occurring with radio-immunotherapy is clinically controllable and acceptable in a population of usually rituximab + chemotherapy pretreated patients. A longer follow-up and a larger number of relapsed FL patients are required to determine the impact of this regimen on response and long-term duration of event free survival. ■

Endocrinology

Director: **Maria Luisa Appetecchia**

STAFF
Roberto Baldelli
Agnese Barnabei
Giuseppe Pasimeni

Scientific Activity

The Division of Endocrinology's current research involves the evaluation and development of novel clinical and laboratory tools useful in the diagnosis and monitoring of endocrine human cancers. The Endocrinology Division has a long-standing interest in improving the detection and treatment of endocrine cancers. In particular the Division focuses on clinical research and new treatment strategies on thyroid and neuroendocrine tumors. Other fields of interest include the endocrine effects of tumors or of related treatments, such as the Growth Hormone Deficit (GHD) or the hypopituitarism in brain neoplasms, hypogonadism and sexual dysfunction due to gonadal tumors or as a consequence of surgery, chemotherapy or radiotherapy, and their impact on quality of life of patients. Ongoing fields of interest are 1) the relationship between hyperinsulinaemia, diabetes mellitus, growth factors, obesity and cancer; 2) the effects of somatostatin analogs on circulating levels of chromogranin A in hormone-refractory prostatic cancer.

Supportive care

In the field of supportive care, the follozing studies are in progress:

- A study on prostate cancer, in collaboration with the Division of Urology (Director Prof Gallucci), with the aim of evaluating the effects of somatostatin analogs (lanreotide) on circulating levels of chromogranin A in hormone-refractory prostatic cancer. IPSEN.
- An international multicenter trial (HypoCCs), verifying the safety profile and the overall response rate in patients with GHD, treated with Growth Hormone replacement therapy (Studio HypoCCS "The Global Hypopituitary Control and Complications Study. A Global Observational Research Program" (B9R-MC-GDGA).

Gynaecology

Director: **Enrico Vizza**

STAFF

Mario Antonio Congiu
Fabio Francesco Diotallevi
Luciano Mariani
Domenica Mazza
Roberto Sindico
Cristina Vincenzoni
Giuseppe Vocaturo

Scientific Activity

■ The division concentrated largely on screening, diagnosis, treatment and follow-up of gynaecologic cancer. The division is composed of eight permanent medical staff and is composed of an outpatient clinic, a ward with 24 beds and a Minimally Invasive Surgery Unit, dedicated to the application of new minimally invasive technologies in the field of Gynaecologic Oncologic Surgery. Its surgical activity, which is both conventional and mininvasive, requires the most advanced technologies, highly specialized interdisciplinary teams and integrated treatments for advanced stages. In 2006-2007, various clinical research protocols and studies in the biology of tumors were conducted.

SPECIFIC ACTIVITIES

VISION PILOT STUDY (Vascular events In noncardiac Surgery patIents cOhort evaluation) study. A large international multi-center cohort study evaluating major vascular events in patients undergoing noncardiac surgery.

A total of 4500 patients at the Institute undergoing noncardiac surgery will participate. All patients who undergo noncardiac surgery are eligible if they are ≥ 45 years of age and receive a general or regional anaesthetic (i.e., plexus block, spinal, or epidural). We will exclude patients undergoing noncardiac surgery who do not require at least an overnight hospital admission after surgery or who only receive infiltrative (i.e., local) or topical anaesthesia. After obtaining written informed consent from eligible patients or their family members, research personnel will interview and examine patients and review their charts to obtain information on patient characteristics that are potential predictors of major perioperative vascular events. The preoperative patient characteristics recorded include: age, coronary artery disease, recent high-risk coronary artery disease, recent and non-recent coronary artery revascularization, cerebrovascular disease, peripheral vascular disease, critical aortic valvular stenosis, congestive heart failure, atrial fibrillation, diabetes treated with insulin or an oral hypoglycemic agent, hypertension, hypercholesterolemia treated with drug therapy, smoking history, and mild or moderate to severe renal insufficiency. All patients will have a troponin T drawn 6 to 12 hours postoperatively and on days 1,2 and 3 after surgery. An ECG is undertaken immediately after an elevated troponin measurement is detected. If a troponin measurement is elevated but the patient's ECG does not fulfil the criteria for myocardial infarction we will recommend that the patient have an echocardiogram. Research personnel will follow patients throughout their time in hospital, personally evaluate patients, review

patients' medical records, ensure study protocols have been followed and note any primary or secondary outcomes. The research personnel will contact patients by phone at 30 days and 1 year post-surgery. If patients indicate that they have experienced an outcome, the study nurse will contact their physicians to obtain the appropriate documentation.

ENDOMETRIAL SURVEILLANCE OF PATIENTS WHO RISK CONTRACTING ENDOMETRIAL CARCINOMA

The aim of the study is to ensure the early diagnosis of endometrial carcinoma in patients at risk (BMI>30, Tamoxifen-users, familiarity for breast, colorectal, ovarian and endometrial cancer). An endometrial test using scraping must be periodically carried out and through the use of ThinPrep-slide cytology we are able to carry out both immunocitochemical as well as morphological tests. Positive or suspicious endometrial cytology is referred to hysteroscopy and biopsy for hystologic diagnosis. Other aims of the study include the possibility of finding an advantageous and efficacious diagnostic path and a forecast marker able to predict neoplasm transformation.

RANDOMIZED PHASE III STUDY COMPARING STANDARD COMBINATION CHEMOTHERAPY VERSUS EXTREME DRUG RESISTANCE ASSAY-SORTED CHEMOTHERAPY FOLLOWING UPFRONT DEBULKING SURGERY IN ADVANCED EPITHELIAL OVARIAN CANCER

The division of Gynaecologic Oncology is participating, together with the division of Oncology A, in a multicentric international prospective phase III study. After surgical debulking, the results of an *in vitro* assay for drug resistance is used to individually select chemotherapy for the patient in order to avoid ineffective treatments, needless toxicity, and loss of quality of life. Patients are randomly assigned to receive the TP regimen (paclitaxel at a dose of 175 mg/m^2 as a 3-hour infusion followed by carboplatin AUC=6) or the EDRA-sorted regimen (carboplatin, cisplatin, paclitaxel, topotecan, doxil, etoposide, gemcitabine, cyclophosphamide: single-drug vs multidrug therapy is open to discussion). Stratification factors will include the treating institution, the FIGO stage (IIB–C, III, or IV). This study hopes to demonstrate that it is feasible to use an *in vitro* assay in routine clinical practice to eliminate ineffective chemotherapeutic agents.

ITALY-USA PROJECT ON PHARMACO-GENOMIC

This study is structured in two parts: 1) determination of protein serous pattern alterations correlated with pathogenesis, prognosis using Proteomic spectra generated by mass spectroscopy 2) identification of specific clusters of proteins, which predict early diagnosis and on which the treatment is tailored using fosfoproteomic. In this prospective study, the protein serous pattern alteration of 200 patients collected at the time of first diagnosis of ovarian cancer is analysed comparing the proteomic spectra with that obtained from the serous belonging to 200 cancer-free women used as a control. The study is ongoing and 40 cases have been enrolled to date.

IORT IN CERVICAL CANCER

IMMUNOGENICITY AND SAFETY OF PROPHYLACTIC QUADRIVALENT HPV (TYPES 6,11,16,18) VIRUS-LIKE PARTICLES

Research shows that women who are infected with these strains of HPV have a tendency to develop cervical dysplasia (L-SIL, H-SIL and ca. *in situ*) which, if left untreated, may further develop into invasive cervical cancer. The division of Gynaecologic Oncology is participating in a multicentric prospective, randomized, Phase III study whose aim is to demonstrate the efficacy of immunization against HPV 6, 11, 16, 18 to prevent the onset of cervical carcinoma. The study also evaluates the safety of the vaccine. Healthy, non-pregnant, females between the ages of 16 and 23 years of age have been enrolled in this study and immunuzed against HPV 6, 11, 16, 18.

LAPAROSCOPIC STAGING AND RESTAGING OF GYNAECOLOGIC TUMORS

Continuing worldwide interest demonstrates that laparoscopic techniques are now a standard tool for any gynaecological oncologist. Therefore, there has been a considerable effort to introduce laparoscopic and related mininvasive techniques in staging and surgery of gynaecologic tumors. The main fields of exploration and application are: extraperitoneal and transperitoneal lymphadenectomy pelvic and lomboartic as a staging or a restaging procedure; differential diagnosis in carcinosis; selection of patients in need of primary citoriductive surgery in advanced ovarian cancer; surgical intensive staging of cervical and endometrial cancer laparoscopically; restaging of borderline ovarian tumors after primary incomplete surgery. ■

Head and Neck Surgery

Director: **Giuseppe Spriano**

STAFF
Roberto Baldinetti
Giovanni Cristalli
Valentina Manciocco
Raul Pellini
Barbara Pichi
Raffaele Roselli
Paolo Ruscito

Scientific Activity

■ Ear, nose and throat and maxillofacial oncological surgery has a range of experience ranging from treating fairly common head and neck cancers to more complicated and difficult cases. Highly specialized surgical protocols and/or procedures are performed by the staff and every decision regarding clinical cases is submitted to the Head and Neck Disease Management Team, which includes specialists in surgery, radiation oncology, medical oncology, endocrinology, radiology, pathology, speech therapy, plastic and reconstructive surgery, dental and maxillofacial prosthetics, nutrition, and pain management. The group meets weekly and works together to meet their patients' diverse needs.

LARYNX SURGERY

Patients diagnosed with squamous cell carcinoma of the larynx are treated following more advanced organ preservation protocols to avoid, if possible, total laryngectomy. Subtotal and partial laryngectomy is performed even in recurrence persistence after RT in selected cases. In total laryngectomy, voice prosthesis and or voice rehabilitation programs are implemented to enable voice restoration.

EXTENDED THYROID SURGERY

Thyroid surgery combined with radionuclotide therapy offers the most challenging opportunities in treatment of thyroid cancer. Surgery should only be recommended if the condition cannot be adequately treated medically; if cancer is suspected or found by cytology the operation is absolutely necessary. Minimally invasive video assisted (MIVA) thyroidectomy and paratyroidectomy is applied in selected cases. The removal of the entire gland and (according to the guidelines) the lymph nodes of the median compartment of the neck (VI level) or in the lateral neck, if involved, must be meticulous to avoid injury to the inferior laryngeal nerve and to preserve parathyroids glands. In our center more advanced cases are treated. In these cases more extensive surgery is necessary and the resection of the larynx is required, as well as oesophagus, trachea, hypopharynx followed by flap reconstruction.

ORAL CANCER SURGERY

Advanced oral cancer surgery is routinely performed. In these cases, after tumor resection it is necessary to restore the anatomical and functional defect. The type of mandible reconstruction is performed according to the patient's characteristics by ostecutaneus microvascular fibula flap, iliac crest microvascular flap, ostecutaneus scapula flap, to combine the reconstruction of the bone of the mandible and the mucosa of the mouth.

IORT (INTRAOPERATIVE RADIATION THERAPY) IN HEAD AND NECK CARCINOMA

Three pilot studies are ongoing, to examine the value of IORT in advanced head and neck carcinoma. The three protocols are: anticipated boost, boost in irradiated recurrent tumors and in tumors infiltrating the mandible or those close to it. ■

Histology and Cytopathology

Director: **Raffaele Perrone Donnorso**

STAFF

Barbara Antoniani
Paolo Avetrani
Maria Benevolo
Simonetta Buglioni
Paola Canalini
Mariantonia Carosi
Beatrice Casini
Vera Chiari
Giuseppina Chichierchia
Renato Covello
Francesca De Santis
Anna Di Benedetto
Maria Diodoro
Cristiana Ercolani
Valentina Federici
Maria Assunta Fonsi
Anna Maria Giannotti
Angela Lattanzi
Sabrina Lori
Ferdinando Marandino
Mirella Marino
Robert Martucci
Elisa Melucci
Mario Moauro
Marcella Mottolese
Patrizia Palmarelli
Chiara Paolemili
Arianna Papadantonakis
Anna Rita Pennetti
Letizia Perracchio
Francesca Rollo
Patrizia Scordati
Steno Sentinelli
Paolo Visca
Assunta Vitale
Amina Vocaturo

Scientific Activity

■ Research projects of this department focused on molecular techniques aimed at investigating biological markers which could have great potential for diagnosis, prognosis and response to conventional and molecular targeted therapy in solid tumors, lymphomas, central nervous tumors and sarcomas. The efforts made to improve the organization of the frozen tumor tissue biobank, leading to the development of well standardized technical operative procedures, strongly strengthened our national and international scientific collaborations and greatly contributed to the majority of our scientific results. Concerning cytological specimens, we demonstrated that chromogenic in situ hybridization (CISH) is a valuable and reproducible alternative to FISH for selecting HER2 amplified breast cancer (BC) patients for Trastuzumab therapy, not only in histological, but also in cytological specimens. Moreover, in a large series of thyroid nodules we compared fine-needle aspirates processed by liquid-based cytology and conventional smears showing that ThinPrep technique could yield a better nuclear detail thus increasing the diagnostic accuracy. With regard to our studies on prognostic and predictive biomarkers, we analyzed COX2 overexpression in the context of the recent described molecular BC classification (luminal, basal-like, HER2 subtypes) demonstrating the impact of this enzyme on the clinical outcome of patients with a luminal A and basal-like phenotype. We stud-

ied the intra/intercellular changes occurring during the epithelial mesenchimal transition (loss of E-cadherin, Focal Adhesion Kinase and hMena overexpression) demonstrating their association with biological factors of known prognostic value. We investigated the prognostic and predictive role of a novel estrogen receptor, ERb, evidencing that there was a trend for the ERb positive/PgR negative cases to have a worse outcome within the group of N+ BC patients treated with chemotherapy (CT). Moreover, we studied the critical signaling molecules involved in the Trastuzumab (T) response/resistance network suggesting that T efficacy is partially dependent on the expression level of PTEN, p-Akt, MAPK pathway in BC patients receiving T + CT as first-line treatment. In advanced rectal cancer we studied a series of patients pretreated with radio-CT

showing that immunohistochemistry (IHC) is a suitable method to determine the correlation between thymidilate synthase expression and response to therapy or to tumor regression grade (TRG). Through IHC we also analyzed MSH2, MLH1, MSH6 expression in adenomas and CRC of patients and relatives bearing a hereditary non polyposis colorectal cancer, further validating immunostaining as a surrogate of DNA mismatch repair enzymes alterations. We investigated surviving IHC expression, a tumor marker with prognostic and therapeutic implications, in a retrospective series of early-stage non small cell lung cancer (NSCLC). Multivariate analyses identified nuclear expression of surviving as independent predictors of OS suggesting that prognosis of early stage of NSCLC can be linked to the cellular pattern of distribution of this antiapoptotic protein. In order to evaluate morphological modifications of prostatic carcinoma occurring after radio/chemotherapy we analyzed a prospective series of prostatic sextant needle biopsies. This study was integrated by IHC analyses evaluating variations in PSA,PSAP, high molecular weight cytokeratins (34betaE12) P63 and Ki67 proliferation index. FISH analysis is of particular relevance in soft tissue sarcomas, a histologically and genetically heterogeneous group of tumors. Their morphologic features frequently overlap due to poor differentiation or limited sampling in small biopsies. We studied a prospective series of soft tissue tumors focusing on detection of recurrent and specific chromosomal translocations. Results obtained indicated that detection of translocation events by FISH in sarcomas provides an important objective tool for confirmation of diagnosis and disease monitoring, relevant to plan accurate therapeutic approaches. FISH methodology was also used to identify specific chromosomal alterations, as translocations and polysomy, in extranodal non-Hodgkin lymphomas. We investigated the clinical behaviour and the Helicobacter Pylori (HP)-dependency of MALT lymphomas, finding a high number of patients with numerical abnormalities of chromosome 18 independent of the presence or absence of HP infection microscopically detected. In glioblastoma multiforme (GMB) patients with low tumor O6-methylguanine-DNA methyltransferase (MGMT) activity, due to promoter methylation, may be more likely to respond to alkylating treatment. We evaluated the relationship between MGMT hypermethylation, detected by methylation-specific PCR, and clinical response to treatment. We established a wide national and international collaborative network to gain insight into the pathogenetic mechanisms in thymoma and in autoimmunity thymoma-related. Our preliminary data indicated that EGFR IHC expression in advanced chemorefractory thymoma seems to be associated with clinical responsiveness to the anti-EGFR agent Cetuximab. Moreover, we found that the AIRE gene, which regulates the negative selection of self-reactive T cells, was poorly expressed in thymoma epithelial cells and that thymomas contained reduced numbers of regulatory (T reg) cells.

The imminent commercial availability of the preventive human papillomavirus (HPV) vaccine led us to study different epidemiological aspects of HPV infection. We examined the prevalence and the genotype distribution of HPV DNA in: 1) male sex partners of CIN affected women, 2) female migrants in Rome. We performed the HPV DNA detection by Hybrid Capture 2 (HC2), a hybridization assay which detects 13 of the most common HR HPV type. Our data suggested that men could represent an important means of HPV transmission between sex partners and that the migrating female population is at a higher risk of developing cervical cancer.

We have recently demonstrated that $p16^{INK4a}$, a cyclin-dependent kinase inhibitor, is associated with high-grade precancerous cervical lesions and carcinomas and may be a useful biomarker in identifying HR-HPV infected low-grade lesions. Therefore, we analyzed a large series of cervicovaginal smears confirming that $p16^{INK4a}$ may identify patients with high grade lesions but in cytological specimens we found a weak correlation between $p16^{INK4a}$ overexpression and HR-HPV infection. ■

DEPARTMENT OF CRITICAL AREA

Intensive Care, Pain Therapy and Palliative Care

Director: **Edoardo Arcuri**

STAFF
Antonio Calamaro
Felice Centulio
Francesco Ciocca
Loriana Di Emidio
Gianfranco Fusco
Lamberto Laurenzi
Lorella Pelagalli

Scientific Activity

■ The work of the department is centered on three major issues:

a) Intensive Care of Cancer Patients
The Department's recent research concerns the identification of biological risk factors as a consequence of antineoplastic therapies. In the last year we have drawn up our ethic-clinical guidelines, regarding the admission of neoplastic and incurable patients at the Intensive Care Unit (ICU), with particular attention paid to those with neurological and respiratory disease.
An agreement with the "Sacred Heart" Hospice has been initiated for the admission of patients "out of therapy". This agreement has been extended to an area of research in pain therapy, psychosocial problems and care of incurable patients.

b) Cancer Pain Therapy
Over the last few years we have elaborated an hypothesis regarding a possible tumor interference on opioid treatment efficacy, based on the observation of thousands of patients treated by the Pain Therapy Unit. This effect is due to the presence of specific opioid receptors on neoplastic cells: the binding of opioid drugs to these receptors may decrease their analgesic activity. This "pseudotolerance" characterizes many situations in a clinical setting of oncologic pain therapy of poor opioid responsiveness.

c) Palliative Care
An agreement between the Institute and the "Sacred Heart" Hospice proposed research of a new model of residential hospice care, very different from the current model (where residence is exclusively reserved for terminal patients). Currently, in the "Sacred Heart" Hospice, a limited number of beds are reserved for patients needing supportive care and pain therapy and who are able to receive assistance during a few hours (Day Hospice) or, for a longer period, to resolve prolonged side effects due to antineoplastic treatments (Restorative Care). A Ministry of Health research project has recently evaluated the clinical, psychosocial and economic impact of this new kind of assistance, extended to patients with incurable chronic disease. ■

Laboratory Animal Center

Director: **Gennaro Citro**

STAFF
Giuseppe Bertini
Giancarlo Cortese
Andrea Enrico
Barbara Nuvoli
Pierino Piccoli
Enrico Spugnini

Scientific Activity

The work carried out by the division has largely been in support of the clinical as well as experimental departments of the facility, using animal models for the development of completed and current research projects.

The availability of animal breading models permits the study of drug kinetics and drug distribution, supplying important information such as: the activity and duration of the effect of a drug; metabolic end of a molecule in an individual; early signs of further intervention to be undertaken in a treatment protocol in the case of an unsatisfactory response of a patient; the eventual interference of drug effects, administered in combination/association with other drugs.

Furthermore, specific antiserum is produced in rabbits through the synthesis of peptides that reproduce the protein antigenic activity sites of biological interest. These reagents help to highlight both the presence of natural antigens in the biological areas where they are expressed as well as to determine the quantity. Moreover, the possibility of using the reagents as drugs in new therapeutic strategies from the peptides produced is experimentally evaluated. In particular synthetic peptides, the amino-acid sequence of which is able to inhibit the links with oncogene products and other functional proteins, are produced.

CURRENT RESEARCH PROJECTS

- 2007/2008 Italy-USA Program on Cancer Pharmacogenomics € 250.000,00
- 2006/2008 Coordinator Italian Network Animal Models Facilities € 110.000,00
- 2007/2010 FIRB/MIUR project "New antineoplastic drugs N° RBIP06LCA9_009 € 150.000,00

Reviewing and Tutor FISR PROJECT "Food Quality and Well-being" Intra-Ministerial Commission MEF.

Medical Oncology “A”

Director: **Francesco Cognetti**

STAFF

Paolo Carlini
Fabiana Letizia Cecere
Anna Ceribelli
Alessandra Fabi
Virginia Ferraresi
Gianluigi Ferretti
Alain Gelibter
Vanessa Graziano
Michele Milella
Carmen Nuzzo
Paola Papaldo
Domenica Pellegrini
Enzo Maria Ruggeri
Michelangelo Russillo
Antonella Savarese
Massimo Zeuli

Scientific Activity

■ The Division of Medical Oncology A has a long-standing commitment to improve the detection and treatment of solid cancer. In 2007 more than 1600 new patients with solid cancer visited our Division, which has one of the largest referral programs for the disease.

The Division’s clinical activity guarantees the treatment and assistance for cancer patients requiring drug administration and clinical follow up. In particular, the Division is developing clinical research and new treatment strategies on solid tumors, especially gastrointestinal, lung, breast, gynecologic tumors and melanomas, using either biological response modulators or drugs, molecularly aimed at specific biologic targets for different tumors, in addition to the classic antineoplastic drugs. The Division adopts regimens with optimal efficacy and with a low toxicity profile, such as the continuous infusion regimens which produce a lower burden of individual toxicity and offer the patient an acceptable quality of life. Several study protocols, each devoted to a single tumor, have been designed with this aim. Other fields of interest include the treatment of cancers which require a wide experience in medical oncology (e.g. gonadal or extragonadal germinal cell tumors and soft tissue sarcomas). Team members provide state-of-the-art diagnosis and treatment to patients with solid cancers, and are able to follow the patient by continually adding to a database that tracks the patient. An important advantage of this database is the ability to gain knowledge in order to help each patient avoid unnecessary surgery, chemotherapy, and radiation therapy, and help predict outcome.

The main research topic of the Division of Medical Oncology A has been the study of new drugs, their combinations and/or sequence and new strategies of integrated treatments. During the past year the Division of Medical Oncology A has produced 54 indexed publications. ■

Medical Oncology “B”

Director: **Massimo Lopez**

STAFF

Maria Ida Abate
Antonella Amodio
Silvia Carpano
Carolina Cauchi
Francesca Conti
Serena Corsetti
Marina Della Giulia
Luigi Di Lauro
Silvia Ileana Sara Fattoruso
Paolo Foggi
Filippo Greco
Giancarlo Paoletti
Laura Pizzuti
Massimo Rinaldi
Luigi Carmine Roma
Valentina Rossi
Domenico Sergi
Irene Venturo
Patrizia Vici
Giuditta Viola

Scientific Activity

Among the fields of interest of the Division of Medical Oncology B (MOB) is to create and maintain liaisons with other oncological associations, universities, and to cooperate with the pharmaceutical industry in areas of mutual interest. Clinical research activities are centered on the evaluation of new treatment strategies for solid tumors, with a special regard to new molecularly targeted drugs and their synergistic interactions with antiblastic chemotherapy. Research activities conducted during 2007 have concerned a number of oncologic fields, with the main areas of interest being breast cancer, gastrointestinal tumors and NSCLCs.

BREAST CANCER

The Division of MOB served as the coordinating center of various breast cancer clinical trials in neoadjuvant, adjuvant and advanced settings, in collaboration with other Italian oncologic centers.

NEOADJUVANT

We designed a multicenter trial with a regimen of epirubicin 80mg/m2 and docetaxel 80 mg/m2, every 3 weeks, with G-CSF support, followed whenever possible by surgery or, in the case of no change or progression, by radiotherapy, and a subsequent adjuvant regimen with vinorelbine, 25 mg/m2 d 1,8 and mitomycin C 10 mg/m2 d 1, every 4 weeks. Estrogen receptor positive patients receive, at the end of chemotherapy, hormone treatment. Enrolment continued during 2007.

ADJUVANT

In this setting we coordinated a phase III multi-center randomized trial to test the efficacy of 4 cycles of epirubicin/cyclophosphamide regimen versus the same regimen preceded by 4 cycles of docetaxel in node positive breast cancer patients. This is the first study testing the sequence taxanes->anthracyclines as adjuvant treatment. Accrual was completed in October 2005 with 750 patients enrolled onto the study (147 in MOB). Treatment

was feasible in both, with a major incidence of neutropenia, usually brief, in the sequencial part. Data on efficacy are pending.

TREATMENT OF ADVANCED BREAST CANCER

In 2007 we continued enrolment for the prospective multi-center randomized study of Epirubicin/vinorelbine versus pegylated liposomal doxorubicin/vinorelbine.
The department is particularly interested in the clinical evaluation of new drugs and new combinations, and we designed and started several clinical trials, most of which were multi-centric, in advanced breast cancer patients.
Literature data and the excellent results obtained from our previously published trial "High activity of salvage treatment with biweekly paclitaxel-gemcitabine combination in heavily pretreated breast cancer patients Vici P et al. J Exp Clin Cancer Res. (2006)" prompted us to design a phase I-II clinical trial, with the bi-weekly combination of infusional gemcitabine with paclitaxel, in anthracycline-pretreated advanced breast cancer; in the phase I study, 18 patients were enrolled and the dose of gemcitabine recommended for the phase II study was 1200 mg/m2 with paclitaxel at 150 mg/m2. Enrollement into this study is almost complete, with 36 patients able to be evaluated for the phase II; preliminary data has been submitted to ASCO 2008.

In 2007 we continued enrolment for another phase II multi-center randomized trial of docetaxel 75 mg/m2 d 8 and gemcitabine 1000 mg/m2 d 1, 8 versus docetaxel 75 mg/m2 d 1 and capecitabine 1,250 bid d 1‡14, with cycles repeated every 3 weeks, as first-line treatment for advanced disease, in patients previously treated with adjuvant anthracyclines.
Several efforts have been devoted, during 2007, to the development of new combinations of molecularly targeted treatments of several cancers, particularly breast cancer. In this disease, particular attention has been focused on DNA methyltransferase and histone deacetylase inhibitors.
Another field of interest has been to investigate tamoxifen resistant mechanisms, and breast cancer patients, treated with tamoxifen in adjuvant setting, were evaluated in terms of clinical outcome and expression of some Erb-family receptors on primary tumors, in collaboration with other clinical divisions of the Institute and with the Department of Experimental Oncology. The paper has been submitted and will be published in 2008 on PLoS ONE.
The Division of OMB is also involved in several other trials in lung cancer, gastric cancer, colorectal cancer, melanoma, and soft tissue sarcomas. Among these, sarcomas, gastrointestinal stromal tumors, (GIST) have received particular attention. ■

Medical Oncology “C”

Director: **Edmondo Terzoli**

STAFF
Emilio Bria
Carla Campanella
Federica Cuppone
Carlo Garufi
Fiorentino Izzo
Paolo Marolla
Cecilia Nisticò
Angela Torsello
Francesco Tropea
Barbara Vanni

Medical personnel
Paolo Asquini
Maria Camilli
Paola Decrestina
Paolo Di Ridolfi
Emanuele Esposito
Wioletta Faltyn
Giuseppina Palombi
Rosario Pimpinella
Massimo Quartuccio
Daniela Tavano

Secretary
Eleonora Bertini

Scientific Activity

The division’s scientific activity in 2007 was focused largely on breast, colorectal and lung cancer. A methodological field of research regarding meta-analyses and surrogate end-points was successfully carried out.
In particular, the development of weekly chemotherapy and the chronomodulated infusion of chemotherapy in breast and colorectal cancer respectively, reflected the extensive experience of the Division directed by Prof. Terzoli, and the division as a cultural and scientific treasure for the Institute. As has been the case with drugs in the past, new chemotherapeutic and molecular targeted agents will be developed for weekly and chronomodulated application. Both these lines of research have been enriched by several international peer-reviewed publications and at meetings attented by national and international opinion leaders.
In order to update oncological knowledge and research, our division has also organised several courses held weekly. These courses involve doctors from all over the country actively participating in discussions regarding the most recent clinical studies and their application to real clinical cases. In other words, the courses represent an ideal and skilled form of training constituted by research and clinical practice. International consensus, regarding weekly chemotherapy for breast cancer, has been recently achieved in several phase III trials and has been shown to be beneficial in activity and efficacy when compared to conventional 3-weekly chemotherapy. These important results, considered alongside the department’s experience and the number of enrolled patients undergoing weekly chemotherapy for breast cancer, make our division one of the biggest and highly skilled in the world. This further confirms the importance of the Institute in clinical research.
Our Division is considered a national and international center of reference for the chronomodulated infusion of chemotherapy in the treatment of colorectal cancer. As a EORTC member, our division coordinates international trials.
All research conducted in the Division is

continuously supported by the Scientific Director of the Institute.

BREAST CANCER

Together with the dose-dense theory, the weekly administration of antiblastics represents one of the emerging issues in the optimization of chemotherapy delivery. From a theoretical point of view, the exposure to a more sustained antineoplastic administration should inhibit tumor regrowth between each cycle, and decrease the onset of chemotherapy-resistant cancer clones. Paclitaxel has become the most studied drug for weekly administration, as preclinical and clinical evidence has revealed interesting possibilities. In fact, while the "laboratory bench" had suggested pro-apoptotic and neo-angiogenetic pathways involved in the weekly paclitaxel mechanism of action, the "patient bed" demonstrates a higher activity and efficacy for such a schedule when compared to 3-weekly administration in both advanced and neo-adjuvant treatment settings. On a sustained weekly basis, paclitaxel is able to increase the dose-intensity as in the dose-dense approach. However, the exploited cytotoxicity appears not to be related solely to this, but also to the increased "rhythmic" administration, which involves interesting pathways different from the 3-weekly schedule. This theory has been demonstrated in the direct comparison with a 3-weekly regimen with the same dose-intensity.

The fact that paclitaxel can be easily and safely administered on a sustained weekly basis and, at the same time, can provide an increased dose-intensity, is a characteristic which is not shared by all drugs. In fact, we previously demonstrated that the sustained administration of weekly docetaxel did not provide any significant dose-intensity increase and, moreover, the expected toxicities appeared at a threshold level.

The chemotherapy intensification provided by both dose-dense or a weekly schedule becomes possible with the concurrent use of hematopoietic growth factors such as G-CSF. The maintenance of this continuous sustained frequency obtained with G-CSF support is crucial for the activity of weekly paclitaxel. A previous report demonstrated that a less intensive G-CSF administration modulated on growth factor half-life, chemotherapeutics, pharmacokinetics and bone marrow function, is equally active when compared to a classical schedule in breast cancer in avoiding toxicity and dose-intensity maintenance. Along similar lines, we have previously demonstrated that a 2-day G-CSF administration is able to reduce neutropenia and also maintain dose-intensity in a sustained weekly schedule. A one unit delay per month translates into a 25% dose-intensity reduction.

Since 1990, our Division has worked on the development of weekly schedules in metastatic breast cancer. In 2004, a phase II study was completed in which patients affected by previously untreated advanced or metastatic breast cancer underwent weekly combination chemotherapy with epirubicin and paclitaxel. Furthermore, two ancillary studies were also conducted to evaluate the cardio- and neuro-toxicity of such a schedule from a clinical, serological and instrumental point of view.

Two research projects were set up (year 2000). Firstly, we initiated a phase I-II study with weekly docetaxel without any rest period in pretreated patients with advanced breast cancer. Secondly, we started a phase II study with weekly epirubicin plus vinorelbine in locally advanced breast cancer. Anthracycline-resistant or refractory patients were enrolled in a phase II study with weekly gemcitabine and paclitaxel. Gemcitabine was also administered at a fixed dose rate (FDR) of 10 mg/m2/min, owing to the extremely recent pharmacokynetic and clinical news regarding this drug.

In terms of results achieved with monoclonal antibodies, patients expressing HER +++ or FISH positive, were treated with weekly trastuzumab in combination with epirubicin and paclitaxel in 1st line or with gemcitabine and paclitaxel if resistant or refractory to anthracyclines.
Regarding the adjuvant treatment of breast cancer, our Division participated in the trials coordinated by the Italian Breast Group (GIM). Node-positive patients after surgery for early stage breast cancer have continued to be randomized in the GIM 2 protocol, while node-negative patients have been included in the GIM 1 trial. During 2007, our division was involved in the GIM 4 and 5 exploring the role of the new aromatase inhibitors and their pharmacogenomic findings as adjuvant treatment for breast cancer.

COLORECTAL CANCER

Together with the Thoracic Surgery Division and the Department of Medical Oncology, a unique research project has been designed and coordinated. A panel of clinical and molecular prognostic factors has been scheduled to be analyzed in one of the most modern and largest patient' samples ever published or presented. In particular, the role of the removal of mediastinal nodes in patients affected by stage I-IIIA non small cell lung cancer who have undergone surgery, was presented at the World Lung Cancer conference, and will be published next year. In the same population, a model of molecular factors will be analyzed.

Today, medicine is evidence-based and the strongest contribution to care is provided by recommendations from large randomized clinical trials (RCTs) or meta-analyses. Therefore, our Division has started a research plan in this methodological field. The experience accumulated by our investigators in collaboration with the Columbia University of New York, has become a clinical project in our Institute, involving a panel of medical doctors and statisticians from the Department of Medical Oncology. A method, by which results can be pooled from conflicting and controversial data from randomized clinical trials, has been developed. In 2007 issues addressed included: the use of combination chemotherapy for advanced pancreatic cancer and the benefit of adjuvant trastuzumab for early breast cancer. Another research field that has been investigated in the last year is clinical trial design and in particular 2 issues: 1) the use of surrogate end-points and 2) subgroup analysis. In this regard, the surrogate end-points for drug or strategy approval by regulatory agencies has been extensively analyzed for early breast cancer, by studying taxanes, aromatase inhibitors and trastuzumab. Moreover, the use of surrogates for early non-small cell lung cancer has also been studied. The most important achievement for the group was the oral presentation at the American Society of Medical Oncology meeting, held in Chicago (US), which considered a comparison of different methods of performing meta-analyses, representing a presentation at the highest level for the medical oncology scientific community worldwide. ■

Medical Physics and Expert Systems

Director: **Marcello Benassi, Anna Maria Di Nallo** (2008)

STAFF
Physicists:
Armando Abate
Marcello Benassi
Vicente Bruzzaniti
Anna Maria Di Nallo
Giovanna Evangelisti June 2007 to July 2008
Giuseppe Iaccarino
Valeria Landoni
Simona Marzi
Luis Pedro Ordonez
Antonella Soriani
Lidia Strigari

Technicians:
Giampiero Carlino, Sandro Carpino
Stefano Luppino, Aleandro Menghi
Sandro Nocentini, Massimo Pedrini
Francesco Quagliani, Berardino Tiberi

Researchers in training (residents):
Chiara D'Ambrosio "Sapienza" University, Rome
Erminia Infusino "Sapienza" University, Rome
Katia Pasciuti "Sacred Heart Catholic" University, Rome

Scientific Activity

■ The Laboratory of Medical Physics and Expert Systems (LMP) technically supports different departments using ionizing and non-ionizing radiations. In particular it supports the Radiotherapy Department in innovative treatments such as:

Intra-operative radiotherapy (IORT)
Studies based on a multidisciplinary approaches for: Prostate cancer. A dose-escalation study in patients with intermediate risk prostate cancer, after radical prostatectomy.

Breast cancer. A study in which patients who underwent conservative surgery for small mammary carcinomas have been randomized to receive IORT on the tumor bed or conventional EBRT to evaluate the local recurrence rate and second ipsilateral tumors, as well as the local recurrence free interval.
Head and neck. A study in which patients with head and neck cancers which recurred after radiation therapy received Salvage Surgery, IORT and External Beam Radiation Therapy (EBRT) or patients after surgery randomized to receive an early boost on locally advanced head and neck cancers.

Intensity modulated radiotherapy (IMRT)
Studies on Head and Neck cancer evaluating the real efficacy of this technique in reducing xerostomia and improving patient comfort. Treatment plan optimization is based on multi-modality imaging and on biological criteria derived from clinical outcomes.

Mono and or multi-institutional hypofractionated studies:. The studies evaluated the toxicities and tumor control in groups of patients who underwent radiotherapy using different schedules in respect to conventional treatments on prostate cancer, partial breast irradiation and primary lung tumor. The LMP supports the Nuclear Medicine Department in innovative systemic treatments for Patient Specific Dosimetry, which allows the Division to optimize activity to improve tumor control and normal tissue sparing (red marrow, liver, kidneys, etc.). The LMP developed a dosimetric tool, based on dose-point kernels, derived from Monte Carlo simulations, to calculate 3D dose distributions, and developed predictive radiobiological models for tumor control. In order to reach and to maintain a high standard of medical image quality and to minimize the risks for patients, the LMP co-operates with Radiotherapy, Nuclear Medicine and the Radiology Department in the maintenance and optimization of a quality assurance program in the field of ionizing and non-ionizing radiations. The main activity within the radio-diagnostic field regards ROC analysis to study low dose TC images and studies based on functional imaging techniques to improve diagnostic capability. The laboratory applies a Bayesian analysis of dynamic Magnetic Resonance breast images to extract information concerning tumor morphology and pathophysiological features in patients. The applications of colour and power Doppler ultrasound is in progress to study hypervascularisation areas for morphologic-functional diagnoses on prostate cancer. The Laboratory also co-operates on the evaluation and optimization of the radiology department's computer network. ■

Nuclear Medicine

Director: **Carlo Ludovico Maini**

STAFF
Serenella Bergomi
Anna Di Luca Sidozzi
Costanza Mazzone
Rosella Pasqualoni
Sandra Rea
Michele Roccotelli
Luisa Romano
Rosa Sciuto
Monica Tuccimei

Scientific Activity

The activities of the Nuclear Medicine Division focused on clinical research directed towards therapy and diagnostics in the main field of oncology. Therapy, as the primary field of clinical activity, includes the radionuclide treatment of thyroid carcinoma and pain from bone metastases. Innovative treatments with new radiopharmaceuticals available for targeted therapy were also implemented such as radioreceptorial therapy of neuroendocrine tumors, selective internal radiation therapy of liver tumors and radio-immunotherapy of lymphoma. Studies of the biological optimization of radiation dose were performed using new algorithms and modified Montecarlo protocols to evaluate heterogeneous dose distribution in tumor lesions. Good clinical practice procedures on radiopharmaceutical preparations have been drafted according to national guidelines. Training on innovative treatments with new radiopharmaceuticals were performed and clinical protocols validated.
Diagnostics include, besides routine oncological studies, radioreceptorial scintigraphy with 111In-octreotide, sentinel node mapping and cardiac gated-SPET scanning. In addition, a PET- CT section was begun and over 1100 oncological exams were performed, including non FDG radiotracers.
In 2006 - 2007 over 40,000 therapeutic and diagnostic procedures were performed with more than 900 cancer radionuclide treatments.

THYROID CANCER

The main clinical and research interest of the Nuclear Medicine Division, since 1992, has focused on thyroid cancer management and the role of radioiodine therapy. Over 500 in-patients per year have been treated with radioiodine therapy and over 2500 patients with DTC have been followed in aftercare at our Division, which is one of the largest referral centers in Italy for this disease. This large accrual of patients with DTC, which have been homogeneously treated on a long term basis, has produced a large amount of data. This has facilitated the investigation of a variety of interesting topics, according to EBM criteria. Clinical research has been based on the analysis of clinical presentation of DTC in the last 15 years and on the refocusing of the disease approach both in therapy and in follow-up, considering the increasing tumor incidence and the continual improvement of diagnostic modalities. New therapeutic strategies using recombinant human TSH instead of hormone withdrawal are currently ongoing to improve the quality of life of patients and minimize discomfort related to hypothyroidism. Extensive experience in the use of recombinant

human TSH for diagnostic purposes obtained in over 600 patients has led to technical improvements in the procedure. All data has been submitted for publication as part of a more extensive analysis. In addition, our experience has been formalized in:

- the "I.R.E. Guidelines for differentiated thyroid cancer management" in the framework of the relative Disease Management team
- a CME teaching course ("Integrated treatment of differentiated thyroid cancer – from guidelines to clinical pathway") aimed at developing clinical governance.

A new radiobiological approach using molecular imaging was also designed and an experimental protocol is now in progress to assess biological and dosimetric optimization for individualizing radioiodine treatment in advanced thyroid carcinoma using ^{124}I PET/CT.

SKELETAL METASTASES FROM SOLID TUMORS

Further activity has included more than 500 treatments for bone metastases performed with the three available bone seeking radioisotopes (^{89}Sr; ^{186}Re; ^{153}Sm) using the same clearly defined criteria for treatment and for response evaluation. This rigorous standardized and reproducible methodology has produced a wealth of comparable data leading to impressive original contributions in this field. The results contribute both to a clarification of clinical indicators using standard procedures and the exploration of innovative strategies through a series of clinical trials. An original dosimetric model to validate the clinical choice of individual doses was also recently implemented and published.

NEURO-ENDOCRINE TUMORS (NET)

Neuroendocrine tumor diagnosis and therapy has been an important field for the Division. Today, our group is considered as a referral center for radioreceptorial scintigraphy having the largest series of NET patients (> 600 pts.) in Italy with a mean accrual of 12 new patients/per month. Somatostatin receptor scintigraphy (SRS) is considered the 'gold standard' imaging procedure in patients with NET and our contributions in this field have been crucial in confirming the diagnostic accuracy of SRS, both in the preoperative work and in the follow-up of these tumors. Apart from our considerable experience in the diagnostic use of radiolabelled somatostatin receptor, we have also performed radioreceptorial therapy with 111-In-OCT in a selected number of neurodocrine tumors (medullary thyroid carcinoma and Merkel tumors). Preliminary results and dosimetric evaluations have demonstrated that this innovative therapy is safe and feasible, while efficacy is currently under evaluation. Our Division has also been involved in an Italian multicentric trial that started in 2007, aimed at evaluating the validity of Somatostatin receptor scintigraphy (SRS) for non functioning GEP tumor management.

SELECTIVE INTERNAL RADIATION THERAPY (SIRT) IN LIVER METASTASES FROM COLORECTAL CANCER

Selective Internal Radiation Therapy (SIRT) is a technique that began development in 1987 in Australia and has been administered to over 600 patients. SIRT consists of embolising radioactive SIR-Spheres® into the arterial supply of the liver, following which the SIR-Spheres® preferentially lodge in the vasculature of the tumor. The spheres deliver high doses of ionising radiation to the tumor component while maintaining radiation to the normal liver at a tolerable level. The radiation half-life of yttrium-90 is 62 hours. High response rates have also been reported using SIR-Spheres® combined with chemotherapy in the regional treatment of liver metastases without any severe complications. Currently, a preliminary study to evaluate the efficacy of the SIRT procedure used only in the loco-regional treatment of liver metastases from colorectal cancer is in progress and an initial study on neuroendocrine liver metastases and hepatocarcinoma is also ongoing.

LYMPHOSCINTIGRAPHY IN BREAST, VULVAR AND COLON CANCER

The sentinel node (SN) procedure has emerged as an alternative to systematic lymphadenectomy in various cancers, reducing treatment-related morbidity. In our Division we have acquired considerable experience in a broad spectrum of tumors, obtaining excellent results. The range of these tumors include breast, colon, vulvar, penile and head-neck in addition to melanoma.

PERFUSIONAL TREATMENT OF MELANOMA AND SARCOMA

Effectiveness of perfusional treatment with tumor necrosis factor alpha (TNF alpha system) in soft-tissue limb sarcoma and in-transit melanoma metastasis treatment was evaluated in preliminary pilot studies. Methodology was first implemented and validated and preliminary clinical results are now available. ■

Neurology

Director: **Bruno Jandolo**

STAFF

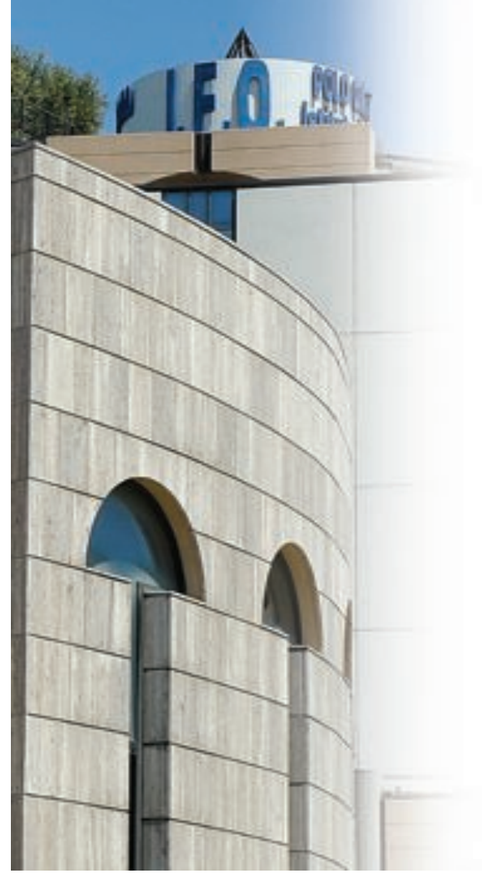
Rita Casillo
Di Lorenzo Cherubino
Antonietta De Fulviis
Anna De Sena
Angela Di Canio
Maurizio Di Lelio
Loredana Di Napoli
Antonella Gagliardo
Edvina Galiè
Lara Guariglia
Maria Luisa Guerra
Giuliana Graziano
Tatiana Koudriavtseva
Marta Maschio
Andrea Minnetti
Fabio Moscatelli
Laura Osnato
Andrea Pace
Gianluca Petreri
Marzia Piccoli
Alberto Pietrangeli
Antonia Sorrentino
Valeria Veneziano
Alessia Zarabla
Alessia Zizzari

Scientific Activity

■ Seizures affect 20-40% of patients with primary brain tumors or brain metastases and significantly alter their quality of life. Furthermore, anticonvulsant treatment is complicated by pharmacological interactions between antiepileptic drugs (AED) and chemotherapeutic agents. We have studied the safety and efficacy of three newer AEDS, Oxcerbazepine, Leveriracetam and Topiramate, that have favourable pharmacokinetics: all three drugs were shown to be safe and effective in patients with brain tumors.
Adjuvant chemotherapy has a significant but limited impact on survival of malignant gliomas. However, the role of salvage chemotherapy (CT) at recurrence after first line treatment is still debated and there is little data about the real benefit and toxicity of second and third-line CT. We analysed PFS, response to CT and toxicity in 127 patients affected by malignant gliomas treated at the Institute with second and third-line CT after recurrence. Our data shows that salvage chemotherapy may be considered an effective treatment option only in a subset of patients with chemosensitive tumor.
Neoplastic meningitis (NM) is the result of the diffuse or multifocal localization of cancer cells in the cerebral spinal fluid (CSF). NM is more often a late complication of solid tumor or lymphoproliferative malignancies. Given that NM is a cancer complication that can spread throughout the entire subarachnoid space, chemo-therapy, whether intrathecal or systemic, is currently considered the best treatment option, but optimal treatment is still controversial. At our Institute there is an ongoing study to evaluate the usefulness of treatment with Depo-Cyte.

In a global project focusing on all oncological problems, both a good therapeutic goal and good quality of life must be achieved. In our project, supported by the Alliance Against Cancer and the Ministry of Health, we studied, with a multidisciplinary approach gathering together many specialists, two fields of QoL, sex-

uality and fatigue. These are often involved in antineoplastic treatments. Sexual dysfunction following surgery for pelvic tumors occurs in 25% - 100% of patients. Toxic neuropathies and "fatigue" follow many antineoplastic treatments (taxol, cisplatin, oxalipiatin, vincristine). Neurophysiological techniques have been employed in recent years to evaluate these complications. The aim of the study was therefore to evaluate the occurrence of sexual dysfunction and fatigue from both a clinical point of view and by means of neurophysiological tests in patients submitted to antineoplastic treatments. The association between lung tumors and neurological paraneoplastic syndromes is well known, but its exact prevalence is not well defined. A study has therefore begun to evaluate the prevalence of damage to the peripheral nervous system and neuromuscular junction in patients affected by lung carcinoma, before any chemotherapy, and the possible correlations between clinical, neurophysiological and serologic data. Sixty eight (68) patients were recruited (56 males, 12 females, age 42-80; 10 with microcitoma, 21 with squamous carcinoma, 20 with adenocarcinoma, 17 with large cells carcinoma). Peripheral neurotoxicity is a well recognized effect of cisplatin chemotherapy that can result in severe disability and represents a major dose-limiting factor. Several studies have recently investigated the role of vitamin E as neuroprotectant in the prevention of cisplatin-induced peripheral neurotoxicity and ototoxicity. An Italian randomized, placebo-controlled, double-blind multicentric study is ongoing to confirm the role of vitamin E supplementation in the prevention of neurotoxicity and ototoxicity induced by cisplatin. Patients involved in cisplatin chemotherapy were randomised to either vitamin E supplements (∂-tocopherol 400 mg/day) or to placebo.
Patients were evaluated by neurological and neurophysiological examination before and after treatment. Although the poor prognosis of malignant brain tumors has not been substantially modified in recent years by anticancer treatment, palliative care in neuro-oncology has received very little attention.



Since October 2000, we have followed a palliative home care program for patients affected by malignant brain tumor after hospital discharge, with the financial support of the Regional Health Service.
The aims of this assistance model are to meet patients' care needs during the evolution of the disease, to provide rehabilitation at home, to improve the patients' quality of life with palliative care, and to facilitate death at home.
Evaluation of Kinetics of Oxcarbazepine and Topiramate in Patients with Epilepsy Related to Brain Tumor Treated with Temozolomide. The aim is to verify, if possible, modifications of the kinetic of Oxcarbazepine and Topiramate which can cause infective treatment with chemotherapeutic agent (Temozolomide) in patients with the epilepsy related to brain tumors. Moreover, the aim is to verify if alteration in the plasmatic levels can worsen the tolerability to these antiepileptic drugs. ■

Neurosurgery

Director: **Emanuele Occhipinti, Alfredo Pompili** (2008)

STAFF
Carmine Maria Carapella
Fabrizio Caroli
Fabio Cattani
Enzo Morace
Piero Andrea Oppido
Laura Raus
Stefano Telera

Research collaborations with graduate and undergraduate students:
Marzia Piccoli Regione Lazio

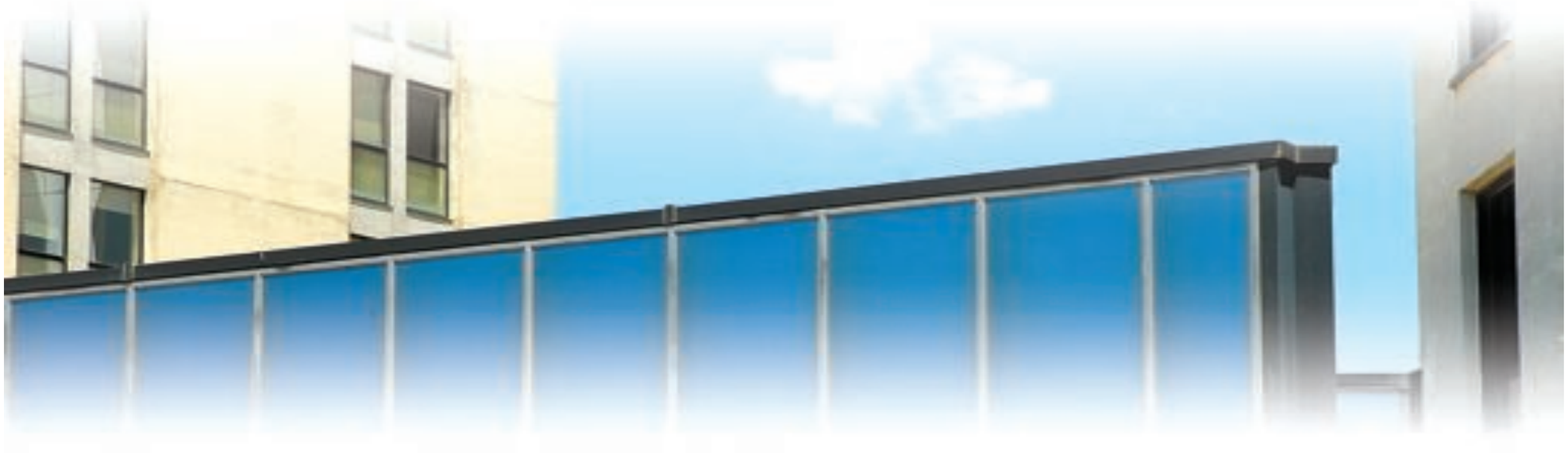
Scientific Activity

The research activity of the Division of Neurosurgery is focused on the study of new diagnostic and therapeuticapproaches in the integrated treatment of primitive and secondary tumors of the nervous system. Research is focused on new, innovative and safer surgical techniques and on clinical neuro-oncology. During 2007, cooperative studies with national and international institutions were started and are now being carried out in these fields.

In 2007 patients affected with glioma of different grading were treated, both those with newly diagnosed tumors, and recurrences with clinical/radiological progression. Patients have been followed by the different structures of the Neuro-Oncological Disease Management Team that weekly discusses relevant clinical cases, and proposes new scientific studies and projects. Furthermore the Center is closely connected with other regional structures; the activity of our DMT has favoured the accrual of patients in clinical studies. This has facilitated the collection of interesting data and its evaluation for presentations at national and international meetings, and for scientific papers.
New therapeutic protocols have been initiated after surgical procedures (biopsy and/or microsurgical removal), radiation treatment and chemotherapy with different therapeutic schedules, both as first line (mainly Temozolomide), and second (largely with Fotemustine). In addition, the increasing efficacy of new therapeutic strategies (microsurgical resection, with second surgical look and intratumoral antiblastic treatment, conformal radiotherapy, with eventual focal boost, adjuvant and/or concomitant chemotherapy) has allowed, in selected patients, second and, in some cases, third line chemotherapy.

Currently the department is involved in the correlation of data regarding tumor bio-molecular characteristics using patient clinical data. In this area a definition of predictive markers of the potential efficacy of different therapeutic approaches is of significant interest; better clinical, radiological, histological, immuno-histochemical and bio-molecular knowledge could contribute to the definition of more selective and efficient diagnostic-therapeutic strategies, facilitating a more defined stratification of patients accrued in new clinical trials.

Different authors (including those in this department) describe a series of prognostic markers, as expression of p53, ampli-



fication and over expression of EGFR, 10q LOH in astrocytic gliomas, and 1p and 19q LOH in oligodendrogliomas, methylation of methyltransferase, determining chemo resistance to methylating and alkylating agents. This research is part of the Ministry of Health. A new research program, in cooperation with the Besta Neurological Institute, has been approved and financed by the Alliance against Cancer, on the "Evaluation of immunotherapy with EGFR inhibitors in the treatment of glioblastoma multiforme". The definition of the study is in progress.

New protocols for the combined treatment of malignant gliomas have been activated, including new modalities of drug delivery, which have been based largely on the direct infusion of new drugs and toxins into the tumoral and peritumoral region, utilising techniques of convection enhanced delivery, that allow the limits linked to the presence of BBB to be overcome.

1 A phase I-II study of prolonged gemcitabine infusion as radiosensitizer for glioblastoma multiforme (approved by EC and activated; in 2005 phase I was concluded, determining the MTD, and the results published in J Neuro-Oncol; on this basis phase II has been activated and the accrual is presently in progress: 22 patients)
2 A phase III multicenter study of intratumoral/interstitial therapy with transmid compared to best standard care in patients with progressive and/or recurrent, non-resectable glioblastoma multiforme (approved by EC and activated; one patient has been accrued and submitted to two courses of this treatment; the accrual has been concluded and results are pending).
3 Unconventional temozolomide chemotherapy, with increased dose-intensity, in the treatment of anaplastic gliomas and progressive or recurrent low grade gliomas (approved and activated).
4 Primary chemotherapy with temozolomide vs. radiotherapy in patients with low grade gliomas after stratification for genetic 1p loss: a phase III study. (EORTC study, approved by EC and activated).
5 The regional project "Continuative home care for brain tumor patients" begun in 2000. At present this Grant is sponsored by the Lazio Region until 2009 for 250.000 euro per year. It involves cooperation with the neurological staff of the Institute. More than 400 patients have been enrolled during the period. An average of 45 patients per month have been assisted.
6 Evaluation of the impact of neurosurgical treatment of glial tumors on cognitive functions, quality of life, depression and pain. (Grant approved and financed by the Scientific Director and presently activated).

A second relevant research activity is involved in the evaluation of new surgical strategies in the treatment of spinal and vertebral tumors; of pituitary adenomas and tumors of the sellar region; in the treatment of infratentorial secondary tumors; centered on defining the role of new technologies and mini invasive approaches.

- Brain metastases: conventional surgery, unconventional approaches; new intra-operative technologies (129 patients enrolled 2004-2007).
- mini-invasive supraorbital approach for sellar and suprasellar region tumors (18 patients enrolled 2004-2007).
- unilateral approach for the resection of intradural spinal tumors (47 patients patients enrolled 2004-2007).
- transphenoidal removal of pituitary adenomas with microsurgical and endoscopy - assisted techniques (92 patients enrolled 2004-2007).
- One stage vertebrectomy with reconstruction and stabilization for metastatic spinal tumors (23 patients enrolled 2004-2007).
- One stage removal and reconstruction for primary spinal tumors (10 patients enrolled 2004-2007).
- The use of new technologies in the removal of spinal tumors (41 patients enrolled 2004-2007).

Our clinical research activity is divided into four fields: advanced studies on integrated treatment of brain gliomas; microneurosurgical and endoscopy-assisted pituitary surgery; new surgical approaches with mini invasive techniques in the resection of brain and spinal tumors; surgical procedures of removal and reconstruction in the treatment of primary and secondary vertebral tumors.

Dermatology Oncology

Director: **Pasquale Frascione**

STAFF
Marcello Casale
Paolo Piemonte

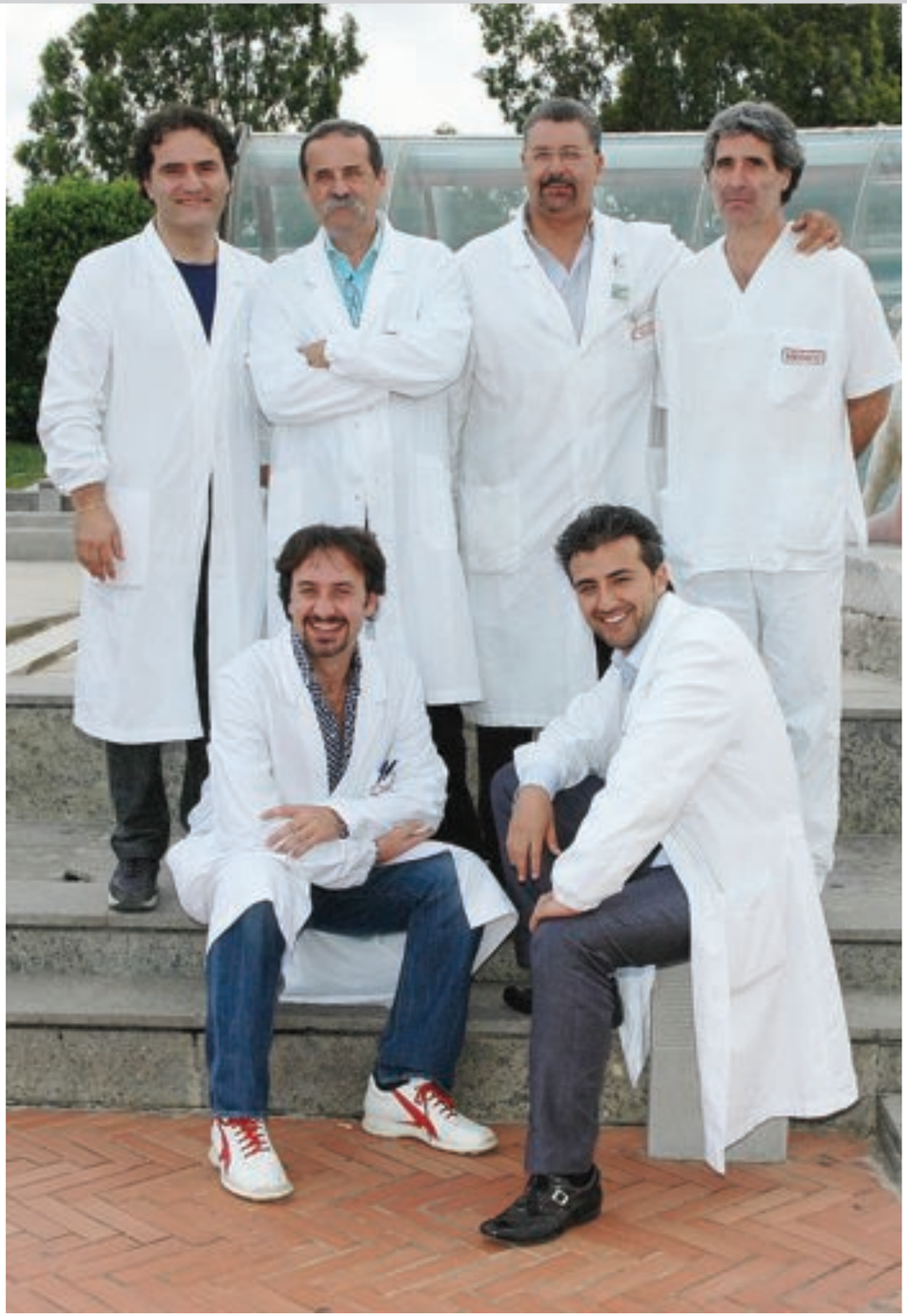
Scientific Activity

The clinical activity performed by the Division of Dermatology Oncology guarantees the diagnosis of skin cancers by clinical evidence and digital technology and their treatment by surgery or systemic and topic therapies.

Professor Frascione and Dr. Piemonte are authors and co-authors of numerous publications and conferences.

Orthopaedics

Director: **Roberto Biagini**



ONCOLOGICAL ORTHOPAEDIC STAFF:
Leonardo Favale
Nicola Salducca

MUSCULAR-SKELETAL TISSUE BANK STAFF:
Fabio Erba Director
Umberto Prencipe
Giuseppe Teori
Carmine Zoccali

Scientific Activity

The Orthopaedics Unit is involved in the diagnosis and therapy of primitive and metastatic muscular-skeletal tumors. It also manages septic and mechanical complication onset in oncological patients.

The Unit has been operative since February 2005; during 2007 they were 139 patients and the Unit performed 129 surgical operations for muscular-skeletal tumors.

RESEARCH ACTIVITY

The Unit joined with Osteosarcoma ISG Protocol and with the Soft Tissue Regina Elena Institute Protocol.

MUSCULAR-SKELETAL TISSUE BANK

During 2007 the bank was active.
It commenced training for personnel involved as well as a quality assessment.

Plastic and Reconstructive Surgery

Director: **Roy De Vita**

STAFF
Maurizio Costantini
Stefano Feliciano
Marcello Pozzi
Antonio Varanese

Scientific Activity

BREAST RECONSTRUCTIVE SURGERY

■ Immediate and delayed breast reconstructive surgery is performed in the Department using highly specialized techniques. Microsurgery is a routine method in our Plastic Surgery Department, especially for delayed reconstruction such as post-QUART and radical mastectomy after radiotherapy.
Since 2002 there have been almost 500 surgical treatments for breast reconstruction performed every year. As first choice, both with immediate and delayed reconstruction, we prefer using tissue expanders. Even though it is a two stage procedure, there is very limited physical and psychological stress for the patient. For cases where tissue expanders cannot be used, we are able to choose from a very large spectrum of surgical methods, from myocutaneous pedicle flaps such as the Latissimus dorsii flap or TRAM flap, to microsurgical free flaps such as TRAM, DIEP or SIEA. Clinical research in breast cancer surgery points towards a more conservative operation versus an aggressive one. Starting from this concept, in cooperation with the General Surgery Department and only in selected cases, we have begun to perform a very conservative form of surgery for breast cancer in which we preserve not only the skin, but also the nipple-areola complex. This technique is called Nipple Sparing Mastectomy.

STEM CELL IN RECONSTRUCTIVE SURGERY

Adult adipose tissue is currently the most important source of multipotent cells in the human body. As extraction needs a modified liposuction, the plastic surgeon is the ideal facilitator of this. The effect we would like to achieve in reconstructive surgery is not only 'filling', but also the regenerating capacity of these cells over the damaged tissues. Our preferred target is the radiated tissue, looking for the microvascular pattern resuscitation. After this treatment, tissues are suitable for reconstructive surgery with implants.

HEAD AND NECK RECONSTRUCTION

Reconstruction of head and neck wounds due to surgical resection for cancer or head and neck trauma creates numerous challenges for the microsurgeon.
Advances in microsurgical transplantation have improved reconstructive efforts considerably. The complex anatomy of the head and neck area creates numerous functional mechanisms involved in:

- Speech
- Swallowing
- Sensation
- Oral continence
- Airway protection
- Facial expression

The goal of reconstruction is to preserve and protect all these mechanisms as much as possible while obtaining reasonable restoration of function and morphological reconstruction.

SKIN CANCER

Skin cancers are known to be the most frequent of human cancers and can be very aggressive. BCC excisions are usually considered as minor surgery and performed in the out-patient department. Unfortunately large underestimated tumors that required major surgery can still be observed. In such cases local flaps are usually employed and when not sufficient, the surgeon uses free micro-surgical flaps. With malignant melanoma, the lesion excision is made according to international protocols and when the Breslow classification of the lesion is equal or over 1 mm, a control is made of the sentry node closest to the skin excision. An important collaboration is ongoing with the Departments of Dermatology, Epidemiology and Radiotherapy for best treatment and follow-up of patients. ■

Psychology

Director: **Patrizia Pugliese**

STAFF

Alessandro Bonucci
Maria Condoleo
Roberta Di Filippo
Chiara Falcicchio
Luana Fotia
Gabriella Maggi
Marzia Malagola
Rossella Milana
Maria Perrone
Claudia Seralessandri
Paola Torti
Santina Travo
Dora Vigliotti

Scientific Activity

The primary aim of oncology is to guarantee care for both patients and their families. In order to realize this care a multi-dimensional team, working in the clinical and research fields, with the objectives of prevention, cure and rehabilitation, is necessary.

All the Division's activities followed a multidisciplinary approach and concentrated on the integration of clinical, research and formative aspects. In this approach the psychologist is integrated within a core multi-disciplinary team. The regular presence of a psychologist within the colorectal, breast, ovarian, prostate, brain or ematologic disease management team (DMT) is important for the majority of the psychological research activities.

Following a multidisciplinary approach, research activities were based primarily on the Quality of Life (QoL) longitudinal studies, early diagnosis and prevention studies as well as studies focused on rehabilitation. Another field of interest included information and communication studies aimed at finding the best communication modality, based on patient information needs and their defence mechanisms.

All these research activities are aimed at detecting the most troubled psychosocial issues in every disease phase and in different neoplasms to carry out new clinical activities targeted to improve these issues.
6841 clinical and research initiatives (psychotherapy, clinical interview and tests) were carried out.
The clinical activity of our Service provides different psychological strategies (counselling, integrated physician-psychologist intervention, individual, couple, family and group psychotherapy).

Pulmonary Physiopathology

Director: **Vincenzo Cilenti**

STAFF
Marina Bonaccorsi
Eliuccia Mastropasqua
Maria Papale
Giorgio Piperno
Carmelina Pronesti
Antonio Scappaticci

Scientific Activity

The mission of the Physiopathology Respiratory Unit is aimed at the prevention, diagnosis, cure and rehabilitation of pulmonary diseases and is achieved through its research activity. The Unit's activity includes: primary and secondary prevention in the field of pneumology through education (above all concerning addiction to smoking), the participation and programming of screening, clinical-functional diagnostics, respiratory therapy and rehabilitation for both in-patients and out-patients, participation in research programs and internal and external work groups of the Institute and the organization of conferences, congresses and participation in courses. During 2007 10672 (9531 during 2006) services (i.e visits, consultations, instrumental tests) were conducted on patients coming from different units of the Institute. Cooperation with the Department of Surgery, above all with Thoracic and Abdominal surgery, for a more accurate identification of surgical risks, has been particularly productive.

There have been a total of 8028 (7940 during 2006) services (visits, instrumental tests) conducted for out-patients who came either for treatment of pulmonary oncology and others diseases or for the treatment of smoking or else in need of respiratory rehabilitation.

Respiratory rehabilitation is offered to patients who have to undergo major thoracic or abdominal surgery or have already undergone pulmonary resection for cancer. The objective is both to improve quality of life and increase knowledge of an area of respiratory rehabilitation which requires further study. In 2007, 419 patients were treated, equivalent to 1701 services.

Approximately 180 individuals sought the help of the Center for the treatment of smoking (referral Center for the Observation of Smoke, Alcohol and Addiction of the I.S.S.). Cooperation with the Institute's Unit of Psychology continues with the aim of evaluating the validity of integrated pharmacologic treatment (substituted with nicotine or bupropione or with vareneclina from August 2007) and behavioural treatment of addiction to smoking. The results of this activity were presented verbally in courses and conferences.

With regard to preventative work, the focus was aimed at educational and didactic intervention for young people in schools.
The "Smoke- free Hospital" continues

with didactic initiatives and monitoring activities.
The study "St.O.Ria. Observational Study regarding the new acute stage of COPD." is also continuing.
The Respiratory Physiopathology Unit also participates in the I-ELCAP Project headed by the Unit of Radiology and Imaging Diagnostic of the Institute which intends to validate the methods of screening for pulmonary cancer in asymptomatic smokers by low-dose spiral CT.

Cooperation with the Unit of Radiotherapy continues:

- "Study of phase I/II regarding the use of radiotherapy hypofractional in patients who have small scale pulmonary cancer"
- Observational study "The role of transforming growth factor plasmatico as factor predictor of pulmonary toxicity in patients affected by pulmonary cancer and radiotherapeutic treatment"
- "Study of a radiotherapy hypofractional plane in patients undergoing surgical treatment, conservative for mammary carcinoma".

The Unit also takes part in the "USA-Italy seroproteomic project for early diagnosis of lung cancer". Within this project the Unit will enrol smokers and nonsmokers with negative spiral CTscan. A 10ml sample of blood will be collected from each participant which will be examined to identify the proteomic profile and to discover novel molecular targets and potential cell-specific biomarkers for increasing the rates of early stage lung cancer detection.

The Unit also takes part in the biennial project: "Primary or Secondary prevention in high risk subjects for development of lung cancer" cooperating with Italian League Against Cancer and preliminary results were presented at the XXXIX Congress National AIPO (2007) "Level monitoring of antibodies p53 on serum of COPD persons with lung cancer risk as early marker of malignant tumor". ■

Radiology and Diagnostic Imaging

Director: **Marcello Crecco**

STAFF

Stefano Canitano
Livio Carpanese
Mauro Caterino
Francesca Romana Ferranti
Salvatore Giunta
Marcello Greco
Ramy Kayal
Annelisa Marsella
Giuseppe Pizzi
Alfonso Russo
Elena Saracca
Mirella Teramo
Giulio Vallati
Antonello Vidiri

Scientific Activity

The activity of the Division of Radiology is organized according to areas concerning organ pathology as well as multidisciplinary areas such as pre-hospitalization or interventional radiology.
During 2007 approximately 57.000 radiological examinations were carried out.

ABDOMINAL IMAGING

A study on the use of superparamagnetic iron-oxide in the evaluation of liver colorectal metastases has been closed. The purpose of the study was to compare the results obtained with iron-oxide MR and plain-MR with that of spiral CT in order to select patients for liver resection; intraoperative-echothomography being the gold standard.
Another study on the evaluation of primitive and secondary liver lesions with Mn-DPDP contrast medium was carried out. The purpose of this study was to recognize small dimension lesions (5 mm) and also a possible characterization. Regarding liver metastases, a study comparing bidimensional versus 3D measurements with volumetric spiraI CT was performed. Our preliminary results show the superiority of 3D measurements (in particular in lesions with irregular edges). Since November 2003, our Radiology Service has been taking part in an Italian multicenter study promoted by SIRM (the Italian Society of Medical Radiology), aimed at identifying hepatic metastasis through echothomographic contrast medium, involving 18 groups, selected from 50 university and hospital centers.
Specific studies on hepatic metastases in colorectal disease are being carried out on patients treated with different chemotherapeutic agents, looking at the drugs used and the method of administration such as bolus or chronomodulated.
In collaboration with the Department of Abdominal Surgery a study is underway to evaluate hepatic vascular anatomy with CT angiography in liver transplant patients when compared with digital angiography.
The Radiology Service is taking part in the following research:

1) Impact of biological profile, chemoradiation and surgery of rectal cancer on downstaging and quality of life.
2) Clinical impact of an innovative strategy of adjuvant therapy in high risk Aster Coller B2 colorectal cancer patients selected by biological profiles.
3) The role of new biomolecular markers of liver cancer on cirrhosis in relation to the efficiency of different therapies.
4) Neuroendocrine tumors of the digestive system: clinical-pathological coordinator. Recently a cooperation was initiated with Sapienza University concerning virtual colonoscopy with spiral CT.

ANGIOGRAPHY IMAGING AND INTERVENTIONAL RADIOLOGY

In 2007, in accordance with current trends and technological and material developments, 9 patients with non-surgical lung and renal tumors were enrolled in a clinical study that consisted of RF percutaneous ablation treatment. RF treatment was also used in 8 selected patients with osteoid osteoma in accordance with orthopaedic surgical indications, a more invasive surgical treatment which is therefore avoided in very young patients.

During the last year, in cooperation with the Urology Department, a new therapeutic approach to kidney neoplasms of small dimensions, detected in the periferic portion, has been achieved. Videolaparoscopic treatment of these neoplasms, which were previously embolized through trans-arterial supereselective catheterism, was performed in a selected group of patients. Angiographic trans-arterial embolization of small kidney hypervascularized tumors allows the ischemic treatment of neoplasia, associated with a devascularization of the surrounding parenchyma. The subsequent decrease in haemorrhagic complications and reduced operative time, favours this type of intervention in single kidney patients with conservative postoperative clear renal function. Thanks to this surgical intervention a 100% technical success was achieved in a total of 92 patients with an average age of 69 years.

In September 2007 a prospective clinical trial to evaluate the role of SIRT (Selective Internal Radiation Therapy) hepatic trans-arterial 90Y administration in patients with un-resectable colorectal liver metastases (progressing as only relapsing site after two lines of neo-adjuvant chemotherapy) was concluded. This was performed in collaboration with four other important Italian Cancer Centers, the Department of Nuclear Medicine of IRE and the Colorectal Cancer Disease Management Team of IRE, under the supervision of SITILO (Italian Society of Local Regional Liver Therapies). 18 patients were treated while the total sample size was 48 patients. Statistical analysis included the valuation of clinical response time to liver progression, toxicity, median survival and quality of life of the patients.

The results obtained enabled the multicentric group to be recognized at an important international conference with a consistent number of abstracts accepted and presented. This professional collaboration with Nuclear Medicine also enabled the possibility of opening up a new clinical research study field regarding the application of such a new therapy in 26 patients with unresectable hepatocellular carcinoma and in 4 patients with neuro endocrine tumors. This ongoing study is showing a good control of the disease in 75% of patients and is now playing an important role as a further application in the clinical practice of a new therapy of focal liver lesions.

Another new evaluative study applied to clinical practice concerns the role of Drug Eluting Beads (DC beads) in the treatment of focal liver lesions. DC Beads comprise a range of biocompatible hydrogel microspheres, non resorbable, precisely calibrated and capable of loading chemotherapeutic agents. These kinds of particles embolize vessels supplying malignant hypervascularized tumors and deliver locally, in the liver, a controlled sustained dose of cht exclusively in a superselective mode. This new therapy is routinely used both for primary and for secondary liver lesion. In 2007 this therapy was performed in 17 patients with HCC and in 2 with colorectal liver metastases. Disease control was seen in 85% of patients, with a strong lowering of side effects and a good quality of life.

SENOLOGIC IMAGING AND BREAST MINI INVASIVE DIAGNOSTICS

In the department of Diagnostic and Mini-invasive Senology mammograms and ultrasound exams are performed with oncologic patients in follow-up and with patients who need an integrated diagnosis (mammograms, ultrasound exams and FNA) to evaluate suspicious or doubtful cases previously selected in other hospitals.

Since November 2007 mammograms have been performed with a direct digital technique that allows lower X-rays doses and reduces examination time and also allowing their archiving on optical disks.

In cases of non palpable breast nodules US guided biopsies are performed. We use semi-automatic needles (caliber 13G) or Mammotome system (11G). US guided biopsies reduce surgical biopsies and in positive cases identify the recectorial state.

For opacity, identification of clusters of microcalcifications or parenchymal distortions, identified on X-ray mammography is proceeded by stereotactic guided biopsies with Mammotome system using 11G and 8G needles. 8G needles are preferred since a greater quantity of material is able to be obtained with less samples. Stereotactic Mammotome allows the diagnosis of many unexpected in situ ductal carcinomas.

Patients with a non palpable nodule undergo a surgical biopsy. With US or X-rays we perform the mammogram of the surgical sample.

We have a screening project for the identification and prevention of breast tumors in subjects at high genetic risk including mammograms, breast US and pelvic US.

NEURORADIOLOGY HEAD AND NECK IMAGING

In collaboration with the Department of Medical Physics, Neurosurgery, Radiotherapy Neurology Clinical Oncology A and Histology, two studies are underway regarding the role of CT-Perfusion in the evaluation of malignant glial tumors and metastases. A quantitative analysis of the dynamic contrast between enhanced CT imaging and the ROC analysis of the perfusion parameters was applied. The technique was utilized to evaluate the microvascular characteristics of brain tumors with the aim of contributing to the non-invasive assessment of tumor malignancy grading, correlating the CBV (Cerebral Blood Volume) to the grade of tumors. CT perfusion was utilized to evaluate the effects of monitoring treatment, particularly radiation necrosis versus relapse.

A study is underway in collaboration with the Department of Neurosurgery, Radiotherapy, Neurology and Clinical Oncology A, evaluating MR in patients, affected

by GBM, treated with concurrent chemo-radiotherapy. In the present phase I study we investigated the association of gemcitabine with radiotherapy in the first line treatment; phase II of the study is currently underway.
A study in collaboration with the Department of Radiotherapy regarding the fusion of MR and CT stereotactic imaging in glial brain tumors is in progress, with the aim of obtaining more precise and limited irradiation fields. To this aim, 100 patients have been enrolled in the study. Another study of the fusion of MR and CT stereotactic imaging is underway to evaluate nasopharynx tumors.
A study in collaboration with the Department of Maxillo-Facial Surgery and Histology is underway regarding the comparative evaluation of the clinical, MR and pathological data regarding T-Stage tumors of the oral cavity and base of the tongue. 60 patients have been enrolled in the study. T-stage accuracy of both clinical examination and MR was found to be respectively 62% and 82%. The assessment of the mandibular status is crucial in the treatment planning of oral cavity carcinoma. Another study regarding the correlation between clinical, CT, MR and pathological data to evaluate the involvement of the mandible is underway and 33 patients have been enrolled.
Another study, in collaboration with the Maxillo-Facial Surgery, Histology and Nuclear Medicine Departments of the Institute is underway, regarding the comparative evaluation of the morphologic (US and MRI) and metabolic (PET) study of the lymph nodes in patients affected by head and neck tumors undergoing chemo-radiotherapy; the results will be correlated with phatological data in patients undergoing planned neck dissection; 27 patients have been enrolled in this study.

UROLOGIC AND GYNAECOLOGIC IMAGING

The interdisciplinary prostate work group coordinated by Prof. Arcangeli continued to optimize radiotherapy for prostate carcinoma with conformation techniques. Diagnostic radiology in the detection of nodules and grading use a multicore method with transrectal echothomography, which allows for a large number of prostatic biopsy samples (up to 20) with greater accuracy.
With CDUS we evaluate the vascularization of the prostate before and after radiotherapy, to study its modifications in order to predict relapse.
A trial regarding the study of the T-stage of bladder tumors with spiral CT with air instillation was concluded. Another study for the evaluation of cervical tumors before and after chemo-radiation therapy with MR is ongoing.
A new study has been started in patients affected by endometrial cancer. These patients undergo careful RM and TVUS study in order to perform more conservative surgery. We are monitoring active protocols to study different therapies in patients affected by advanced renal cell carcinoma.

THORACIC IMAGING

To date the study "Low Dose TC Spiral in the early diagnosis of Lung Cancer in subjects at risk" has enrolled 1450 subjects; 1015 subjects have undergone first annual repeat screening and 363 have undergone second annual repeat screening.
31 NSCLC and 1 SCLC were detected, 25 of these at the baseline and 7 at annual repeat screening, 24 of the cancers detected were at stage I.
Since 2002, the Regina Elena Cancer Institute has participated in I-ELCAP (The International Early Lung Cancer Action Program) together with another 35 Institutes.
In a study performed by the International Early Lung Cancer Action Program on 31,567 people at risk of lung cancer, the screening resulted in a diagnosis of lung cancer in 484 participants. Of these, 412 (85%) had clinical stage I lung cancer. The estimated 10 year survival rate was 88% in this subgroup. Among the 302 participants with clinical stage I cancer who underwent surgical resection within 1 month after diagnosis, the survival rate was 92%. The 8 participants with clinical stage I cancer who did not receive treatment died within 5 years after diagnosis. ■

Radiotherapy

Director: **Giorgio Arcangeli**

STAFF

Fabrizio Ambesi Impiombato
Stefano Arcangeli
Laura Carpino
Rosaria Del Vecchio
Giuseppe Giovinazzo
Sara Gomellini
Laura Marucci
Adriana Micheli
Maria Alessandra Mirri
Maria Grazia Petrongari
Paola Pinnarò
Valentina Pinzi
Rita Rambone
Biancamaria Saracino

Physicians in training

Alessandro Di Marzo
Giampiero Parca

Scientific Activity

The recent installation of high technology equipment in our division allowed us to start several clinical studies to implement and validate new radiation therapy techniques in the treatment of various cancer sites.

IORT STUDIES

This technique was implemented thanks to a dedicated, movable linear accelerator installed in the operating room.

Prostate cancer: we concluded a phase II study that is the object of a paper in press on IJROBF.

PROSTATE CANCER

An institutional, multidisciplinary project with the participation of the Radiation Oncology, Urology, Radiology, Pathology and Gastroenterology Divisions, has already been conducted in our Institute with the aim of establishing standard evaluation methods and criteria for all the future planned studies on prostate cancer.

Phase II randomized trial of hypofractionation versus standard fractionation radiotherapy in unfavourable risk prostate cancer:
The study started in February 2003 and finished in September 2007. It has since moved to a multicenter, randomized phase III study with the participation of the Free University of Brussels and Ghent. The objective is the enrolment of 320 patients.

Pilot trial on feasibility of Dose Escalation with Intensity Modulated Radiotherapy (IMRT) for patients with intermediate risk prostate cancer:
To evaluate the impact on rectal and genitourinary system toxicities and on local control using the Intensity Modulated Radiotherapy technique (IMRT). The study started in April 2005, requiring an enrolment of sixty patients. Thirty five patients have been selected so far.

BRAIN TUMORS

Phase II trial of concomitant radiotherapy and gemcitabine in the treatment of malignant gliomas:
This study was started in 2004 and is still ongoing.

LUNG TUMORS

Multicentric Phase II trial of concomitant radiotherapy and chemotherapy with gemcitabine after induction cisplatinum based chemotherapy in the management of inoperable stage IIIA-B lung tumors
The aim of this study is to evaluate the toxicity and efficacy in local control and

survival of an integrated regimen consisting of 2 courses of chemotherapy with Gemcitabine (1000 mg/mq day 1 and 8) and CDDP (75 mg/mq day 8) followed by concomitant Radiotherapy (64-66 Gy/2 Gy Fr) and CT (Gemcitabine 300 mg/mq 1day/weekly).
Total number of patients: **2**

Observational study on the role of transforming growth factor beta (tgf-beta) as a predicting factor of lung radiation-induced toxicity in curative thoracic radiotherapy:
The purpose of the study is to assess the utility of transforming growth factor-beta-1 (TGF-beta1) together with dosimetric (V20/V30) and tumor parameters as a predictor for radiation pneumonitis (RP) in the course of curative radiotherapy (≥ 54 Gy) for lung tumors. Baseline, at the end of RT and quarterly for twelve months serial blood samples for TGF beta concomitantly with PFR and CT evaluations were performed.
Total number of patients: **10**

Phase II trial of neoadjuvant alimta plus cisplatin followed by surgery and radiation in the treatment of pleural mesothelioma:
The aim of this Phase II, multi-center study is to determine the effectiveness and feasibility of a multimodality approach in the treatment of malignant pleural stage I to III mesothelioma. Patients undergo ALIMTA plus cisplatinum based chemotherapy followed by extrapleural pneumonectomy, and post-operative radiation therapy (50,4 Gy /28 fractions).
Pathological complete response rates and clinical response rates are measured by radiological assessment. Disease free survival, median survival and overall toxicity are evaluated.
Total number of patients: **8**

Phase I/II trial of hypofractionated conformal radiotherapy (hcrt) in the management of small primary or metastatic lung tumors:
The purpose of this study is to evaluate acute/late toxicity, LC and DFS and OS of a hypofractionated radiotherapy course (40 Gy/5Fr/2.5 w) in patients affected by small primary or metastatic lung tumors.
Total number of patients: **41** (43 lesions)

Xerostomia evaluation in patients treated with Intensity Modulated Radiation Therapy (IMRT) for nasopharyngeal cancer:
The study has been closed and the relative manuscript submitted for publication.

Feasibility study on the use of intra-operative radiation therapy (IORT) as an early boost in locally advanced head and neck cancer:
The study is now closed and the relative manuscript has been submitted for publication.

Two parallel randomized phase II studies on the use of 3D radiotherapy and Amifostine or Intensity Modulated Radiation Therapy (IMRT) to reduce xerostomia in patients treated with radical or adjuvant radiotherapy for head and neck cancer:
The study is ongoing.

GEM-OXAÆGEM/RT

Induction Chemotherapy with Gemcitabine-Oxaliplatin (GEM-OXA) followed by concomitant weekly Gemcitabine plus Radiotherapy (GEM/RT) in Locally Advanced, Unresectable Pancreatic Cancer: A Phase II Study
This study is ongoing.

Thoracic Surgery

Director: **Fancesco Facciolo**

STAFF
Gabriele Alessandrini
Sandro Carlini
Virna Cerasoli
Felicita Corzani
Massimo Filippetti
Enrico Melis

Scientific Activity

■ The division participated in the International Early Lung Cancer Action Program (I-ELCAP). This study was performed to study and develop low-dose CT screening for lung cancer. The institutions participating in this study have been using a common protocol for the screening (though different entry criteria) so that the resulting data can be pooled to provide up-to-date information for protocol updates, and on the resulting diagnostic distribution (primarily in terms of stage and size) of the diagnosed cases of lung cancer. After a sufficiently lengthy follow-up of the diagnosed cases it will be possible to assess the curability of the screen-diagnosed lung cancers.

SERUM-PROTEOMIC PROJECT (ITALY-USA PROJECT)

The behaviour and outcome of lung cancer is highly variable. The molecular basis of this variability is unknown; neither standard histopathology nor currently available molecular markers can predict these characteristics. The identification of novel biomarkers to differentiate tumors from normal cells and predictors of tumors' behaviour, such as pathological stage, response to chemotherapy and site of relapse, is very important in clinical practice. To date, none of the hundreds of single markers evaluated have provided a significant clinical utility, but by surveying thousands of genes with the use of microarrays or proteomic technologies, it is now possible to read the molecular signature of an individual tumor.

Proteomics-based approaches allow the examination of expressed proteins of a tissue or cell type, complementing the genome initiatives, and are increasingly being used to address biomedical questions. Increased knowledge of the close connection between apoptosis and cancer has lead to a vast number of researches around apoptotic induction with chemotherapeutic agents and small molecule inhibitors; the chemotherapeutic agent paclitaxel (Taxol) activates mitogen-activated protein kinase (MEK), extracellular signal-regulated kinase, and combined, with MEK inhibition, synergistically enhances apoptosis.

The most effective treatment for lung cancer remains surgical resection of early stage disease; however, sporadic lung cancer is rarely diagnosed in its earliest stages. The potential of screening technologies to increase rates of detecting early stage lung cancer, and thus the expectation of more treatable cases, has driven considerable research and development of screening technologies.

Randomized controlled trials (RCTs) of CXR and sputum cytology have failed to demonstrate a mortality benefit for

either technique, and we do not recommended screening with serial CXR or sputum cytology for asymptomatic individuals or individuals without a history of cancer.
LDCT scanning is a promising technology due to its sensitivity and ability to assess growth of nodules, and ongoing studies may provide additional information about the costs and benefits of screening with this technology.

A proteomic approach, using two-dimensional gels coupled with mass spectrometry to identify altered proteins in primary lung tumors with matched adjacent normal tissue, could be the right way to identify the proteomic profile, allowing us to discover novel molecular targets and potential cancer cell-specific biomarkers.

An Italy-USA serum-proteomic project for lung cancer enrols smokers and non smokers with NSCLC, divided into four groups:

P1: smokers and non smokers with NSCLC histologically proved (100 patients);
P2: smokers and non smokers with negative spiral CT scan (300 patients);
P3: people with spiral CT scan suspicion for lung cancer followed by negative histologic findings (20 patients);
P4: people with spiral CT scan suspicion for lung cancer and positive histologic findings (100 patients);

Phase II Trial of Neoadjuvant ALIMTA plus Cisplatin followed by Surgery and Radiation in the Treatment of Pleural Mesothelioma:
The aim of this study is to assess whether preoperative CT followed by surgery and postoperative radiotherapy improves survival for stage I-III (T1-3 N0-2) malignant pleural mesothelioma. This phase II study includes an induction chemotherapy with pemetrexed + cisplatin for 3 cycles (q 21 days) followed by extrapleural pneumonectomy and postoperative hemithoracic radiotherapy (54 Gy). Until now we have enrolled 8 patients.

An open randomised, prospective, multi center, parallel-group trial to compare efficacy and safety of TachoSil versus standard surgical treatment in patients undergoing pulmonary lobectomy for lung malignancy and requiring treatment for air leakage.
Tachosil is a ready-to-use absorbable haemostatic developed for intra-operative local haemostasis and tissue sealing. The product was developed under the research code name TachoComb S, and it obtained positive feedback from the scientific committee (CHMP) of the European Agency for the Evaluation of Medical Products (EMEA), leading to its approval by the EU Commission. Tachosil is a surgical patch, which consists of an equine collagen sponge coated with solid components of fibrin glue: human fibrinogen and human trombin. This fixed combination is applied directly to the wound surface. Upon contact with blood, body fluids or physiological saline, the mechanism of this system mimics the final stage of the coagulation cascade, in which thrombin converts fibrinogen into fibrin. The intention of the present clinical development program is to expand the therapeutic indication to include surgical tissue sealing.

At the end of the study we enrolled 41 patients, 37 randomized to tachoSil or standard surgical treatment and 4 screening failure.

A randomized phase III trial of preoperative versus postoperative chemotherapy with cisplatin and gemcitabine in stage Ib-IIIa non-small cell lung cancer (nsclc).

CLINICAL ACTIVITY

Our activity involves all the general thoracic surgery procedures with a particular interest in surgery for NSCLC, malignant pleural mesothelioma, extended chest wall resections and mediastinal tumors. In 2007, 782 patients were referred to our department, 465 males and 317 females; we performed 510 surgical interventions and we also performed 450 diagnostic endoscopic procedures. ■

DEPARTMENT OF ONCOLOGICAL SURGERY

Urology

Director: **Michele Gallucci**

STAFF

Ruggiero Cantiani
Giuseppe Cusumano
Piero De Carli
Salvatore Guaglianone
Luciano La Manna
Andrea Loreto
Giovanni Mainiero
Vincenzo Pompeo
Giuseppe Simone
Sarah Volpe

Scientific Activity

1. A STUDY ON THE EFFECT OF SETUP ERRORS AND ORGAN MOTION ON PROSTATE CANCER TREATMENT WITH IMRT

To assess the influence of setup errors and organ motion in terms of the probability of tumor control and normal-tissue complications by tumor control probability and normal-tissue complication probability. Twelve patients were treated for prostate cancer with intensity-modulated radiation therapy. Two orthogonal portal images were taken daily. All patients underwent three computed tomography scans during the 8-week treatment time (i.e. baseline, intermediate and final). The original treatment plans were re-evaluated, taking into account setup errors and organ motion.
The mean shifts +/- standard deviation of the whole patient population in the lateral, anterior-posterior, and craniocaudal direction were 1.0 +/- 1.5 mm, 0.9 +/- 2.1 mm, and 1.9 +/- 2.1 mm, respectively. In most of the recalculated dose-volume histograms, the coverage of clinical target volume was granted despite organ motion, whereas the rectal wall histograms were often very different from the planned ones.
We studied the impact of prostate and rectum motion, as well as setup errors, on dose-volume histograms. The estimation of these effects may have implications for predictive indications when planning intensity-modulated radiation therapy treatments on prostate.

2. METABOLOMIC ANALYSIS APPLIED TO SURGICALLY TREATED PROSTATE CANCER

Metabolomics can be understood as the comprehensive and simultaneous systematic determination of metabolite levels in whole organisms and their changes over time in response to stresses like disease, toxic exposure, or dietary change. Low molecular weight metabolites present in the cells, tissues, organs and biological fluids may be viewed as the end products of gene expression or of protein activity (enzymes), thus defining the biochemical phenotype of an integral biological system, including human. Likewise the metabolome, assessed via biological fluids (such as urine and plasma) reflects a person's history, including age, gender, lifestyle, nutritional status, interactions with the environment, possible pathological conditions and the effect of drug therapies. Urine and plasma are easily

obtained, essentially non-invasively, and can therefore be readily used for disease diagnosis and in a clinical trial setting for monitoring drug therapy. One of the principal analytical techniques that is employed for metabolomic studies is based on NMR spectroscopy.
In this research, we propose applying metabolomic techniques on biological fluids (such as plasma and urine) to the study of disease diagnosis and therapy monitoring in prostate cancer, the most common cancer affecting older men in developed countries and a significant cause of death in elderly men. As therapeutic possibilities are limited and only one marker is commonly estimated (PSA, Prostate specific antigen), the application of metabolomics could provide new biomarkers and therapeutic targets.

Objective
The aim of the study is to analyse urine and plasma from patients at the moment of clinical diagnosis, before performing radical prostatectomy, and after 3 months and subsequently 6 months and 1 year from the surgical procedure applying 1H-NMR based metabolomics. In this way, it is possible to understand intersubject variability and to predict the metabolism and effect of a dosed substance. Collected data will be analyzed in order to find significant associations between metabolomics biomarkers and oncologic results (serum-PSA value, imaging, and standard exams of follow-up schedule).
Follow-up schedule includes serum PSA evaluation at 3-month intervals for the first two years, bone scan yearly and digital rectal examination at each visit (3-month intervals for the first year and at 6-month intervals thereafter). Strict follow-up, including PSA at 3-month intervals, bone scan and coline PET-CT scan, will be scheduled for patients who show increasing PSA levels.

Samples will be taken by nurses at the Department of Urology and then sent to the pharmacogenomic unit where they will be collected, prepared and frozen as described below.
Frozen samples will be sent for analysis to the Department of Physical Chemistry in Biotechnology where NMR spectroscopic analyses will be performed and the collected data analyzed.

Samples
Venous blood will be taken into heparin tubes to provide plasma for analysis and then frozen at –80°C. Each of the samples of the first void urine collections will be mixed with 0.25 ml of water and then will be frozen at –80°C until analysis.

1H NMR spectroscopy
1H NMR spectra will be acquired on a Bruker Avance DRX 500 spectrometer operating at 500.13MHz 1H frequency. Samples will be measured in 5-mm-od NMR tubes at 300 K.

Plasma
Plasma samples will be thawed and 300 ml aliquots will be added to 300ml of H2O:D2O(90%:10%). For each sample, a 1D spectrum will be acquired using a standard pulse sequence with suppression of the water peak (1DNOESY presat). In addition to the Standard 1D spectrum, Carr–Purcell–Meiboom–Gill (CPMG) spectra, will be acquired to attenuate broad signals arising from protein and lipoproteins.

Urine
Each of the samples will be allowed to thaw at room temperature, mixed with 0.375ml of 0.1M buffer, centifugated at 1000g and analyzed by 1HNMR spectroscopy after transferring a 600-ml aliquot to 5 mm NMR tubes. For each sample, a 1D spectrum will be acquired using a standard pulse sequence with suppression of the water peak (1DNOESY presat).

Data Reduction
Spectral binning. The collected spectra will be reduced into spectral bins with widths ranging from 0.01 to 0.03 ppm by using the ACD intelligent bucketing method that sets the bucket divisions at local minima (within the spectra) in a way to ensure that each resonance is in the same bin throughout all spectra.
The area under each bin will be integrated and normalized. The resulting data will be used as input variables for multivariate analysis. The reduced and normalized NMR spectral data will be imported into MATLAB (version 7.1, The Mathworks, Natick, MA), mean centred and unit variance scaled, and analysed using in-house routines.

Multivariate analysis
One common objective in metabolomic analysis is to classify a sample, based on the identification of inherent patterns of peaks in a dataset (usually a spectrum) and secondly to identify those spectral features responsible for the classification. This approach can also be used for reducing the dimensionality of complex datasets, to enable easy visualization of any clustering or similarity of the various samples. Alternatively, with what are known as 'supervised' methods, multiparametric datasets can be modelled so that the class of separate samples (a 'validation set') can be predicted based on a series of mathematical models derived from the original data or 'training set'.

Chemometrics methods
The multivariate pattern recognition method will be Principal Component Analysis (PCA) and Orthogonal-Projection to Latent Structure (O-PLS), an extended version of PLS.
PCA belongs to the class of unsupervised methods and is used both to investigate the dimensionality of the data and to project the original data into a least squared optimised orthogonal space. Its application to 1H-NMR spectra is a well established technique.
OPLS-Discriminant Analysis (OPLS-DA) has attracted increasing interest in the metabonomic field due to the possibility of obtaining models that allow a thorough interpretation of the results. This is achieved by separate modelling of predictive (variation of interest) and class-related (variation not related to the responses) variation in the X-matrix (the spectral descriptors, being metabolite concentrations or buckets) through the identification of Y-orthogonal variation (being Y matrix).
Because PCA is capable of identifying gross variability and is not necessarily capable of distinguishing 'among groups' and 'within groups' variability, when the within groups variability dominates the among groups variability, PLS (or OPLS and PLS-DA) will necessarily perform better.

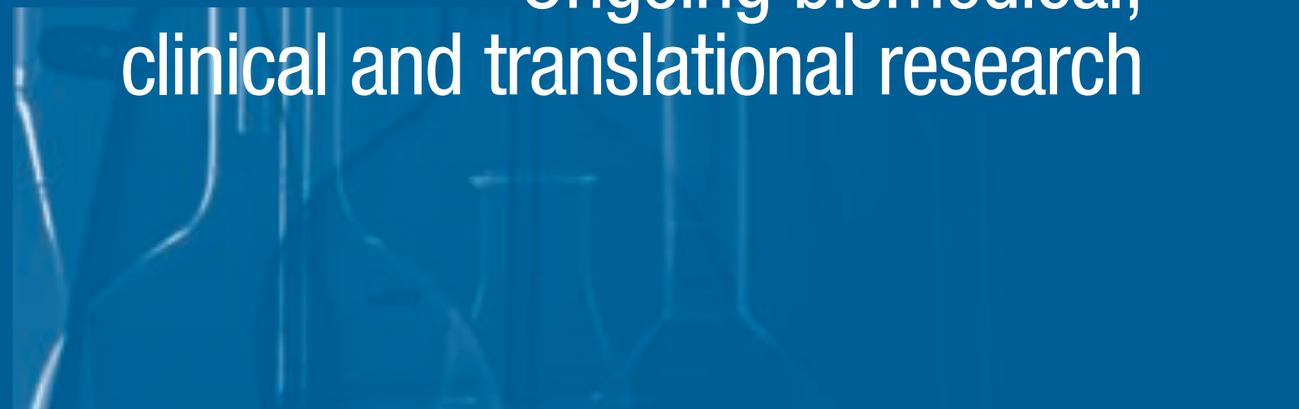
3. PROTEOMIC STUDY
Object: a scientific report on the ITA-USA project about sieroproteomic and prostate cancer 2007-2008.
After a start-up meeting in March 2007 in Turin, the prostate cancer study and sieroproteomic was begun at the Institute. Our Institute has had previous experience with sieroproteomic studies for other diseases and for this reason no particular problems, in terms of organization, were experienced.
We enrolled the majority of patients belonging to group 2 and 3 because most of these patients came to our hospital with a previous diagnosis of prostate cancer or for follow-up after surgery for prostate cancer. For the women's group, we asked patients' relatives to participate.
Methods: we informed patients about the project, collected all data required for the study and the head nurse organized the blood collection. Blood collected for the study was sent to the laboratory of pharmacokinetic. Storage of samples and code registration was done according to ITA-USA Protocol.

Patients data enrolled at our Institute:

GROUP	PATIENTS	SAMPLES
1	8	8
2	48	57
3	31	31
4	0	0
5	4	4
TOT	**91**	**100**

We are now checking data in order to send it to ISS as soon as possible.

Ongoing biomedical, clinical and translational research



TITLE

The role of the tumor stem cell marker CD133 in initiating and the progression of thyroid differentiated cancer

COORDINATOR

Maria Luisa Appetecchia
Endocrinology

SENIOR INVESTIGATORS

Iole Cordone
Clinical Pathology

Ferdinando Marandino
Surgical Pathology

Giuseppe Spriano
Head & Neck Surgery

JUNIOR INVESTIGATORS

Anna Lisa Bilotta
Endocrinology

Angela Maria Di Francesco
Clinical Pathology

Background

■ 1. Several studies have demonstrated that only a minority of cancer cells appear to be tumor-initiating and possesse the metastatic phenotype. These cells are self-renewing, can differentiate into any cell within the tumor population, and can migrate, establishing cancer metastasis. Given the similarities between tumor-initiating cells and normal stem cells, the tumor-initiating cells have been termed “cancer stem cells” (CSC). Biologically distinct populations of CSC have been identified in cancers within the hematological malignancies and in most solid tumors, including colon cancer. Although a universal marker for cancer stem cells has not been identified, several studies have suggested that the CSC fraction within a variety of human cancers, including colon and liver cancers, may be identified by the expression of the CD133 surface marker. The specific CD133 function remains unclear, but its expression has been normal as well as CSC in human tissues (12). Thus, CD133 has been used to isolate and characterize CSC from several human tumors. Indeed, it has been recently reported that expression of the cell surface marker CD133 identifies a subpopulation of cells within human colon cancers. These cells show specific features of CSC, being able to initiate tumor growth and to reproduce human colon cancers in immunodeficient mice.

The aim of our study was to verify the presence of CD133+ cells in a subset of human solid tumors (colon, liver metastasis from colon cancer and thyroid) and to acquire more information on the biology of these cells.

2. Fine needle aspiration cytology (FNAC) of the thyroid is a non-invasive, cost-effective screening procedure that is valuable for distinguishing neoplastic lesions from non-neoplastic nodules with a sensitivity and specificity for detecting neoplasia of >90%, and a percentage of false positives or negative results of 5%. Some follicular cells that exhibit some of the features of papillary carcinoma could be observed in a cytology slide of thyroiditis, leading to a diagnostic pitfall, as well as cellularity and overlapping cytological criteria in hyperplasia might lead to a false diagnosis.
Flow cytometry immunophenotyping (IF) is a mandatory technique for the diagnosis and monitoring of haematological malignancies in routine clinical practice. Moreover, this approach significantly increases diagnostic accuracy when applied to fine needle aspiration cell suspension from lymphoid tissues (Dey P, Thasneem A et al 2006). Nevertheless, to date no biological data has been published from the flow cytometry IF analysis of fresh single cell suspension from FNAC of thyroid nodules.

Methods

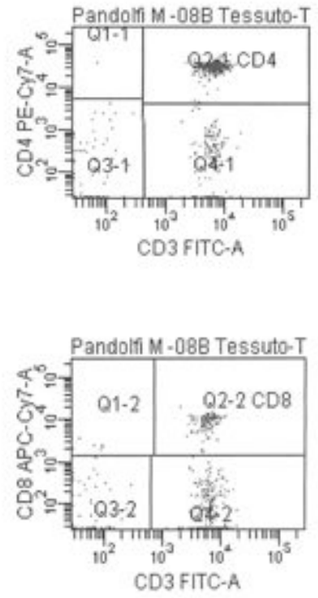
1. Samples (tumor and normal tissue) were obtained from consenting patients undergoing surgery for primary or metastatic to the liver colon cancers. Samples were mechanically fragmented and then digested by 0,2% collagenase I at 37°C, for 90 min. Cell suspensions (from tumor and normal tissue) were filtered through cell strainers and characterized by flow cytometry, using antibodies against CD133, CD45 and CD31.
Then, CD133+ and CD133- cells were isolated using CD133 Cell Isolation Kit. Briefly, cells were labeled with micro beads CD133/1-conjugated, then sorted by the manufacturer's isolation kit. The purity of the isolated cells was evaluated by flow cytometry using PE-conjugated anti-CD133/2.
Purified CD133+ and CD133- cells were suspended in soft agar in 6-well culture plates at a density of 5000 cells/well and cultured for 2 weeks. Finally, colonies were counted under the microscope.

2. In this study, in addition to conventional cytology, we assessed the value of six colour flow cytometry IF in FNA samples of 2 cases of thyroid disease. After FNA, a mononuclear cell suspension was obtained for flow cytometry analysis. Flow cytometry characterization of the cell suspension was preformed with a number of >10.000 valuable cells, in all the samples.
The sample for FC immunophenotype was processed within 2 – 4 hours from collection, in order to avoid loss of cells and maximize cell collection. After gentile tissue dissociation in a Petri dish containing 2 to 3 ml of phosphate buffer solution (PBS), the single cell suspension obtained from FNA was washed twice in PBS for 5 minutes at 1600 revolution per minute. The supernatant fluid was discarded and the pellet of cells was suspended in PBS for flow cytometry characterization.
The cell suspension was incubated with 5 ml of directly conjugated monoclonal antibody (McAb) of interest. We combined the following 6 colour McAb utilising B, T/NK and monocytic lineage specific markers (Becton Dickinson Bioscience): CD3 Fitc / CD56 Pe / CD45 PerCP / CD4 PE-Cy7 / CD19 APC / CD8 APC-CY7. Prior to any tube sample acquisition, a flow cell cleaning with distilled water was performed, for 1 to 3 minutes, to avoid any contamination or carry over of samples. Events were acquired utilising a FACSCanto flow cytometer (Becton Dickinson Bioscience). A minimum of 10.000 events for each cell marker was acquired. Six colour flow cytometry analyses were performed utilising the FACSDiva software, by a haematologist unaware of the cytological results.

Results

1. We collected 17 primary colon cancer and 8 metastasis. The median age of study patients was 61.3 years. First, in order to quantify the percentage of CD133+ cells in tumor samples, with respect to normal tissues harvested from the same patient, we analyzed (by flow cytometry) the cell suspensions derived from dissociation of both neoplastic and normal surrounding tissues. The percentage of CD133+ cells was consistently higher in tumor samples when compared to healthy tissues in the majority of samples analyzed and the difference between the two groups was pertinent ($p<0.01$).
Then, to determine whether CD133+ tumor cells were more tumorigenic than their CD133- counterparts, we compared their anchorage-independent growth using the soft agar colony formation assay. Purified CD133+ and CD133- cells were suspended in soft agar and colonies were counted after two weeks. CD133+ cells displayed a significantly higher ability to form colonies in soft agar which were also larger in size than the ones obtained from CD133- cells.

2. A significant infiltration of CD3 reactive T lymphocytes, from 20% to 40% of the CD45 positive leukocyte, was documented, sided by a minority of CD4 dim / positive monocytes. The 6 colour IF allowed a sub-characterization of the T lymphocytes according to the CD4/CD8/CD56 expression. Moreover a small percentage of CD45 negative cells, compatible with thyroid cells, were documented.
FNAC yielded adequate material in all cases. After gentle dissociation, a cell suspension was obtained for flow cytometry analysis. Diagnostic characterization of the leukocyte population was performed, with a number of >10.000 valuable CD45 positive events, in all the samples. According to the 6 colour screening panel of the tube n° 1 (CD3 Fitc / CD56 Pe / CD45 PerCP / CD4 PE-Cy7 / CD19 APC / CD8 APC-CY7) three different populations were identified: the leukocyte population (CD45/SSC), the lymphoid CD19 positive B and CD3 positive T lymphocytes, sub-divided according to CD4, CD8 and CD56 expression (Fig. 1) and a sub-population of monocytes.
A small percentage (9%) of CD4 dim monocytes was documented. In one case, a significant infiltration of T CD3 CD4 positive lymphocytes was observed, with a prevalence of CD4+ lymphocytes. By contrast, the peripheral blood T subpopulation showed a normal CD4/CD8 ratio.



Flow cytometry sub-characterization of the CD3 positive lymphoid population in a case of FNA of thyroid nodule.

Conclusions

1. Our preliminary data confirm a significantly higher proportion of CD133+ cells in human primary and metastatic colon cancers samples with respect to healthy tissue. Also, our findings confirm that these cells are able to form colonies in soft agar *in vitro*, which correlates with the ability to form tumors *in vivo*.
Further studies will be conducted to identify CD133+ CSC population in thyroid cancer samples and to evaluate their *in vitro* biological behaviour.

2. From our preliminary results, we characterize a sub-population of reactive T leukocytes in two FNA of thyroid nodules. T and NK lymphocytes play an important role in the regulation of the immune response and the mediation of dominant immunologic tolerance. Several reports have described an important infiltration of T and NK lymphoid cells in different kinds of solid tumor, playing a crucial role in the control of tumor immune responses and tumor associated macrophage has been correlated to poor prognosis. To date, no data has been published on the FC characterization of the reactive side population in thyroid solid tumor.

The presence of non-malignant, reactive immune response has been demonstrated in a variety of solid and haematological tumors and a relationship between the amount of tumor infiltrating T lymphocytes and outcome has been shown in solid and haematological tumors.

Flow cytometry IF can potentially represent a powerful, reliable and rapid technique for the characterization of FNAC and can improve the diagnostic approach and therapeutic strategies of thyroid tumors. ■

TITLE

Fasting Glucose as a Predictive Marker of HER/neu Expression and Time to Development of Resistance to Trastuzumab in Breast Cancer Patients (GHERB)

COORDINATOR

Maddalena Barba
Epidemiology

Iole Cordone
Clinical Pathology

SENIOR INVESTIGATOR

Marcella Mottolese
Anatomical Pathology

Patrizia Vici
Oncology B

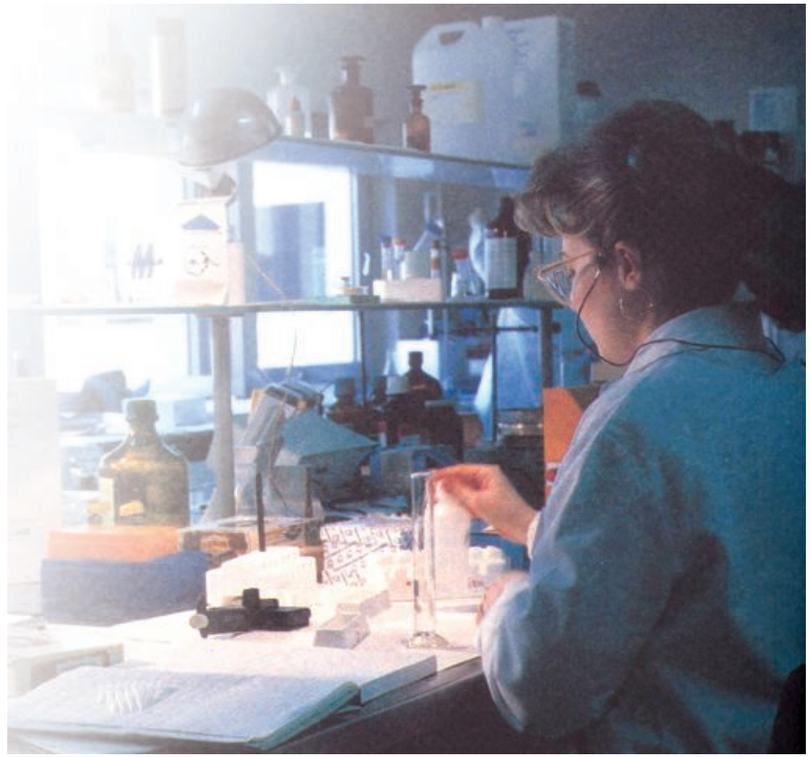
Months 1-2

Over the initial two-month period, we first organized, wrote and presented all relevant documentation to the IFO Ethical Board for approval. We then planned a series of meetings to present the details of the GHERB project to the potential collaborating Departments at the Institute. We discussed the feasibility of the study in the context of how the daily activities carried out by any single Department would significantly contribute to the study.

We were and fully supported by the Pathology Department, and by Medical Oncology B Department. We are also working with Dr Mottolese, pathologist, who has outstanding experience in the field of breast diseases, and Dr Patrizia Vici, a medical oncologist with exceptional experience in breast cancer. They both revised the documentation pertinent to the project and significantly contributed to the protocol implementation.

Months 3-5

Dr Giuseppina Caolo, with a background in sociology and statistics, joined the research team at the Institute. With the Principal Investigator (PI), Dr Caolo defined the study algorithm and enrolment procedures. Dr Caolo also contributed to the implementation of the study questionnaire, which was tested for



demographics and specific study variables with volunteers. She then developed the corresponding database using an appropriate software (Access 2007). The database will be further implemented in the following months by using a visual basic ed SQL-server for automatic control and data standardization.

Months 6-12

Dr Caolo started working at recruitment and the retrieval of clinical records.

1. Recruitment: Dr Caolo personally invited the following two groups of women, diagnosed with breast cancer, to join our study:
   a. breast cancer patients currently attending the Medical Oncology Day Hospital for a Trastuzumab-based therapy.
   b. breast cancer patients currently attending the Ambulatory for follow up. These women were treated with Trastuzumab between 2000 and the present.

   Twenty-seven (27) women were invited and accepted, a 100% participation rate. They consequently signed an informed consent form and donated approximately 40 minutes of their time to answer the questionnaire. We required and obtained the medical records pertinent to the variables of interest and study outcomes. For validation purposes, the information reported by the patients at the time of recruitment will be compared with the corresponding medical reports.
2. Clinical records: Dr Caolo has identified an adjunctive group of sixty-three (63) eligible breast cancer patients based on the consultation of the updated lists provided by the Medical Oncology and Pathology Departments. This group of women includes the following sub-categories of breast cancer patients:
   a. twenty-three (23) patients who have recently (within 4-8 weeks) developed brain metastases
   b. thirty-five (35) patients who have

been recently (within 4-8 weeks) diagnosed with a early breast cancer

c. six (6) patients who have been recently (within 4-8 weeks) diagnosed with locally advanced breast cancer.

These are breast cancer patients tested positive for HER-2 overexpression /amplification and currently in need of a Trastuzumab-based chemotherapy. For women who belong to any of these 3 subcategories, we have required and obtained the corresponding medical records.

The routine attendance of the Medical Oncology Day Hospital will briefly allow Dr Caolo to invite these women to join our study and to collect the self-reported information needed.

The retrieval of clinical records has further led to the identification of a group of 15 women with the following characteristics:

- breast cancer diagnosis
- HER-2 overexpression/amplification
- Trastuzumab-based chemotherapy

Unfortunately, they have died from the disease over the past months/years. For these patients, medical records will be made exclusively available.

In summary, the GHERB study is currently driven by a highly collaborative spirit among the Epidemiology, Medical Oncology B and Pathology Departments. This has facilitated the following steps:

- active enrolment of 28 breast cancer patients (for whom the existing medical records have also been obtained)
- medical records retrieving for 63 further women who will shortly be invited to join the study and for whom self reported information will be made available
- identification of a group of 15 eligible women who had died from the disease and whose medical records will be made available to our research team. ■

TITLE

Evaluation of the impact of new neurosurgical procedures in the combined treatment of brain gliomas, with regard to cognitive functions, quality of life, and peri-operative pain

COORDINATOR

Carmine Maria Carapella
Neurosurgery

CO-RESEARCHERS

Edoardo Arcuri
Intensive Care, Pain Therapy and Palliative Care

Fabio Cattani
Neurosurgery

Alessandra Fabi
Medical Oncology

Bruno Jandolo
Neurology

Marta Maschio
Neurology

Rossella Milana
Psychology

Alessandra Mirri
Radiotherapy

Enzo Morace
Neurosurgery

Emanuele Occhipinti
Neurosurgery

Piero Oppido
Neurosurgery

Andrea Pace
Neurology

Alfredo Pompili
Neurosurgery

Patrizia Pugliese
Psychology

Francesca Sperati
Epidemiology

Stefano Telera
Neurosurgery

Irene Terrenato
Epidemiology

Walter Tirelli
Intensive Care, Pain Therapy and Palliative Care

Antonello Vidiri
Radiology

Aims of the study

■ The present study will evaluate the impact of new neurosurgical procedures in the combined treatment of brain gliomas, also considering the influence of peri-operative loco-regional treatments, not only on patient survival. In particular this research intends to explore the influence of these therapeutic strategies on the functional neurocognitive status, quality of life (QOL), emotive status – mainly in the field of depression, and post-operative pain, in adult patients affected with primary brain gliomas.

For this reason a combined test battery, easy to administer, has been prepared with the aim of exploring different items in the neurocognitive area, as well as in the emotional and quality of life areas. All patients have to be examined with standard tests in the following cognitive domains: 1) attention and executive functions, 2) short-term and working memory, 3) verbal and nonverbal memory, 4) word fluency, and 5) visuoconstruction. The QOL is globally evaluated with EORTC QLQ-C30 questionnaire in association with the Brain specific module EORTC QLQ-BN20 questionnaire, and the FACT-An scale specifically directed to the fatigue domain; the affective disorders in the field of depression will be explored with BDI score.

Patients receive a pre-treatment baseline evaluation, and on-treatment immediately after surgical procedure (first week after surgery), and before the next course of therapy, at least every three months, until they leave the study because of progressive disease.

The data regarding progression-free survival and overall survival will be compared with an historical data set obtained from the data base of our Department of Neuroscience, regarding more than 500 patients affected with primary brain tumors.

This information could allow the detection of changes induced by neurosurgical procedures, mainly considering new imaging-guided microsurgical resection and loco-regional treatments, as well as tumor progression. The minimal survival benefit of existing treatments highlights the need for other measures of patient

outcome, including influence on cognitive functions and quality of life (QOL). Net clinical benefit of cancer therapy includes (a) survival benefit, (b) time to treatment failure and disease-free survival, (c) complete response rate, (d) response rate, and (e) beneficial effects on disease-related symptoms and/or quality of life. In the case of brain gliomas, characterized by a progressive impaired mental function, a beneficial treatment may be one that stabilizes or slows the progression of worsening symptoms, whether or not overall survival is extended; on this basis, this type of assessment could offer the potential to better define the relative risks versus benefits of unconventional treatment regimens, particularly when they exhibit small differences in terms of survival benefit.

Report of the first year activity

In the first year we had to preliminarily detect the possible administration modality of different tests, mainly in the first post-operative week, when anticonvulsant drugs as well as post-operative brain edema could interfere with the level of consciousness of the patients, limiting their attention and time-space orientation. During this period we also submitted to pre- and post-operative evaluation patients affected by different pathologies, such as extra-axial brain tumors like meningioma, to differentiate the effect of the treatment from the specific alterations induced by highly infiltrating tumor growth, typical of brain gliomas.
At the first interim analysis, performed at the end of August, since March 2008 24 patients (mean age 54.8; range 20-79) have been enrolled and included in the study; of them, 18 were re-evaluated after surgical treatment and 3 during the follow-up, at least 3 months after the first evaluation. Having been evaluated, patients affected by brain gliomas of different malignancy grading, including glioblastoma multiforme, anaplastic astrocytoma, as well as well differentiated glioma, were submitted to microsurgical resection, under MRI-guided neuronavigation, or stereotactic biopsy; the different therapeutic choice was made after considering the extension and topographical location of the tumor, as well as the patient's clinical condition. For this reason it is not correct to stratify the eventual modifications observed in the cognitive and QOL evaluation of these patients on the basis of the pathology grading and/or the specific therapeutic procedure.
At baseline, QOL assessment through EORTC QLQ-C30 questionnaire has showed higher mean scores in social functioning (mean score= 65.7), and physical functioning (mean score= 63) than Global QOL functioning (mean score =57.6), role functioning (mean score= 58.5), and emotional functioning (mean score= 48.7).
In the EORTC symptomatic scale the variable most impaired by the disease seems to be fatigue (mean score= 36.9).
After surgery some functional scales of QOL show improvement, as Global QOL (T0 = mean score 57.6 vs. T1 = mean score 70.8), emotional functioning (T0= mean score 48.7 vs. T1= 62.5), and physical functioning (T0= mean score 63 vs T1 = mean score 68.6).
Conversely, a worsening has been observed on the symptomatic scales, such as pain (T0 = mean score 19.8 vs T1 = 29.1), insomnia (T0 = mean score 42.3 vs. T1 = mean score 58) appetite loss (T0 = mean score 5.5 vs. T1 =16.6), constipation (T0 = mean score 15.3 vs T1 = mean score 37).
Moreover, on brain-specific module EORTC QLQ-BN20, the most impaired area was communication deficit (T0 = mean score 50 vs T1 = mean score 43); conversely, the area of future uncertainty seemingly improved (T0 = mean score 63 vs T1 = mean score 67).
The BDI questionnaire showed lower depression scores than the cut-off value (16), both at baseline (mean score =11) and after surgery (mean score =11.3); suggesting a mild depressed attitude, probably influenced by hospitalization.
At baseline Mini Mental State Examination (MMSE) showed that 73% (mean score= 29.3) of patients did not exhibit cognitive impairment, 18% (mean score= 21.6) of patients showed a moderate cognitive impairment, and only in 9% (mean score=18.6) a severe cognitive impairment was observed.
After surgery 86.6% (mean score= 24.1) of patients showed an absence of cognitive impairment, 13.3% (mean score= 8.9) of them severe cognitive impairment.
The present data has to be confirmed in a less restricted sample, and sequentially analysed during the follow-up, to confirm the influence of different therapeutic procedures on relevant functional items, eventually modifying the present treatment protocol.
We intend, during the second year of the project, to extend the analysis sample as well as the length of clinical follow up, allowing a more consistent evaluation of the potential role of new surgical procedures. The results will be presented in scientific meetings and will be submitted as original work for publication. ■

TITLE

Cerebrospinal fluid flow cytometry immunophenotyping for a more sensitive and specific approach to diagnosis and monitoring of neoplastic meningitis and brain tumor infiltration

COORDINATOR
Iole Cordone Clinical Pathology

SENIOR INVESTIGATORS
Maria Carmine Carapella Neurosurgery
Mariantonia Carosi Histopathology
Raffaella Coccia Virology
Federico De Marco Virology
Alessandra Fabi Medical Oncology "A"
Bruno Jandolo Neurology
Mirella Marino Histopathology
Marta Maschio Neurology
Alessandra Mirri Radiotherapy
Emanuele Occhipinti Neurosurgery
Piero Oppido Neurosurgery
Andrea Pace Neurology
Raffaele Perrone Donnorso Histopathology
Maria Concetta Petti Haematology
Francesco Pisani Haematology
Antonello Vidiri Radiology
Gaetano Vitelli Clinical Pathology

JUNIOR INVESTIGATORS
Maria Laura Dessanti Haematology
Serena Masi Clinical Pathology
Alessia Pasquale Clinical Pathology
Cristiano Spadaccio Virology
Francesca Sperati Epidemiology

First year report

■ Neoplastic meningitis (NM) is a common problem in neuro-oncology occurring in up to 15% of cancer patients. After acute leukemias and high-grade non-Hodgkin lymphomas, breast, lung and melanoma are the most common solid tumors that metastasize to the leptomeninges.
Morphological evaluation of cerebrospinal fluid (CSF) by light microscopy is considered the diagnostic "gold standard" for the detection of CNS involvement in patients with NM; however, this is a low sensitive method, with a reported false-negative rate of up to 60%. Moreover, the first diagnostic lumbar puncture is negative in up to 45% of cases and 25-30% of cytological positive CSF had a leukocyte count <4 cell uL, hampering a molecular or immunological analysis by standard methods.
Flow cytometry immunophenotyping (IF) of blood and marrow is an essential tool in the diagnosis and monitoring of hematological malignancies in routine clinical practice and is increasingly utilized to detect hematological CSF infiltration. It has been demonstrated that CSF IF is more sensitive than cytology in a significant proportion of cases (Hegde U et al; 2005). However, no studies have been reported wich evaluate the role of CSF flow cytometry for the monitoring of minimal residual disease (MRD) after treatment and its potential role for the diagnosis of solid tumors NM.

The primary end point of this translation research project is to assess the value of multiparametric flow cytometry IF in CSF samples for the diagnosis of NM in patients with different types of solid tumors and haematological malignancies and to com-

pare the sensitivity of flow cytometry with conventional cytology and magnetic resonance imaging (MRI) techniques.
In this regard, this project is characterized by a highly multidisciplinary approach, involving both basic and clinical researchers.

During this first year we focused principally on:

1) Assessing the value of the flow cytometry approach for the diagnosis of neoplastic meningitis
2) Confirming the reliability of the flow cytometry characterization to hypocellular CSF samples
3) Establishing the marker combination to be utilised by flow cytometry for follow-up analysis and MRD monitoring after treatment
4) Characterising the subpopulation of reactive CSF leukocyte (lymphoid and myeloid / macrophage populations) in NM
5) Organizing files to keep all biological information.

Patient enrolment

Patients with suspected haematological CSF infiltration and confirmed diagnosis of breast cancer NM were enrolled for flow cytometry characterization.
Clinical characteristics and number of FC studies are illustrated in Table 1.

Materials and Methods

CELL COUNT AND MORPHOLOGICAL ANALYSIS

Cell count was performed using the Turk reagent and a Nageotte chamber. Morphological examination was done on cytospin preparation stained with May-Grunwald Giemsa and analysed by two experienced observers, unaware of the flow cytometry analysis. Morphological diagnosis was then compared with flow cytometry study to confirm the diagnosis of NM.

FLOW CYTOMETRY

The CSF sample for FC immunophenotype was processed within 2 – 4 hours from collection, in order to avoid cell loss and maximize cell collection. 2 to 9 millilitres of CSF were collected in a tube without any transport medium. After centrifugation at 1600 revolution for 10 minutes, the pellet of cells was suspended in phosphate buffer solution (PBS) and a 6 colour flow cytometry characterization was performed utilising a FACSCanto flow cytometer (Becton Dickinson Bioscience).

The cell suspension was divided between 2 to 3 tubes and incubated with 5 ml of directly conjugated monoclonal antibody (McAb) of interest. The following directly conjugated Abs were utilised: CD2 Pe-Cy7, CD3 Fitc, CD4 Pe-Cy7, CD5 APC, CD8 APC-Cy7, CD56 Pe as T/NK cell markers, CD19 APC, CD20 APC-Cy7, CD22 APC, CD79b APC, sIg Kappa Pe, sIg Lambda Fitc, CD138 Pe for B cells, HLA.DR APC-Cy7, CD38 Pe, CD45 PerCP, CD10 Pe, CD15 Fitc, CD34 Pe-Cy7 and CD133 Pe as myeloid and non lineage specific markers (Becton Dickinson Bioscience, Coulter, Miltenyi-Byotec). After McAb dispensation the incubation was performed utilising the "Lyse and Wash Assistant" (Becton Dickinson Bioscience) according to the Single Lyse and Wash program. Prior to any tube sample acquisition a flow cell cleaning with distilled water (for 2 to 4 minutes run) was performed to avoid sample carry over. A cluster of more than 25 events fulfilling criteria for malignant population was classified as positive. Events were acquired utilising a FACSCanto flow cytometer (Becton Dickinson Bioscience). 6-colour flow cytometry analysis was performed utilising the FACSDiva software, by a haematologist unaware of the cytological results. The side leukocyte population was utilised as positive/negative internal control.

PATIENT CHARACTERISTICS	NUMBER OF CASES	POSITIVE AT DIAGNOSIS BY FACS	N° OF FOLLOW UP STUDIES
Sex			
Male	16	3	
Female	34	11	
Median age (years, range)	55 (19 – 79)		
Diagnosis			
NHL	32	3	4
Multiple myeloma	5	2	1
Acute leukaemia	5	1	4
Breast cancer	8	8	16

Table 1. Patient characteristics

Preliminary results

6-colour flow cytometry IF of CSF samples from 42 patients with suspected haematological malignancies and 8 breast cancer NM were analysed and compared with the sensitivity of conventional cytology.

HAEMATOLOGICAL SAMPLES

Forty-two haematological patients entered the study: 32 non-Hodgkin Lymphomas (NHL), 5 multiple myelomas (MM) and 5 acute leukaemias (AL) with suspected CSF infiltration.
Despite the low absolute cell number (5 cell / ml, range 1–21), a median of 1648 (range 274 – 6511) valuable events was characterized in all the samples. CSF infiltration was diagnosed in 6 cases: 2 mantle-cell lymphomas (MCL), 2 MM, 1 Primary Central Nervous System Lymphoma (PCNSL) and 1 case of dendritic cell leukaemia. A proportion of CD38 CD138 CD28 CD117 CD56 Kappa positive plasma cells were identified in the CSF samples of the 2 MM positive cases. A percentage of CD19 CD20 CD22 CD5 positive, CD10 negative, surface Ig k light chain positive B cells were identified in the 2 MCL cases and in the PCNSL and an infiltration by HLA.DR CD4 CD138 positive leukaemic cells was documented in one case of dendritic cell leukaemia. No FC negative/morphology positive cases have been observed. Leukaemic immunophenotype identified in the first sample of each positive case was utilised as a patient specific molecular asset for follow-up analysis. In 3 cases evaluated after chemotherapy, MRD was positive by FC, although negative by conventional cytology. Tumor cells were sided by a significant infiltration of reactive T CD3 positive lymphocytes, in a proportion from 8% to 85% of CD45 positive cells, with a prevalence of CD4 positive lymphocytes (CD4/CD8 ratio = 4.51) in all cases.

BREAST CANCER NM

CSF localization was diagnosed if samples were positive by cytology. Eight patients with breast cancer NM were analysed by 6-colour flow cytometry IF at diagnosis. Samples were obtained from lumbar CSF and from ventricular CSF via an indwelling Ommaya reservoir. For follow-up analysis, samples from 16 patients treated by multiple intrathecal chemotherapy infusion were collected and analysed both by conventional cytology and flow cytometry.
Despite the low absolute cell number (29 cell / ml, range 1–182), a median of 30.743 (range 393 – 181.000) valuable events were characterized in all the samples.
According to 6-colour screening panel (CD3 Fitc / CD56 Pe / CD45 PerCP / CD4 PE-Cy7 / CD19 APC / CD8 APC-CY7) three different populations were identified: a prevalence (60%, range 1 – 96) of CD45 negative large cells representative of the breast cancer infiltration, a proportion (1 - 65%) of CD3/CD4 positive reactive T lymphocytes and a minority (2 - 13%) of CD4 dim monocytes. The FC study identified a proportion of CD15 positive/CD45 negative breast cancer cells in 3 of the 4 cases analysed for this marker. In follow-up analysis, 4 cases with MRD by FC were negative by conventional cytology.

Discussion

From our preliminary results, several end points of this project have been addressed in this first year of study.

Six-color FC IF of CSF samples has shown to be a sensitive and specific approach for NM diagnosis and monitoring in hematological malignancies and breast cancer NM (**primary end-point**). Leukemic immunophenotype identified in the first sample of each positive case was utilised as patient-specific immunological asset for follow up analysis. This approach allowed the identification of residual tumor cells in patients with low-volume disease after treatment (**secondary end-point**).
In a significant proportion of cases a brain biopsy, especially for deeply-located lesions, is at risk of developing neurological complications. The CSF identification of tumor cells by flow cytometry could reduce the need for brain biopsy, therefore limiting severe complications. From our results, a diagnosis of PCNSL has been performed by FC CSF characterization within a few hours from sample collection, with no need for a brain biopsy, allowing the rapid identification of the best treatment strategy.
Furthermore, due to high sensitivity and specificity, FC appears a new, powerful, and reliable approach in identifying new markers with potentially high prognostic value.
Regarding the characterization of breast cancer patients, a population of CD45 negative CD15 positive cells was documented at diagnosis and facilitated follow-up monitoring after treatment. Initial adhesion of cancer cells to endothelium can be mediated by CD15. CD15 expression of breast cancer tumor cells in the CSF samples could explain one of the mechanisms responsible for the tumor cells migration from the primitive localization to the brain, representing a new marker with a potentially high prognostic value.
Moreover, we characterized a subpopulation of reactive CSF T leukocytes in breast cancer NM. T and NK lymphocytes play an important role in the regulation of immune response and the mediation of dominant immunologic tolerance. Several reports have described an important infiltration of T and NK lymphoid cells in different solid tumors, playing a crucial role in the control of tumor immune response, suggesting a correlation with poor prognosis. To date, no data has been published on FC characterization of the reactive side population in solid tumor NM.

The proposed research introduces new ideas and methods substantially different from existing approaches, innovative in monitoring hematological meningitis, the diagnosis and monitoring of breast cancer NM and for the identification of new prognostic markers.

The aims of future studies are to:
1) confirm the data of this first year of study increasing the number of NM cases
2) expand the number of antigen identified by FC on breast cancer tumor cells for the identification of new diagnostic and prognostic markers and to confirm the value of CD15 expression in breast cancer NM
3) identify a panel of molecules able to distinguish between different types of CSF tumor infiltrating cells and to assess the value of CD56 bright/CD45 negative population as a diagnostic marker for neuroectodermal/neuroendocrine tumors
4) compare the expression of CSF infiltrating tumor cells with the IHC results of the primary or metastatic tissue
5) evaluate the expression of the stem cell marker CD133 in the CSF tumor cell of patients with solid cancer NM. ■

TITLE
A prospective study of feasibility of Nipple Sparing Mastectomy (NSM) in breast cancer patients and its related aesthetic and psychological outcomes

COORDINATOR
Roberta Di Filippo
Psychology

CO-RESEARCHERS
Claudio Botti
Franco Di Filippo
Pasquale Perri
Angelo Psaila
General Surgery "A"

Roy De Vita
Pier Paolo Gullo
Marcello Pozzi
Antonio Varanese
Plastic and Reconstructive Surgery

Gabriella Maggi
Patrizia Pugliese
Psychology

Marcella Mottolese
Letizia Peracchio
Anatomical Pathology

Francesca Sperati
Irene Terrenato
Epidemiology

First year report

■ The aim of the project is to evaluate the prognostic factors involved in Nipple – Areola Complex (NAC) infiltration and the possible post – surgery complications that could affect the feasibility of the technique. Moreover the aim of the project is to investigate the psychologic and aesthetic outcomes of this surgical technique, compared with breast reconstruction mastectomy and radical mastectomy.
The project involved the following Operative Units: Department of General Surgery "A", Service of Plastic and Reconstructive Surgery, Service of Psychology, Service of Anatomopathology, BioStatistic and Epidemiology Department.
Several phases have been necessary in order to realize this project.
The Project's complexity, the number of Operative Units involved and the limited nature of received funds, have all necessitated a **first phase**, to optimize the availability of resources. It was decided to finance a collaboration contract for a Psychologist who would coordinate all the Operative Units and perform the psychological assessment of enrolled patients.
A number of staff meetings were planned with the aim of optimizing the project coordination and improving the management of human resources employed in the process. Within this phase, in order to promote the patients' participation, a specific activity was performed. A "one day global evaluation" provided advantages in terms of time and resources for the patients.
The second phase of the study included the patients' enrollment.
Since March 2008, 42 breast cancer patients have been enrolled and 31 patients have been included according to the eligibility criteria (median age=51, range 39-71.); 16 patients underwent the second evaluation before the beginning of medical treatment.
Psychological variables assessment at **baseline** evidenced in the EORTC-QLQ C30 questionnaire a good Social functioning and Global Quality of Life functioning (mean score= 88, 78). The mean scores of emotional functioning are lower (mean score= 73). Moreover 80% of

patients showed both a disinvestment of sexual area and low future perspectives (EORTC QLQ BR23 questionnaire).
On the HADs 78% of patients showed anxiety levels higher than the cut off value (>8).
The semi-structured interview showed a high investment both on body image (88% of patients) and on breast (84% of patients) and a low frequency of sexual intercourse (64% low vs. 36% high frequency). Only 16% of women referred to have participated actively in treatment decision-making and 36% to have been fully informed.
Post-surgery psychological evaluation through a EORTC-QLQ C30 questionnaire showed a worsening in Quality of Life Areas (Social functioning 69 mean score vs. 88, Global Quality of Life functioning 64 mean score vs. 78, Emotional functioning 69 mean score vs. 73) and increased anxiety levels on the HADs (10 mean score). The disinvestment of the sexual area and low future perspectives were still present in most of the patients (EORTC-QLQ BR23).
The semi structured interview showed a stable investment on body image (85%) and on breast (71%) and further worsening in the sexual intercourse frequency (71% low vs. 29% high frequency).
The early evaluation phase does not facilitate the assessment of possible presence of loco regional and systemic recurrence, the correlation between the biomolecolar factors and disease course, nor the aesthetic outcome. ■

TITLE

Female and age: A different way of conducting Phase I pharmacokinetic study based on analysis of previous studies with targeted molecules

Susanna Di Segni
Pharmacokinetic Unit
Alain Gelibter
Medical Oncology "A"

Background

■ The goal of a new target-based approach is to improve the efficacy and selectivity of cancer treatment by developing agents that specifically block the pathogenic mechanisms that account for malignant transformation. Pharmacokinetic parameters are usually not sufficiently correlated with patient characteristics, such as age, gender, or excretory organ function, or with outcome measures, such as the severity of common toxic effects; female sex has been shown to be a risk factor for clinically relevant adverse drug reactions.
Our study could be the first to investigate the role of the correlation between sex, age and drug pharmacokinetic profile of targeted agents in cancer patients.
Understanding the impact of age and gender on toxicity could led to a change in conception, design and conduction of phase I trials using targeted molecules.

Aims

To retrospectively evaluate the impact of age and gender on the variability of PK parameters in Phase I studies with targeted therapies. To build PhaseI/PK database with the main trials selected as described below and verify if there is a rationale in the selection of enrolled patients related to age and gender. This study will also evaluate if toxicity and outcome are sex and age correlated. Starting from obtained results to build and validate a new method of designing phase I/PK studies.

Methods

The data for this analysis came from 2 sources: abstracts reporting on phase 1 cancer treatment trials drawn from a systematic review of journal articles that resulted from these trials, between 2000 and 2007.

Pub Med Research

- Key word: Targeted molecules
- Key word: Phase I/II studies or Phase I/II and Pharmacokinetic studies (for each of the agents)

A database with 254 phase I and II trials and with 63 targeted agents has been created:

CLASS OF AGENTS	TOT
SM small molecule	50
AS antisense oligodeoxynucleotide	5
AB antibody	8
Tot	63

INCLUSION CRITERIA

- Trials conducted between 2000 and 2007
- Phase 1 and 2 of oncology trials of solid tumors
- Nonpediatric trials (age> 18 years)
- Trials reporting pharmacokinetic analysis
- Trials enrolling Male and Female population
- Targeted Agents (SM: small molecules; AS: Antisense Oligodeoxynucleotide; AB: Antibody)

EXCLUSION CRITERIA

- Trials of Radiation therapy only
- Trials of Hematological malignancies
- Trials of cytotoxic chemotherapeutic agents
- Trials of Gender related pathology (ovarian cancer, prostate cancer etc).

After revision of inclusion/exclusion criteria 160 phase I and II trials and 48 targeted agents were selected

NUMBER OF STUDIES AND AGENTS FINALLY SELECTED FOR DATABASE

	N° OF AGENTS	N° OF TRIALS
SM	39	135
AS	5	14
AB	4	11
tot	48	160

SM: Small molecules; AS: Antisense oligonucleotides; AB:antibody

TRIALS WERE ANALYZED FOR:

- Number of patients treated
- Number of Male and Female with PK analysis
- Age (median and range)
- Pk analysis

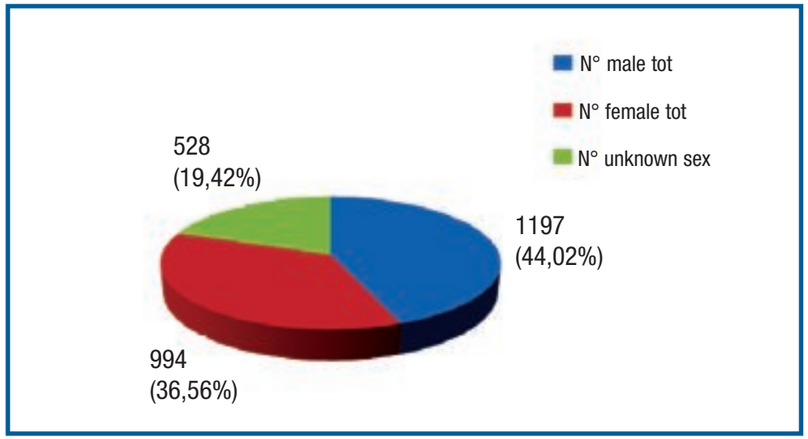
PRELIMINARY DATA:

CATEGORY	N° AGENTS	N° TRIALS
SM	16	56
AB	4	11
AS	3	7
tot	23	74

To date 74/160 trials have been analyzed:

Data have been evaluated for
- Number of total patients
- Number of Male and Female enrolled for each trial and for each single study
- Number of unknown gender patients and unknown gender pk trials
- Prevalence of men over women (or viceversa) in the selected studies
- Age of the enrolled patients

NUMBER OF PATIENTS ENROLLED FOR PK ANALYSIS



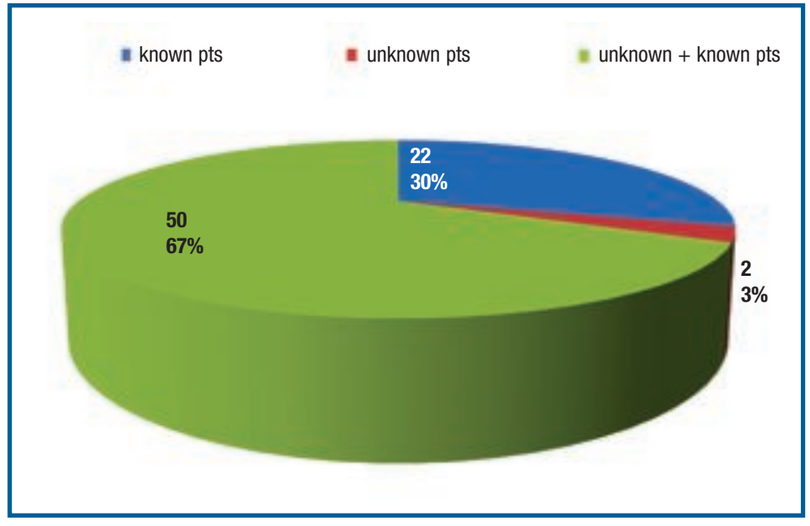
NUMBER OF MALE, FEMALE AND UNKNOWN GENDER PATIENTS: DATA REPORTED FOR:

AGENT	CLASS	N OF STUDIES	PK PATIENTS		UNK PK PTS	TOT MF PER AGENT (M+F+UNK)	%UNK PTS
			MALE	FEMALE			
AG013736	SM	1	16	20	0	36	0,0
BEVACIZUMAB	AB	2	8	17	28	53	52,8
BMS-214662	SM	5	52	42	88	182	48,4
C225-cetuximab	AB	6	147	158	67	372	18,0
CCI-779 temsirolimus	SM	4	80	50	0	130	0,0
CEP701	SM	1	unk	unk	10	10	100,0
CI-1033	SM	5	46	33	86	165	52,1
CI-1040	SM	1	0	0	76	76	100,0
CI-994	SM	1	15	11	0	26	0,0
COL-3 metastat	SM	2	46	24	0	70	0,0
CP-724714	SM	1	0	30	0	30	0,0
E7070-indisulam	SM	7	184	152	24	360	6,7
EMD72000-matuzumab	AB	2	24	16	0	40	0,0
Endostatin	SM	5	74	62	0	136	0,0
Enzastaurin	SM	3	22	24	68	114	59,6
GW 572016 Lapatinib	SM	4	61	85	12	158	7,6
ISIS 3521-affinitak	AS	4	60	34	0	94	0,0
ISIS 5132	AS	2	13	9	29	51	56,9
ISIS2503	AS	1	16	11	0	27	0,0
OSI774 -erlotinib	SM	7	130	63	19	212	9,0
PS-341-Bortezomib	SM	2	10	4	12	26	46,2
TRATUZUMAB-Herceptin	AB	1	17	2	0	19	0,0
ZD1839-gefitinib	SM	7	176	147	9	332	2,7

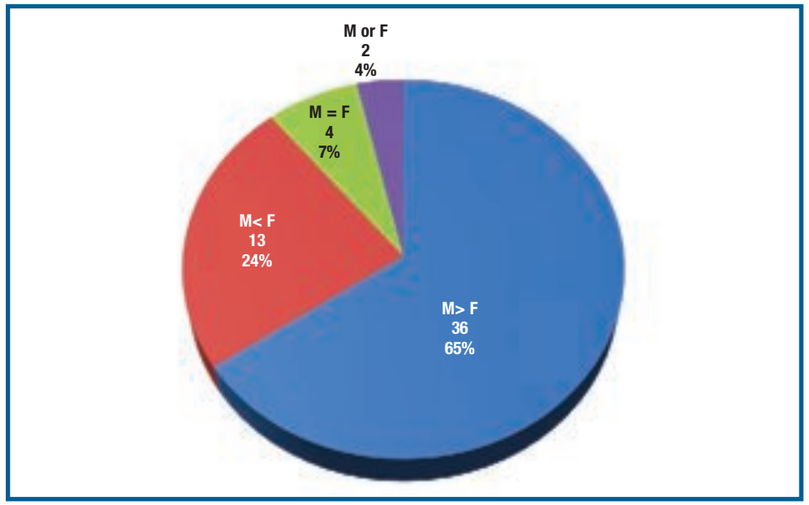
PERCENTAGE OF UNKNOWN GENDER POPULATION IN PK TRIALS: DATA REPORTED FOR SINGLE:

AGENT	CLASS	N OF STUDIES	UNK TRIALS	%UNK TRIALS
AG013736	SM	1	0	0
BEVACIZUMAB	AB	2	1	50
BMS-214662	SM	5	3	60
C225-cetuximab	AB	6	2	33,33
CCI-779 temsirolimus	SM	4	0	0
CEP701	SM	1	1	100
CI-1033	SM	5	3	60
CI-1040	SM	1	1	100
CI-994	SM	1	0	0
COL-3 metastat	SM	2	0	0
CP-724714	SM	1	0	0
E7070-indisulam	SM	7	1	14
EMD72000-matuzumab	AB	2	0	0
Endostatin	SM	5	0	0
Enzastaurin	SM	3	2	67
GW 572016 Lapatinib	SM	4	1	25
ISIS 3521-affinitak	AS	4	0	0
ISIS 5132	AS	2	1	50
ISIS2503	AS	1	0	0
OSI774 -erlotinib	SM	7	1	14
PS-341-Bortezomib	SM	2	1	50
TRATUZUMAB-Herceptin	AB	1	0	0
ZD1839-gefitinib	SM	7	1	14

NUMBER AND PERCENTAGE OF STUDIES WITH SPECIFIED AND UNSPECIFIED GENDER



PREVALENCE OF MALE OVER FEMALE (OR VICEVERSA) IN ANALYZED STUDIES (NUMBER AND PERCENTAGE OF TRIALS REPORTED)



PREVALENCE OF M AND F IN ANALYZED STUDIES: DATA REPORTED FOR SINGLE AGENT

AGENT	CLASS	N STUDY	M=F; M>F; M<F*	NUMEROSITÀ M	F
AG013736	SM	1	1M<F	16	20
BEVACIZUMAB	AB	2	1M<F	8	17
BMS-214662	SM	5	2M>F	52	42
C225-cetuximab	AB	6	4M<F	147	158
CCI-779 temsirolimus	SM	4	3M>F; 1M=F	80	50
CI-1033	SM	5	2M>F	46	33
CI-994	SM	1	1M>F	15	11
COL-3 metastat	SM	2	2M>F	46	24
CP-724714	SM	1	1F		30
E7070-indisulam	SM	7	3M>F; 3M<F	184	152
EMD72000-matuzumab	AB	2	1M=F; 1M>F	24	16
Endostatin	SM	5	1M=F; 3M>F;1M<F	74	62
Enzastaurin	SM	3	1M<F	22	24
GW 572016 Lapatinib	SM	4	2M>F; M<F	61	85
ISIS 3521-affinitak	AS	4	3M>F; M=F	60	34
ISIS 5132	AS	2	1M>F; 1M<F	13	9
ISIS2503	AS	1	1M>F;	16	11
OSI774 -erlotinib	SM	7	5M>F; 1M	130	63
PS-341-Bortezomib	SM	2	1M>F	10	4
TRATUZUMAB-Herceptin	AB	1	1M>F	17	2
ZD1839-gefitinib	SM	7	5M>F; 1M<F	176	147

*data from unknown gender trials are not reported

AGE

- Median age: 58 years (range: 19-90, 2191)
- Broad Range : 63 years
  - range: 27-90 years, 36M and 33F
  - range: 22-85 years, 18M and 22F
- Narrow Range: 23 years (39-62, 3M and 5F)

DISCUSSION

What emerges from our analysis is:

- 44.02%, 36.5% and 19.04% of the population enrolled for PK analysis are respectively male, female, unknown gender
- Only 2.7% trials have enrolled exclusively male or female population:
- 65% vs 24% of the trials have male preponderance (unknown gender not considered)
- Authors do not report the number of male and female if only a group of patients enrolled in the trial submitted to pharmacokinetic analysis (25.6% of the analyzed studies)
- 2.7% (2/74) of the trials have reported the number of enrolled patients < 65 years:
  - range 40-73, 19M and 12F (21pts<65 years);
  - range 28-75, 25M and 39F (50pts<65years)
- 94.6% (70/74) of the trials enrolled patients > 65years
- 16.21% (12/74) of the trials enrolled patients >80years (295M and 213F)
- PK analysis is related to dose, route of administration and concomitant chemotherapy
- Only 4 papers have specified the number of male and female for each dose level
- Toxicity and Efficacy are not gender or age related.

Future development

During the following months data will be completed and critically revised. A selected target agent with consolidated data will be chosen to obtain individual patient data in order to evaluate whether results will be confirmed by such analysis. ■

TITLE

Psycho-social Variables, Circadian Rhythms, Circulating Cytokines and Disease Recurrence in Patients Affected by Colon-Rectum Neoplasm: a Longitudinal Study

COORDINATOR

Chiara Falcicchio Psychology

CO-RESEARCHERS

Maria Perrone
Patrizia Pugliese
Psychology

Carlo Garufi
Massimo Zeuli
Medical Oncology C, A

Marialuisa Appetecchia
Roberto Baldelli
Endocrinology

Giovanni Cigliana
Giuseppe Vercillo
Clinical Pathology

Holger Schünemann
Francesca Sperati
Epidemiology Department

Introduction

■ The aim of the project is to investigate: (**a**) the possible prognostic role of psycho-social factors and Quality of Life (QoL) variables on Progression Free Survival (PFS) of patients affected by stage III colon-rectum cancer who will be treated with adjuvant chemotherapy; (**b**) the presence of alterations both of circadian rhythms and biological factors (hormons and cytokines), and their possible relationship with psycho-social and demographic variables.

In order to achieve these aims, it was necessary to estabilish, before the project commenced, a temporary biological bank for storage at -80°C of the biological samples (serum, plasma, urines, saliva) with which project-related diagnostics analysis would be subsequently carried out.

The Operative Units of Medical Oncology, Psychology, Clinical Pathology, Endocrinology and Epidemiology are involved in this project.

The Project involved several phases:

First phase: due to the Project's complexity, the number of Operative Units involved and the exiguity of funds received, several meetings were needed to optimize the availability of resources.
It was decided to allocate the total amount of financing to a collaboration contract for a Psychologist who would coordinate all the Operative Units and perform the psychological assessment of enrolled patients. Moreover, several contacts have initiated with pharmaceutical companies in order to receive an estimated budget for the acquisition of the kits necessary for the project development. The actual cost of kits or disposable material necessary for biological sampling has used residual funds of a previous Institute project (QoL), coordinated by Service of Psychology.
Subsequently, a pathway has been set up in order to facilitate all the Operative Units activities and the patients' compliance.
A research-dedicated database was established in order to organize and handle all data coming from psycho-social variables, biological parameters and circadian rhythms.
Finally various internet contacts were initiated with the authors of PAC (Psychological Adjustement in Cancer) questionnaire, concerning both questionnaire utilization and potential collaboration for validating an Italian version. In this context the contacted authors debated the limitations of this instrument, and subsequently it was decided to replace PAC with a MAC questionnaire.

Second phase: Enrollment and evaluation of study patients.
Since February 2008, 22 patients have been enrolled, 18 (10 males, 8 females; mean age 64, range 36-75) met the inclusion criteria. Psychological variables assessment at baseline showed: the presence of an adequate social support (especially practical support) in 80% of patients (MOS questionnaire); high levels of anxiety and depression in 75% (POMS questionnaire); disadaptive copying strategies in 60% (COPE questionnaire). Furthermore, in 78% of patients, quality of life mean scores were seemingly lower than normative ones, especially regarding emotional functioning area (EORTC-QLQ C30 questionnaire). Finally, 85% of patients show both a disinvestment of sexual area and low future-perspectives (EORTC QLQ CR38 questionnaire).
Considering the large size of the sample needed to carry out the study, and the small number of patients affected by colon-rectum cancer attending the Institute (as already emphasized by Prof. Terzoli, Coordinator of Colo-Rectal Disease Management Team), project staff decided to propose a collaboration with other centers. To date, INT in Milan, Fatebenefratelli Hospital and Biomedical Campus in Rome have agreed to collaborate. ■

TITLE
Biological and dosimetric optimization for individualizing radioiodine treatment in advanced thyroid carcinoma using ^{124}I PET/CT

COORDINATOR
Carlo Ludovico Maini
Nuclear Medicine

CO-RESEARCHERS
Marcello Benassi
Medical Physics
Giuseppe Iaccarino
Medical Physics
Luisa Romano
Hematology
Rosa Sciuto
Nuclear Medicine
Francesca Sperati
Epidemiology
Lidia Strigari
Medical Physics
Giulio Vallati
Radiology
Antonello Vidiri
Radiologia

Introduction

■ The ^{124}I dosimetry has been proven in recent preliminary studies to be feasible and useful. The use of ^{124}I for absolute quantification requires expertise and a well-designed software package. ^{124}I bioimaging could be a promising approach in patients suffering from advanced differentiated thyroid cancer and could represent a new tool to optimize a reliable procedure for exact individual patient dosimetry and for patient specific organ dose–limited therapy.

The aim of this study is to validate a method of patient-specific dosimetry using ^{124}I PET/CT in patients with advanced thyroid carcinoma in order to maximize the therapeutic index of ^{131}I therapy. There are two specific endpoints of the study:
The first endpoint is the validation of dosimetry methodology that includes:
1. bioimaging with pre-treatment ^{124}I PET/CT and post-treatment ^{131}I SPECT with registration of CT/PET and SPECT images; 2. dose calculation method, based on the EGSnrc (electron-gamma shower) Monte Carlo code, used for calculating the absorbed dose distribution in 3 dimensions. This method accurately accounts for density and atomic number variations in tissue by direct electron- and photon-transport calculations and scoring of the energy depositions from each particle in a voxel-based representation of the patient.
The second endpoint is to verify the efficacy of this method for patient specific dosimetry in a randomized clinical trial comparing standard treatment using ^{131}I fixed activities versus patient-specific calculated ^{131}I activities.

Work in progress

The research included two different phases:

FIRST PHASE – VALIDATION AND FEASIBILITY

The first phase of the research (from October 2007 to March 2008) focused on the first end-point: validation of dosimetry methodology.

The validation of dosimetry included a phase of preclinical evaluation and afterwards clinical evaluation of feasibility.

a) Preclinical evaluation with phantom

A number of preliminary activities were performed in order to optimize the procedure and calibrate the instrumentation:

- Calibration of activity calibrator with ELIZA cell and of well calibrator (ATOMLAB 950)
- Setting acquisition parameters for PET – CT scanner. In fact high resolution tomographs (SIEMENS, Biograph HI-REZ) present a different sensitivity versus standard 2D tomograph requiring a different parameter setting to increase signal-noise ratio. A phantom containing 6 hot spheres, filled with ^{124}I and with a sphere-to-background ratio of 6:1, was scanned repeatedly at different times using the same time interval chosen for patients: emission and transmission scan durations of 6min/BED and 3 min/scan, respectively. Sphere-to-background ratios in the reconstructed images were determined and noise equivalent count (NEC) rates of ^{124}I only were measured over time. The settings giving the highest NEC rate and contrast were used for the clinical scans to obtain the highest quality images. An activity ranging from 111-148 MBq was adequate for patient imaging.

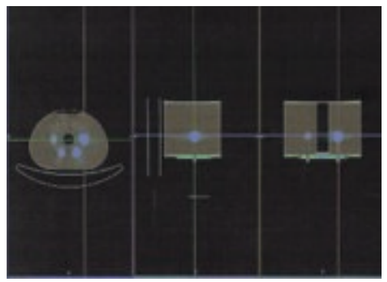


Figure 1: Emission and transmission scan of the phantom containing 6 hot spheres for sphere-to-background analysis.

The result of this phase was the optimitazion of administered activity and technical procedure.

b) Clinical evaluation:

Two patients were enrolled to validate bio-imaging protocol.
The patients selected had evidence of advanced disease and persistant distant macro-metastases after repeated treatments. A baseline ^{131}I WB scan was obtained documenting extensive bone metastatic disease. Before ^{124}I imaging and ^{131}I therapy, patients were prepared with rhTSH stimulation on the basis of cardiovascular risk.
Oral administration of 111 MBq of ^{124}I was carried out at day 0 and serial images of the patients were obtained. Serial images were obtained according to the protocol at approximately 4 - 6, 20, and 44 hours after radioiodine administration; additional early and late images were obtained to identify the best time for imaging. Blood samples and whole-body counts were obtained as required by this new thyroid imaging protocol for blood dosimetric evaluation.
For each scan, volumetric ROI of tumor volume and organs at risk (OARs) were delineated to calculate the volume and minimum/medium/maximum activity. About 400 images were delineated for each scan.

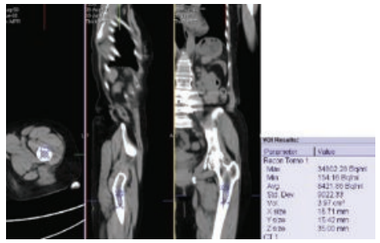


Figure 2: ROI delineation of a lesion

Blood samples were also measured and the corrected activity was obtained using a reference standard. A dataset of results was obtained for each patient, reporting the percentage of ^{124}I biodistribution. All data were corrected for the physical half-life and used for dosimetric evaluation.

The results of this preliminary analysis were as follows:

- PET imaging was sub-optimal for diagnostic aims so the tumor localization was assessed on TC imaging only
- tumor uptake was very low with respect to normal tissue, contrary to that experienced on the basis of 131-I whole-body uptake in the same patients

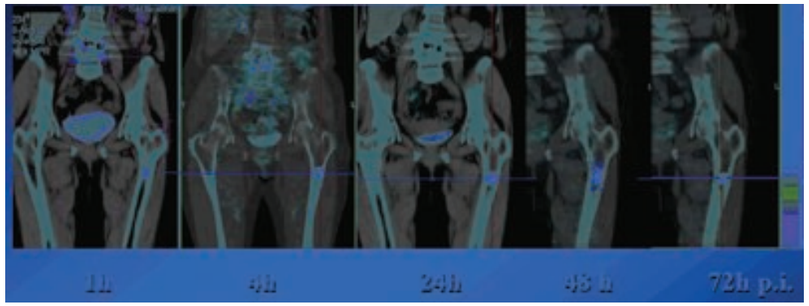


Figure 3: Co-registered PET/CT images over time in a patient

What is the reason for the observed sub-optimal ^{124}I uptake? The question was intriguing as preliminary literature suggests a better radiotracer performance in thyroid cancer imaging.

The actions adopted to answer the question were:

1. Quality control checks of the scanners that confirmed the correct performance of the instruments
2. Consulting manufacturers and application specialists to further check parameter settings. The adopted parameters were confirmed
3. Investigation of patients characteristics and method of preparation: the hypothesis of an influence of preparation modality (rhTSH) on the uptake was formulated.

This hypothesis was tested on a third of patients after informed consent. The patients underwent two ^{124}I studies: the first after rhTSH stimulation and the second after hormone withdrawal. The results obtained from the second study were completely different and optimal for diagnostic and dosimetric study. This issue has not been reported until now in published papers. This experience will be published as a case report to underline the important limitation of the ^{124}I imaging method.

SECOND PHASE: RANDOMIZED TRIAL

After method validation the second phase of the research was started: the randomized study.

From April 2008 to July 2008, four patients with bone metastases fulfilling inclusion criteria after the baseline valuation were enrolled, adequately prepared with hormone withdrawn and randomized in two groups:

Patients from Group A received a fixed dose of ^{131}I (180 mCi) according to clinical setting:

Patients from Group B underwent dosimetric evaluation according to the validated procedure. A prescribed mean dose of a 15 Gy to each lesion was chosen as reference to obtain tumor control while a maximum dose of 2 Gy to red marrow was assumed safe.

Dosimetric results are reported in TABLE 1

Patients	Administered Activity	Lesion localization	dose to tumor (Gy)		Tumor volume	Red marrow dose (Gy)
			Average	Maximum		
1.	250	D-2	21.2	124.2	26.4 cc	0.75
2.	270	L- 3	22.5	98.5	4 cc	0.17
		Left femur	16.3	36.6	1 cc	0.17

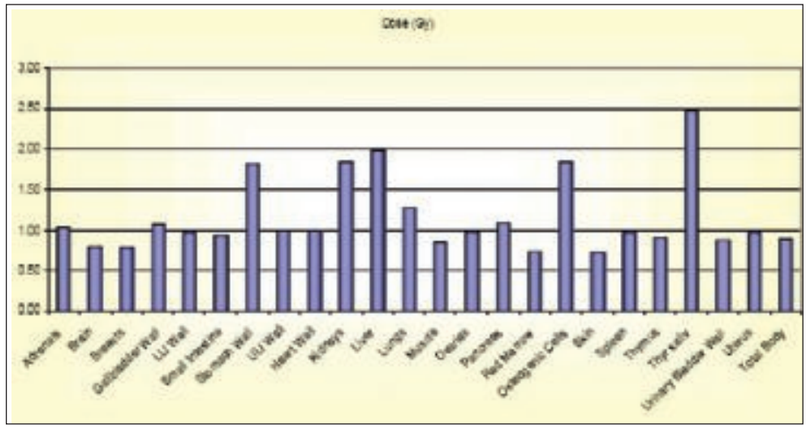


Figure 4: doses to OARs in patient n. 2

Analytical dose report for OARs (organs at risk) was obtained for each patient as reported in Figure 4.
Follow-up for efficacy and late toxicity is ongoing in both groups. No early toxicity was observed in patients.
In September 2008 a third patient was enrolled in Group B and is scheduled for ^{124}I dosimetry for November 2008. ■

TITLE
Individual susceptibility, genetic differences and bio-molecular mechanisms connected to regression/progression of hpv-correlate lesions

COORDINATOR
Luciano Mariani
Gynaecology

CO-RESEARCHERS
Maria Benevolo
Histology and Cytopathology
Federico De Marco
Virology
Ferdinando Marandino
Histology and Cytopathology
Marcella Mottolese
Histology and Cytopathology
Francesca Sperati
Epidemiology
Aldo Venuti
Virology
Enrico Vizza
Gynaecology
Amina Vocaturo
Histology and Cytopathology

COLLABORATORS
Paola Di Bonito
Italian Ministry of Health
Colomba Giorgi
Italian Ministry of Health
Aldo Morrone
San Gallicano Institute
Antonella Testa
ENEA, Casaccia Rome

First year report

1. INTERACTION BETWEEN GST GENOTYPES AND SMOKING HABITS AND THE RISK OF CERVICAL CANCER

■ The development and progression of cervical cancer (CC) is mainly due to infection with high-risk human papillomavirus (HR-HPV), and infection with HR-HPV types may be transient or can persist and cause acute low-grade squamous intraepithelial lesions (LSILs). If not treated, these lesions may progress to high-grade squamous intraepithelial lesions (HSILs), and to CC. It is also well recognized that most HPV-infected women do not develop CC and a small percentage of CC does not have evidence of HPV infection. Therefore, factors other than HPV, such as smoking, must contribute to the induction of CC. Smoking maintains cervical HPV infection for a longer period. Cigarette smoke carcinogen-specific products, such as N-nitrosamines and nicotine, have been found to concentrate in cervical mucus.
Glutathione S-transferases (GSTs) are a family of phase 2 enzymes involved in the detoxification of various exogenous as well as endogenous reactive species. Among these, *GSTM1*, *GSTT1*, and *GSTP1* enzymes play an important role in the detoxification of metabolites of carcinogens in tobacco smoke. Polymorphisms in these enzymes have been associated with a higher risk of different types of tumors in heavy smokers, suggesting a gene-environment interaction for cancer outbreak. The *GSTM1* and *GSTT1* genes exhibit a deletion polymorphism, which in the case of homozygosity (*GSTM1* null and *GSTT1* null) leads to the absence of phenotypic enzyme activity. A polymorphism in the *GSTP1* gene, in which valine (Val) replaces isoleucine (Ile) at position 105, affects the activity of the enzyme. Individuals with *GSTP1* Ile/Ile genotype have an increased risk of invasive CC, and this risk is higher among smokers.
The aim of this study is to investigate whether some GST polymorphisms could influence the risk of developing CC, either separately or in combination with a smoking habit, in a cohort of HR-HPV infected Italian women.

Table 1. Baseline characteristics of cases and controls

	CASES	CONTROLS
Sample size	81	111
Age (years)	41.7±12.3	36.3±10.1
Smoking habit		
YES	28	32
NO	34	45
no data available	19	34
HR-HPV infection	YES	NO

The study population comprises 81 women infected with HR-HPV and 111 controls, negative for HPV and free of any kind of malignancy. Cases and controls were recruited from the Regina Elena Cancer Institute, the San Gallicano Hospital and the Umberto I Hospital, Rome (Italy). All cases were classified according to the 2001 Bethesda classification guidelines.
Our results suggest that the risk of cervical cancer may be related to the *GSTM1* null, *GSTT1* null, and *GSTP1* AA genotypes in combination with HPV infection, but independently of smoking habits. These results, with respect to current literature, may strengthen the idea that a multifactorial mechanism is involved in the development of cervical cancer. However, larger sample sizes are needed to confirm these findings and to understand the possible interactions between the different GSTs polymorphisms and the different cervical lesions (LSIL, HSIL and CC) with respect to smoke and HPV infection.

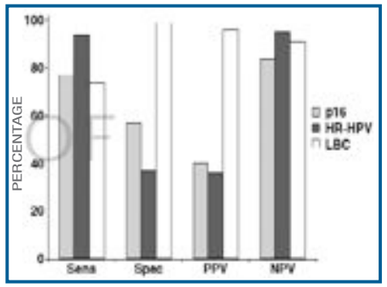
2. MOLECULAR METHODS AND PERSISTENT HPV-INFECTION

More than 300 samples were analyzed with different techniques to determine the genotype and the expression of HPV in cervix samples: discordant results among the utilized techniques were recorded in the same sample. The observed differences among the analyzed techniques with respect to analytical sensitivity, ability to discriminate between different HPV types, and ability to recognize multiple infections suggest that the existing assays still fail to identify all of the possible HPV types involved.
This justifies the development of new and improved HPV typing assays

- **Pilot study of the expression profile in clinical specimens**
  SPP1: secreted phosphoprotein 1 or Osteopontin, extracellular glycosylated bone phosphoprotein. Recently OPN was found expressed in many neoplastic tissues, such as colon, breast, prostate and lung carcinomas by oligonucleotide microarrays. We have set-up a quantitative RT-PCR assay for the detection in cervix carcinoma.
- **Gene expression profile and cell opoptosis**
  From previous studies conducted in the Virology Laboratory we know that E5 (an oncogene expressed in the first stages of infection/transformation of a part of the HPV16) interacts with the cell apoptic pathway. Therefore, we evaluated the expression profile of a genetic cluster connected to this pathway in the HaCaTE5 cell line, through the RT-PCR and RT Profile PCR Array Human Apoptosis (the table shows the genes investigated).
- **Analysis of the physical changes to virus**
  The W12 cell line was derived from a low-grade HPV16 positive lesion of the cervix and had been previously characterized as containing multiple copies of the HPV16 genome. Utilizing the multiple PCR for the E2 and E6 gene of HPV 16 we were able to detect the virus in a monomeric episomal form in the early stages (W12E) and integrated into the cell genome in later stages (W12G). As shown in the figure, the lack of band corresponding to the E2 gene indicates the loss of this gene during integration. Note that this integration with the loss of the E2 gene is a frequent event in cervix tumor progression.

3. ROLE OF P16^{INK4A} EXPRESSION IN LIQUID-BASED CERVICAL CYTOLOGY

p16INK4a is overexpressed in high-risk human papillomavirus (HR-HPV)–infected preneoplastic and neoplastic lesions of the uterine cervix. Our aim was to verify whether p16 is a diagnostic marker in cervical liquid-based cytology. We performed p16 immunocytochemical analysis and the Hybrid Capture 2 (HC2) test (Digene, Gaithersburg, MD) for HR-HPV infection, in 471 ThinPrep-processed (Cytyc, Boxborough, MA) cervicovaginal samples and correlated the results with histologic findings.



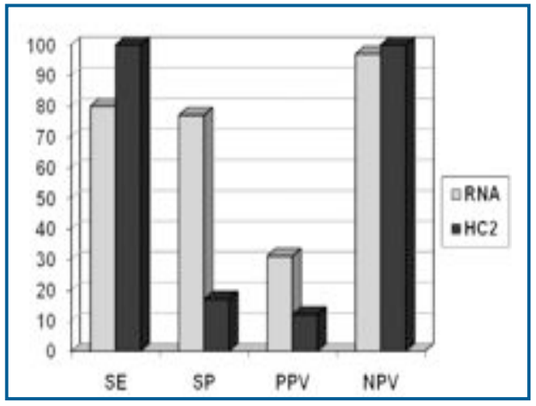
A total of 32.3% of the specimens showed p16 immunoreactivity, whereas the HC2 test was positive in 41.2% of the cases (65.2% concordance rate).
Correlating the cytologic, p16, and HPV results with histologic findings revealed HC2 as the most sensitive test for a diagnosis of cervical intraepithelial neoplasia 2 or worse, whereas cytologic examination was the most specific. The positive predictive value was significantly higher for cytologic examination than for p16 and HR-HPV testing. This data suggests that p16 evaluation in ThinPrep samples does not have a better clinical effectiveness for identifying high-grade lesions when compared to conventional morphologic examination and HPV-testing.

4. E6/E7 MRNA EXPRESSION AND DNA HPV TESTING VS CYTOLOGY IN LIQUID-BASED CYTOLOGY MEDIUM

The genes E6 and E7 of HR HPV are considered fundamental for conversion to and maintenance of malignancy. The detection of their RNA transcripts might be more useful, when compared to mere DNA tests, as a risk evaluation factor for active and potentially persistent infections. Moreover, the relative expression levels of E6 and E7 mRNA have been found to increase, together with the cervical disease severity. The aim of this study was to compare the detection of E6/E7 mRNA

	mRNA+	mRNA-	TOT
HC2+	127	246	373
HC2-	4	68	72
TOT	31	314	445

Human Papillomavirus (HPV) with detection of DNA HPV and to investigate whether E6/E7 mRNA could represent a more specific biomarker in identifying high grade cervical lesion, compared to HR-HPV DNA testing.
The study included 445 ThinPrep cervico-vaginal samples from patients sent to the Institute because of a clinical and/or a colposcopic suspicion of HPV infection or a previous abnormal Pap-test.



Cervicovaginal samples were taken by cytobrush (Cytyc) and plastic Ayre's spatula (Cytyc) and stored in the *PreservCyt* solution (Cytyc). The liquid-based cytology (LBC) Pap-Tests were performed by means of the Thin Prep 2000 System (Cytyc).
Testing for HR HPV was performed by means of the HC2 technique (Digene Corporation, Italy), a semi-quantitative signal-amplified hybridization assay for the chemiluminescent detection of the 13 most common HR HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68.
The PreTect HPV Proofer technology amplifies full-length E6/E7 mRNA of HPV types 16, 18, 31, 33 and 45.

The results are reported below:

HR-HPV DNA:
SENSITIVITY: 100%
SPECIFICITY: 17%
PPV: 12%
NPV: 100%

RNA-HPV:
SENSITIVITY: 80%
SPECIFICITY: 77%
PPV: 31%
NPV: 97%

These findings suggest that mRNA expression, evaluated in cervico-vaginal ThinPrep samples, although less sensitive than HR-HPV DNA testing, may be a promising biomarker in detecting HSIL specimens.
Larger studies are necessary to evaluate the predictive values of the mRNA HPV testing.

5. ANTIBODY RESPONSE TO HUMAN PAPILLOMAVIRUSES (HPVS) AS MEASURED BY A NOVEL ELISA TECHNIQUE

Seroreactivity is not a valuable parameter for the diagnosis of Human Papillomavirus (HPV) infection but it is potentially valuable as a marker of viral exposure in elucidating the natural history of this infection. More data is needed to assess the clinical relevance of serological response to HPV.
The objective of this sub-area was to assess the clinical/epidemiological correlates of HPV seroreactivity in a cohort of HIV-negative and HIV-positive women. Seroreactivity of 96 women, evaluated in an ELISA test based on denatured HPV16 late (L) and early (E) antigens, was correlated with their clinical and epidemiological data previously collected for a multi-centre Italian study, HPV-Pathogen ISS study.
No significant correlation was found between HPV DNA detection and seroreactivity. Female, smokers showed significantly less seroreactivity to L antigens as compared with non-smokers. HIV-positive women showed significantly less (66.7%) antibody response as compared with HIV-negative women (89.3%), with a particularly impaired response to L antigens. Women, HIV-positive and smokers, showed by far the lowest seroprevalence (33.3%) as compared to 75.9% among all other women (OR = 0.158; 95%CI 0.036–0.695, p = 0.014; Fisher's exact test). Importantly, this association did not lose its significance when controlled for confounding from age (continuous variable) in multivariate analysis or using Mantel-Haenszel test for age-groups.
HIV-positive smokers make up a special high-risk group, with a highly impaired immunological response that could prevent eradication of persistent HPV infections and thus contribute to the development of CIN3/CC.

6. HPV-PREVENTIVE STUDY IN MIGRANT WOMEN IN ROME (WITH SAN GALLICANO HOSPITAL)

The study was scheduled in order to organize a program of prevention against cervical cancer in female migrants in Rome, and therefore to facilitate access to appropriate preventive oncological facilities for discriminated women. The study will also investigate the risk factors and social conditions (HPV-subtypes, sexual behavior, smoking habits) of such women since their migration to Italy. Using a mother tongue questionnaire (with a cultural mediator) it will be possible to achieve data on social conditions and lifestyle. An HPV-testing (HC2) combined with Pap-test (with further genotype distribution) will be performed in all women enrolled in the study. Further diagnostic/therapeutic decisions will depend on the results of both tests.
Up-to-now we have enrolled 114 women. Scientific results, expected in the next few months, will increase cancer prevention awareness among female migrant populations.
The aim of the present study is focused on culturally appropriate intervention strategies aimed at limiting the disparities that migrants usually suffer in most of the developed western nations with respect to their native counterparts. ■

TITLE
Molecular screening for hereditary forms of breast, ovary and colon carcinoma: integrated evaluation and reclassification of genetic variants of uncertain clinical significance

COORDINATOR
Aline Martayan
Biologist

CO-RESEARCHERS
Anna Maria Cianciulli
Clinical Pathology
Maria Grazia Diodoro
Anatomical Pathology
Elisabetta Falvo
Clinical Pathology
Patrizio Giacomini
Immunology
Marcella Mottolese
Anatomical Pathology
Lupe Sanchez Mete
Digestive Endoscopy
Antonella Savarese
Medical Oncology "A"
Francesca Sperati
Epidemiology
Vittoria Stigliano
Digestive Endoscopy
Irene Terrenato
Epidemiology
Gaetano Vitelli
Clinical Pathology

First year report

■ Important cancer susceptibility genes are found in about 7% of breast cancer, 10% of ovarian cancer and 5-10% of colorectal cancer. Susceptibility is caused by specific mutations mainly in the following cancer susceptibility genes: BRCA1, BRCA2, MUTYH, APC and MMR genes. BRCA1 and BRCA2, involved in breast and ovary cancer, have multiple biological functions including participation in a pathway that mediates error-free repair of double strand DNA breaks by homologous recombination; MMR (MLH1, MSH2, MSH6 and PMS2) genes are implicated in DNA mismatch repair; MUTYH encodes a key glycosylase of the base-excision repair system (BER); APC has a key role in cell adhesion and also regulates colonic mucosa cell growth. Pathogenic mutations in these genes are responsible for: (a) Hereditary Breast and Ovary Cancer (HBOC), (BRCA1 and BRCA2 genes); (b) Colon cancer associated with multiple colorectal adenomas, including FAP (an autosomal dominant condition mostly due to mutations in the APC gene) and MAP (an autosomal recessive condition caused by mutations in the MUTYH gene); and (c) Hereditary Non Polyposis Colorectal cancer (HNPCC – now known as Lynch syndrome) caused by mutations primarily in MLH1, MSH2 and MSH6 genes. Genetic testing identifies different kinds of mutations that may be classified as follows: (a) pathogenic variants, (b) variants with no clinical significance, (c) variants with unknown clinical significance, (d) new variants. Whereas (a) and (b) allow complete diagnosis, (c) and (d) requires additional investigation. Despite the significant increase in sensitivity achieved with current tests, parameters such as predictivity, reliability and specificity remain to be optimized.

PROJECT AIMS

The general aim of the project was to devise a multidisciplinary approach integrating novel risk evaluation strategies for patient enrollment, novel molecular diagnosis pathways for genetic screening, and novel instruments for proper manage-

ment of this information. The primary aims were:

1) To adopt thorough genetic screening strategies such as: (a) full-length direct sequencing (sensitivity of 98%) of critical susceptibility genes (BRCA1, BRCA2, APC, MUTYH and MMR genes) in order to dramatically improve genetic testing sensitivity, (b) perform MLPA to search for medium to large size rearrangements in BRCA1, BRCA2, APC and MMR genes. Recently published data shows that this technique allows for the identification of large genetic alterations in approximately 10-20% of the cases tested negative for standard tests.
2) To use an integrated model that includes the analysis of epidemiological data in combination with an evolutionary approach to enable a more precise and accurate characterization of variants of unknown significance and facilitate a meta-analysis of the available case studies. This integration is possible thanks to new groundbreaking bioinformatics software.
3) To characterise the biological effect of variants of unknown significance by using either structural RNA analysis (mRNA expression of splice variants through RT- and QRT-PCR) or specific functional assays meant to compare the activity of the missense mutants versus WT genes.
4) To assess genomic instability by assessing MSI (Microsatellite Instability) in patients with suspected Lynch syndrome.
5) To identify, in the same patients, defects in the expression of specific MMR genes through the immunohistochemical analysis of colon cancer tissues.
6) To re-classify hereditary carcinomas with known BRCA1 and BRCA2 mutations and clinical history, by IHC staining of tumor lesions in our rich retrospective archival collection. The prognostic relevance of this classification and its possible relationship with specific mutations would then be assessed. Primary objectives would then be the starting point for strategies aimed at achieving the following secondary objectives:
   - new patient classification and recruitment strategies; improved and more accurate genetic screening approaches;
   - improved immunohistochemical and surrogate molecular markers of genetic predisposition to cancer;
   - more rationale decision-making strategies for patient management and follow-up (particularly for low to intermediate risk cohorts);
   - identification and characterization of new disease variants.

ACHIEVED GOALS

Patient recruitment

- For the enrollment of probands with diagnosis suspicion of HBOC, improved recruitment criteria have been used: probands were ranked depending on their genetic risk according to the current criteria of Modena and statistical probability models (Gail, Claus, Frank, BRCAPro), developed to estimate the likelihood of bearing mutations in BRCA1/2, were used to help both the counsellor and the proband in making genetic testing decisions. T**able 1.**
- Patients with features of the Lynch syndrome, according to Amsterdam II criteria, were considered for immunohistochemical evaluation (IHC) to search for MMR gene expression defects. **Table 2.**
- Patients with diagnosis suspicion of FAP or AFAP were recruited according to their medical history and clinical characteristics. **Table 3.**

Improved molecular testing procedures

- Full-length sequencing has so far almost completely replaced our former SSCP screening technique except for BRCA1 and BRCA2 genes due to the huge size of their coding sequences.
- MLPA technique has been successfully used for the detection of exon/s deletion in the APC gene: two new genomic rearrangements have been identified. This technique, as we expected, has therefore been crucial in the identification of the molecular defect in two unsolved cases of "classical FAP". All first degree relatives are now being tested to ascertain whether they have inherited the same genomic rearrangement. During the coming months other samples from subjects with diagnosis suspicion of Lynch Syndrome, hereditary breast and ovary cancer and FAP, with so far non-detected molecular defects, will be tested with MLPA technique.
- We started using the "Align GVGD analysis" for the characterization of variants of BRCA1 and BRCA2 of unknown clinical significance with encouraging results that are in accordance with the most recent information obtained from scientific literature.
- In the case of intronic and exonic variants virtually affecting a splicing site or a consensus motif, innovative bioinformatics tools are being extensively used, such as the following: NetGene2serve (http://www.cbs.dtu.dk/services/NetGene2/), GeneScan (http://genes.mit.edu/GENSCAN.html) and SpliceView (http://l25.itba.mi.cnr.it/webgene/wwwspliceview.html).
- The data from the above in silico testing tools have been verified by RT-PCR in order to detect and approximately quantify aberrant mRNA splicing products in the test sample. In the coming months QRT-PCR will be per-

P.n.	Age	Sex	Affected proband	Diagnosis	Gene tested 1	Gene tested 2	Test 1	Test 2	Test 3	Results
Br1	48	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	BRCA2+
Br2	50	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	BRCA2+
Br3	50	F	Y	HBOC	BRCA1	-	DS	-	-	BRCA2+
Br4	62	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	BRCA1+
Br5	51	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	UV
Br6	52	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	BRCA2+
Br7	71	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	UV
Br8	34	F	N	HBOC	-	BRCA2	DS	-	-	BRCA2+
Br9	38	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	UV
Br10	31	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	UV
Br11	48	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	UV
Br12	64	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	UV
Br13	58	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	BRCA2+
Br14	52	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	P
Br15	71	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	BRCA1+
Br16	62	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	NAF
Br17	35	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	BRCA2+
Br18	40	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	P
Br19	51	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	P
Br20	48	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	P
Br21	31	F	N	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	P
Br22	40	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	P
Br23	47	M	Y	HBOC	BRCA1	-	DS	-	-	BRCA1+
Br24	49	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	NAF
Br25	52	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	UV
Br26	59	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	NAF
Br27	48	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	UV
Br28	49	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	P
Br29	37	F	N	HBOC	BRCA1	BRCA2	DS	-	-	P
Br30	53	M	Y	HBOC	-	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	P*
Br31	51	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	P*
Br32	44	F	N	HBOC	-	BRCA2	DS	-	-	NAF
Br33	56	M	Y	HBOC	-	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	P*
Br34	62	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	UV*
Br35	55	M	Y	HBOC	-	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	UV*
Br36	49	M	Y	HBOC	-	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	P*
Br37	47	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	UV*
Br38	57	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	UV*
Br39	56	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	P*
Br40	66	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	P*
Br41	62	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	UV*
Br42	48	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	UV*
Br43	47	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	UV*
Br44	42	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	-	-	BRCA2+
Br45	46	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	UV*
Br46	52	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	P*

Table 1
Cases analysed for BRCA1 and BRCA2 genes pathogenic alterations

P.n.	Age	Sex	Affected proband	Diagnosis	Gene tested 1	Gene tested 2	Test 1	Test 2	Test 3	Results
Br47	51	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	UV*
Br48	42	F	N	HBOC	-	BRCA2	DS	-	-	NAF
Br49	42	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	P*
Br50	60	F	Y	HBOC	BRCA1	BRCA2	SSCP/DS	(MLPA)	(RT-PCR)	P*
Br51	63	F	Y	HBOC	BRCA1	BRCA2	DS	-	-	UV
Br52	38	M	N	HBOC	BRCA1	BRCA2	DS	-	-	UV
Br53	43	M	N	HBOC	BRCA1	BRCA2	DS	-	-	P
Br54	47	M	N	HBOC	BRCA1	BRCA2	DS	-	-	P
Br55	49	F	N	HBOC	-	BRCA2	DS	-	-	BRCA2+
Br56	29	F	N	HBOC	-	BRCA2	DS	-	-	BRCA2+
Br57	30	F	N	HBOC	-	BRCA2	DS	-	-	BRCA2+

DS: direct sequencing
MLPA: Multiple Ligation Dependent Primer Amplification
RT-PCR: Reverse Transcription PCR
SSCP: Single Strand Conformation Polymorphism
NAF: No Alteration Found
P: Polymorphism/s
N: Not affected
Y: Affected
UV: Unclassified Variants
* : Partial result, testing of another gene in progress
HBOC: Hereditary Breast and Ovary Cancer syndrome

P.n.	Age	Sex	Affected proband	Diagnosis	Gene tested 1	Gene tested 2	Gene tested 3	test 1	test 2	test 3	Results
Ly1	68	F	Y	LYNCH	MLH1	-	-	SSCP/DS	-	-	NAF
Ly2	61	F	N	LYNCH	MLH1	-	-	SSCP/DS	-	-	P
Ly3	68	M	N	LYNCH	MLH1	-	-	SSCP/DS	-	-	P
Ly4	81	F	Y	LYNCH	MLH1	-	-	SSCP/DS	-	-	P
Ly5	50	F	Y	LYNCH	MLH1	-	-	SSCP/DS	-	-	P
Ly6	34	M	Y	LYNCH	MLH1	-	-	SSCP/DS	-	RT-PCR	MLH1 +
Ly7	54	F	N	LYNCH	MLH1	-	-	SSCP/DS	-	RT-PCR	MLH1 +
Ly8	31	F	Y	LYNCH	MLH1	-	-	SSCP/DS	-	RT-PCR	MLH1 +
Ly9	35	M	Y	LYNCH	MLH1	-	-	DS	-	-	MLH1 +
Ly10	68	F	Y	LYNCH	MLH1	-	-	DS	-	-	UV
Ly11	61	M	Y	LYNCH	-	MSH2	-	DS	-	-	MSH2+
Ly12	52	M	Y	LYNCH	MLH1	-	-	DS	-	-	NAF
Ly13	61	M	Y	LYNCH	MLH1	-	-	DS	-	-	P
Ly14	51	M	Y	LYNCH	MLH1	MSH2	MSH6	DS	(MLPA)	(RT-PCR)	P*
Ly15	49	M	N	LYNCH	-	MSH2	MSH6	DS	(MLPA)	(RT-PCR)	NAF*
Ly16	64	M	N	LYNCH	-	MSH2	MSH6	DS	(MLPA)	(RT-PCR)	P*
Ly17	38	M	N	LYNCH	-	MSH2	MSH6	DS	(MLPA)	(RT-PCR)	P*
Ly18	51	M	Y	LYNCH	-	MSH2	MSH6	DS	(MLPA)	(RT-PCR)	P*
Ly19	21	M	N	LYNCH	-	MSH2	MSH6	DS	(MLPA)	(RT-PCR)	UV*
Ly20	24	F	N	LYNCH	-	MSH2	MSH6	DS	(MLPA)	(RT-PCR)	P*

DS: direct sequencing
MLPA: Multiple Ligation Dependent Primer Amplification
RT-PCR: Reverse Transcription PCR
SSCP: Single Strand Conformation Polymorphism
NAF: No Alteration Found
P: Polymorphism/s
N: Not affected
Y: Affected
UV: Unclassified Variants
* : Partial result, testing of another gene in progress

Table 2
Cases analysed for MLH1, MSH2 and MSH6 genes pathogenic alterations

formed to quantify precisely the aberrant versus wild type mRNA molecule in the test sample.

- Neoplastic lesions of patients with a diagnosis suspicion of Lynch syndrome have been pre-screened for the expression of the major four MMR gene products (MLH1, MSH2, PMS2 and MSH6) by Institute pathologists. The lack of expression of either of the MMR genes has defined which gene to analyse in order to identify the inherited genetic defect underlying the Lynch syndrome. Germline mutations in MLH1 and MSH2 account for 90 percent of Lynch syndrome, while mutations in MSH6 and, to a lesser extent, PMS2 are responsible for the remaining 10 percent. We therefore started with MLH1 and MSH2 genes. In a few cases, however, we were unable to find any molecular defect in these genes despite their lack of expression in the tissue sample tested. In the case of MLH1 this might be due to epigenetic modifications such as promoter methylation. To address this issue PCR-based methylation testing will be performed. Besides, since MSH2 and MSH6 functionally interact, a simultaneous loss of both proteins in IHC is usually expected. Immunohistochemical analysis on sections of neoplastic lesions showed a lack of expression of both MSH2 and MSH6 genes in approximately 40% of the samples tested. MSH6 gene mutations have been mostly observed in atypical HNPCC families, characterized by a weaker tumor family history, higher age at disease onset, and low degrees of microsatellite instability and according to recent data, they may have a larger contribution than previously recognized. We therefore started screening for molecular defects in MSH6 gene in a subgroup of candidate patients.

P.n.	Age	Sex	Affected proband	Diagnosis	Gene tested 1	Gene tested 2	Test 1	Test 2	Test 3	Results
Fa1	54	F	N	FAP	APC	-	DS	-	-	NAF
Fa2	30	F	Y	FAP	APC	-	SSCP/DS	MLPA	RT-PCR	APC Del ex 9-10-10A
Fa3	53	F	Y	FAP	APC	MUTYH	SSCP/DS	MLPA	RT-PCR	APC Del ex 14*
Fa4	34	F	Y	FAP	APC	MUTYH	DS	MLPA	-	NAF
AF5	61	M	Y	AFAP	APC	MUTYH	SSCP/DS	MLPA	-	P
AF6	75	M	Y	AFAP	APC	MUTYH	DS	MLPA	-	MUTYH +/+
AF7	39	F	Y	AFAP	APC	MUTYH	DS	MLPA	-	P
AF8	65	F	Y	AFAP	APC	MUTYH	DS	MLPA	-	P
Fa9	32	M	Y	FAP	APC	-	DS	MLPA	-	NAF
AF10	24	F	Y	AFAP	APC	MUTYH	DS	MLPA	-	P
AF11	14	M	N	AFAP	-	MUTYH	DS	-	RT-PCR	MUTYH+/-
AF12	12	M	N	AFAP	-	MUTYH	DS	-	RT-PCR	MUTYH+/-
AF13	66	F	Y	AFAP	-	MUTYH	DS	-	-	P
AF14	60	F	Y	AFAP	-	MUTYH	DS	-	-	NAF
AF15	73	M	Y	AFAP	-	MUTYH	DS	-	-	NAF
Fa16	33	M	N	FAP	APC	-	DS	MLPA	-	NAF
Fa17	41	M	Y	FAP	APC	-	DS	-	-	IN PROGRESS
AF18	60	M	Y	AFAP	-	MUTYH	DS	-	-	MUTYH+/+
AF19	40	M	N	AFAP	-	MUTYH	DS	-	-	IN PROGRESS
AF20	65	F	Y	AFAP	APC	MUTYH	DS	(MLPA)	-	IN PROGRESS
AF21	41	F	N	AFAP	-	MUTYH	DS	-	-	MUTYH+/-
Fa23	57	M	Y	FAP	APC	-	DS	(MLPA)	-	IN PROGRESS
Fa24	64	F	Y	FAP	APC	MUTYH	DS	(MLPA)	-	IN PROGRESS

DS: direct sequencing
MLPA: Multiple Ligation Dependent Primer Amplification
RT-PCR: Reverse Transcription PCR
SSCP: Single Strand Conformation Polymorphism
NAF: No Alteration Found
P: Polymorphism/s
N: Not affected
Y: Affected
* : Partial result, testing of another gene in progress
FAP: Familial Adenomatous Polyposis
AFAP: Attenuated Familial Adenomatous Polyposis

Table 3
Cases analysed for APC and MUTYH genes pathogenic alterations

GOALS

- About 70 new patients have been enrolled so far (see tables 1-3) and approximately 30 to 40 new patients with diagnosis suspicion of hereditary cancer syndromes will be recruited for genetic testing within the next 6 months.
- We aim to finish genetic screening of the first group of patients within a few weeks and are planning to start

recruiting new patients in the next weeks.

- MSI analysis has just started in collaboration with the Department of Anatomical Pathology and the results will help us to make the correct decision in those cases where no genetic alterations in candidate MMR genes were found. If MSI is detected then we will look further at other MMR genes or with other analytical procedures (e.g., MLPA)
- PCR-based methylation testing in samples with a lack of expression of MLH1 in IHC and with no detected genetic alteration, will also help us to make the correct decision on whether to proceed further with molecular screening or to classify the MLH1 gene in that sample as epigenetically modified.
- In the case of BRCA1 and BRCA2 genes almost 40% of the genetic alterations detected are of unknown clinical significance; as already mentioned, a great effort has to be made for the characterization of these genetic defects in order to provide more effective tailor-made treatment and follow-up strategies. The first data arising from "Align GVGD analysis" is very interesting and it would be critical to test our results in functionality assays. To this end, we have already prepared the experiment design and assessed which cell lines, cloning devices and plasmids to use for each cancer susceptibily gene. We envisage starting this in the coming months.
- As far as reclassification of hereditary carcinomas with known BRCA1 and BRCA2 mutations is concerned, we intend to start in collaboration with the Anatomical Pathology Unit in the coming months.
- From the beginning of 2009 we will focus on the preparation of scientific manuscripts on the considerable amount of interesting data that has arisen from our studies; to this end we already have a case report in progress. ■

TITLE

Molecular stratification to classify colorectal and non-small-cell lung cancer patients for clinical outcome after treatment with epidermal growth factor receptor tyrosine kinase inhibitors

COORDINATOR

Roberta Merola
Cytogenetic Clinical Pathology

SENIOR INVESTIGATORS

Anna Maria Cianciulli
Cytogenetic Clinical Pathology

Carlo Garufi
Medical Oncology "C"

Diana Giannarelli
Biostatistics

Michele Milella
Medical Oncology "A"

Marcella Mottolese
Anatomical Pathology

JUNIOR INVESTIGATORS

Carla Campanella
Medical Oncology "C"

Carmen Nuzzo
Medical Oncology "A"

Giulia Orlandi
Cytogenetic Clinical Pathology

Isabella Sperduti
Epidemiology

First year report

■ A tremendous increase in the understanding of cancer biology has led to the strategic discovery of targets for the treatment of cancer. Identification of these targets has enabled the discovery of drugs that effectively inhibit those targets. The major challenges remaining in the area of targeted agents include patient selection strategies and the optimal use of these compounds in combinations.

Molecular alterations along the EGFR pathway have been proposed as being the major determinant of clinical outcome in response to EGFR tyrosine kinase inhibitors (TKI) in NSCLC patients and to monoclonal antibodies (mAbs) that target the extracellular domain of EGFR in colon cancer.

Therefore, this project is characterized by a highly multisciplinary approach, including both basic and clinical research.

We aimed to comparatively analyze a number of molecular, cytogenetics and phenotypic parameters to identify those markers that should improve individual therapeutic targets.

This project was divided into the following aims:

A) To obtain an overview of genetic variables (EGFR amplification, Chromosome 7 polysomy, gene copy number) and to determine the levels of protein expression in immunohistochemical assays in advanced lung and colon tumors;
B) To compare the individualized molecular cytogenetics characteristics of these tumors with protein expression, mutations and AKT activation status;
C) To associate these variables with clinical outcome (objective response, progression disease, median survival);
D) To identify those patients who benefit from target-therapy on a molecular basis.

The overall goal of this translation research project is to perform a molecular stratification of NSCLC and colorectal patients for therapy with EGFR antagonists.

During this first year we principally focused on:

- Enrollment and patient follow-up;
- Sample collection to set up conditions scheduled for next year;
- Organization of all paper work (personal files) necessary to maintain all clinical information;
- Protocol standardization for molecular assays;
- Assessment of genetic and phenotypic variables characterizing each patient enrolled (milestone A);

Experimental plan (milestone A) for the first year should provide preliminary information about the scientific development of other milestones (B, C, D) in the following year.

Results

PATIENT'S ENROLLMENT

In the first year a prospective series of 188 lung and 101 colorectal cancer patients with a pathologically confirmed diagnosis were enrolled for genetic and phenotypic characterization (*estimated sample size =340*). The clinical characteristics of patients are illustrated in Tables 1 and 2.

Patient characteristics	Number	%
Sex		
Male	112	59.6
Female	76	40.4
Median age (years range)	61 (31-81)	
Stage of disease		
III	35	18.6
IV	153	81.4
Histology		
Adk/BAC	125	66.5
Squamous	17	9
Other	46	24.5
Smoking History		
Non smoker	60	31.9
Current smoker	56	29.8
Former smoker	55	29.3
Not available	17	9

Table 1. Lung cancer patient's characteristics

Patient characteristics	Number	%
Sex		
Male	63	62.4
Female	36	35.6
Median age (years range)	63 (26-80)	
Performance Status		
0	61	71
1	23	27
>1	2	2
Primary tumor site		
Colon	79	78.2
Rectum	22	21.8
Liver metastases	79	78.2
Number of metastatic sites		
1	77	76.2
>1	24	23.8

Table 2. Colon cancer patient characteristics

Patients will receive standard treatment as first line chemotherapy and antagonist EGFR at progression disease.

STATISTICAL ANALYSIS

From separate single unit databases a new Access 2006 Database has been created, summarizing pertinent study information.

SAMPLE COLLECTION

To provide adapted material for molecular assays, on the basis of published guidelines (J Clin Onc 26:983-994,2008) we paid particulary attention to pre-analytical phase parameters:

- *tumor heterogeneity* (at least 3 representative areas were assessed for tumor section if possible)
- *sample type (*sample type was recorded such as excision, biopsy)
- *minimum sample size (*for FISH analysis at least 100 assessable tumor cell nuclei were evaluated)
- *sample collection and storage* (fixation and paraffin embedding were standard; samples were stored as a block, not as precut slides, since long term storage of cut sections resulted in decreased quality of FISH analysis)

If these parameters were not observed, the samples were considered not-evaluable.

PROTOCOL STANDARDIZATION FOR MOLECULAR ASSAYS

Cytogenetic Evaluation

The aim of this unit is to exploit our expertise and background to address specific issues relevant to identifying different patient subgroups, based on variation in genetic patterns and to identify new molecular markers of stratification, to classify colorectal and non-small-cell lung cancer patients for clinical outcome after treatment with EGFR-targeted therapies.

In order to evaluate chromosome 7 and EGFR gene *status*, the cytogenetic unit staff essentially concentrated on improving the FISH (fluorescence in situ hybridization) protocol on formalin-fixed paraffin-embedded section and, on the basis of cut-off values, on establishing the FISH criteria in two evaluated neoplasias.

FISH was performed using the EGFR assay kit (Vysis, Inc., Downers Grove, IL), which includes two directly labeled DNA probes: a locus specific probe for EGFR gene labeled with SpectrumOrange (LSI EGFR) and an alpha satellite probe targeting the centromere region of chromosome 7 labeled with SpectrumGreen (CEP 7). The assay was performed according to the manufacturer's instructions.

Fluorochrome signals were captured individually and images were generated via computer with Quips Genetic Workstations and Imaging Software (Vysis). The slides were observed at 100x magnification. At least 100 well-defined nuclei were scored for each hybridization. Clumps, overlapping nuclei and tumor infiltranting leucocytes were disregarded. Only nuclei with unambiguous chromosome 7 centromeric hybridization signals were scored for the EGFR signal numbers. FISH results included hybridization signal counts from at least 100 tumor cells that meet the morphologic and quality criteria for evaluation.

We created a FISH scoring system including EGFR gene copy number, chromosome 7 copy number alterations and the average signals of gene to chromosome 7 (ratio). This counting system stratifies our results as follows:

- $r \geq 2.0$ amplified
- $2 < r < 4$ low amplified
- $4 < r < 10$ moderate amplified
- $r > 10$ high amplified

As regards chromosome 7 numerical status, we defined a sample as monosomic or polysomic when cancer cell population showed single or multiple (>3) 7 centromere signals, respectively.

It is important to note that an increased EGFR gene copy number most frequently results from chromosome 7 polysomy, whereas true EGFR amplification, which is defined as the presence of tight EGFR gene clusters and a gene copy number to chromosome 7 centromere number ratio of ≥ 2, occurs less often. For this reason polysomic specimens are further stratified as:

- $3 < x < 5$ signals/cell low polysomy 7
- $x > 5$ signals/cell highpolysomy 7

Immunohistochemistry Analysis
Immunohistochemical staining was carried out on 5-µm-thick paraffin-embedded tissues. Sections were harvested on SuperFrost Plus slides (Menzel-Glaser, Braunschweig, Germany) and were incubated in a heater at 70°C for 20'.
EGFR expression was assessed by indirect immunoperoxidase staining with EGFR-pharmDx-kit (Dako, Milan, IT) which includes a Proteinase k, the peroxide solution, the EGFR antibody and a sensitive polymer-HRP system with DAB chromogenic substrate.
The deparaffinized and rehydrated sections were pretreated in a enzime solution (Proteinase-k) at room temperature for 5' and after the block of peroxidase sections were incubated 30' with the anti-EGFR (IgG1, monoclonal antibody). The immunoreactions were revealed by a sensitive polymer-HRP system for 30' using DAB as a chromogenic substrate. All sections were slightly counterstained with Mayer's hematoxylin and mounted in aqueous mounting medium (UCS Diagnostics, Rome, IT). EGFR was evidenced in the membrane of neoplastic cells and was scored considering a percentage of staining with a cut off of 10%. An intensity score was also taken into account (1+, 2+, 3+). Evaluation of the immunohistochemical results was carried out independently and blinded by two investigators.

SECTION PRELIMINARY RESULTS

In this report, we divide the results of the genetic and phenotypic characterization into two specific sections for lung and colon cancer:

Section 1. Lung cancer
In Table 3 we present the genetic and immunohistochemical results obtained in lung cancer evaluation. In 128 (68.1%) of analysed samples EGFR was not amplified, 14 (7.4%) showed EGFR gene amplification with ratio>2. On the basis of polysomy values we identified two groups of tumors:
- 97 (51.6%) tumors with EGFR gene signals between 3- 5 copies
- 20 (10.6%) tumors with EGFR gene signals > 5

In Fig.1 we show an example of advanced non-small cell lung carcinoma showing EGFR gene amplified with ratio = 5 (moderate amplification).
EGFR protein expression showed the higher percentage for score 3 (29.8%) [Fig. 2].

PATTERN FISH	N	%
EGFR Amplification		
No	128	68.1
Yes	14	7.4
ne	46	24.5
EGFR Polysomy		
No	14	7.4
Low Polysomy (3 - 5 copies)	97	51.6
High Polysomy (>5 copies)	20	10.6
ne	57	30.3
EGFR PROTEIN EXPRESSION SCORE (IHC)		
0	64	34
1	13	6.9
2	55	29.3
3	56	29.8

Ne: not evaluable

Table 3

2.5.2. Section 2. Colon cancer
As illustrated in Table 4, on the basis of established criteria, EGFR gene amplification was seen only in 4/101 patient tumor samples (4%).
In 61/101 (60.4 %) of colon cancer specimens, polysomy 7 was present: 41 (40.6%) with low polysomy (from three to five EGFR gene signals number/cell), while 20 (19.8%) with high polysomy (more than five EGFR gene signals number/cell) [Fig. 3].
EGFR protein was overexpressed in 78 out of 101 patients (89.1%), 22 with a 1+ staining score, 40 with 2+ score and 28 with 3+ score [Fig. 4].

PATTERN FISH	N	%
EGFR amplification		
No	79	78.2
Yes	4	4.0
ne	18	17.8
EGFR Polysomy		
No	28	27.7
Low Polysomy (3 - 5 copies)	41	40.6
High Polysomy (> 5 copies)	20	19.8
ne	24	11.9
EGFR PROTEIN EXPRESSION SCORE (IHC)		
0	9	0.9
1	22	21.8
2	40	39.6
3	28	27.7

Ne: not evaluable

Table 4

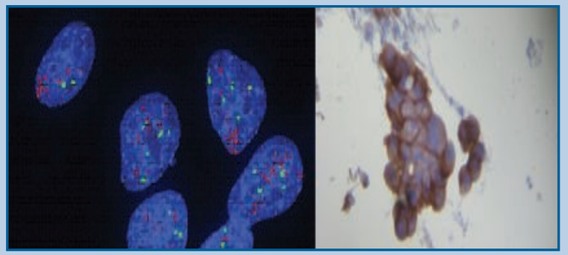


Fig. 1

Fig. 2

Fig. 1 FISH image:
CEP 7 (green) and EGFR gene (red) showing moderate amplification (ratio=5) in NSLC cancer. (100X)

Fig. 2
Lung cancer sample showing a group of neoplastic cells strongly positive for EGFR (20x)

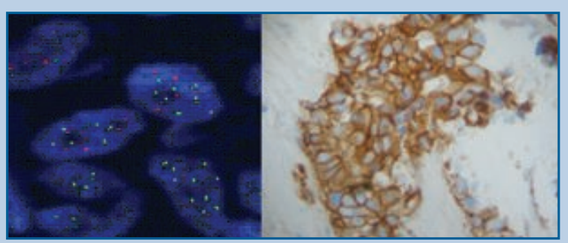


Fig. 3

Fig. 4

Fig. 3 FISH image:
CEP 7 (green) and EGFR gene (red) showing high polysomy in advanced colon cancer. (100X)

Fig. 4
Primary colon cancer displaying an intense plasma membrane immunostaining for EGFR (20X)

3. OBJECTIVES

This prospective study is currently ongoing:

- We are planning to recruit a sufficient number of patients (a minimum of 51) to achieve the estimated sample size of 340 patients in the next few months;
- We are creating a large DB collecting all information on EGFR pathway alterations patients, to identify the molecular determinants of response to clinical outcome;
- Statistical analysis will validate which EGFR biomarkers are considered as prognostic and/or predictive factors for cancer treatment and Multiple Corrispondence Analysis will be used to identify distinct biological profiles. ■

TITLE

Gene expression and phenotypic profiles in breast cancer and adjacent non-involved tissue as markers for early detection of malignant transformation

COORDINATOR

Marcella Mottolese Histology and Citopathology

CO-INVESTIGATORS

Department of Pathology:
Barbara Antoniani Technician
Anna Di Benedetto Biologist
Ferdinando Marandino Pathologist
Elisa Melucci Biologist
Flavia Novelli Biologist
Letizia Perracchio Pathologist
Steno Sentinelli Pathologist
Paolo Visca Pathologist

Department of General Surgery "A":
Claudio Botti Surgical Oncologist
Franco Di Filippo Surgical Oncologist

Laboratory of Immunology:
Pier Giorgio Natali Immunologist

Department of Medical Oncology:
Alessandra Fabi OMA, Medical Oncologist
Cecilia Nisticò OMC, Medical Oncologist

R.O.C.:
Giovanni Blandino Molecular Biologist
Silvia D'Agostino Molecular Biologist

Department of Epidemiology:
Francesca Sperati Biostatistician

First year report

■ The aims of this project, characterized by a highly multidisciplinary approach, were:

a) Identification, through Affymetrix technology, of gene expression and microRNA profiling patterns in morphologically normal peritumoral tissues (PTTs) and the companion malignant breast cancer (BC) classified according to the novel molecular BC taxonomy.

b) Evaluation of Estrogen ∂ (ER∂) and ß (ERß) and Progesterone receptors (PgR) modulation in BC and autologous PTTs sampled from premenopausal BC patients

c) Prognostic role of ER∂ in BC classified according to molecular subtypes

d) Determination of the role of cyclooxygenase-2 (COX2) in the regulation of BC apoptosis

e) Identification, by immunohistochemistry (IHC) of changes in proliferation (Ki-67, HER-2, EGFR, Cyclin-D1) and apoptosis related molecules (p53, bcl-2, Fas system) in PTTs in comparison with non-containing cancer normal breast and BC molecular subtypes

f) Predictive relevance of altered Fas/FasL system in BC and in CD3+ infiltrating lymphocytes analyzing BC sampled from patients submitted to adjuvant therapy.

During the first year we focused on the following issues which concerned the first three points (a,b,c) planned in our grant proposal:

A. TISSUE SAMPLE COLLECTION

An accurate tissue sample collection (frozen breast cancer tissues and paraffin embedded tissues) which is the essential and necessary basis on which to carry out the project, represented one of the main activities during this first year.

Frozen tissue collection

1. According to the sample size calculation reported in our project, we collected 130 frozen tissue specimens from BC patients (premenopausal and postmenopausal patients) surgically treated at the Institute during the last 12 months. For each patient we sampled two different biopsies, one from the tumor itself and one from the uninvolved PTT area.
   Moreover, biopsies from 35 healthy breast tissues obtained from reductive mammoplasty were obtained from the surgical department of our Institute.
   When possible, we sampled at least two specimens for each tumor. Biopsies were frozen in liquid nitrogen immediately after surgical excision and the specimens were stored at –80° until their use.

Paraffin embedded tissue collection

2. We collected paraffin embedded tissues from 66 premenopausal breast cancer patients and autologous PTTs together with 33 benign lesions. All patients were women under 50 years whose menstrual cycle phase was accurately determined through multiple serum progesterone evaluation.
3. We selected a large series of BC patients with complete follow up data in order to verify whether phenotypic and molecular alterations eventually found in PTTs can also be used to identify BC in groups differing in biology, behaviour, response to therapy and outcome, focusing on ER∂, ERß and PgR.
4. We set up a dedicated database in which we collected pathological and biological data from the patients. In particular, we focused on hormonal receptor and HER2 status in order to accurately stratify the BC patients according to their molecular characteristics. Therefore through immunohistochemical methods, using a few protein biomarkers (eg, ER, PgR, HER-2, basal cytokeratins), we were able to identify Luminal A, Luminal B, Triple Negative and HER2 subtypes.

B. RNA PREPARATION AND MICRORNA QUALITY CONTROL

To carry out the first aim, we performed RNAs and genomic DNAs extraction from tumor and autologous PTT biopsies of 50 Luminal, 8 Basal-like and 9 HER2 BC patients in collaboration with Dr. Giovanni Blandino and collaborators.
RNAs and genomic DNAs were prepared from breast biopsies (stored at –80°C) using TRIzol extraction method (Invitrogen).
Small RNA quality was evaluated by:

a) spectrophotometer analysis (using NanoDrop)
b) quantitative RT-PCR analysis of snRNA-U6 expression. Quantitative RT-PCR analysis was performed using TaqMan MicroRNA assays (Applied Biosystem). This method requires just 10 nanograms of purified total RNA to reliably quantify the expression of miRNAs.
c) Small RNA assay on the Agilent 2100 bioanalyzer. The Small RNA assay can:
   - Visualize miRNA, Small RNA, oligo nucleotides from 6 - 150 nt for verifying sample integrity
   - Quantify miRNA component (in the concentration range of 50 - 2000 pg/µL) among all small RNAs (pre-miRNA, 5S, ribosomal RNA, etc) relative to an external standard, for verifying sample enrichment and purity
   - Automate sample quantitation, sizing and purity determination

C. EVALUATION OF P53 STATUS

p53 expression was evaluated by IHC on all BC patients. Moreover, p53 sequencing is ongoing to characterize the kind of mutations in TP53 gene, if present, in order to investigate its implication in microRNAs' expression patterns in breast specimens.

In particular, P53 status will be assessed by sequencing exons 5 to 8, holding the hot spot nucleotides for mutations. *P53* gene is amplified by PCR from genomic DNA using M13-tagged oligonucleotides drawn in the intronic regions surrounding the exons 5, 6, 7 and 8. Both sense and antisense strands are submitted to sequencing.

D. MICRORNA EXPRESSION PROFILING

The Human miRNA Microarray from Agilent contains probes for 723 human and 76 human viral microRNAs from the Sanger database v.10.1.

Each slide is an 8 x 15K format (~15,000 features printed in an 8-plex format, eight individual microarrays on a 1" x 3" glass slide) printed using Agilent's 60-mer Inkjet Technology, which, unlike competing platforms, synthesizes 40–60-mer oligonucleotide probes directly onto the array, resulting in high-purity, high-fidelity probes.

This miRNA platform requires small amounts of total RNA—in the 100 nanogram range—because it uses a high-yield labeling method, and does not require size fractionation or amplification steps that may introduce undesired bias during miRNA profiling.

E. WITH REGARD TO THE AIM WE FOCUSED ON TWO MAIN ISSUES:

1. Hormonal receptors distribution in BC and companion PTTs in our series of premenopausal patients
2. Prognostic and predictive role of ERß during BC progression

1. Modulation of estrogen and progesterone receptors in benign and malignant breast epithelium from premenopausal women

In premenopausal women, normal breast tissue usually displays a low content of hormonal receptors, phisiologically varying during the menstrual cycle (ER 5-10%, PGR 15-25%). Since it has been hypothesized that an increase in ER and PGR expression may be a BC risk factor, for the purpose of our study we analyzed, by immunohistochemistry, the ER∂, ERß (a second type of estrogen receptor which has prompted the re-evaluation of the model of estrogen action) and PGR expression in 66 invasive BC in parallel with the autologous adjacent uninvolved epithelium and in 33 benign lesions. Results obtained demonstrated that both benign breast lesions and autologous PTTs showed a physiological downregulation, from the follicular to the luteal phase, of the median values of ER∂ (24% vs 12%; 26% vs 12%) and PgR (18% vs 10%; 21% vs 13%) whereas no variation was observed for ERß (24% vs 20%; 27% vs 25%). In BC ER∂, PgR and ERß did not show any variation from the follicular to the luteal phase (ER∂: 33.4% vs 35.8%; PgR: 34.4% vs 43.4%; ERß: 31.9% vs 34.2%). Also of interest, BC PTTs did not display any significant variation of ER∂ expression during the menstrual phase (p=0.09) (Figure 1a and b).

These findings, although preliminary, suggested that there were no differences in ERß expression in relation to the menstrual cycle, neither benign lesions, breast cancer or PTTs. In contrast, ER∂ expression but not PgR, although physiological decreasing during the luteal phase in benign tissues, did not show any downregulation in BC PTTs, the percentage being as high as in the follicular phase. These findings indicate a need for better understanding of the role and regulation of ER∂, ERß and PgR in mammary carcinogenesis of premenopausal women and suggest that the expression of ER∂ and PGR in BC adjacent tissues, appearing morphologically uninvolved, may represent a biopathological marker reflecting a preinvasive stage of the disease.

2. Role of Estrogen receptor-beta in node-positive and node-negative breast cancer, classified according to molecular subtypes

Estrogen receptor-alpha (ER∂) and progesterone receptor (PgR) are consolidat-

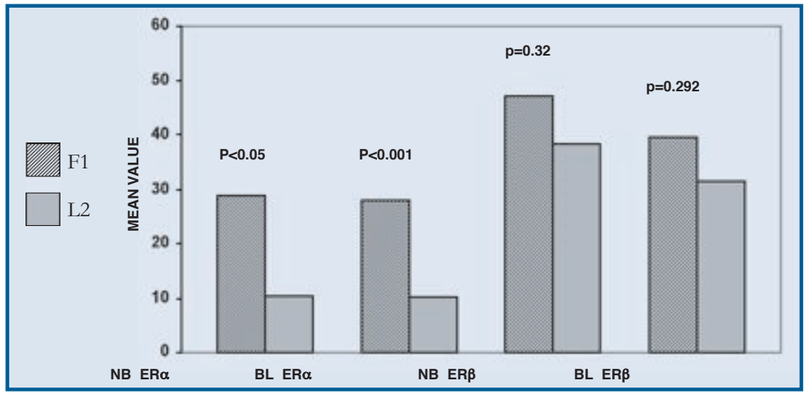
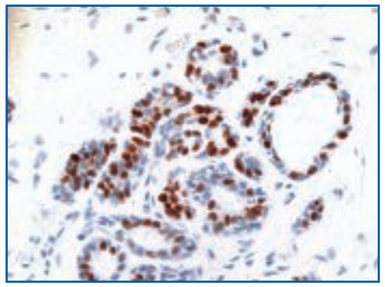
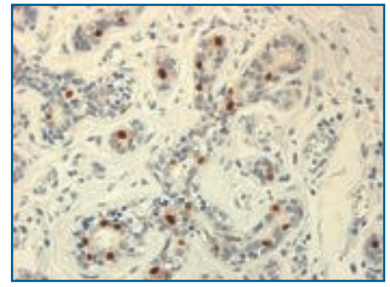


Figure 1a - Comparison between ERα and ERβ in normal breast (NB) and benign lesion (BL): follicular phase (F1) vs luteal phase (L2)



ERα follicular phase: benign lesions



ERα luteal phase: benign lesions

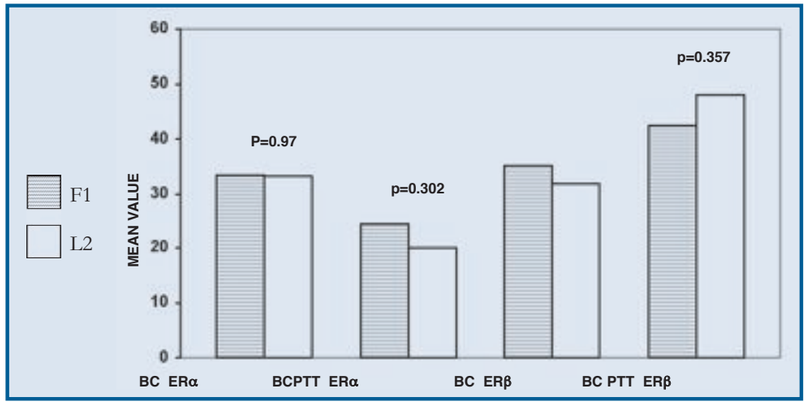
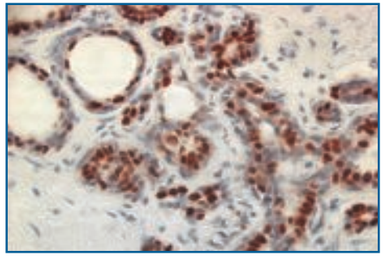
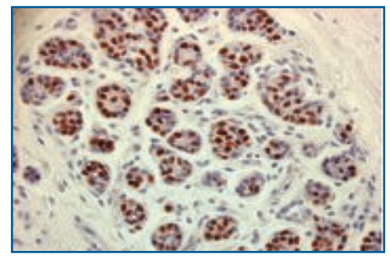


Figure 1b - Comparison between ERα and ERβ in breast cancer (BC) and breast cancer peritumoral tissue (BC PTT): follicular phase vs luteal phase



ERβ follicular phase: benign lesions



ERβ luteal phase: benign lesions

ed predictors of response to hormonal therapy (HT). In contrast, little information regarding the role of estrogen receptor-beta (ERß) in various breast cancer risk groups treated with different therapeutic regimens is available. In particular, there is no data concerning ERß distribution within the novel molecular breast cancer subtypes luminal A (LA) and luminal B (LB), HER2 (HS), and triple-negative (TN). To this end we conducted an observational prospective study using immunohistochemistry to evaluate ERß expression in 936 breast carcinomas. Associations with conventional biopathological factors and with molecular subtypes were analyzed by multiple correspondence analysis (MCA), while univariate and multivariate Cox regression analysis and classification and regression tree analysis were applied to determine the impact of ERß on disease-free survival in 728 patients with complete follow-up data. ERß evenly distributes (55.5%) across the four molecular breast cancer subtypes, confirming the lack of correlation between ERß and classical prognosticators **(see Figure 2).** However, the relationships among the biopathological factors, analyzed by MCA, showed that ERß positivity is located in the quadrant containing more aggressive phenotypes such as HER2 and TN or ERϑ/PgR/Bcl2^{-} tumors. Kaplan-Meier curves and Cox regression analysis identified ERß as a significant discriminating factor for disease-free survival both in the node-negative LA (P = 0.02) subgroup, where it is predictive of response to HT, and in the node-positive LB (P = 0.04) group, where, in association with PgR negativity, it conveys a higher risk of relapse **(see Figure 3)**.

Data indicated that, in contrast to node-negative patients, in node-positive breast cancer patients, ERß positivity appears to be a biomarker related to a more aggressive clinical course. In this context, further investigations are necessary to better assess the role of the different ERß isophorms in the early phase of neoplastic transformation

Figure 2

Estrogen receptor-beta (ERß) evenly distributes (55% to 56%, P = 0.99) across the four molecular subtypes(**a)**, whereas the percentages of p53 **(b)** and Ki67^{+} **(c)** tumors significantly increased (P <0.0001) and the percentage of Bcl2 significantly decreased (P <0.0001) moving from the luminal A (LA) phenotype to luminal B (LB), triple-negative (TN), and HER2 (HS) **(d)**.

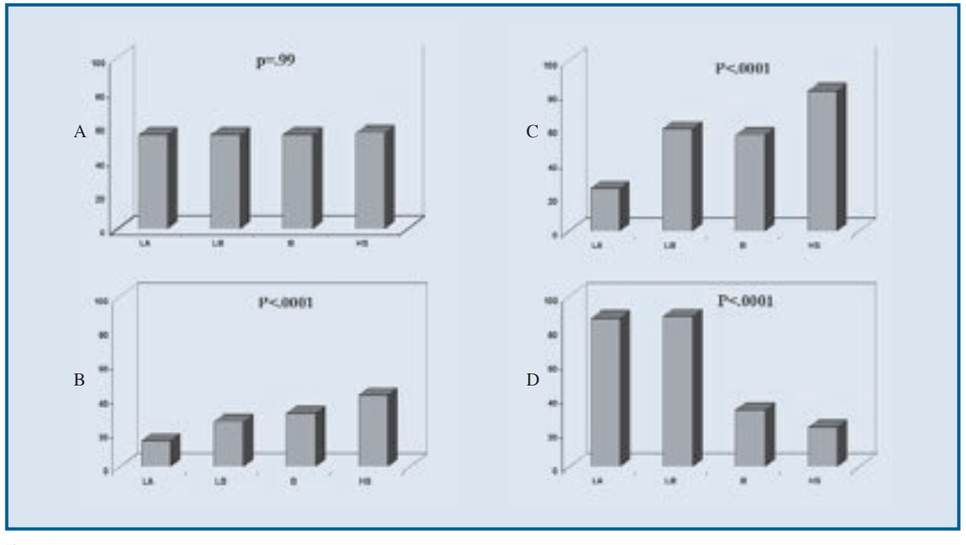


Figure 2

Figure 3

Kaplan-Meier estimates of disease-free survival for estrogen receptor-beta (ERß) status within the molecular subtypes and according to negative (N^{-}) or positive (N^{+}) nodal status, respectively, in each subgroup: luminal A (LA) **(a,e)**, luminal B (LB) **(b,f)**, HER2 (HS) **(c,g)**, and triple-negative (TN) **(d,h)**. P values were calculated using the log-rank test. ■

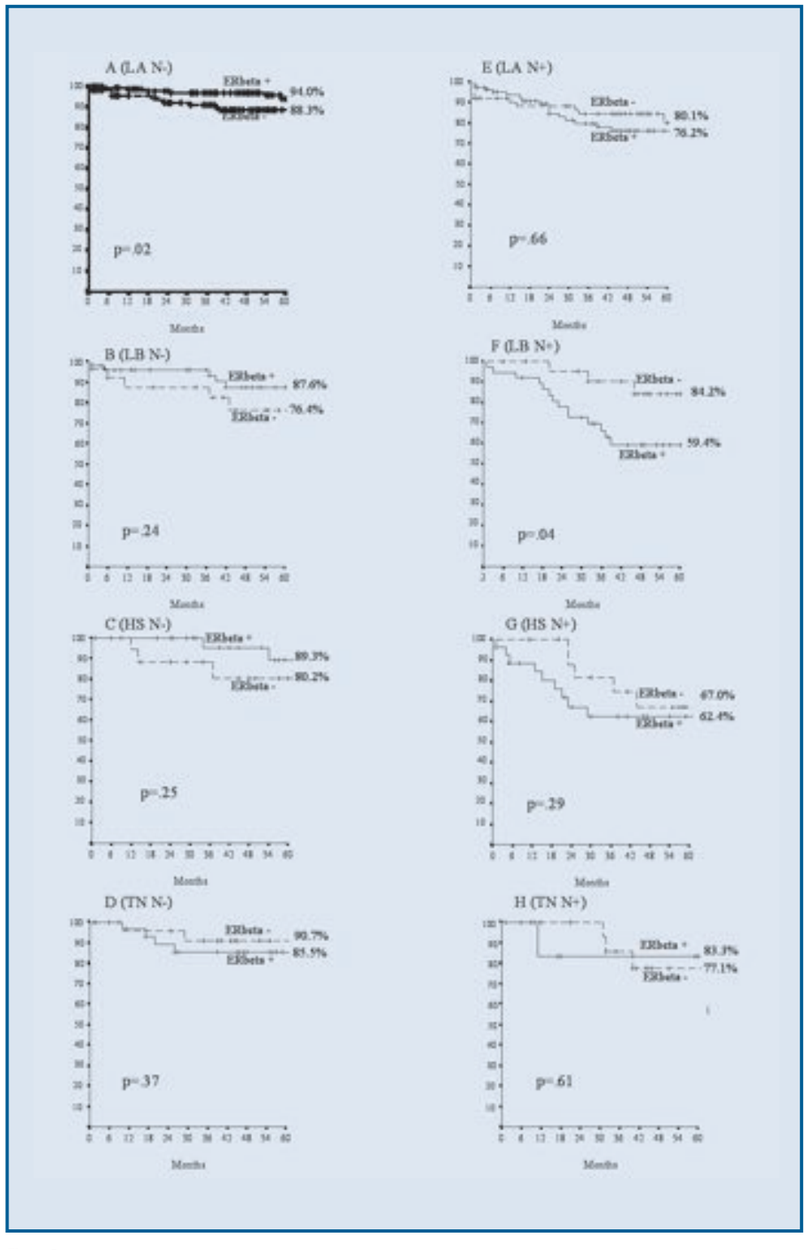


Figure 3

TITLE

Exploring new biomarkers involved in clinical outcome and therapeutic response in patients with locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy

COORDINATOR

Angela Torsello
Medical Oncology "C"

SENIOR INVESTIGATORS

Fabrizio Ambesi Impiombato
Radiotherapy

Maurizio Cosimelli
Surgery

Maria Grazia Diodoro
Histology and Cytopathology

Giulia Fontemaggi
R.O.C.

Carlo Garufi
Medical Oncology "C"

Carlo L. Maini
Nuclear Medicine

Marcella Mottolese
Histology and Cytopathology

Giulia Piaggio
Experimental Oncology

Rosa Sciuto
Nuclear Medicine

Francesca Sperati
Epidemiology

Irene Terrenato
Epidemiology

First year report

■ The overall goal of this study is to identify new biomarkers, at the level of microRNA and protein expression, as predictors of clinical outcome and therapeutic response in rectal cancer patients treated with neoadjuvant chemoradiotherapy (CRT). Altered miRNA expression provide complex fingerprints that may serve as molecular biomarkers for tumor diagnosis, prognosis of disease-specific outcomes, and prediction of therapeutic responses. However, the accuracy of miRNAs to classify cancers and its prognostic power remains to be explored in large cohorts of patients. Some studies have evidenced a possible correlation between wild type p53 activity and miRNA expression in colorectal cancer. Since mutant p53 expression identifies high risk patients in rectal cancer, it may be important to investigate whether a correlation exists between mutant p53 and miRNA expression.
The primary objectives of the study are the following:

1) to investigate the profile of microRNA expression in normal and tumoral rectal tissues.
2) to study the correlation between microRNA profiles and mp53/NF-Y target gene expression and their relevance as prognostic factor in locally advanced rectal cancer.
3) to assess possible molecular biomarker modifications induced by neoadjuvant CRT and their correlation with tumor regression.

The secondary objective is to investigate whether PET-scan modification can been used as an early predictor of neoadjuvant CRT response and its correlation with molecular biomarkers.

PATIENTS AND CLINICAL CHARACTERISTICS

In the first year of this project, we started enrolment of 67 patients with locally advanced rectal cancer requested according to sample size calculation. We considered 30 patients of whom 10 were prospective (recruited from September 2007 to September 2008) and 20 were retrospective cases. Retrospective cases were selected for two reasons: 1) to assess the feasibility of molecular techniques; 2) to assess the clinical relevance in patients with a longer follow-up. We created a database for the collection of patient characteristics and molecular data.
Patients had the following clinical characteristics: median age 63 years (35-78 y), male/female 18/12, cT_{3-4} N_0 /anyT N_{1-2} 11/19.
According to the protocol, before and after neoadjuvant CRT patients will be imaged with: computed tomography, magnetic resonance, endoscopic ultrasonography. For prospective cases we also performed the PET-scan before, during and after 2-3 weeks of neoadjuvant CRT completion.

TREATMENT AND TOXICITY

Neoadjuvant chemoradiotherapy (CRT) consists of:

- radiotherapy: 45 Gy are delivered to the whole pelvic at 1.8 Gy daily, 5 times per week for 5 weeks; 5.4 Gy are delivered to the mesorectum, to a total dose of 50.4 Gy for all patients.
- four schedules of chemotherapy were delivered according to different clinical conditions during the whole duration of RT: (1) 5-Fuorouracil 200/mg/mq/die i.c.: 2 patients; 2) Capecitabine 1700 mg/mq/die: 10 patients; 3) Oxaliplatin 100 mg/mq days 1, 15, 30+ Capecitabine 1300mg/mq/die: 17 patients; 4) 5-Fluorouracil 200 mg/mq/die+ Oxaliplatin 60 mg/mq/week+ Panitumumab 6 mg/Kg q2w: 1 patient.

Treatments were generally well tolerated and major G3 toxicities were rectal tenesmus (20%) and diarrhoea (16%).

MOLECULAR ANALYSIS

We are collecting histological samples for molecular analysis. For prospective cases biopsies were performed on normal and tumoral tissue during rectoscopy before neoadjuvant CRT. During surgery both tumor and distal normal tissue were collected. All samples were rapidly embedded in paraffin blocks for analyses. For retrospective cases tissue samples were identified from the archival files of the Institute's Department of Pathology and a pathologist reviewed all cases and confirmed the diagnosis.
We are starting to analyze molecular data on available samples.

Immunoistochemistry. Tissues are being evaluated to detecte the expression of the following mp53/NF-Y target genes: cyclin A, cyclin B1, cdk1, and cdc25C. Moreover, some samples have been also analysed for p53 and NF-Y.
Rectal cancers with high levels of cyclin A, cyclin B1, cdk1, cdc25C, p53 and NF-Y immunoreactivity have been used as positive controls. Negative controls have been obtained by omission of the primary antibody.
The collection of immunoistochemical data is in progress and has been completed for 16/30 patients enrolled.

microRNA. To investigate whether a different microRNA expression profile exists in normal and tumoral rectal tissue, both in the biopsies and surgical specimens, microdissected cores of paraffin block sections should be obtained using a Laser Capture Microdissection (LCM) technology. In order to optimize this analysis on our samples, especially considering the small size of the biopsies, we are performing control experiments to set up the entire strategy.
1) microRNA expression profiling. The ROC platform is setting up the human miRNA microarray technology from Agilent. It contains probes for 723 human and 76 human viral microRNAs from the Sanger database v.10.1. Each slide is an 8 x 15K format (~15,000 features printed in an 8-plex format, eight individual microarrays on a 1" x 3" glass slide) printed using Agilent's 60-mer_Inkjet Technology, which, unlike competing platforms, synthesizes 40–60-mer oligonucleotide probes directly onto the array, resulting in high-purity, high-fidelity probes.
2) RNA extraction and quality control. To obtain RNA preparations useful for studies of microRNA expression profiling we tested on a few samples the RecoverAll™ total Nucleic Acid Isolation kit (Ambion). This kit is specifically used for RNA extraction from formalin-fixed, paraffin-embedded (FFPE) tissues. The quality and quantity of obtained RNA has been, until now, evaluated by spectrophotometer analysis (using Nan-

oDrop) and we have now designed the strategy to perform quantitative RT-PCR analysis of snRNA-U6 expression on these samples. Next we will test these RNAs using the Small RNA assay on the Agilent 2100 bioanalyzer. Indeed, the Small RNA assay can visualize miRNA, Small RNA, oligo nucleotides from 6-150 nt for verifying sample integrit. Moreover it can quantify miRNA component (in the concentration range of 50-2000 pg/μL) among all small RNAs (pre-miRNA, 5S, ribosomal RNA, etc) relative to an external standard, for verifying sample enrichment and purity.
Next we will perform Quantitative RT-PCR analysis using TaqMan MicroRNA assays (Applied Biosystem). This method requires just 10 nanograms of purified total RNA to reliably quantify the expression of miRNAs.
The control experiments conducted over this year, after initial difficulty in RNA extraction from formalin-fixed/paraffin-embedded (FFPE) tissues, finally resulted positive in term of feasibility and reproducibility, and therefore the system will be used for the analysis of all rectal cases.

NEXT STEP

In the second year of the project we will complete patient enrolment and sample collection. Then we will complete the molecular analysis of the samples for statistical analysis. ■

TITLE

VISION - Vascular event In noncardiac Surgery patients cOhort evaluation study

COORDINATOR

Enrico Vizza
Gynaecology

INVESTIGATORS

Ermelinda Baiocco
Gynaecology

Giacomo Bruscoli
Gynaecology

Giacomo Corrado
Gynaecology

Emanuela Mancini
Gynaecology

Holger Schünemann
Epidemiology

Cristina Vincenzoni
Gynaecology

First year report

■ Our research activity has been focused on the realization of the VISION (**Vascular events In noncardiac Surgery patients cOhort evaluation**) study. The VISION study is a large international multicenter cohort study evaluating major vascular events in patients undergoing noncardiac surgery. The primary objectives of the Study are to determine: 1) incidence of major vascular events (i.e., vascular death, non-fatal myocardial infarction, non-fatal cardiac arrest, and non-fatal stroke) at 30 days after surgery; 2) optimal clinical model to predict major vascular events at 30 days after surgery; 3) proportion of patients 30 days after surgery with perioperative myocardial infarctions that may go undetected without troponin monitoring; and 4) relationship between postoperative troponin measurements and the 1 year risk of vascular death. Several countries around the world (Canada, Brazil, China, Colombia, India, Malaysia, Spain, Hong Kong, Italy) are participating in the VISION Study. Our Institute is the only Center participating in Italy. All centers will recruit 2000-4000 patients over a 2 year period. Patients are eligible if they undergo noncardiac surgery requiring overnight hospital admission, if they are > 45 years of age and if they receive a general or regional anaesthetic.
In the enrolled patients the VISION Study will determine the current incidence of major vascular events following non-cardiac surgery, and evaluate whether troponin measurements after surgery can identify myocardial infarctions that are likely to go unrecognized and predict vascular death at one year, thereby facilitating appropriate timely interventions. Moreover, it is important to emphasize that beyond the established scientific value of this study, VISION has a role in the management of the patient in the post-operative period. In particular, in our experience, the troponin measurements of enrolled patients have provided support to the cardiologists in excluding greater cardiological complications in the post-operative period, in case of increased blood pressure and atrial fibrillation.
Despite a delay in beginning the Study (April 2008) and the difficulties in the coordination of the different surgical Departments, up to now we have enrolled more than 200 patients. We have also produced an abstract with the data of the pilot study to be presented to the SIGO Congress in Torino.

STUDY DESIGN

All centers will recruit 2000 – 4000 patients in a 2 year period. Patients are eligible if they undergo noncardiac surgery requiring overnight hospital admission, if they are > 45 years of age and receive a general or regional anaesthetic. All patients will have a troponin T drawn between 6 and 12 hours postoperatively and on the 1st, 2nd, and 3rd day after surgery. An ECG is undertaken immediately after an elevated troponin measurement is detected. If a troponin measurement is elevated but the patient has no ECG changes, ischemic symptoms, or pulmonary edema to fulfill the diagnostic criteria for myocardial infarction, then the patient will undergo an echocardiographic study.

PREPARATORY WORK

The Hamilton Health Sciences McMaster University Medical Center has successfully undertaken a pilot cohort study whose results have demonstrated a 6-8% event rate for major perioperative vascular events. The rate for major perioperative vascular events evidenced by the pilot is higher than 1.4% of major perioperative vascular events demostrated by Lee et. al (the only study that included relatively unselected patients > 50 years of age undergoing elective noncardiac surgery with an expected length of stay > 2 days).

IMPORTANCE OF THE STUDY

Despite its limitations, the pilot study suggests that the incidence of major perioperative vascular events may be increasing and highlights the need for a large multicenter, international, prospective cohort study to establish the current incidence.
The VISION Study will determine the current incidence of major vascular events following noncardiac surgery, and will identify the optimal clinical risk prediction model. This study will also evaluate whether troponin measurements after surgery can identify myocardial infarctions that are likely to go unrecognized and predict vascular death at one year, thereby facilitating appropriate timely interventions. ■

New Idea Awards

SCIENTIFIC PROJECTS AWARDED BY INTRAMURAL CALLS

Experimental Studies

TITLE
Proteomic and Red-ox proteomic analysis of high grade HPV related dysplastic lesions

RESEARCHER
Federico De Marco Virology

CO-RESEARCHERS
Carla Blarzino Virology
(La Sapienza University, Rome)
Maria Benevolo Histology and Cytopathology
Raffaella Coccia Virology
(La Sapienza University, Rome)
Silvio Flamini Virology
Ferdinando Marandino Histology and Cytopathology
Luciano Mariani Gynaecology
Marcella Mottolese Histology and Cytopathology
Francesca Paolini Virology
Marzia Perluigi Virology
(La Sapienza University, Rome)
Holger Schünemann Epidemiology
Irene Terrenato Epidemiology
Aldo Venuti Virology
Enrico Vizza Gynaecology
Amina Vocaturo Histology and Cytopathology

Abstract

■ *Uteri cervix* carcinoma is the second most diffuse tumor among women worldwide. It affects women aged 31-50 at the top of their human and professional potential with a heavy economical and emotional impact on individuals and on the community. It is now unanimously accepted that the infection with certain types of Human Papillomavirus (HPV) represents the etiological agent of cervical cancer (IARC 1995; zur Hausen 1996; Walboomers 1999). However viral oncogenes expression is not *per se* sufficient to induce cervical cancer and other factors, presently unknown, are needed to drive the viral infected cell along the multi-step process of neoplastic progression.
The identification of such factors is a fundamental step in the development of improved strategies for prevention, early diagnoses and treatments of dysplastic lesions. To this end a number of factors have been proposed. However, despite intensive experimental work, our present understanding of dysplastic progression remains largely unsatisfactory. Oxidative Stress (OS) represents an attractive candidate in this context: it is constantly generated during respiratory chain as well as during inflammation, immune reactions, toxic chemicals, mechanical stress or UV irradiation. Epithelial tissues, the elective target for HPV infection, are heavily exposed to all named stresses. Nonetheless the role of Oxidative Stress (OS) in HPV driven carcinogenesis has been surprisingly neglected so far. The recent development of computer assisted methodologies for the analysis of protein expression in highly complex samples, the proteomics, enables us to describe the global array of proteins expressed in tissues under specific conditions, making the systematic search for new molecular factors involved in cancer progression more productive. In a preliminary study conducted on *in vitro* tissue cultures we described the proteomic profile of transformed cells and identified a number of OS related proteins potentially involved in neoplastic progression.
With the present project we aim to extend the analysis to High Grade dysplastic lesions. The study will combine the proteomic approach, for the description of the global protein profile, with the red-ox proteomic tool to describe the pattern of oxidatively modified proteins. These data combined with virological, biochemical and cyto-histological analyses will contribute to identify new molecular factors involved in neoplastic progression and to describe a "proteome signature" of dysplastic lesions providing new topics to the bio-molecular dissection of neoplastic progression as well as the bases for improved diagnostic tools, prognostic markers and therapeutic procedures. ■

TITLE
Sensitivity to tyrosine kinase receptors targeted therapy in pancreatic cancer: Role of hMena isoforms and Epithelial to Mesenchimal transition

PRINCIPAL INVESTIGATOR
Paola Nisticò Immunology

CO-INVESTIGATORS
Francesca Di Modugno
Laboratory 'A' of Preclinical Experimental Chemotherapy
Michele Milella
Simona Pino
Division of Medical Oncology 'A'
Marcella Mottolese
Letizia Perracchio
Paolo Visca
Histology and Cytopathology

INTERNATIONAL COLLABORATION
Frank Gertler
Associate Professor, Massachusetts Institute Of Technology, Cambridge, Ma.

Abstract

■ Cancer of the exocrine pancreas is the fourth leading cause of cancer related-death worldwide and even though the current frontline agent, the deoxycytidine analogue gemcitabine, provides symptomatic improvement it does not extend median survival beyond six months.
Thus, the development of more effective, targeted-based therapies is desperately needed.
In recent years the epidermal growth factor receptor (EGFR), has been demonstrated as a therapeutic target in pancreatic cancer. However, considering the disappointing clinical experience with gefitinib and other EGFR antagonists in cancer patients, we questioned whether or not tumor heterogeneity might limit the number of primary tumors that could potentially respond to EGFR-directed therapy.
Human pancreatic cancer is a model system in which tumor cells often represent a minor population due to an intense host-stromal response, a hallmark of this aggressive neoplasia. A systematic immunohistochemical analysis of pancreatic cancer microenvironment in parallel with the expression of EMT markers (E-Cadherin, NCadherin, Vimentin) will be performed. Since it is well known that epithelial to mesenchymal transition (EMT) is related to can-

cer invasiveness and aggressiveness and based on our preliminary data showing that EMT markers may predict TKI sensitivity, we will investigate the role of EMT in determining the sensitivity to EGFR TKI.
During EMT cytoskeleton proteins are strongly deregulated and we have recently isolated, in breast tumors, hMena, a key actin regulatory molecule, and two splice variants hMena+11a and hMenaΔv6 over-expressed in a mutually exclusive manner in tumor cell lines. hMena+11a characterizes an epithelial phenotype, whereas, hMenaΔv6 is expressed in tumor cells with a mesenchymal phenotype and is correlated to the epithelialmesenchymal transition (EMT), We will evaluate whether in pancreatic tumor cell lines the different pattern of hMena isoform expression might identify an epithelial or mesenchymal phenotype. Moreover, hMena+11a is phosphorylated following EGF treatment and is involved in EGF-driven proliferation of breast cancer cell lines, thus indicating that it couples tyrosine kinase signalling to the actin cytoskeleton. One of the aims of this project is to place hMena and its isoforms along the EGFR signalling pathways in pancreatic tumors. We will also evaluate the contribution of hMena isoforms as signalling elements downstream from these relevant therapeutic molecular targets. From a clinical point of view we will analyze *in vitro* and *in vivo* the role of hMena isoforms' overexpression as a predictive marker of the therapeutic response of EGFR family member targeted therapies.
The goal of this study is to identify predictive markers that could help clinicians to prospectively select pancreatic cancer patients who are most likely to respond to the EGFR antagonists. ■

TITLE

Pre-invasion behavior and dormancy in melanoma: epidemiological, clinical, pathological, molecular and immunological aspects

PRINCIPAL INVESTIGATOR
Pier Giorgio Natali Immunology

Abstract

■ In this application, we challenge the view of one-way, irreversible, multi-step tumor progression. Building on epidemiological, clinical and pathological observations, we postulate that cells of the nevomelanocytic lineage (normal adult melanocytes, common acquired nevi, pigmented and non-pigmented lesions, primary melanomas, in transit as well as distant metastases) enter and egress competent stages of invasion. We propose that all these lesions must be cDNA profiled to detect common recurrent signatures of progression. This model is relevant to several issues, including dormancy of tumor cells, immune surveillance, and prognostication of melanoma outcome. ■

TITLE

Endothelin A receptor pathways and related factor in ovarian cancer chemoresistancy: therapeutic implications

PRINCIPAL INVESTIGATOR
Anna Bagnato
Laboratory 'A' Associated to the Surgical Oncologic Department

CO-INVESTIGATORS
Valentina Caparra
Valeriana Di Castro
Aldo Lupo
Stefano Masi
Maria Rita Nicotra
Laura Rosanò
Francesca Spinella
Laboratory "A" Associated to the Surgical Oncologic Department

Pier Giorgio Natali
Immunology

Donatella Del Bufalo
Experimental Preclinical Chemotherapy

Michele Milella
Medical Oncology "A"

Marcella Mottolese
Histology and Cytopathology

Abstract

■ The lack of specific markers of early diagnosis makes epithelial ovarian cancer the fifth leading cause of cancer related mortality in western world women. Although during the last decades overall survival has improved due to the use of new chemotherapy regimens, the majority of patients will relapse and develop resistant disease. Therefore, new therapeutic strategies for ovarian cancer treatment are continuously explored. There is growing evidence that interactions between tumor and microenvironment-derived factors affect response rate to chemotherapy. In this context in ovarian cancer cells, endothelin-1 (ET-1) selectively through the binding on ETA receptor (ETAR) regulates the dynamic interactions of tumor microenvironment including changes in intercellular communications, cell–cell and cell–matrix adhesion molecules, by modulating the switching in cadherin profiles, inactivation of connexins, activating matrix-metalloproteinases (MMPs), and cross-talk in integrin- and hypoxia-mediated signaling so as to cooperatively engage transcriptional programs leading to epithelial to mesenchymal transition (EMT), tumor progression, and metastasis. Therefore we will evaluate the expression of ETAR as predictor of chemoresistance on ovarian cancer tissues collected from patients at diagnosis and in persistent/recurrent disease after chemotherapy as well as in ovarian cancer cell lines sensitive and resistant to standard chemotherapy (taxol and platinum). Recent evidences point to a critical role of EMT in endowing cancer cells with drug resistance, that may occur following the onset of alternative escaping signalings. To better understand the underlying molecular mechanisms that occur in EMT after ETAR activation, in this project we will evaluate whether ß-arrestin, which serves as regulator and scaffold of G-protein-coupled receptor (GPCR), may function as cytoplasmic chaperone to drive ß-catenin-mediated cell invasion. Moreover in this study we will explore whether ß-arrestin could function as nuclear messenger of ETAR to mediate epigenetic signaling that controls ß-catenin transcriptional

programs leading to EMT. Moreover, the implication of ET-1 axis in hypoxia-driven signaling prompted us to consider whether it could be involved in the regulation hypoxia inducible factor (HIF)-1∂ and of cyclooxygenase (COX)-1/-2, which are expressed at higher levels in ovarian carcinoma and correlate with poor prognosis and drug resistance. The identification of regulatory mechanisms which are at the basis of the interplay between microenvironmental hypoxia and ET-1 axis may have significant implications for developing a targeted therapy which overcomes chemoresistance. The confirmation of ETAR as a new molecular target, may allow us to expand the therapeutic repertoire of ETAR blockade by exploring the efficacy of ZD4054, a novel highly selective ETAR antagonist, in the regulation of ovarian cancer cell progression and drug resistance. In this context, the ETAR-mediated interconnected signaling network could represent a possible mechanism of chemoresistance. Recent studies have provided evidence of a functional correlation between EMT-related gene expression and drug sensitivity, suggesting that E-cadherin and other microenvironmental-related genes, such as (HIF)-1∂, beside being potential markers of response to chemotherapy, may also play a role in the mechanism underlying drug response. With the aim of identifying such mechanisms, we will evaluate whether pharmacologic blockade of ETAR with specific ETAR antagonist can predict and influence the response to chemotherapeutic agents. The identification of the molecular changes that occur during ovarian tumor progression and chemoresistance will allow the design of integrated therapies which incorporated ZD4054 in combination with carboplatin plus paclitaxel. Considering that new ETAR targeting strategies are in clinical development, the combination therapy with ETAR antagonist provides different mechanisms to increase the sensitivity to current chemotherapeutic agents, and new insights for improving therapeutic approaches in ovarian cancer treatment. ■

TITLE

Telomere maintenance mechanisms as targets for anticancer drug development

PRINCIPAL INVESTIGATOR

Annamaria Biroccio
Laboratory "A" of Preclinical Experimental Chemotherapy

CO-PARTICIPATING RESEARCHERS

Barbara Benassi
R.O.C.

Gennaro Citro
Laboratory Animal Center and Pharmacokinetic Unit

Antonella Farsetti
Laboratory "C" of Molecular Oncogenesis

Carlo Leonetti
Laboratory "A" of Preclinical Experimental Chemotherapy

Marcella Mottolese
Histology and Cytopathology

Abstract

■ Telomere maintenance is important to all dividing cells, including cancer cells. Possible targets for the disruption of telomere maintenance are specific DNA structures, such as G-quadruplexes, that can form from telomeric sequences. Results from our group and others indicate that G4 ligands might target telomeres and disrupt telomere maintenance in cells making these compounds attractive potential anticancer agents. The molecular mechanisms of this new class of potential antineoplastic agents are not understood entirely and one crucial question remains the selectivity of these molecules towards normal cells. Moreover, currently there is little known about the antineoplastic activity and toxicity of this class of compounds and their interaction with cytotoxic in preclinical models.

Here we will study the molecular mechanism(s) by which RHPS4, one of the most effective and selective G4 ligands, rapidly uncaps telomeres; moreover, we will characterize in detail the events that take place at the RHPS4 binding to the telomeres in order to identify molecular markers of tumor response and compelling molecules that may have a synergistic effect in tumor response, offering a new opportunity for cancer therapy. The project will go one step further by studying the toxicological profile and antitumor activity of RHPS4 in comparison with standard antineoplastic agents on xenografts of different tumor histotype. Moreover, the interaction between RHPS4 and chemotherapeutics will be evaluated both *in vitro* and *in vivo*. These studies will be extremely informative in helping to guide rational clinical telomere therapeutic strategies.

More telomere structures could also be valuable targets, especially those related to telomere protection against DNA damage response. In this context, we have recently demonstrated that disruption of the telomeric protein TRF2 limits tumorigenicity of human melanoma cells, validating TRF2 as interesting targets in chemotherapy. Here will conduct an in-depth evaluation of the telomeric protein TRF2 in xenografted human tumors, including its effects on metastasis, angiogenesis and immune response. In order to better understand the role of TRF2 in human tumorigenicity and to identify relevant drug targets within the TRF2 protein, we will define the mechanisms by which TRF2 inhibition limits malignancy, using tamoxifen- inducible TRF2 expression vectors to probe the anti-cancer effects of this telomeric component *in vivo*. In addition, we will establish a new drug screening procedure named SHP (SUMO-1 – Heptapeptide – Protein transduction domain or PTD), aimed at the identification of short anti-TRF2 peptide motifs. We will examine in this project whether a previously established collection of SHPs against TRF2 can reduce the growth of different human tumors xenografts in mice. ■

TITLE

Significance of ErbB-family expression in natural history of cervical cancer and HPV-related disease

PRINCIPAL INVESTIGATOR

Aldo Venuti Virology

CO-INVESTIGATORS

Francesca Paolini Virology

CO-RESEARCHERS

Anna Cianciulli Clinical Pathology
Federico De Marco Virology
Silvio Flamini Virology
Massimo Lopez Medical Oncology B
Ferdinando Marandino Histology and Cytopathology
Luciano Mariani Gynaecology
Marcella Mottolese Histology and Cytopathology
Irene Terrenato Epidemiology
Patrizia Vici Medical Oncology B
Enrico Vizza Gynaecology
Amina Vocaturo Histology and Cytopathology

Abstract

■ The EGFR (epidermal growth factor receptor) family consists of four ErbB tyrosine kinase receptors (also named EGFR-1 or HER-1, HER-2, HER-3, HER-4) involved in a complex network of signal transduction pathways, playing a key role in regulating cell proliferation, differentiation, motility, invasion, angiogenesis and survival. Co-expression of these receptors favors omo-hetero-dimerization among them, enhancing tyrosine-kinase activity promoting the phosphorylation of several tyrosine residues which leads to a complex signalling cascade.

The presence of EGFR and ErbB 2,3,4 receptors has been associated with accelerated tumor progression and resistance to therapy for various types of malignancies, including cervical cancer. Some reports indicate EGFR-1 frequently over-expressed in cervical cancer, and expres-

sion seems to correlate also with disease progression, while ErbB-4 overexpression in some reports has been linked to a good prognosis and longer disease-free-survival.
An increased expression of ErbB-2 receptors has also been detected with CIN1 to CIN3 progression.

Few studies have evaluated the prognostic significance of multiple ErbB family receptors in cervical cancer, and some reports indicate an important role of ErbB-2 regulation by HPV-16 E6 gene in oncogenic transformation of cervical cells. Other viral genes have been implicated in the growth factor signalling regulation during the viral replication or transformation and therefore there is the need to ascertain *in vitro* the relationship of all these viral oncogenes with the ErbB-family receptors.
The study will be developed by two parallel tasks *in vitro* and *in vivo* to validate any *in vitro* (cell lines) results by a verification of their plausibility in clinical samples and vice versa to find a biological *in vitro* explanation for possible host/virus interactions detected *in vivo* (clinical samples). The causal relationship of this receptor network to disease progression and resistance to therapy as well as to the viral transformation could provide a rationale for future targeting this signalling pathway with innovative and promising "target-therapies". ■

TITLE

Bcl-2 and bcl-xL involvement in tumor angiogenesis: role of hypoxia

PRINCIPAL INVESTIGATOR
Donatella Del Bufalo
Laboratory "A" of Preclinical Experimental Pathology

CO-INVESTIGATORS
Antonio Candiloro
Marianna Desideri
Chiara Gabellini
Daniela Trisciuoglio
Laboratory 'A' of Preclinical Experimental Pathology
Maria Benevolo
Histology and Cytopathology
Frauke Goeman
R.O.C.
Michele Milella
Division of Medical Oncology "A"

Abstract

■ There is now an increasing body of data supporting our previous studies suggesting a role for bcl-2 and bcl-xL other than that of regulating the mitochondrial pathway of apoptosis. In particular, we have previously demonstrated that the overexpression of the antiapoptotic protein bcl-2 in human melanoma synergizes with hypoxia to increase angiogenesis, and that treatment of melanoma cells with bcl-2/bcl-xL antisense oligonucleotides reduces angiogenic activity. Moreover, bcl-2 overexpression in melanoma cells increases vascular endothelial growth factor (VEGF) expression and hypoxia inducible factor-1 (HIF-1) transcriptional activity through both phosphatidylinositol 3-kinase- and mitogen-activated protein kinase-dependent pathways. The ability of bcl-xL, another antiapoptotic gene, to induce angiogenesis has also been demonstrated by our group in glioblastoma and melanoma cells through upregulation of the interleukin 8 (CXCL8). The aim of this project is to evaluate the molecular mechanism(s) by which bcl-2 and bcl-xL increases angiogenesis. Preclinical models of human melanoma, breast carcinoma and glioblastoma will be used for *in vitro* and *in vivo* experiments. Using bcl-2 and bcl-xL gene constructs carrying point mutations or missing a coding sequence for functional domains, we will evaluate which region(s) of bcl-2 and bcl-xL is required for their proangiogenic activity and, in particular, for bcl-2-induced VEGF expression and HIF-1 activity, and for bcl-xL-induced CXCL8 expression. Moreover, through chemical and genetic inhibitors, the possible involvement of Ras/Raf-1 pathway in bcl-2 and bcl-xL-induced angiogenesis will be investigated and pull-down experiments will be also performed to identify possible molecules interacting with bcl-2 or bcl-xL proteins. Chemical and genetic inhibitors will be also used to analyze the involvement of Nuclear Factor –kappa B (NF-kB) in CXCL8 induction by bcl-xL. Another goal of the proposed study is to identify, by ChIP-on-Chip, specific *in vivo* binding of HIF-1 transcription factor to a broad range of DNA target sequences, both in parental and in bcl-2 or bcl-xL overexpressing cells under normoxic or hypoxic conditions. This information could prospectively bring new information on the molecular mechanisms by which bcl-2, bcl-xL and hypoxia modulate tumor angiogenesis and could lead to the identification of some genes that are modulated by bcl-2, bcl-xL and hypoxia, conditions frequently present in solid tumors which could be responsible for drug resistance. Moreover, the definition of the molecular mechanisms by which angiogenesis is activated could help to identify new targeted therapies.
Finally, on the basis of preclinical evidences from our group indicating that trastuzumab downregulates bcl-2 expression and potentiates apoptosis induction by bcl-2/bcl-xL bispecific antisense oligonucleotides in HER-2 gene amplified breast cancer cells, and that mTOR inhibition by rapamycin derivatives exerts potent anti-angiogenic effects, correlative studies aimed at evaluating differences in endpoints (i.e. circulating angiogenic factors, targeted molecules expression) induced by the addition of mTOR inhibitors to a standard, widely used, trastuzumab-based combination regimen will be performed. ■

TITLE

HIPK2, a potent inducer of apoptosis: characterization of molecular pathways, role in tumorigenesis and tumor resistance to anticancer therapies

PRINCIPAL INVESTIGATOR
Silvia Soddu
Laboratory "C" of Molecular Oncogenesis

CO-INVESTIGATORS
Francesca De Nicola
Laboratory Associated to the Medical Oncology Department
Mirella Marino
Marcella Mottolese
Histology and Cytopathology

Abstract

■ The p53 tumor suppressor, which is mutated or inactivated in the majority of human cancers, functions as a master regulator of cell response to several types of stress including DNA damage and oncogenic stimuli. To mediate these functions, p53 protein must be activated by post-translational modifications, which require interactions with specific enzymes. In the past few years, we have shown, along with other groups, that HIPK2 (Homeodomain-Interacting Protein Kinase 2) is one of such enzymes. HIPK2 binds to and activates the apoptotic function of p53 by specifically phosphorylating it at Ser46, a modification that promotes changes in p53 affinity for different promoters with a shift from cell cycle arrest-related genes to apoptosis-related ones. In addition, HIPK2 depletion by RNA interference induces a strong resistance to different anticancer treatments by inhibiting p53 dependent and independent apoptosis. Our recent studies indicate the existence of different mechanisms of HIPK2 inactivation in human thyroid and breast cancers (e.g., allele-specific LOH,

forced cytoplasmic re-localization). These observations strongly support the hypothesis that HIPK2, like other apoptosis activators or p53 regulators, is a tumor suppressor gene in its own right and deserves a mechanist characterization of its pathways, whether dependent on or independent of p53. To this aim, we propose to investigate three different aspects of HIPK2 function:

I) identification of the mechanism(s) of HIPK2 activation in response to genotoxic damage;

II) characterization of the molecular and functional consequences of the physical interaction of HIPK2 with *f*Np63 and Che-1, we had previously identified in co-precipitation studies;

III) screening of primary tumors with different status of the endogenous *TP53* gene to evaluate the loss/inhibition of HIPK2 activity.

This proposal will contribute to the characterisation of HIPK2 as a novel player in tumor development and/or progression and carries a high probability of identifying a new marker for prognostic evaluation and novel targets for anticancer therapy. ■

TITLE

Maintenance of Genomic Stability at CpG Sites

PRINCIPAL INVESTIGATOR

Alfonso Bellacosa
Laboratory "A" Associated to the Medical Oncology Department

COLLABORATORS

Serena Buontempo
Gennaro Citro
Laboratory Animal Center and Pharmacokinetic Unit
Maurizio Fanciulli
Laboratory "B" Associated to the Medical Oncology Department
Patrizio Giacomini
Laboratory "B" of Immunology
Lionel Larue

CLINICAL COLLABORATORS

Marcella Mottolese
Histology and Cytopathology
Vittoria Stigliano
Digestive Endoscopy

Abstract

■ Up to one-third of all cancer mutations are G:C to A:T transitions in the context of CpG sites.

However, the mechanisms leading to increased instability of CpG sites during tumorigenesis are poorly understood. Spontaneous deamination of cytosine and 5-methylcytosine creates G:U and G:T mismatches, and is generally considered a major mechanism. Several mammalian base excision repair (BER) enzymes are thought to prevent mutagenesis caused by spontaneous deamination at CpG sites. We recently identified one such enzyme, human MED1 (also known as MBD4). Both MED1 and the previously characterized enzyme TDG display uracil and thymine DNA N-glycosylase activity specific for G:U and G:T mismatches in the context of CpG sites. We are among those who have previously inactivated the *Med1* gene in the mouse germline; inactivation of *Med1* increases mutagenicity at CpG sites, but only 3-fold, suggesting that Tdg may provide a redundant function in repairing deamination events. To study the functional role of TDG, we recently generated conditional Tdg knock-out mice. While Med1 knock-out mice are viable, homozygous inactivation of Tdg in the mouse germline leads to multiple developmental defects and midgestation lethality, but the molecular mechanisms are unknown. Experiments in this proposal will test the hypothesis that the two BER enzymes, MED1 and TDG, cooperate in counteracting the mutagenic and tumorigenic consequences of different types of DNA damage at CpG sequences; thus, combined inactivation of these BER enzymes might be necessary to uncover full mutagenicity of CpG sites. Specifically, the requirement of TDG during development will be investigated; mutation frequency of Tdg single- and Med1/Tdg double-mutant mice will be examined in crosses with BigBlue mice as a reporter system; the role of Med1/Tdg in tumorigenesis will be examined by conducting crosses with animal models of colonic adenomas (ApcMin mice) and melanoma (tyrosinase-Cre, Tyrosinase-N-Ras mice); finally, TDG expression in human colorectal and melanoma specimens will be assessed to evaluate the involvement of TDG in human disease. These studies will provide new insights into mechanisms of endogenous mutagenesis by deamination, and their link to cancer. ■

TITLE

The NF-Y/mutp53-microRNAs Axis in Cancer Progression and Chemiotheraphy Resistance

PRINCIPAL RESEARCHER

Giulia Piaggio
Laboratory "C" of Molecular Oncogenesis

CO-RESEARCHERS

Fabrizio Ambesi Impiombato
Radiotherapy
Maurizio Cosimelli
General Surgery B
Silvia Di Agostino
R.O.C.
Marcella Mottolese
Histology and Cytopathology
Francesca Sperati
Irene Terrenato
Epidemiology
Angela Torsello
Medical Oncology C

Abstract

■ Mechanisms involved in the microRNA (miRNA) biogenesis are known to cause aberrant expression of miRNA s in cancer. Less clear is the contribution of mammalian transcription factors in the regulation of miRNA expression. Recently a possible correlation between wild-type p53 (wtp53) transcriptional activity and miRNA expression has been shown, but data about mutant p53 are not available yet. We have previously shown that the transcription factor NF-Y brings mutant p53 (mutp53) onto CCAAT-boxes containing promoters in response to DNA damage. The oncogenic cooperation of these two major regulators allows cells to override cellular fail-safe programs, thus permitting tumor progression in response to chemotherapy.

The first aim of this research is to investigate whether the NF-Y/mutp53 axis modulates the expression of specific miRNAs in response to DNA damage, directly regulating their gene transcription. This aim will be addressed by both *in silico*, genome wide and functional experimental approches: a) the presence of the CCAAT cis-regulatory motif in a set (input) of upstream pre-microRNA human sequences will be investigated by computational methods; b) the binding of the NF-Y/mutp53 complex on these sequences will be investigated by ChIP on chip experiments before and after DNA damage; c) NF-Y and mutant p53 will be knocked down via RNAi, and the expression profiles of microRNAs will be measured using miRNA microarray analysis, before and after DNA damage. The integrated analysis of the results coming from these three approches allow us to define

a pattern of miRNAs whose expression is directly regulated by the NF-Y/mutp53 complex before and after DNA damage.
The second aim of this research is to investigate whether the specific pattern of NFY/mutp53 dependent miRNAs, defined in the first aim, plays a critical role in the mutp53 gain of function activity in response to DNA damage. This aim will be addressed by functional experiments overexpressing and knocking out single or couple miRNAs in culture cells and analysing the biological effect on cell proliferation after DNA damage.
To investigate the molecular mechanism through which these miRNAs exert their activity, we will use bioinformatic tools and biochemical analysis to identify putative target genes of the miRNAs that will have an effect on cell proliferation.
Our preliminary evidence demonstrates that an oncogenic cooperation between NF-Y and mutp53 exists in rectal cancer of patients treated with neoadjuvant chemoradio-therapy (CRT).
This cooperation upregulates NF-Y target genes involved in the progression of cell cycle permitting tumor progression. Here, we hypothesize that NF-Y/mutp53 complex functions through miRNA expression to promote progression to a chemotherapy resistant phenotype. Thus, a secondary aim of this study will be to address whether a correlation exists between the presence of NF-Y/mutp53 and a specific profile of miRNAs expression in rectal cancer biopsys from patients treated with neoadjuvant CRT.
To address this aim, samples will be selected by laser capture microdissection (LCM) to isolate normal and tumoral tissue and expression profiles of the pattern of miRNAs characterized in the first aim will be measured by Q-PCR. ■

TITLE

Validation of RALT/MIG6 as tumor suppressor in human neoplastic disorders: from pathological analysis to cellular modelling

PRINCIPAL INVESTIGATOR
Sergio Anastasi
Laboratory "B" of Immunology

COLLABORATORS
Anna Maria Cianciulli
Clinical Pathology
Yuri Frosi
Postdoc, Laboratory "B" of Immunology
Carlo Garufi
Medical Oncology C
Marcella Mottolese
Histology and Cytopathology
Cecilia Nisticò
Medical Oncology 'C'
Letizia Perracchio
Histology and Cytopathology
Edgar Allan Pogna
Post-doc, Laboratory "B" of Immunology
Oreste Segatto
Laboratory "B" of Immunology
Silvia Soddu
Laboratory "C" of Molecular Oncogenesis
Irene Venturo
Medical Oncology C

Abstract

■ Aberrant signalling by receptor tyrosine kinases of the ErbB family has a causal role in a number of human tumors, including breast, lung and colorectal carcinomas, head and neck squamous cell carcinomas, glioblastomas. This has propelled an enormous interest in drug-discovery programs aimed at developing ErbB-targeted therapeutics. Thus, understanding the pathogenesis, biology and molecular genetics of ErbB-driven tumors is of paramount importance, since this knowledge is critical for improving targeted therapeutics of tumors with high incidence and aggressive behaviour. Our laboratory is interested in investigating negative signalling to ErbB RTKs as a means to discover novel pathways of tumor suppression. We have identified RALT/MIG6 as a feedback inhibitor of ErbB RTKs. RALT is capable of suppressing ErbB signalling via two sequentially acting mechanisms, namely suppression of receptor kinase function and receptor downregulation. This is an essential function, as loss of RALT is sufficient to cause aberrant ErbB-driven proliferation and tumor formation in the mouse.
The investigation of the tumor suppression activity of RALT in human tumors has been hampered by the lack of reagents suitable for large scale immunohistohemical studies of human tumors. We have developed monoclonal antibodies against RALT that allow for sensitive and specific detection of RALT in paraffin-embedded tissues. Here we propose to investigate whether loss of RALT occurs in human tumors displaying aberrant ErbB signalling and, if so, whether it correlates with clinical prognosis and/or responsiveness to ErbB-targeted therapeutics. Finally, studies in genetically defined cellular model systems will model the impact of RALT loss on oncogenic ErbB signalling. ■

TITLE

Molecular processes underlying Human Papillomavirus-induced cellular transformation and cervical carcinoma progression

PRINCIPAL INVESTIGATOR
Anna Maria Mileo
Laboratory 'C' Associated to the Prevention and Diagnosis Department

CO-INVESTIGATORS
Claudia Abruzzese
Emanuele Bellacchio
Stefano Mattarocci
Marco Paggi
Laboratory 'C' Associated to the Prevention and Diagnosis Department
Mariantonia Carosi
Paolo Visca
Histology and Cytopathology
Antonella Bavarese
Medical Oncology 'A'
Giuseppe Cutillo
Enrico Vizza
Gynaecology

Abstract

■ In the last two decades, the "viral hypothesis" of cancer has been considerably strengthened by the notion that a number of small DNA viruses, such as Adenovirus, Human Papillomavirus (HPV) and SV40, can profoundly interfere with complex cellular processes as cell cycle, differentiation, senescence, apoptosis, gene expression and - finally - transformation. In this field, considerable interest is raised by the so-called "high-risk" HPV strains, as HPV-16 and -18, which are the causative agents of at least 90% of cervical cancers, conferring to such viral infection a pivotal role.
We aim to help reconstruct the network of effects elicited by high-risk HPVs during the infective or transforming processes targeted to human cells. To this end, we are designing a "hypothesis-driven" transcriptome analysis of dysplastic/neoplastic cervical diseases, in order to reveal molecular signatures for these pathologies, as well as diversity among cancers. The selection of the genes whose expression will be assayed springs from public data (published literature and public databases) and from the specific laboratory know-how and experimentation on HPV and other small DNA tumor viruses. The study will be essentially, but not uniquely, retrospective, i.e. gene expression analysis, via quantitative real-time polymerase chain reaction, of the selected genes in archival formalin-fixed, paraffin-embedded samples from patients with a well-recognized clinical history. Thus a robust statistical approach will validate a panel of genes, chosen among those assayed, whose expression can be effectively correlated with clinical parameters. Indeed, this effort is done with the aim to identify new taxonomic procedures with diagnostic, prognostic and possibly therapeutic significance. ■

TITLE

Transcriptional control of DeltaN-p73 gene expression in hematopoietic neoplasms

PRINCIPAL INVESTIGATOR

Maria Rizzo
Laboratory "C" of Molecular Oncogenesis

CO-INVESTIGATORS

Iole Cordone
Clinical Pathology

Giulia Fontemaggi
R.O.C.

Maria Concetta Petti
Hematology

Holger Schünemann
Epidemiology

Francesca Sperati
Epidemiology

Abstract

The p53 paralog, TPp73 gene gives rise to multiple, functionally distinct proteins involved in the control of growth arrest, apoptosis and differentiation. Multiple TA (transactivation competent, proapoptotic and anti-proliferative) p73 carboxy-terminal splicing isoforms (α, β, γ, δ, ε, ζ, η and η1) are expressed from the P1p73 promoter. A second intragenic promoter, P2p73, controls the expression of N-terminally deleted proteins (ΔN-p73) that lack the amino-terminal transactivation domain and acts as dominant-negative repressors of p53- and p73-dependent apoptosis.

Interestingly, alterations in TAp73 and ΔN-p73 expression, rather than inactivating mutations within the TP73 gene, have been described in many human cancers. In particular, some hematological diseases as acute lymphoid leukemia (ALL), Burkitt's lymphomas, B-non-Hodgkin's lymphomas (B-NHLs), and multiple myeloma (MM) display CpG island hypermethylation of the TA promoter as important and predominant mechanism in regulating p73 expression. Instead, acute myeloid leukaemias (AMLs) have hypermethylation of the ΔN-p73 promoter and a relative enrichment of shorter TAp73 isoforms. In addition, we have previously shown that leukemic blasts from patients with acute promyelocytic leukemia (APL) present a peculiar lack of ΔN-p73 supporting a role of p73 in the pathogenesis of APL in an unusual manner. Altogether these findings suggest that non-mutational mechanisms, such as promoter hypermethylation or aberrant expression of specific isoforms (ΔN-p73), rather than structural alterations, may contribute to the pathogenesis of some hematopoietic neoplasias.

We identified the PML/RAR? fusion protein as a direct regulator of the ΔN-p73 transcription and showed that retinoic acid (RA) treatment relieves P2p73 repression *in vitro* and restores ΔN-p73 expression in APL patients *in vivo*. At the functional level, we found that restoration of ΔN-p73 expression in APL cells induces a number of differentiation markers and cooperates with RA-induced differentiation *in vitro*, further supporting the relevance of ΔN-p73 inhibition in hematopoietic tumors.

Based on these considerations, we propose here to investigate the molecular regulatory mechanism(s) of ΔNp73 expression in specific haematological malignancies such as APL and MM.

We propose to address the following specific aims:

I) characterization of the molecular mechanism(s) governing PML/RAR?-mediated transcriptional repression of ΔN-p73.
II) signature of differentiation-associated miRs linked to ΔN-p73 in granulocytic differentiation.
III) analysis of ΔN-p73 expression in MM patient samples at diagnosis.

Overall, this proposal aims to contribute to a better understanding of the APL leukemogenesis together with novel targets for prognostic assessment and diagnostic correlations.

TITLE

Role of Che-1 in DNA damage response

PRINCIPAL INVESTIGATOR

Maurizio Fanciulli
Laboratory "B" associated to the Medical Oncology Department

RESEARCHERS INVOLVED IN THE PROJECT

CLINICAL AREA

Maurizio Cosimelli
General Surgery B

Marcella Mottolese
Histology and Cytopathology

Vittoria Stigliano
Gastroenterology

EXPERIMENTAL AREA

Cinzia Rinaldo
Laboratory "C" of Molecular Oncogenesis

Tiziana Bruno
Agata Desantis
Laboratory "B" associated to the Medical Oncology Department

COLLABORATORS

Bruno Amati
(IEO/IFOM, Milan, Italy)

Gianluca Bossi
Laboratory "C" of Molecular Oncogenesis

Elisabetta Mattei
(CNR/European Brain Research Institute, Rome, Italy)

Abstract

In response to diverse genotoxic stresses, cells activate DNA damage checkpoint pathways to protect genomic integrity and promote survival of the organism. Depending on DNA lesions and context, damaged cells with activated checkpoint can be eliminated by apoptosis or silenced by cellular senescence, or can survive and resume cell cycle progression upon checkpoint termination. DNA damage response machinery (DDR) is constitutively activated in early, premalignant lesions of major types of human solid tumors and defects in DDR components probably contribute to the pathogenesis of all types of human cancer. In the past few years we could show that DNA damage leads to stabilization and accumulation of Che-1 an RNA polymerase II-binding protein that plays an important role in transcription activation of p53 and in maintenance of the G2/M checkpoint. Furthermore, our recent studies indicate that Che-1 is down-regulated during the apoptotic process by its interaction with MDM2 and NRAGE. These observations strongly support the hypothesis that Che-1 is an anti-apoptotic gene involved in DDR and deserves a mechanist characterization of the pathways in which it is involved. To this aim, we propose to investigate two different aspects of Che-1 function

1. Characterization of the physiological role/s played by Che-1 in the oncogene-induced DNA damage response in incipient tumor cells;
2. Evaluation of the contribute of Che-1 to DNA damage response and DNA repair.

Overall, the results of this project could have a significant impact in cancer research. Indeed, detailed understanding of the pathways underlying checkpoint response, as well as identification and characterization of the participating proteins, will significantly advance the ability to unravel the complex processes leading to the development of cancer. Furthermore, understanding of the mechanisms underlying checkpoint response after DNA damage will benefit existing therapeutic modalities and likely contribute to the development of novel cancer-treatment approaches. ■

TITLE

Transcriptional regulation and signalling networks of ∂6ß4 integrin that contributes to tumor progression

PRINCIPAL INVESTIGATOR

Rita Falcioni

Laboratory "C" of Molecular Oncogenesis

CO- INVESTIGATORS

Giulia Bon

Laboratory "C" of Molecular Oncogenesis

CO- RESEARCHERS

Stefania Dell'Orso

R.O.C.

Alessandra Fabi

Medical Oncology A

Marcella Mottolese

Histology and Cytopathology

Abstract

■ The ∂6ß4 integrin is up regulated in different tumor types of epithelial origin where it participates in signalling pathways that contribute to malignancy. The ∂6ß4 combines with and enhances the signalling function of several receptor tyrosine kinases such as ErbB-2, ErbB-3, EGFR and Met by facilitating key functions of carcinoma cells including their ability to migrate, invade, and evade apoptosis. The mechanism involves a profound ∂6ß4 effect on specific signalling pathways, especially the PI3-K/Akt pathway. In the past few years, we have shown that ∂6ß4 integrin associates with ErbB-2 oncogene in mammary tumor cells and promotes PI3K-dependent invasion. PI3-K activation is in part dependent on the ability of ∂6ß4 to regulate ErbB-3 expression that results in an increase of ErbB-2/ErbB-3 heterodimerization and, as consequence, an increase of Akt-activated tumor survival. In breast cancers, amplification of ErbB-2 oncogene occurs in almost 25% of tumors. It is associated with poor patient outcome and even though Trastuzumab induces clinical response in these tumors, some breast cancers escape trastuzumab treatment. In these patients, ErbB-2/ErbB-3 heterodimer is the strongest stimulator of the PI3K/Akt pathway that can abrogate responsiveness to Trastuzumab treatment. Our previous data suggests a possible involvement of ∂6ß4 integrin in the mechanism of Trastuzumab resistance in ErbB-2-overexpressing mammary tumors. On the other hand, the expression of ∂6ß4 integrin is reduced in basal cell carcinoma of the skin and prostate cancer. These findings clearly indicate that ∂6ß4 integrin could have complex dual functions. A clear understanding of the transcriptional regulation of ß4 could explain the meaning of this dual function. It has been reported that ß4 expression is regulated, at least in part, at the transcription level. In order to elucidate the mechanism responsible for ß4 gene expression and the transcriptional basis for cancer invasion and metastasis, we obtained preliminary results indicating that HIPK2 (Homeodomain-Interacting Protein kinase 2) participates in the regulation of ß4 integrin transcription.

Within the present grant proposal with the aim to clarify the role of ß4 integrin in the progression of human cancers we propose to investigate the following aspects:

1. Analysis of the role of ∂6ß4 and ErbB-3 functional interaction in the mechanism of resistance to Trastuzumab treatment in ErbB-2-overessing breast cancer cells and tumors.
2. Characterization of the transcriptional regulation of ∂6ß4 integrin in tumors; This proposal will contribute to characterize ∂6ß4 integrin as an important player in the regulation of tumor development and progression. ■

TITLE

p53 family interaction network as a target of anti-tumoral peptide therapy

PRINCIPAL INVESTIGATOR

Sabrina Strano

R.O.C.

CO-RESEARCHERS

CLINICAL AREA

Marcella Mottolese

Histology and Cytopathology

Cecilia Nisticò

Medical Oncology C

Holger Schünemann

Epidemiology

EXPERIMENTAL AREA

Gennaro Citro

Animal Facility and Pharmacokinetic Unit

Barbara Benassi

Silvia Di Agostino

R.O.C.

Abstract

■ The therapeutic efficacy of anti-cancer agents depends strongly on their ability to trigger apoptosis in target tumor cells. Many physical and chemical DNA damaging agents used routinely in cancer therapy are potent p53 activators. 50% of all human tumors overexpress mutant forms of p53 due mainly to missense mutation of the p53 gene. A growing number of studies suggest that the nature of a p53 mutation in a cell can impact upon cellular properties, clinical responses to therapy and prognosis of a tumor. Thus, it is conceivable to propose that at least certain mutant forms of p53 possess gain of function activity, whereby they contribute positively to cancer progression. The molecular mechanism underlying gain of function of mutant p53 remains to be elucidated. To this end, we may depict two possible scenarios. The first one relies on the assumption that mutant p53 can either activate or repress target genes through its intact transactivation domain, while the second is based on the binding and sequestering of proteins whose function is required for efficient apoptosis in response to anti-cancer agents. Two new p53 homologues, p73 and p63 have recently been identified. As expected for p53 like proteins, p73 and p63 recapitulate p53 functions including growth arrest, apoptosis, and differentiation when overexpressed exogenously in p53-/- and p53+/+ tumor cells. We and others have recently reported that human

tumor derived p53 mutants can engage in a physical and functional interaction with p73 isoforms. Thus, p73 and probably p63 can be considered target proteins for inactivation of gain of function p53 mutants. Furthermore, proteins interacting either with mutant p53 or p73 might also interfere with the biological outcome of the entire network. The last decade has brought increasing attention to identify small molecules and gene targeting approaches against the specific molecular abnormalities that create and drive cancer. Among the new molecules, an increasing number of the new medicinal products concern peptides for therapeutic or diagnostic use.

The main objective of the proposed research proposal is the *in vitro* and *vivo* evaluation of the therapeutic potential of short interfering peptides (Short Interfering Mutantp53 Peptides/SIMPs) capable to disassemble the oncogenic protein complexes mutantp53/ p73 in tumor cells.

To this end, we propose an experimental approach along the following steps: **Firstly,** we will analyze in depth, at biochemical and functional level, how short peptides interfere with the protein-protein interactions between mutant p53, p73 and p63. We will analyze the functional implications of the association between mutant p53, p73 and p63 in the tumor response to conventional anticancer treatment such as cis-platin and adriamycin. A special focus will be given to the impact of short interfering peptides on p73 transcriptional activity (gene profiling and ChIP on chip analysis). **Secondly**, we will investigate the combination of the pre-treatment of short interfering peptides capable of disrupting the protein complex mutantp53/p73 with new target agents such as monoclonal antibodies and small molecules with EGFR-TK inhibitor activity. Trastuzumab, a recombinant humanized anti-HER2 monoclonal antibody, and lapatinib, a tyrosine kinase ErbB1 and Erb2 inhibitor, showed remarkable activity in patients with breast cancer expressing ErbB1 and/or overexpressing ErbB2 which are frequently associated with high frequency of p53 mutations.

Thirdly, we will investigate the impact of SIMPs activity *in vivo* by measuring tumor growth in nude mice and evaluating the efficacy of the combination of SIMPs with anticancer drugs (cisplatin, adriamycin, Trastuzumab and Lapatinib) in the treatment of breast-tumor xenograft-bearing BALB/c nude mice. ■

Graphs

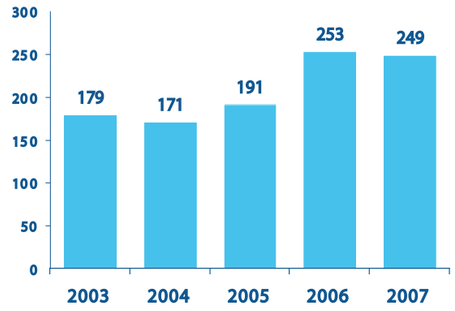
Scientific Activity

PUBLICATIONS

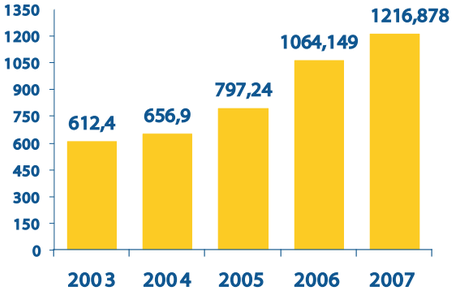
Total Number of Publications, according to both raw and Normalized Impact Factor, from 2003 to 2007

Year	No. of Publications	I.F. raw	I.F. normalized
2003	179	612,4	616,6
2004	171	656,9	576,7
2005	191	797,24	706,1
2006	253	1064,149	920,5
2007	249	1216,878	935,5

Peer-Reviewed Publications from 2003 - 2007

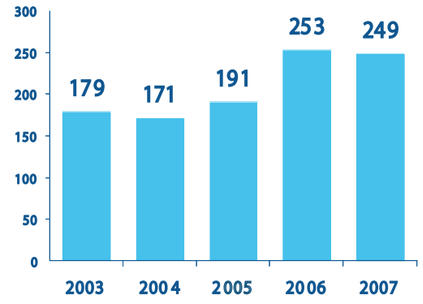


No. of Publications by year

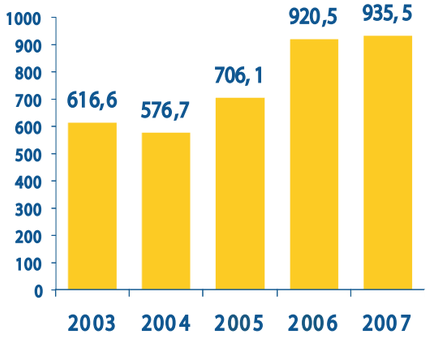


I.F. (raw)

Peer-Reviewed Publications from 2003 - 2007

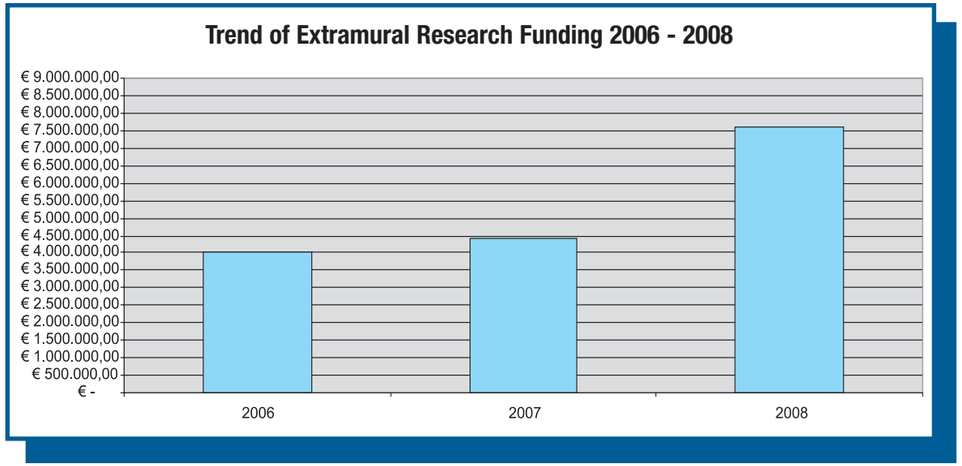


No. of Publications by year



I.F. (normalized*)

* Normalized according to Italian Ministry of Health criteria


Trend of Extramural Research Funding 2006 - 2008
€ 9.000.000,00
€ 8.500.000,00
€ 8.000.000,00
€ 7.500.000,00
€ 7.000.000,00
€ 6.500.000,00
€ 6.000.000,00
€ 5.500.000,00
€ 5.000.000,00
€ 4.500.000,00
€ 4.000.000,00
€ 3.500.000,00
€ 3.000.000,00
€ 2.500.000,00
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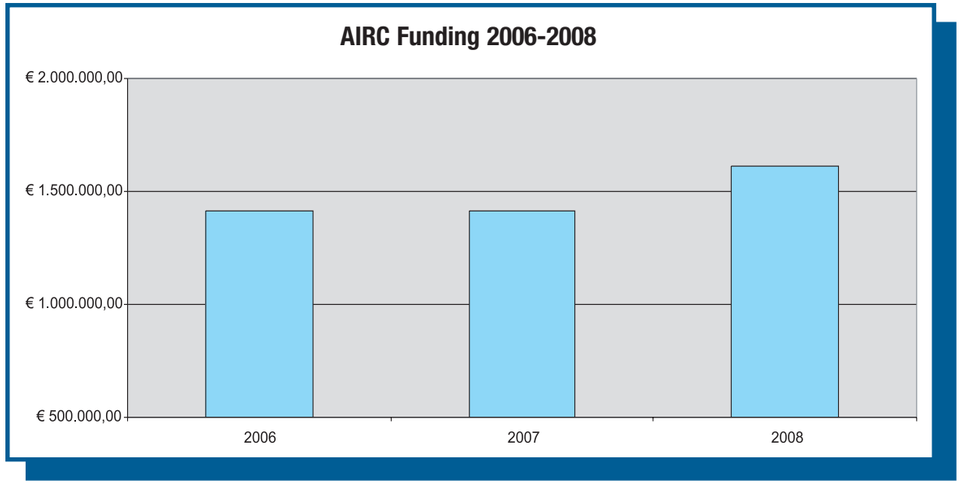
€ 1.500.000,00
€ 1.000.000,00
€ 500.000,00
€ -
2006
2007
2008


AIRC Funding 2006-2008
€ 2.000.000,00
€ 1.500.000,00
€ 1.000.000,00
€ 500.000,00
2006
2007
2008

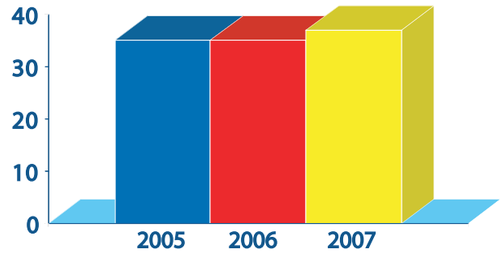

2007 - Clinical Research by Supporting Companies
Roche S.p.A. 9
Pfizer Italia s.r.l. 9
Astra Zeneca S.p.A. 5
Novartis Farma S.p.A. 4
Eli Lilly S.p.A. 4
Bristol Myers Squibb S.r.l. 4
Amgen S.p.A. 4
Glaxo Smithkline S.p.A. 3
Oncotech 2
Nycomed Italia s.r.l. 2
Merck Sharp & Dohme S.p.A. 2
Italfarmaco S.p.A. 2
Wyeth Lederle S.p.A. 1
Serono International, S.A. 1
Schering-Plough S.p.A. 1
Schering S.p.A. 1
Pierre Fabre s.r.l. 1
Laboratorios Dr. Esteve, SA 1
KS Biomedix Ltd. 1
Janssen Cilag S.p.A. 1
Helsinn Healthcare SA 1
Fresenius Biotech GmbH 1
Dompè Biotec S.p.A. 1
0 1 2 3 4 5 6 7 8 9 10

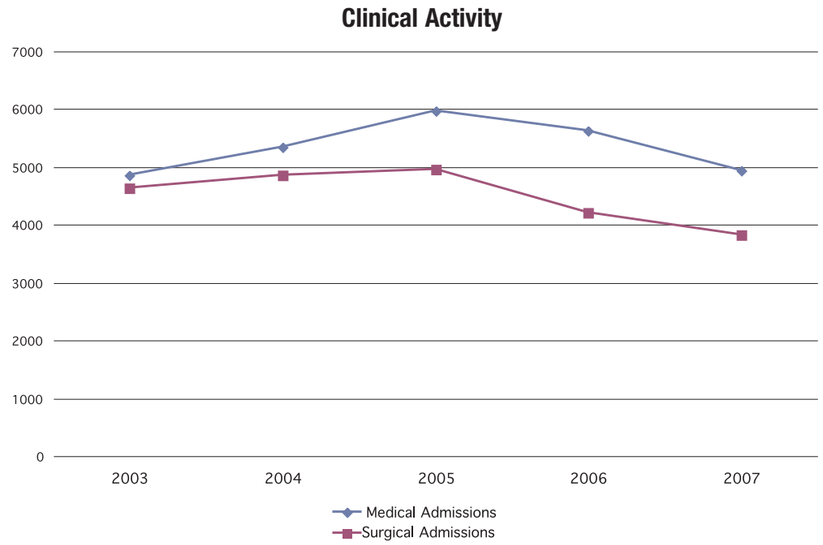
Clinical Trials and Patients Enrolled: 2005-2007

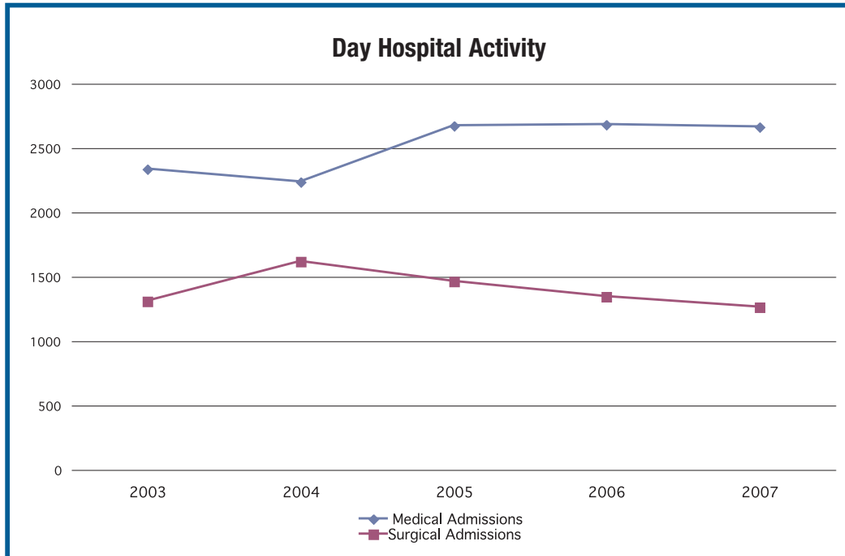
	TOTAL TRIALS	TOTAL PATIENTS
2005	199	1896
2006	200	1538
2007	218	1763

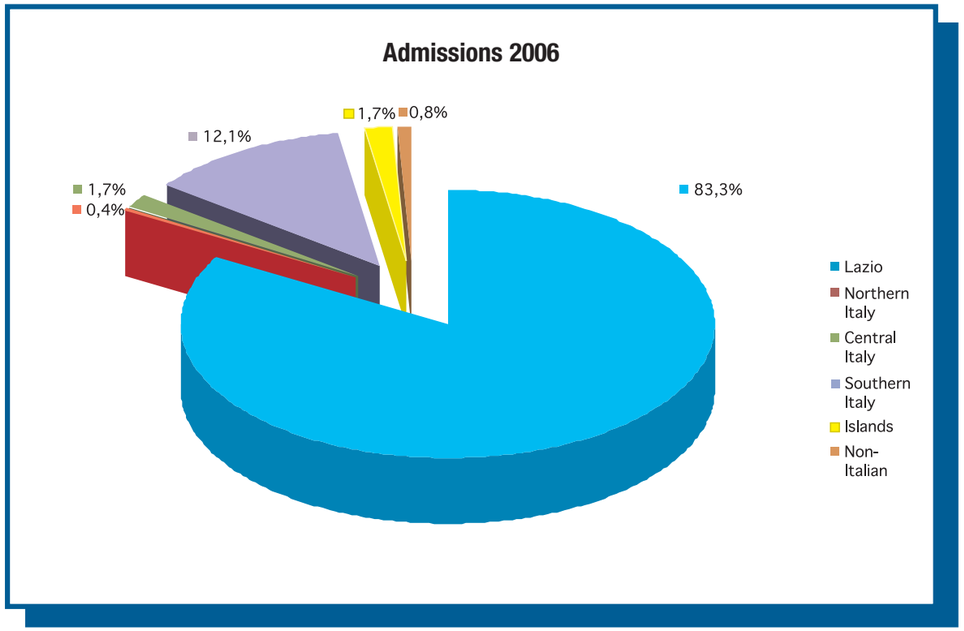
I.R.E.: Clinical Trials by year

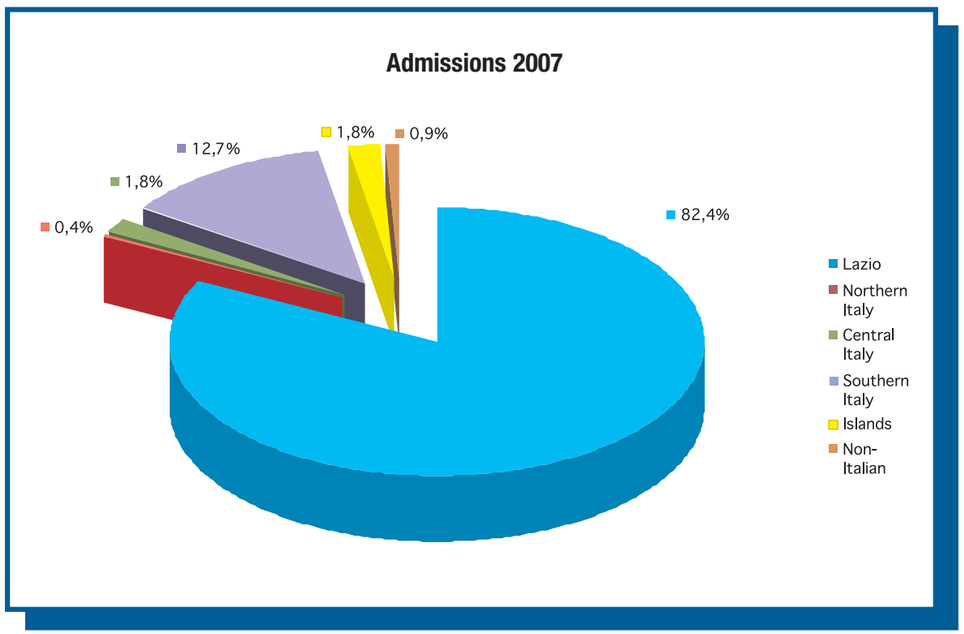
35 Active Studies 2005
35 Active Studies 2006
37 Active Studies 2007

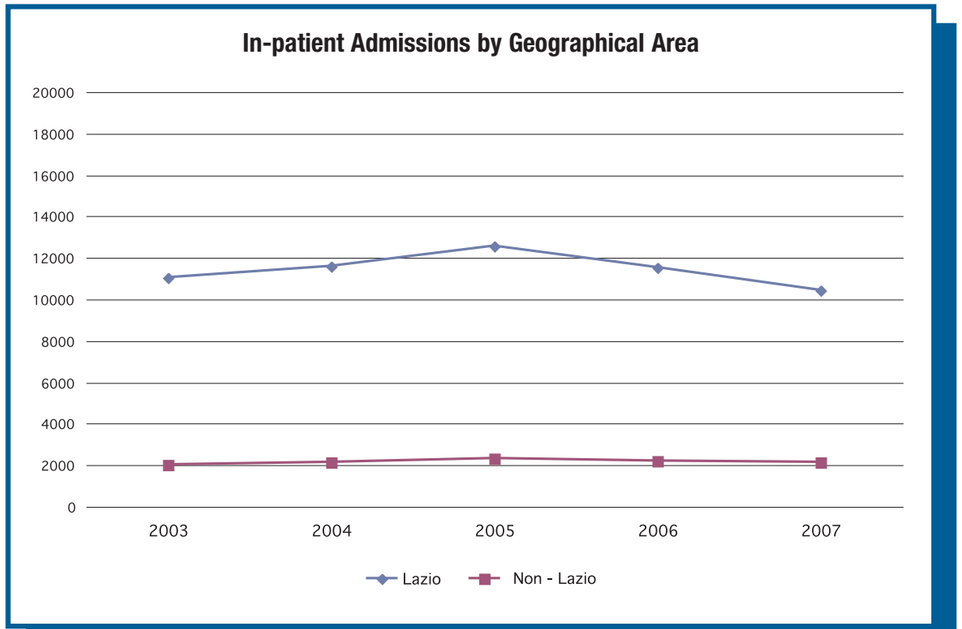


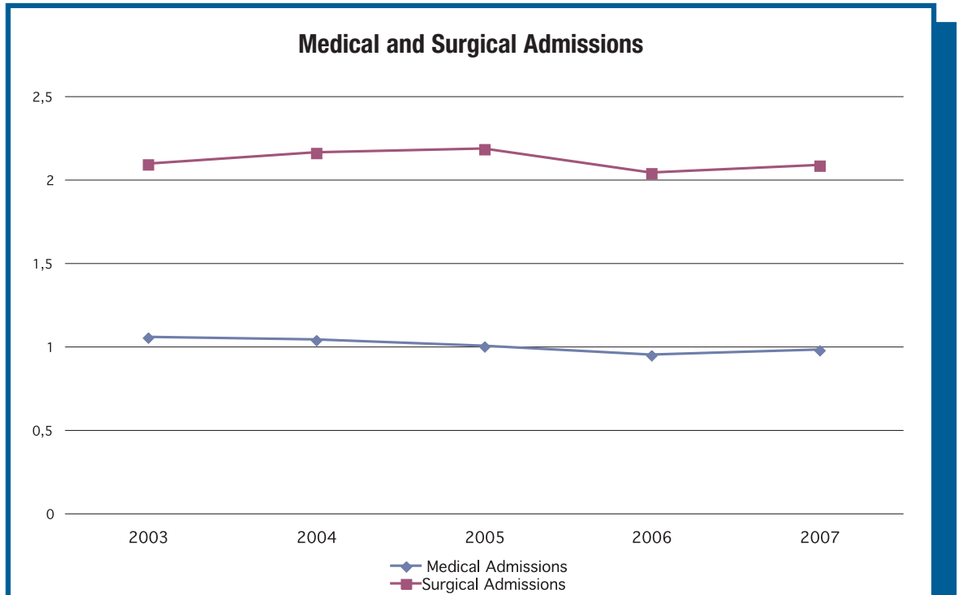




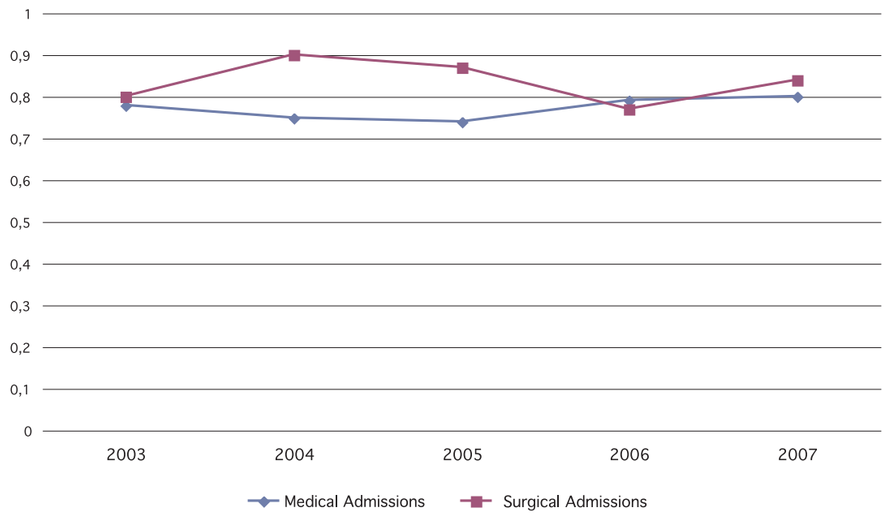

Admissions 2006
1,7%
0,8%
12,1%
1,7%
0,4%
83,3%
Lazio
Northern Italy
Central Italy
Southern Italy
Islands
Non-Italian


Admissions 2007
1,8%
0,9%
12,7%
1,8%
0,4%
82,4%
Lazio
Northern Italy
Central Italy
Southern Italy
Islands
Non-Italian

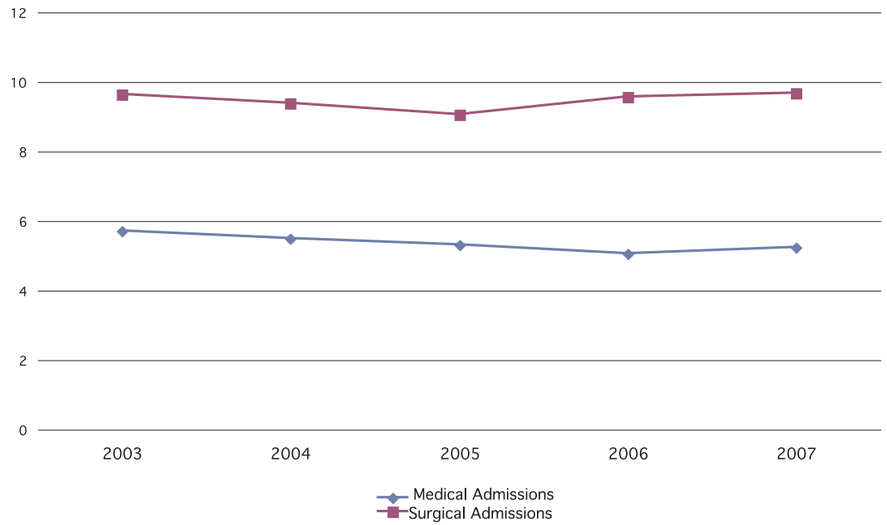

In-patient Admissions by Geographical Area
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
2003
2004
2005
2006
2007
Lazio
Non - Lazio


Medical and Surgical Admissions
2,5
2
1,5
1
0,5
0
2003
2004
2005
2006
2007
Medical Admissions
Surgical Admissions

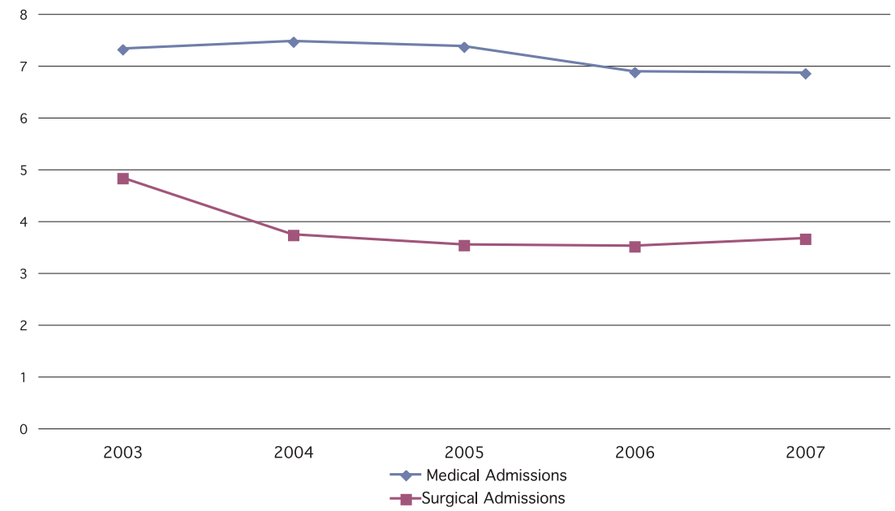
Day Hospital



In-patients



Average Length of Stay



Publications

Publications in Referenced Journals 2007

1. Akl E.A., Grant B.J., Guyatt G.H., Montori V.M., Schünemann H.
A decision aid for COPD patients considering inhaled steroid therapy: development and before and after pilot testing.
BMC MEDICAL INFORMATICS AND DECISION MAKING 2007; 15;7:12.

2. Akl E.A., Kamath G., Kim S.Y., Yosuico V., Barba M., Terrenato I., Sperati F., Schünemann H.
Oral anticoagulation for prolonging survival in patients with cancer.
COCHRANE DATABASE OF SYSTEMATIC REVIEWS 2007; 18(2):CD006466.

3. Akl E.A., Karmath G., Kim S.Y., Yosuico V., Barba M., Terrenato I., Sperati F., Schünemann H.
Oral anticoagulation may prolong survival of a subgroup of patients with cancer: a cochrane systematic review.
JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH 2007; 26(2):175-84.

4. Akl E.A., Kamath G., Yosuico V., Kim S.Y., Barba M., Terrenato I., Sperati F., Cook D., Schünemann H.
Anticoagulation for thrombosis prophylaxis in cancer patients with central venous catheters.
COCHRANE DATABASE OF SYSTEMATIC REVIEWS 2007; 18(3):CD006468.

5. Akl E.A., Maroun N., Guyatt G., Oxman A.D., Alonso-Coello P., Vist G.E., Devereaux P.J., Montori V.M., Schünemann H.
Symbols were superior to numbers for presenting strength of recommendations to health care consumers: a randomized trial.
JOURNAL OF CLINICAL EPIDEMIOLOGY 2007; 60(12):1298-305.

6. Akl E.A., Maroun N., Major S., Afif C., Chahoud B., Choucair J., Sakr M., Schünemann H.
Why are you draining your brain? Factors underlying decisions of graduating Lebanese medical students to migrate.
SOCIAL SCIENCES AND MEDICINE 2007; 64(6):1278-84.

7. Akl E.A., Maroun N., Major S., Chahoud B., Schünemann H.
Graduates of Lebanese medical schools in the United States: an observational study of international migration of physicians.
BMC HEALTH SERVICES RESEARCH 2007; 5;7:49.

8. Akl E.A., Mustafa R., Bdair F., Schünemann H.
The United States physician workforce and international medical graduates: trends and characteristics.
JOURNAL OF GENERAL INTERNAL MEDICINE 2007; 22(2):264-8.

9. Akl E.A., Van Doormall F.F., Barba M., Kamath G., Kim S.Y., Kuipers S., Middeldorp S., YosuicoV., Dickinson H.O., Schünemann H.
Parenteral anticoagulation for prolonging survival in patients with cancer who have no other indication for anticoagulation.
COCHRANE DATABASE OF SYSTEMATIC REVIEWS 2007; 18(3):CD006468.

10. Alo P.L., Amini M., Piro F., Pizzuti L., Sebastiani V., Botti C., Murari R., Zotti G., Di Tondo U.
Immunohistochemical expression and prognostic significance of fatty acid synthase in pancreatic carcinoma.
ANTICANCER RESEARCH 2007; 27(4B):2523-7.

11. Anastasi S., Baietti M.F., Frosi Y., Alema S., Segatto O.
The evolutionarily conserved EBR module of RALT/MIG6 mediates suppression of the EGFR catalytic activity.
ONCOGENE 2007; 26/57:7833-46.

12. Arcangeli S., Saracino B., Petrongari M., Gomellini S., Marzi S., Landoni V., Gallucci M., Sperduti I., Arcangeli G.
Analysis of toxicity in patients with high risk prostate cancer treated with intensity-modulated pelvic radiation therapy and simultaneous integrated dose escalation to prostate area.
RADIOTHERAPY AND ONCOLOGY 2007; 84:148-155.

13. Arndt V, Kaatsch P., Steliarova-Foucher E., Peris-Bonet R., Brenner H. Working group (Ramazzzotti V.).
Up-to-date monitoring of childhood cancer long-term survival in Europe: central system tumors.
ANNALS OF ONCOLOGY 2007; 18:1734-42.

14. Arndt V., Lacour B., Steliarova-Foucher E., Spix C., Znaor A., Pastore G., Stiller C., Brenner H. Working group (Ramazzzotti V.).
Up-to-date monitoring of childhood cancer long-term survival in Europe: tumors of the sympathetic nervous system, retinoblastoma, renal and bone tumors, and soft tissue sarcomas.
ANNALS OF ONCOLOGY 2007; 18:1722-33.

15. Azzini E., Bugianesi R., Romano F., Di Venere D., Miccadei S., Durazzo A., Foddai M.S., Catasta G., Linsalata V., Maiani G.
Absorption and metabolism of bioactive molecules after oral consumption of cooked edible heads of Cynara scolymus L. (cultivar Violetto di Provenza) in human subjects: a pilot study.
BRITISH JOURNAL OF NUTRITION 2007; 97(5):963-9.

16. Badaracco G., Rizzo C., Mafera B., Pichi B., Giannarelli D., Rahimi S.S., Vigili M.G., Venuti A.
Molecular analyses and prognostic relevance of HPV in head and neck tumors.
ONCOLOGY REPORTS 2007; 17:931-939.

17. Badaracco G., Venuti A.
Human papillomavirus therapeutic vaccines in head and neck tumors.
EXP. REV. ANTI INFECT THER. 2007; 7(5):753-66.

18. Bagnato A., Rosanò L.
Epithelial-mesenchymal transition in ovarian cancer progression: a crucial role for the endothelin axis.
CELLS TISSUES ORGANS 2007; 185:85-94.

19. Baldelli R., De Marinis L., Bianchi A., Pivonello R., Gasco V., Auriemma R., Pasimeni G., Cimino V., Appetecchia M., Maccario M., Lombardi G., Pontecorvi A., Colao A., Gottoli S.
Microalbuminuria and insulin sensitivity in patients with GH-secreting pituitary tumor.
JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM 2007; 93:3710-3714.

20. Baldelli R., Ettorre M., Vennarecci G., Pasimeni G., Carboni F., Lorusso R., Bernabei A., Appetecchia M.
Malignant insulinoma presenting as metastatic liver tumor. Case report and review of the literature.
JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH 2007; 26(4):603-7.

21. Baldini E., Ulisse S., Marchioni E., Di Benedetto A., Giovannetti G., Petrangeli E., Sentinelli S., Perrone D., Reale M.G., Mottolese M., Gandini L., Lenzi A., D'Armiento M.
Expression of Fas and Fas ligand in human testicular germ cell tumors.
INTERNATIONAL JOURNAL OF ANDROLOGY 2007; 3:.

22. Bassler D., Ferreira-Gonzalez I., Briel M., Cook D.J., Devereaux P.J., Heels-Ansdell D., Kirpalani H., Meade M.O., Montori V.M., Rozenberg A., Schünemann H., Guyatt G.H.
Systematic reviewers neglect bias that results from trials stopped early for benefit.
JOURNAL OF CLINICAL EPIDEMIOLOGY 2007; 60(9):869-73.

23. Bellacchio E., Paggi M.
Protease-mediated arsenic prodrug strategy in cancer and infectious diseases: a hypothesis for targeted activation.
JOURNAL OF CELLULAR PHYSIOLOGY 2007; :.

24. Benassi B., Zupi G., Biroccio A.
Gamma-glutamylcysteine synthetase mediates the c-Myc-dependent response to antineoplastic agents in melanoma cells.
MOLECULAR PHARMACOLOGY 2007; 72:1015-1023.

25. Benevolo M., Mottolese M., Marandino F., Carosi M., Diodoro M., Sentinelli S., Visca P., Rollo F., Mariani L., Vocaturo G., Sindico R., Di Giannuario D., Perrone D., Pellicciotta M., Vocaturo A.
HPV genotypes concordance between sex partners.
JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH 2007; 26(4):609-12.

26. Benevolo M., Vocaturo A., Novelli F., Mariani L., Vocaturo G., Cianciulli A., Marandino F., Perrone D., Giannarelli D., Natali P., Mottolese M.
Prognostic value of HER2 and progesterone receptor expression in endometrial carcinoma with positive peritoneal washing.
ANTICANCER RESEARCH 2007; 27:2839-44.

27. Blandino G., Fanciulli M., Levrero M., Piaggio G.
The post-genomic era: workshop on chromatin immunoprecipitation-related techniques.
CELL DEATH AND DIFFERENTIATION 2007; 14(7):1390-1.

28. Bon G., Folgiero V., Di Carlo S., Sacchi A., Falcioni R.
Involvement of alpha6beta4 integrin in the mechanisms that regulate breast cancer progression.
BREAST CANCER RESEARCH 2007; 9:203.

29. Bosetti C., Rossi M., McLaughlin J.K., Negri E., Talamini R., Lagiou P., Montella M., Ramazzotti V., Franceschi S., LaVecchia C.
Flavonoids and the risk of renal cell carcinoma.
CANCER EPIDEMIOLOGY BIOMARKERS & PREVENTION 2007; 16(1):98-101.

30. Bosetti C., Rossi M., McLaughlin J.K., Negri E., Talamini R., Lagiou P., Montella M., Ramazzotti V., Franceschi S., La Vecchia C.
Micronutrients and the risk of renal cell cancer: a case-control study from Italy.
INTERNATIONAL JOURNAL OF CANCER 2007; 120(4):892-6.

31. Bravi F., Bosetti C., Scotti L., Talamini R., Montella M., Ramazzotti V., Negri E., Franceschi S., La Vecchia C.
Food groups and renal cell carcinoma: a case-control study from Italy.
INTERNATIONAL JOURNAL OF CANCER 2007; 1;120(3):681-5.

32. Breccia M., Avvisati G., Latagliata R., Carmosino I., Guarini A., De Propris M.S., Gentilini F., Petti M., Cimino G., Mandelli F., Lo-Coco F.
Occurrence of thrombotic events in acute promyelocytic leukemia correlates with consistent immunophenotypic and molecular features.
LEUKEMIA 2007; 21(1):79-83.

33. Brenner H., Coebergh J.W., Parkin D.M., Izarzugaza I., Clavel J., Arndt V., Steliarova-Foucher E., Working group, Cancer Registry of Latina Province (Ramazzotti V.).
Up-to-date monitoring of childhood cancer long-term survival in Europe: leukaemias and lymphomas.
ANNALS OF ONCOLOGY 2007; 18(9):1569-77.

34. Brenner H., Steliarova-Foucher E., Arndt V., Working group, Cancer Registry of Latina Province (Ramazzotti V.).
Up-to-date monitoring of childhood cancer long-term survival in Europe: methodology and application to all forms of cancer combined.
ANNALS OF ONCOLOGY 2007; 18(9):1561-8.

35. Bria E., Carlini P., Cognetti F., Terzoli E., Giannarelli D.
Re: Survival with aromatase inhibitors and inactivators versus standard hormonal therapy in advanced breast cancer: meta-analysis.
JOURNAL OF THE NATIONAL CANCER INSTITUTE 2007; 17;99(2):176-7.

36. Bria E., Cuppone F., Cecere F., Milella M., Nisticò C., Cognetti F., Terzoli E.
Adjuvant chemotherapy for non-small cell lung cancer.
JOURNAL OF THORACIC ONCOLOGY 2007; 2(5):7-11.

37. Bria E., Cuppone F., Fornier M., Nisticò C., Carlini P., Milella M., Sperduti I., Terzoli E., Cognetti F., Giannarelli D.
Cardiotoxicity and incidence of brain metastases after adjuvant trastuzumab for early breast cancer: the dark side of the moon? A meta-analysis of the randomized trials.
BREAST CANCER RESEARCH AND TREATMENT 2007.

38. Bria E., Di Maio M., Cuppone F., Nisticò C., Cognetti F., Giannarelli D.
Should subgroup analysis of randomized clinical trials have a direct impact on clinical practice?
JOURNAL OF CLINICAL ONCOLOGY 2007; 10;25(5):605-6.

39. Bria E., Milella M., Gelibter A., Cuppone F., Pino M.S., Ruggeri E., Carlini P., Nisticò C., Terzoli E., Cognetti F., Giannarelli D.
Gemcitabine-based combinations for inoperable pancreatic cancer: Have we made real progress? A meta-analysis of 20 phase 3 trials.
CANCER 2007; 110:525-533.

40. Bria E., Visca P., Novelli F., Casini B., Diodoro M., Perrone D., Botti C., Sperduti I., Facciolo F., Milella M., Cecere F., Cognetti F., Mottolese M.
Nuclear and cytoplasmic cellular distribution of survivin as survival predictor in resected non-small-cell lung cancer.
EJSO 2007.

41. Bryant D., Brozek L., Jaeschke R., Schünemann H., Guyatt G.
Commentary: Can guideline-defined asthma control be achieved and how it influences patients' quality of life – the goal study.
MED PRAKT 2007; 195(5):142-44.

42. Bryant D., Schünemann H., Brozek J., Jaeschke R., Guyatt G.
Patient reported outcomes: general principles of development and interpretability.
POL. ARCH. MED. WEWN. 2007; 117(4):5-11.

43. Browne R.W., Koury S.T., Marion S., Wilding G., Muti P., Trevisan M.
Accuracy and biological variation of human serum paraoxonase 1 activity and polymorphism (Q192R) by kinetic enzyme assay.
CLINICAL CHEMISTRY 2007; 53(2):310-7.

44. Calandrella N., Risuleo G., Scarsella G., Mustazza C., Castelli M., Galati F., Giuliani A., Galati G.
Reduction of cell proliferation induced by PD166866: an inhibitor of the basic fibroblast growth factor.
JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH 2007; 26(3)::405-9.

45. Cannistraci C., Lesnoni La Parola I., Cardinali G., Bolasco G., Aspite N., Stigliano V., Picardo M.
Co-localization of IgA and TG3 on healthy skin of coeliac patients.
JOURNAL OF THE EUROPEAN ACADEMY OF DERMATOLOGY AND VENEREOLOGY 2007; 21(4):509-14.

46. Capocaccia R., Sant M., Berrino F., Simonetti A., Santi V., Trevisani F., EUROCARE Working Group. (Ramazzotti V.).
Hepatocellular carcinoma: trends of incidence and survival in Europe and the United States at the end of the 20th century.
AMERICAN JOURNAL OF GASTROENTEROLOGY 2007; 102(8):1661-70.

47. Caporale A., Brescia A., Galati G., Castelli M., Saputo S., Terrenato I., Cucina A., Liverani A., Gasparrini M., Ciardi A., et al.
Locoregional IL-2 therapy in the treatment of colon cancer. Cell-induced lesions of a murine model.
ANTICANCER RESEARCH 2007; 27(2):985-9.

48. Capurso G., Schünemann H., Terrenato I., Moretti A., Koch M., Muti P., Capurso L., Delle Fave G.
Meta-analysis: the use of non-steroidal anti-inflammatory drugs and pancreatic cancer risk for different exposure categories.
ALIMENTARY PHARMACOLOGY & THERAPEUTICS 2007; 15;26(8):1089-99.

49. Caraglia M., Marra M., Leonetti C., Meo G., D'Alessandro A.M., Baldi A., Santini D., Tonini G., Bertieri R., Zupi G., Budillon A., Abbruzzese A.
R115777 (Zarnestra)/Zoledronic acid (Zometa) cooperation on inhibition of prostate cancer proliferation is paralleled by Erk/Akt inactivation and reduced Bcl-2 and bad phosphorylation.
JOURNAL OF CELLULAR PHYSIOLOGY 2007; 211:533-43.

50. Carducci M., Calcaterra R., Rinaldi M., Mussi A., Viola G., Venturo I., Perracchio L., Franco G., Lopez M., Morrone A.
Paraneoplastic Raynaud phenomenon with digital necrosis associated with hyperhomocysteinemia and antiphospholipid antibodies.
ARCHIVES OF DERMATOLOGY 2007; 143(10):1342-3.

51. Carducci M., Nosotti L., Calcaterra R., Bonifati C., Mussi A., Pelagalli L., Di Emidio D., Laurenzi L., Russo A., Franco G., Toma L., Morrone A.
Early development of disseminated nocardiosis during immunosuppressive treatment for pemphigus vulgaris.
EUROPEAN JOURNAL OF DERMATOLOGY 2007; 17(4):346-7.

52. Caretti E., Devarajan K., Coudry R., Ross E., Clapper M.L., Cooper H.S., Bellacosa A.
Comparison of RNA amplification methods and chip platforms for microarray analysis of samples processed by laser capture microdissection.
JOURNAL OF CELLULAR BIOCHEMISTRY 2007.

53. Cario G., Izraeli S., Teichert A., Rhein P., Skokowa J., Möricke A., Zimmermann M., Schrauder A., Karawajew L., Ludwig W.D., Welte K., Schünemann H., Schlegelberger B., Schrappe M., Stanulla M.
High interleukin-15 expression characterizes childhood acute lymphoblastic leukemia with involvement of the CNS.
JOURNAL OF CLINICAL ONCOLOGY 2007; 20;25(30):4813-20.

54. Carlini P., Bria E., Giannarelli D., Felici A., Papaldo P., Fabi A., Ruggeri E., Milella M., Nuzzo C., Cecere F., Gelibter A., Pino M.S., Nistico' C., Cuppone F., Metro G., Terzoli E., Cognetti F., Ferretti G.
Aromatase inhibitors in post-menopausal metastatic breast carcinoma.
EXPERT OPINION ON INVESTIGATIONAL DRUGS 2007; 16(7):1023-36.

55. Carlini P., Michelotti A., Ferretti G., Ricci S., Giannarelli D., Pellegrini M., Cresti N., Di Cosimo S., Bria E., Papaldo P., Fabi A., Ruggeri E., Milella M., Alimonti A., Salesi N., Cognetti F.
Clinical evaluation of the use of exemestane as further hormonal therapy after nonsteroidal aromatase inhibitors in postmenopausal metastatic breast cancer patients.
CANCER INVESTIGATION 2007; 25(2):102-5.

56. Castaldi L., Serra C., Moretti F., Messina G., Paoletti R., Sampaolesi M., Torgovnick A., Baiocchi M., Padula F., Pisaniello A., Molinaro M., Cossu G., Levrero M., Bouché M.
Bisperoxovanadium, a phospho-tyrosine phosphatase inhibitor, reprograms myogenic cells to acquire a pluripotent, circulating phenotype.
FASEB JOURNAL 2007; 21(13):3573-83.

57. Cavaletti G., Frigeni B., Lanzani F., Piatti M., Rota S., Briani C., Zara G., Plasmati R., Pastorelli F., Caraceni A., Pace A., Manicone M., Lissoni A., Colombo N., Bianchi G., Zanna C., Italian NETox Group.
The Total Neuropathy Score as an assessment tool for grading the course of chemotherapy-induced peripheral neurotoxicity: comparison with the National Cancer Institute-Common Toxicity Scale.
JOURNAL OF THE PERIPHERAL NERVOUS SYSTEM 2007; 12(3):210-5.

58. Cazzaniga M.E., Mustacchi G., Pronzato P., De Matteis A., Di Costanzo F., Floriani I., NORA Study Group., (Carlini P.).
Adjuvant treatment of early breast cancer: do the St Gallen recommendations influence clinical practice? Results from the NORA study.
ANNALS OF ONCOLOGY 2007; 18(12):1976-80.

59. Ceccatelli A., Benassi M., D'Andrea M., De Felice P., Fazio A., Nocentini S., Strigari L.
Experimental determination of calibration settings of a commercially available radionuclide calibrator for various clinical measurement geometries and radionuclides.
APPLIED RADIATION AND ISOTOPES 2007; 65:120-5.

60. Ceribelli A., Pino M., Cecere F.
Gender differences: implications for clinical trials and practice.
JOURNAL OF THORACIC ONCOLOGY 2007; 2(5):15-8.

61. Ceribelli A., Pino M.S., Gelibter A., Milella M., Cecere F., Caterino M., Facciolo F., Mirri M., Cognetti F.
Sequential chemotherapy in nonsmall-cell lung cancer: cisplatin and gemcitabine followed by docetaxel.
CANCER 2007; 109:727-31.

62. Chiaffarino F., Parazzini F., Bosetti C., Franceschi S., Talamini R., Canzonieri V., Montella M., Ramazzotti V., Franceschi S., La Vecchia C.
Risk factors for ovarian cancer histotypes.
EUROPEAN JOURNAL OF CANCER 2007; 43(7):1208-13.

63. Cianciulli A., Merola R., Leonardo C.
Re: Franclim R. Ribeiro, Rui Henrique, Ana T. Martins, Carmen Jerónimo and Manuel R. Teixeira. Relative copy number gain of MYC in diagnostic needle biopsies is an independent prognostic factor for prostate cancer patients.
EUROPEAN UROLOGY 2007; 52(5):1538-9.

64. Conti F., Carpano S., Sibilio L., Sergi D., Di Lauro L., Amodio A., Vici P., Abbate M.I., Ferranti F.R., Viola G., Botti C., Foggi P., Sperduti I., Lopez M.
High-dose CEF (cyclophosphamide, epirubicin, fluorouracil) as primary chemotherapy in locally advanced breast cancer: long term survival data.
LA CLINICA TERAPEUTICA 2007; 158(4):331-41.

65. Conti F., Sergi D., Foggi P., Abbate M.I., Lopez M.
New combination chemotherapy regimens in the primary treatment of operable breast cancer.
LA CLINICA TERAPEUTICA 2007; 158(1):55-75.

66. Coombes R.C., Kilburn L.S., Snowdon C.F., Paridaens R., Coleman R.E., Jones S.E., Jassem J., Van De Velde C.J., Delozier T., Alvarez I., Del Mastro L., Ortmann O., Diedrich K., Coates A.S., Principal and coinvestigators in the IES (Intergroup Exemestane Study), Cognetti F., Carlini P.
Survival and safety of exemestane versus tamoxifen after 2-3 years' tamoxifen treatment (Intergroup Exemestane Study): a randomised controlled trial.
LANCET 2007; 17;369(9561):559-70.

67. Coral S., Sigalotti L., Covre A., Nicolay H.J., Natali P., Maio M.
5-AZA-2'-deoxycytidine in cancer immunotherapy: a mouse to man story.
CANCER RESEARCH 2007; 15;67(6):2900-1.

68. Corneli G., Di Somma C., Prodam F., Bellone J., Bellone S., Gasco V., Baldelli R., Rover S., Schneider H.J., Gargantini L., Gastaldi R., Aimaretti G.
Cut-off limits of the GH response to GHRH plus arginine test and IGF-I levels for the diagnosis of GH deficiency in late adolescents and young adults.
EUROPEAN JOURNAL OF ENDOCRINOLOGY 2007; 157(6):701-708.

69. Cuppone F., Bria E., Verma S., Pritchard K.I., Gandhi S., Carlini P., Milella M., Nisticò C., Terzoli E., Cognetti F., Giannarelli D.
Do adjuvant aromatase inhibitors increase the cardiovascular risk in postmenopausal women with early breast cancer : meta-analysis of randomized trials.
CANCER 2007.

70. D'Andrea M., Iaccarino G., Carpano S., Strigari L., Benassi M.
Primary photon fluence extraction from portal images acquired with an amorphous silicon flat panel detector: experimental determination of a scatter filter.
JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH 2007; 26(1):125-32.

71. Dal Maso L., Zucchetto A., Tavani A., Montella M., Ramazzotti V., Talamini R., Canzonieri V., Garbeglio A., Negri E., Tonini A., La Vecchia C., Franceschi S.
Renal cell cancer and body size at different ages: an Italian multicenter case-control study.
AMERICAN JOURNAL OF EPIDEMIOLOGY 2007; 166(5):582-91.

72. De Marco F., Perluigi M., Blarzino C., Cini C., Coccia R., Venuti A.
UVB irradiation down-regulates HPV-16 RNA expression: Implications for malignant progression of transformed cells.
VIRUS RESEARCH 2007; 130:249-59.

73. De Nicola F., Bruno T., Iezzi S., Di Padova M., Floridi A., Passananti C., Del Sal G., Fanciulli M.
The prolyl isomerase Pin1 affects CHE-1 stability in response to apoptotic DNA damage.
JOURNAL OF BIOLOGICAL CHEMISTRY 2007; 282(27):19685-91.

74. Di Certo M.G., Corbi N., Bruno T., Iezzi S., De Nicola F., Desantis A., Ciotti M.T., Mattei E., Floridi A., Fanciulli M., Passananti C.
NRAGE associates with the anti-apoptotic factor Che-1 and regulates its degradation to induce cell death.
JOURNAL OF CELL SCIENCE 2007; 1;120:1852-8.

75. Di Lauro L., Nunziata C., Arena M.G., Foggi P., Sperduti I., Lopez M.
Irinotecan, docetaxel and oxaliplatin combination in metastatic gastric or gastroesophageal junction adenocarcinoma.
BRITISH JOURNAL OF CANCER 2007; 3;97(5):593-7.

76. Di Modugno F., De Monte L., Balsamo M., Bronzi G., Nicotra M.R., Alessio M., Jager E., Condeelis J.S., Santoni A., Natali P., Nisticò C.
Molecular cloning of hMena (ENAH) and its splice variant hMena+11a: epidermal growth factor increases their expression and stimulates hMena+11a phosphorylation in breast cancer cell lines.
CANCER RESEARCH 2007; 67:2657-6.

77. Di Nallo A., Covello R., Ortenzia O., Ordonez P., Abate A., Benassi M.
The breast dynamic contrast enhanced MRI: preliminary results of a quantitative analysis.
JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH 2007; 26:235-9.

78. Dahm P., Kunz R., Schünemann H.
Evidence-based clinical practice guidelines for prostate cancer: the need for a unified approach.
CURRENT OPINION IN UROLOGY 2007; 17(3):200-7.

79. Deganello A., Manciocco V., Dolivet Gilles, Leemans C. René, Spriano G.
Infrahyoid fascio-myocutaneous flap as an alternative to free radial forearm flap in head and neck reconstruction.
HEAD AND NECK-JOURNAL FOR THE SCIENCES AND SPECIALTIES OF THE HEAD AND NECK 2007; 29:285-91.

80. Dentelli P., Rosso A., Zeoli A., Gambino R., Pegoraro L., Pagano G., Falcioni R., Brizzi M.F.
Oxidative stress-mediated mesangial cell proliferation requires RAC-1/reactive oxygen species production and beta4 integrin expression.
JOURNAL OF BIOLOGICAL CHEMISTRY 2007; 282(36):26101-10.

81. Divet A., Paesante S., Grasso C., Cavagna D., Tiveron C., Paolini C., Protasi F., Huchet-Cadiou C., Treves S., Zorzato F.
Increased Ca2+ storage capacity of the skeletal muscle sarcoplasmic reticulum of transgenic mice over-expressing membrane bound calcium binding protein junctate.
JOURNAL OF CELLULAR PHYSIOLOGY 2007; 213(2):464-74.

82. Errani C., Biagini R., Schuster S., Biagini R., Casadei R., De Paolis M., Bertoni F., Boriani S., Mercuri M.
Reconstruction with fascia lata allograft of the posterior vertebra elements after resection for aneurysmal bone cyst in a child.
EUROPEAN SPINE JOURNAL 2007; 16(9):1531-5.

83. Escudier B., Pluzanska A., Koralewski P., Ravaud A., Bracarda S., Szczylik C., Chevreau C., Filipek M., Melichar B., Bajetta E., Gorbunova V., Bay J.O., Bodrogi I., Jagiello-Gruszfeld A., Moore N., AVOREN Trial investigators. (Work Group, Ruggeri E.)
Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial.
LANCET 2007; 170,22:2103-11.

84. Ettorre M., Douard R., Corazza V., Santoro R., Vennarecci G., Santoro E.
Anatomical basis of liver hanging maneuver: a clinical and anatomical *in vivo* study.
AMERICAN SURGEON 2007; 73(11):1193-6.

85. Ettorre M., Vennarecci G., Santoro R., Boschetto A., Miglioresi L., Corazza V., Mancini P., Lorusso R., Spoletini D., Antonini M., Tacconi G., Santoro E.
Modified liver hanging maneuver during orthotopic liver transplantation with inferior vena cava preservation: results after 120 consecutive applications.
TRANSPLANTATION PROCEEDINGS 2007; 39(6):1881-2.

86. Fabi A., Metro G., Papaldo P., Mottolese M., Melucci E., Carlini P., Sperduti I., Russillo M., Gelibter A., Ferretti G., Tomao S., Milella M., Cognetti F.
Impact of celecoxib on capecitabine tolerability and activity in pretreated metastatic breast cancer: results of a phase II study with biomarker evaluation.
CANCER CHEMOTHERAPY AND PHARMACOLOGY 2007; 6:.

87. Felsani A., Mileo A., Maresca V., Picardo M., Paggi M.
New technologies used in the study of human melanoma.
INTERNATIONAL REVIEW OF CYTOLOGY-A SURVEY OF CELL BIOLOGY 2007; 261:247-86.

88. Ferreira-González I., Busse J.W., Heels-Ansdell D., Montori V.M., Akl E.A., Bryant D.M., Alonso-Coello P., Alonso J., Worster A., Upadhye S., Jaeschke R., Schünemann H., Chiba N., Permanyer-Miralda G., Domingo-Salvany A., Wu P., Mills E.J., Guyatt G.H.
Problems with use of composite end points in cardiovascular trials: systematic review of randomised controlled trials.
BRITISH MEDICAL JOURNAL 2007; 14;334(7596):786.

89. Ferretti G.
Forkhead box P3-positive regulatory T cells as therapeutic target for breast cancer.
JOURNAL OF CLINICAL ONCOLOGY 2007; 10;25(23):29.

90. Ferretti G.
Temsirolimus for advanced renal-cell carcinoma.
NEW ENGLAND JOURNAL OF MEDICINE 2007; 6;357(10):1050.

91. Ferretti G., Fabi A., Carlini P., Papaldo P., Felici A., Cognetti F.
Antitumor effects of clinical dosing regimens of bisphosphonates in experimental breast cancer bone metastasis.
BONE 2007; 41(1):155-6.

92. Ferretti G., Fabi A., Carlini P., Papaldo P., Felici A., Tomao S., Cognetti F.
Zoledronic Acid and angiogenesis.
CLINICAL CANCER RESEARCH 2007; 15;13(22):6850.

93. Ferretti G., Felici A., Carlini P., Cognetti F.
Exemestane or tamoxifen?
LANCET 2007; 12;369(9573):1599.

94. Ferretti G., Felici A., Carlini P., Cognetti F.
Re: Ovarian ablation or suppression in premenopausal early breast cancer: results from the International Adjuvant Breast Cancer Ovarian Ablation or Suppression randomized trial.
JOURNAL OF THE NATIONAL CANCER INSTITUTE 2007; 5;99(17):1344-5.

95. Ferretti G., Felici A., Carlini P., Cognetti F.
Re: Polychemotherapy for early breast cancer: results from the international adjuvant breast cancer chemotherapy randomized Trial.
JOURNAL OF THE NATIONAL CANCER INSTITUTE 2007; 19;99(18):1416.

96. Ferretti G., Felici A., Cognetti F.
Forkhead box P3-positive regulatory T cells in immune surveillance and cancer.
BRITISH JOURNAL OF CANCER 2007; 8;97(7):1015-6.

97. Ferretti G., Felici A., Cognetti F.
Pregnancy levels of estrogen and progesterone: the double-edged sword.
CANCER EPIDEMIOLOGY BIOMARKERS & PREVENTION 2007; 16(3):634.

98. Ferretti G., Felici A., Cognetti F.
Re: Age-related lobular involution and risk of breast cancer.
JOURNAL OF THE NATIONAL CANCER INSTITUTE 2007; 4;99(7):571-2.

99. Ferretti G., Felici A., Cognetti F.
Re: Treatment of human epidermal growth factor receptor 2 overexpressing breast cancer xenografts with multiagent human epidermal growth factor receptor targeted therapy.
JOURNAL OF THE NATIONAL CANCER INSTITUTE 2007; 99(21):1644.

100. Ferretti G., Felici A., Cognetti F.
The other side of p53.
ANNALS OF ONCOLOGY 2007; 18(8):1425-6.

101. Ferretti G., Felici A., Cognetti F.
The protective side of progesterone.
BREAST CANCER RESEARCH 2007; 9(6):402.

102. Ferretti G., Felici A., Cognetti F., Mandalà M.
Transforming growth factor-beta signaling and regulatory T cells.
JOURNAL OF CLINICAL ONCOLOGY 2007; 10;25(29):4695-6.

103. Ferretti G., Felici A., Papaldo P., Fabi A., Cognetti F.
HER2/neu role in breast cancer: from a prognostic foe to a predictive friend.
CURRENT OPINION IN OBSTETRICS & GYNECOLOGY 2007; 19(1):56-62.

104. Ferretti G., Papaldo P.
Granulocyte colony-stimulating factor-associated complications and increase in leukocyte number.
ANNALS OF ONCOLOGY 2007; 18(6):1118-9.

105. Ferretti G., Papaldo P.
Acute myeloid leukaemia or myelodysplastic syndrome following use of granulocyte colony-stimulating factors during breast cancer adjuvant chemotherapy.
BREAST CANCER RESEARCH AND TREATMENT 2007.

106. Floridi A., Fanciulli M.
Che-1: a new effector of checkpoints signaling.
CELL CYCLE 2007; 1;6(7):804-6.

107. Folgiero V., Bachelder R.E., Bon G., Sacchi A., Falcioni R., Mercurio A.M.
The alpha6beta4 integrin can regulate ErbB-3 expression: implications for alpha6beta4 signaling and function.
CANCER RESEARCH 2007; 67:1645-5.

108. Frost M.H., Bonomi A.E., Cappelleri J.C., Schünemann H., Moynihan T.J., Aaronson N.K., Clinical Significance Consensus Meeting Group.
Applying quality-of-life data formally and systematically into clinical practice.
MAYO CLINIC PROCEEDINGS 2007; 82(10):1214-28.

109. Gajewski P., Jaeschke R., Brozek J., Schünemann H.
Can clinical practice guidelines lead astray?
POL. ARCH. MED. WEWN. 2007; 117(4):12-5.

110. Galeone C., Pelucchi C., Talamini R., Negri E., Dal Maso L., Montella M., Ramazzotti V., Franceschi S., La Vecchia C.
Onion and garlic intake and the odds of benign prostatic hyperplasia.
UROLOGY 2007; 70(4):672-6.

111. Galeone C., Pelucchi C., Talamini R., Negri E., Montella M., Ramazzotti V., Zucchetto A., Dal Maso L., Franceschi S., La Vecchia C.
Fibre intake and renal cell carcinoma: a case-control study from Italy.
INTERNATIONAL JOURNAL OF CANCER 2007; 15;121(8):1869-72.

112. Gallucci M., Guaglianone S., Papalia R., Simone G., Forastiere E., Leonardo C., Carpanese L.
Superselective embolization as first step of laparoscopic partial nephrectomy.
UROLOGY 2007; 69(4):642-5.

113. Gallucci M., Vico E., Merola R., Leonardo C., Sperduti I., Felici A., Sentinelli S., Cantiani R., Orlandi G., Cianciulli A.
Adverse genetic prognostic profiles define a poor outcome for cystectomy in bladder cancer.
EXPERIMENTAL AND MOLECULAR PATHOLOGY 2007; 83(3):385-91.

114. Gallucci M., Vico E., Sentinelli S., Leonardo C., Visca P., Orlandi G., Cianciulli A.
Familial papillary renal carcinoma:cytogenetic characterization in normal and tumor tissues in two brothers. A case report.
UROLOGIC ONCOLOGY-SEMINARS AND ORIGINAL INVESTIGATIONS 2007; :.

115. Gallus S., Foschi R., Negri E., Talamini R., Franceschi S., Montella M., Ramazzotti V., Tavani A., Dal Maso L., La Vecchia C.
Dietary zinc and prostate cancer risk: a case-control study from Italy.
EUROPEAN UROLOGY 2007; 52(4):1052-6.

116. Gallus S., Foschi R., Talamini R., Altieri A., Negri E., Franceschi S., Montella M., Dal Maso L., Ramazzotti V., La Vecchia C.
Risk factors for prostate cancer in men aged less than 60 years: a case-control study from Italy.
UROLOGY 2007; 70(6):1121-6.

117. Gambacorta M.A., Valentini V., Coco C., Manno A., Doglietto G.B., Ratto C., Cosimelli M., Miccichè F., Maurizi F. et al..
Sphincter preservation in four consecutive phase II studies of preoperative chemoradiation: analysis of 247 T3 rectal cancer patients.
TUMORI 2007; 93(2):160-9.

118. Gelibter A., Di Segni S., Zeuli M., Milella M., Ferretti G., Nuzzo C., Cognetti F.
Old age: biologic versus chronologic.
JOURNAL OF CLINICAL ONCOLOGY 2007; 20;25(6):E8.

119. Gelibter A., Gamucci T., Ferraresi V., Giannarelli D., Cognetti F., Zeuli M., Pollera C.F., Di Costanzo F., Nuzzo C., Gabriele A., Signorelli C., Gasperoni S., Ferraresi V., Giannarelli D., Cognetti F., Zeuli M.
A phase II trial of gefitinib in combination with capecitabine and oxaliplatin as first-line chemotherapy in patients with advanced colorectal cancer.
CURRENT MEDICAL RESEARCH AND OPINION 2007; 23(9):2117-23.

120. Giacchetti S., Gorlia T., Garufi C., Lèvi F.
Should subgroup analysis of randomized clinical trials have a direct impact on clinical practice? (REPLAY).
JOURNAL OF CLINICAL ONCOLOGY 2007; 25(5):605-6.

121. Giordano A., Bellacchio E., Bagella L., Paggi M.
Interaction Between the Cdk2/Cyclin A Complex and a Small Molecule Derived from the pRb2/p130 Spacer Domain: A Theoretical Model.
CELL CYCLE 2007; 6(21):2591-3.

122. Giorgini S., Trisciuoglio D., Gabellini C., Desideri M., Castellini L., Colarossi C., Zangemeister-Wittke U., Zupi G., Del Bufalo D.
Modulation of bcl-xL in tumor cells regulates angiogenesis through CXCL8 expression.
MOLECULAR CANCER RESEARCH 2007; 5(8):761-71.

123. Giovannetti A., Pierdominici M., Mazzetta F., Marziali M., Renzi C., Mileo A.M., De Felice M., Mora B., Esposito A., Carello R., Pizzuti A., Paggi M., Paganelli R., Malorni W., Aiuti F.
Unravelling the complexity of T cell abnormalities in common variable immunodeficiency.
JOURNAL OF IMMUNOLOGY 2007; 178(6):3932-43.

124. Gorini G., Stagnaro E., Fontana V., Miligi L., Ramazzotti V., Amadori D., Rodella S., Tumino R., Crosignani P., Vindigni C., Fontana A., Vineis P., Seniori Costantini A.
Alcohol consumption and risk of Hodgkin's lymphoma and multiple myeloma: a multicentre case-control study.
ANNALS OF ONCOLOGY 2007; 18(1):143-8.

125. Gorini G., Stagnaro E., Fontana V., Miligi L., Ramazzotti V., Amadori D., Rodella S., Tumino R., Crosignani P., Vindigni C., Fontana A., Vineis P., Seniori Costantini A.
Alcohol consumption and risk of leukemia: A multicenter case-control study.
LEUKEMIA RESEARCH 2007; 31(3):379-86.

126. Green S., Higgins J.P., Schünemann H., Becker L.
Response to paper by Lang A, Edwards N, and Fleiszer A.
JOURNAL OF CLINICAL EPIDEMIOLOGY 2007; 60(6):598-9.

127. Gregorj C., Ricciardi M.R., Petrucci M.T., Scerpa M.C., De Cave F., Fazi P., Vignetti M., Vitale A., Mancini M., Cimino G., Palmieri S., Di Raimondo F., Specchia G., Fabbiano F., Cantore N., Mosna F., Camera A., Luppi M., Mandelli F., Andreeff M., Milella M., Foà R., Tafuri A.; GIMEMA Acute Leukemia Working Party.
ERK1/2 phosphorylation is an independent predictor of complete remission in newly diagnosed adult acute lymphoblastic leukemia.
BLOOD 2007; 15;109(129:5473-6.

128. Gridelli C., Gallo C., Ceribelli A., Gebbia V., Gamucci T., Ciardiello F., Carozza F., Favaretto A., Daniele B., Galetta D., Barbera S., Rosetti F., Rossi A., Maione P., Cognetti F., Testa A., Di Maio M., Morabito A., Perrone F., On behalf of the GECO Investigators.
Factorial phase III randomised trial of rofecoxib and prolonged constant infusion of gemcitabine in advanced non-small-cell lung cancer: the GEmcitabine-COxib in NSCLC (GECO) study.
LANCET ONCOLOGY 2007; 8(6):500-12.

129. Guyatt G., Schünemann H.
How can quality of life researchers make their work more useful to health workers and their patients?
QUALITY OF LIFE RESEARCH 2007; 16(7):1097-105.

130. Hassan C., Zullo A., Winn S., Eramo A., Tomao S., Rossini F.P., Morini S.
The colorectal malignant polyp: scoping a dilemma.
DIGESTIVE AND LIVER DISEASE 2007; 3981:92-100.

131. Iaccarino G., Pinnarò P., Landoni V., Marzi S., Soriani A., Giordano C., Arcangeli S., Benassi M., Arcangeli G.
Single fraction partial breast irradiation in prone position.
JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH 2007; 26(4):543-52.

132. Julien S., Puig I., Caretti E., Bonaventure J., Nelles L., van Roy F., Dargemont C., De Herreros A.G., Bellacosa A., Larue L.
Activation of NF-kappaB by Akt upregulates Snail expression and induces epithelium mesenchyme transition.
ONCOGENE 2007.

133. Latagliata R., Carmosino I., Breccia M., Minni A., Testi A., Iorio N., Lo-Coco F., Avvisati G., Petti M., Mandelli F., Cimino G.
Late relapses in acute promyelocytic leukaemia.
ACTA HAEMATOLOGICA 2007; 117(2):106-8.

134. Leonetti C., Biroccio A., D'Angelo C., Semple Sean C., Scarsella M., Zupi G.
Therapeutic integration of c-myc and bcl-2 antisense molecules with docetaxel in a preclinical model of hormone-refractory prostate cancer.
PROSTATE 2007; 67:1475-85.

135. Leonetti C., Zupi G.
Targeting different signaling pathways with antisense oligonucleotides combination for cancer therapy.
CURRENT PHARMACEUTICAL DESIGN 2007; 13:463-70.

136. Mainardi S., Pelosi A., Palescandolo E., Riccioni R., Fontemaggi G., Diverio D., Testa U., Sacchi A., Grignani F., Lo-Coco F., Levrero M., Blandino G., Rizzo A.
DeltaN-p73 is a transcriptional target of the PML/RARalpha oncogene in myeloid differentiation.
CELL DEATH AND DIFFERENTIATION 2007; 14(11):1968-71.

137. Maltsman-Tseikhin A., Moricca P., Niv D.
Burning mouth syndrome: will better understanding yield better management?
PAIN PRACT. 2007; 7(2):151-62.

138. Mandalà M., Moro C., Labianca, Ferretti G.
Erlotinib in pancreatic cancer patients: do we need more information from the NCIC CTG trial?
JOURNAL OF CLINICAL ONCOLOGY 2007; 20;(25827):4320-1.

139. Manente L., Perna A., Buommino E., Altucci L., Lucariello A., Citro G., Baldi A., Iaquinto G., Tufano M.A., De Luca A.
The Helicobacter pylori's protein VacA has direct effects on the regulation of cell cycle and apoptosis in gastric epithelial cells.
JOURNAL OF CELLULAR PHYSIOLOGY 2007; 214(3):582-7.

140. Mariani L., Morrone A., Preti M., Sbiroli C., Tomao F., Tomao S.
Migrant women and cervical cancer: background of a prevention study.
EUROPEAN JOURNAL OF GYNAECOLOGICAL ONCOLOGY 2007; 29(1):52-6.

141. Marzi S., Arcangeli G., Saracino B., Petrongari M., Bruzzaniti V., Iaccarino G., Landoni V., Soriani A., Benassi B.
Relationships between rectal wall dose-volume constraints and radiobiologic indices of toxicity for patients with prostate cancer.
INTERNATIONAL JOURNAL OF RADIATION ONCOLOGY BIOLOGY PHYSICS 2007; 68:41-9.

142. Maschio M., Dinapoli L., Zarabla, Pompili A., Carapella C., Pace A., Giannarelli D., Occhipinti E., Jandolo B.
Outcome and tolerability of topiramate in brain tumor associated epilepsy.
JOURNAL OF NEURO-ONCOLOGY 2007.

143. Masotti A., Moretti F., Mancini F., Russo G., Di Lauro N., Checchia P., Marianecci C., Carafa M., Santucci E., Ortaggi G.
Physicochemical and biological study of selected hydrophobic polyethylenimine-based polycationic liposomes and their complexes with DNA.
BIOORGANIC & MEDICINAL CHEMISTRY 2007; 1;15(3):1504-15.

144. Massa S., Franconi R., Brandi R., Muller A., Mett V., Yusibov V., Venuti A.
Anti-cancer activity of plant-produced HPV16 E7 vaccine.
VACCINE 2007; 20;25(16):3018-21.

145. Mattei E., Corbi N., Di Certo M.G., Strimpakos G., Severini G., Onori A., Desantis A., Libri V., Buontempo S., Floridi A., Fanciulli M., Baban D., Davies K.E., Passananti C.
Utrophin up-regulation by an artificial transcription factor in transgenic mice.
PLOS ONE 2007; 22;2(1):E774.

146. Mattioni M., Soddu S., Porrello A., D'Alessandro R., Spila A., Guadagni F.
Serum anti-p53 antibodies as a useful marker for prognosis of gastric carcinoma.
INTERNATIONAL JOURNAL OF BIOLOGICAL MARKERS 2007; 2284):302-6.

147. Mauro L., Catalano S., Bossi G., Pellegrino M., Barone I., Morales S., Giordano C., Bartella V., Casaburi I., Andò S.
Evidences that leptin up-regulates E-cadherin expression in breast cancer: effects on tumor growth and progression.
CANCER RESEARCH 2007; 67:3412-21.

148. McCann S.E., McCann W.E., Hong C.C., Marshall J.R., Edge S.B., Trevisan M., Muti P., Freudenheim J.L.
Dietary patterns related to glycemic index and load and risk of premenopausal and post-menopausal breast cancer in the Western New York Exposure and Breast Cancer Study.
AMERICAN JOURNAL OF CLINICAL NUTRITION 2007; 86(2):465-71.

149. McCann S.E., Wactawski-Wende J., Kufel K., Olson J., Ovando B., Kadlubar S.N., Davis W., Carter L., Muti P., Shields P.G., Freudenheim J.L.
Changes in 2-hydroxyestrone and 16alpha-hydroxyestrone metabolism with flaxseed consumption: modification by COMT and CYP1B1 genotype.
CANCER EPIDEMIOLOGY BIOMARKERS & PREVENTION 2007; 16(2):256-62.

150. McCubrey J.A., Steelman L.S., Chappell W.H., Abrams S.L., Wong E.W., Chang F., Lehmann B., Terrian D.M., Milella M., Tafuri A., Stivala F., Libra M., Basecke J.
Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance.
BIOCHIMICA ET BIOPHYSICA ACTA-MOLECULAR CELL RESEARCH 2007; 1773(8):1263-84.

151. Mercadante S., Arcuri E.
Pharmacological management of cancer pain in the elderly
DRUGS & AGING 2007; 24(9):761-76.

152. Metro G., Pino M., Pellegrini D., Sacerdoti G., Fabi A.
Brain radiotherapy during treatment with anticonvulsant therapy as a trigger for toxic epidermal necrolysis.
ANTICANCER RESEARCH 2007; 27:1167-70.

153. Metro G., Sperduti I., Russillo M., Milella M., Cognetti F., Fabi A.
Clinical utility of continuing trastuzumab beyond brain progression in HER-2 positive metastatic breast cancer.
ONCOLOGIST 2007; 12(12):1467-9.

154. Micheli A., Secreto G., Meneghini E., Krogh V., Muti P., Venturelli E., Berrino F.
Re: Endogenous steroid hormone concentrations and risk of breast cancer among premenopausal women.
JOURNAL OF THE NATIONAL CANCER INSTITUTE 2007; 99(5):408-9.

155. Migliano E., Monarca C., Mariani L., Tedesco M., Bucher S.
Vulvar primitive melanoma and sentinel lymph node: case report.
EUROPEAN JOURNAL OF GYNAECOLOGICAL ONCOLOGY 2007; 28(3):229-32.

156. Milella M., Konopleva M., Precupanu C.M., Tabe Y., Ricciardi M.R., Gregorj C., Collins S.J., Carter B.Z., D'Angelo C., Petrucci M.T., Foa R., Cognetti F., Tafuri A., Andreeff M.
MEK blockade converts AML differentiating response to retinoids into extensive apoptosis.
BLOOD 2007; 109(5):2121-9.

157. Minutilli E., Giannarelli D., Anzà M., Garinei R., Perri P., Botti C., Piarulli L., Bruno P., Rea S., Visca R., Mottolese M., Di Filippo S., Di Filippo F.
Sentinel node biopsy in cutaneous melanoma: correlations between melanoma prognostic factors and sentinel node status.
JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH 2007; 26(1):71-6.

158. Moehren U., Papaioannou M., Reeb C.A., Grasselli A., Nanni S., Asim M., Roell D., Prade I., Farsetti A., Baniahmad A.
Wild-type but not mutant androgen receptor inhibits expression of the hTERT telomerase subunit: a novel role of AR mutation for prostate cancer development.
FASEB JOURNAL 2007; 22(4):1258-67.

159. Molfino A., Muscaritoli M., Cascino A., Fanfarillo F., Fava A., Bertini G., Citro G., Fanelli F.R., Laviano A.
Free tryptophan/large neutral amino acids ratios in blood plasma do not predict cerebral spinal fluid tryptophan concentrations in interleukin-1-induced anorexia.
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR 2007.

160. Molica S., Cutrona G., Vitelli G., Mirabelli R., Molica M., Digiesi G., Ribatti D., Ferrarini M., Vacca A.
Markers of increased angiogenesis and their correlation with biological parameters identifying high-risk patients in early B-cell chronic lymphocytic leukemia.
LEUKEMIA RESEARCH 2007; 31(11):1575-8.

161. Mottolese M., Orlandi G., Sperduti I., Merola R., Buglioni S., Di Benedetto A., Pinnarò P., Perracchio L., Venturo I., Cognetti F., Cianciulli A.
Bio-pathologic characteristics related to chromosome 11 aneusomy and cyclin D1 gene status in surgically resected stage I and II breast cancer: Identification of an adverse prognostic profile.
AMERICAN JOURNAL OF SURGICAL PATHOLOGY 2007; 31:247-54.

162. Motzer R.J., Hutson T.E., Tomczak P., Michaelson M.D., Bukowski R.M., Rixe O., et al. working Group, Ruggeri E.
Sunitinib versus interferon alfa in metastatic renal-cell carcinoma.
NEW ENGLAND JOURNAL OF MEDICINE 2007; 11:356(2):115-126.

163. Mustacchi G., Cazzaniga M.E., Pronzato P., De Matteis A., Di Costanzo F., Floriani I.; NORA Study Group.(Contributors Carlini P.)
Breast cancer in elderly women: a different reality? Results from the NORA study.
ANNALS OF ONCOLOGY 2007; 18(6):991-6.

164. Nádasi E., Anga B., Sándor J., Megyesi J., Kelemen D., Mottolese M., Natali P., Hegedus G., Arany I., Ember I.
Prognostic factors in Hungarian breast cancer patients.
ANTICANCER RESEARCH 2007; 27(1A):279-82.

165. Nardinocchi L., Puca R., Sacchi A., D'Orazi G.
HIPK2 knock-down compromises tumor cell efficiency to repair damaged DNA.
BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS 2007; 14;361(1):249-55.

166. Narducci M.L., Grasselli A., Biasucci L.M., Farsetti A., Mulè, Liuzzo G., La Torre G., Niccoli G., Mongiardo R., Pontecorvi A., Crea F.
High telomerase activity in neutrophils from unstable coronary plaques.
JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY 2007; 18;50(25):2369-7.

167. Nie J., Beyea J., Bonner M.R., Han D., Vena J.E., Rogerson P., Vito D., Muti P., Trevisan M., Edge S.B., Freudenheim J.L.
Exposure to traffic emissions throughout life and risk of breast cancer: the Western New York Exposures and Breast Cancer (WEB) study.
CANCER CAUSES & CONTROL 2007; 18(9):947-55.

168. Nisticò C., Bria E., Cuppone F., Carpino A., Ferretti G., Vitelli G., Sperduti I., Calabretta F., Toglia G., Tomao S., Cognetti F., Terzoli E.
Troponin-T and myoglobin plus echocardiographic evaluation for monitoring early cardiotoxicity of weekly epirubicin-paclitaxel in metastatic breast cancer patients.
ANTI-CANCER DRUGS 2007; 18:227-32.

169. Nisticò C., Bria E., Cuppone F., Fornier M., Sperduti I., Carpino A., Pace A., Cognetti F., Terzoli E.
Weekly epirubicin and paclitaxel with granulocyte colony-stimulating factor support in previously untreated metastatic breast cancer patients: a phase II study.
ANTI-CANCER DRUGS 2007; 18:687-92.

170. Omerovic J., Alimandi M., Santagelo L., Puggioni E.M., Marrocco J., Dall'Armi C., Palumbo C., Belleudi F., Di Marcotullio L., Frati L., Torrisi M.R., Cesareni G., Gulino A., Alimandi M.
The E3 ligase Aip4/Itch ubiquitinates and targets ErbB-4 for degradation.
FASEB JOURNAL 2007; 21(11):2849-62.

171. Onori A., Desantis A., Buontempo S., Di Certo M.G., Fanciulli M., Salvatori L., Passananti C., Corbi N.
The artificial 4-zinc-finger protein Bagly binds human utrophin promoter A at the endogenous chromosomal site and activates transcription.
BIOCHEMISTRY AND CELL BIOLOGY-BIOCHIMIE ET BIOLOGIE CELLULAIRE 2007; 85(3):358-65.

172. Pace A., Nisticò C., Cuppone F., Bria E., Galiè E., Graziano G., Natoli G., Sperduti I., Jandolo B., Calabretta F., Tomao S., Terzoli E.
Peripheral neurotoxicity of weekly paclitaxel chemotherapy: a schedule or a dose issue?
CLINICAL BREAST CANCER 2007; 7(7):550-4.

173. Pace A., Parisi C., Di Lelio M., Zizzari A., Petreri G., Giovannelli M., Pompili A.
Home rehabilitation for brain tumor patients.
JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH 2007; 26(3):297-300.

174. Pagan J.K., Arnold J., Hanchard K.J., Kumar R., Bruno T., Jones M.J., Richard D.J., Forrest A., Spurdle A., Verdin E., Crossley M., Fanciulli M., Chenevix-Trench G., Young D.B., Khanna K.K.
A novel corepressor, BCoR-L1, represses transcription through an interaction with CtBP.
JOURNAL OF BIOLOGICAL CHEMISTRY 2007; 18;282(20):15248-57.

175. Parrella P., Seripa D., Matera M.G., Rinaldi M., Signori E., Gravina C., Gallo A.P., Prencipe M., Grandone E., Mariani L., Cordiali P., Di Carlo A., Stentella P., Pachì A., Fazio V.M.
Lack of association between genetic variants in the mannose-binding lectin 2 (MBL2) gene and HPV infection.
EUROPEAN JOURNAL OF EPIDEMIOLOGY 2007; 22(3):159-62.

176. Passananti C., Fanciulli M.
The anti-apoptotic factor Che-1/AATF links transcriptional regulation, cell cycle control, and DNA damage response.
CELL. DIV. 2007.

177. Passananti C., Floridi A., Fanciulli M.
Che-1/AATF, a multivalent adaptor connecting transcriptional regulation, checkpoint control, and apoptosis.
BIOCHEMISTRY AND CELL BIOLOGY-BIOCHIMIE ET BIOLOGIE CELLULAIRE 2007; 85(4):477-83.

178. Pellini R., Pichi B., Ruscito P., Ceroni A.R., Caliceti U., Rizzotto G., Pazzaia A., Laudadio P., Piazza C., Peretti G., Giannarelli D., Spriano G.
Supracricoid partial laryngectomies after radiation failure: A multi-institutional series.
HEAD AND NECK-JOURNAL FOR THE SCIENCES AND SPECIALTIES OF THE HEAD AND NECK 2007.

179. Pelucchi C., Galeone C., Dal Maso L., Talamini R., Montella M., Ramazzotti V., Montella M., Franceschi S., La Vecchia C.
Dietary acrylamide and renal cell cancer.
INTERNATIONAL JOURNAL OF CANCER 2007; 15;120(6):1376-7.

180. Petrucci E., Pasquini, Petronelli A., Saulle E., Mariani G., Riccioni R., Biffoni M.L., Ferretti G., Benedetti Panici P., Cognetti F., Scambia G., Humphreys R., Peschle C., Testa U.
A small molecule Smac mimic potentiates TRAIL-mediated cell death of ovarian cancer cells.
GYNECOLOGIC ONCOLOGY 2007; 105(2):481-92.

181. Pichi B., Manciocco V., Marchesi P., Pellini R., Ruscito P., Vidiri A., Covello R., Spriano G.
Rhabdomyoma of the parapharyngeal space presenting with dysphagia.
DYSPHAGIA 2007.

182. Pierantoni G.M., Rinaldo C., Mottolese M., Di Benedetto A., Esposito F., Soddu S., Fusco A.
High-mobility group A1 inhibits p53 by cytoplasmic relocalization of its proapoptotic activator HIPK2.
JOURNAL OF CLINICAL INVESTIGATION 2007; 117(3):693-702.

183. Pino M.S., Gamucci T., Mansueto G., Trapasso T., Narducci F., Giampaolo M.A., Fariello A.M., Sperduti I., Ceribelli A., Cognetti F.
A phase II study of biweekly paclitaxel (P) and gemcitabine (G), followed by maintenance weekly paclitaxel in elderly patients with advanced non-small cell lung cancer (NSCLC).
LUNG CANCER 2007; :DEC 7.

184. Piselli P., Zanfi C., Corazza V., Ferretti S., Scuderi M., Secchia S.B., Lauro A., Dazzi A., Pinna A., Ettorre M., Vennarecci G., Santoro E., Ferretti G., Gusman N., Berloco P.B., et al..
Incidence and timing of infections after liver transplant in Italy.
TRANSPLANTATION PROCEEDINGS 2007; 39(2):1950-2.

185. Pistritto G., Puca R., Nardinocchi L., Sacchi A., D'Orazi G.
HIPK2-induced p53Ser46 phosphorylation activates the KILLER/DR5-mediated caspase-8 extrinsic apoptotic pathway.
CELL DEATH AND DIFFERENTIATION 2007; 14(10):1837-9.

186. Puhan M.A., Guyatt G.H., Golstein R., Mador J., McKim D., Stahl E., Griffish L., Schünemann H.
Relative responsiveness of the Chronic Respiratory Questionnaire, St. Georges Respiratory Questionnaire and four other health-related quality of life instruments for patients with chronic lung disease.
RESPIRATORY MEDICINE 2007; 101(2):308-16.

187. Puhan M.A., Schünemann H., Wong E., Griffith L., Guyatt G.H.
The standard gamble showed better construct validity than the time trade-off.
JOURNAL OF CLINICAL EPIDEMIOLOGY 2007; 60(10):1029-33.

188. Quaglia A., Capocaccia R., Micheli A., Carrani E., Vercelli M., Working group (Ramazzotti V., Cercato C.).
A wide difference in cancer survival between middle aged and elderly patients in Europe.
INTERNATIONAL JOURNAL OF CANCER 2007; 15;120(10):2196-201.

189. Rasi G., Serafino A., Bellis L., Lonardo M.T., Andreola F., Zonfrillo M., Vennarecci G., Pierimarchi P., Vallebona P.S., Ettorre M., Santoro E., Puoti C.
Nerve growth factor involvement in liver cirrhosis and hepatocellular carcinoma.
WORLD JOURNAL OF GASTROENTEROLOGY 2007; 7;13(37):4986-95.

190. Reni M., Zaja F., Mason W., Perry J., Mazza E., Spina M., Bordonaro R., Ilariucci F., Faedi M., Corazzelli G., Manno P., Franceschi E., Pace A., Candela M., Abbadessa A., et al..
Temozolomide as salvage treatment in primary brain lymphomas.
BRITISH JOURNAL OF CANCER 2007; 26;96(6):864-7.

191. Rinaldo C., Prodosmo A., Mancini F., Iacovelli S., Sacchi A., Moretti F., Soddu S.
MDM2-regulated degradation of HIPK2 prevents p53Ser46 phosphorylation and DNA damage-induced apoptosis.
MOLECULAR CELL 2007; 25:739-50.

192. Rinaldo C., Prodosmo A., Siepi F., Soddu S.
HIPK2: a multitalented partner for transcription factors in DNA damage response and development.
BIOCHEMISTRY AND CELL BIOLOGY-BIOCHIMIE ET BIOLOGIE CELLULAIRE 2007; 85:411-8.

193. Roche H., Yelle L., Cognetti F., Mauriac L., Bunnell C., Sparano J., Kerbrat P., Delord J.P., Vahdat L., Peck R., Lebwohl D., Ezzeddine R., Cure H.
Phase II clinical trial of ixabepilone (BMS-247550), an epothilone B analog, as first-line therapy in patients with metastatic breast cancer previously treated with anthracycline chemotherapy.
JOURNAL OF CLINICAL ONCOLOGY 2007; 10;25(23):3415-20.

194. Rosanò L., Di Castro V., Spinella F., Nicotra M., Natali P., Bagnato A.
ZD4054, a specific antagonist of the endothelin A receptor, inhibits tumor growth and enhances paclitaxel activity in human ovarian carcinoma *in vitro* and *in vivo*.
MOLECULAR CANCER RESEARCH 2007; 6(7):2003-11.

195. Rosanò L., Di Castro V., Spinella F., Tortora G., Nicotra M.R., Natali P., Bagnato A.
Combined targeting of endothelin A receptor and epidermal growth factor receptor in ovarian cancer shows enhanced antitumor activity
CANCER RESEARCH 2007; 67:6351-9.

196. Rossi M., Garavello W., Talamini R., Negri E., Bosetti C., Dal Maso L., Lagiou P., Tavani A., Polesel J., Barzan L., Ramazzotti V., Franceschi S., La Vecchia C.
Flavonoids and the risk of oral and pharyngeal cancer: a case-control study from Italy.
CANCER EPIDEMIOLOGY BIOMARKERS & PREVENTION 2007; 16(8):1621-5.

197. Ruscito P., Pichi B., Marchesi P., Spriano G.
Minimally invasive video-assisted submandibular sialoadenectomy: a preliminary report.
JOURNAL OF CRANIOFACIAL SURGERY 2007; 18(5):1142-7.

198. Salesi N., Russillo M., Marandino F., Ruggeri E., Metro G., Cognetti F., Fabi A.
Bone of the hands as unusual metastastatic site of renal cell carcinoma.
JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH 2007; 26(4):595-7.

199. Salvati E., Leonetti C., Rizzo A., Scarsella M., Mottolese M., Galati R., Sperduti I., Stevens M.F.G., D'Incalci M., Blasco M., Chiorino G., Bauwens S., Horard B., Gilson E., Stopacciaro A., Zupi G., Biroccio A.
Telomere damage induced by the G-quadruplex ligand RHPS4 has an antitumor effect
JOURNAL OF CLINICAL INVESTIGATION 2007; 117:3236-47.

200. Samaritani R., Corrado G., Vizza E., Sbiroli C.
Cyclophosphamide "metronomic" chemotherapy for palliative treatment of a young patient with advanced epithelial ovarian cancer.
BMC CANCER 2007; 15;7:65.

201. Sant M., Allemani C., Sieri S., Krogh V., Menard S., Tagliabue E., Nardini E., Micheli A., Crosignani P., Muti P., Berrino F.
Salad vegetables dietary pattern protects against HER-2-positive breast cancer: a prospective Italian study.
INTERNATIONAL JOURNAL OF CANCER 2007; 15;121(4):911-4.

202. Santoro R., Carboni F., Lepiane P., Ettorre M., Santoro E.
Clinicopathological features and prognosis of gastric cancer in young European adults.
BRITISH JOURNAL OF SURGERY 2007; 94:737-42.

203. Saulle E., Petronelli A., Pasquini L., Petrucci E., Mariani G., Biffoni M., Ferretti G., Scambia G., Benedetti-Panici P., Cognetti F., Humphreys R., Peschle C., Testa U.
Proteasome inhibitors sensitize ovarian cancer cells to TRAIL induced apoptosis.
APOPTOSIS 2007; 12(4):635-55.

204. Scardino A., Alimandi M., Correale P., Smith S.G., Bei R., Firat H., Cusi M.G., Faure O., Graf-Dubois S., Cencioni G., Marrocco J., Chouaib S., Lemonnier F.A., Jackson A.M., Kosmatopoulos K.
A polyepitope DNA vaccine targeted to Her-2/ErbB-2 elicits a broad range of human and murine CTL effectors to protect against tumor challenge.
CANCER RESEARCH 2007; 67:7028-36.

205. Scarpino S., Di Napoli A., Stoppacciaro A., Antonelli M., Pilozzi E., Chiarle R., Palestro G., Marino M., Facciolo F., Rendina E.A., Webster K.E., Kinkel S.A., Scott H.S., Ruco L.
Expression of autoimmune regulator gene (AIRE) and T regulatory cells in human thymomas.
CLINICAL AND EXPERIMENTAL IMMUNOLOGY 2007; 149(3):504-12.

206. Schünemann H.
Salmeterol and fluticasone propionate did not reduce mortality in chronic obstructive pulmonary disease.
EVID. BASED. MED. 2007; 12(4):114.

207. Schünemann H., Castelli M., Muti P.
Systematic reviews for the Journal of Experimental and Clinical Cancer Research: Going where the science takes us
JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH 2007; 26(2):169-174.

208. Schünemann H., Hill S.R., Kakad M., Bellamy R., Uyeki T.M., Hayden F.G., Yazdanpanah Y., Beigel J., Chotpitayasunondh T., Del Mar C., Farrar J., Tran T.H., Ozbay B., Sugaya N., Fukuda K., Shindo N., Stockman L., Vist G.E., Croisier A., Nagjdaliyev A., Roth C., Thomson G., Zucker H., Oxman A.D.
WHO Rapid Advice Guideline Panel on Avian Influenza.
WHO Rapid Advice Guidelines for pharmacological management of sporadic human infection with avian influenza A (H5N1) virus.
LANCET INFECTIOUS DISEASES 2007; 7(1):21-31.

209. Schünemann H., Hill S.R., Kakad M., Vist G.E., Bellamy R., Stockman L., Wisløff T.F., Del Mar C., Hayden F., Uyeki T.M., Farrar J., Yazdanpanah Y., Zucker H., Beigel J., Chotpitayasunondh T., Hien T.T., Ozbay B., Sugaya N., Oxman A.D.
Transparent development of the WHO rapid advice guidelines.
PLOS MEDICINE 2007; 4(5):E119.

210. Schünemann H., Norman G., Puhan M.A., Ståhl E., Griffith L., Heels-Ansdell D., Montori V.M., Wiklund I., Goldstein R., Mador M.J., Guyatt G.H.
Application of generalizability theory confirmed lower reliability of the standard gamble than the feeling thermometer.
JOURNAL OF CLINICAL EPIDEMIOLOGY 2007; 60(12):1256-62.

211. Sergi F., Flammia G.P., Alcini A., Perrone G., Guaglianone S., Forastiere E., Rabitti C., Gallucci M.
Collagen changes in the ureteropelvic junction after failed antegrade endopyelotomy.
JOURNAL OF ENDOUROLOGY 2007; 21(1):103-7.

212. Severino A., Abruzzese C., Manente L., Valderas A.A., Mattarocci S., Federico A., Starace G., Chersi A., Mileo A., Paggi M.
Human papillomavirus-16 E7 interacts with Siva-1 and modulates apoptosis in HaCaT human immortalized keratinocytes.
JOURNAL OF CELLULAR PHYSIOLOGY 2007; 212:118-25.

213. Shrader M., Pino M.S., Brown G., Black P., Adam L., Bar-Eli M., Dinney C.P., McConkey D.J.
Molecular correlates of gefitinib responsiveness in human bladder cancer cells.
MOLECULAR CANCER THERAPEUTICS 2007; 6(1):277-85.

214. Shrader M, Pino M.S., Lashinger L., Bar-Eli M., Adam L., Dinney C.P., McConkey D.J.
Gefitinib reverses TRAIL resistance in human bladder cancer cell lines via inhibition of AKT-mediated X-linked inhibitor of apoptosis protein expression.
CANCER RESEARCH 2007; 15;67(4):1430-5.

215. Sibilio L., Martayan A., Setini A., Lo Monaco E., Tremante E., Butler R.H., Giacomini P.
A single bottleneck in HLA-C assembly.
JOURNAL OF BIOLOGICAL CHEMISTRY 2007; 23:1267-74.

216. Sieri S., Pala V., Brighenti F., Pellegrini N., Muti P., Micheli A., Evangelista A., Grioni S., Contiero P., Berrino F., Krogh V.
Dietary glycemic index, glycemic load, and the risk of breast cancer in an Italian prospective cohort study.
AMERICAN JOURNAL OF CLINICAL NUTRITION 2007; 86(4):1160-6.

217. Simone G., Leonardo C., Papalia R., Guaglianone S., Rabitti C., Forastiere E., Gallucci M.
Case report: laparoscopic ureteral reconstruction with pelvic flap in ureteropelvic junction obstruction of ectopic left kidney.
JOURNAL OF ENDOUROLOGY 2007; 21(9):1041-3.

218. Simone G., Papalia R., Leonardo C., Gallucci M.
Re: Alexander Tsivian, Shalva Benjamin, A. Ami Sidi. A Sealed Laparoscopic Nephroureterectomy: A New Technique.
EUROPEAN UROLOGY 2007; 15:.

219. Soriani A., Landoni V., Marzi S., Iaccarino G., Saracino B., Arcangeli G., Benassi B.
Setup verification and *in vivo* dosimetry during intraoperative radiation therapy (IORT) for prostate cancer.
MEDICAL PHYSICS 2007; 34:3205-10.

220. Sperati F., Agabiti N., Picconi O., Pancioni T., Sperati A., et al.
Epidemiology of femur fracture and characteristics of hospital care in Lazio.
ANN. IG. 2007; 19(1):49-61.

221. Spinella F., Rosanò L., Di Castro V., Decandia S., Nicotra M., Natali P., Bagnato A.
Endothelin-1 and endothelin-3 promote invasive behavior via hypoxia-inducible factor-1alpha in human melanoma cells.
CANCER RESEARCH 2007; 67:1725-34.

222. Spugnini E., Arancia G., Porrello A., Colone M., Formisano G., Stringaro A., Citro G., Molinari A.
Ultrastructural modifications of cell membranes induced by “electroporation” on melanoma xenografts.
MICROSCOPY RESEARCH AND TECHNIQUE 2007; 22(4):302-6.

223. Spugnini E., Baldi F., Mellone P., Feroce F., D’Avino A., Bonetto F., Vincenzi B., Citro G., Baldi A.
Patterns of tumor response in canine and feline cancer patients treated with electrochemotherapy: preclinical data for the standardization of this treatment in pets and humans.
JOURNAL OF TRANSLATIONAL MEDICINE 2007; 5:48.

224. Spugnini E., Campioni M., D'Avino A., Caruso G., Citro G., Baldi A.
Cell-cycle molecules in mesothelioma: an overview.
JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH 2007; 26(4):443-9.

225. Spugnini E., Citro G., D'Avino A., Baldi A.
Potential role of electrochemotherapy for the treatment of soft tissue sarcoma: First insights from preclinical studies in animals.
INTERNATIONAL JOURNAL OF BIOCHEMISTRY & CELL BIOLOGY 2007; 40:159-63.

226. Spugnini E., Citro G., Dotsinsky I., Mudrov N., Mellone P., Baldi A.
Ganglioneuroblastoma in a cat: A rare neoplasm treated with electrochemotherapy.
VETERINARY JOURNAL 2007.

227. Spugnini E.P., Citro G., Mellone P., Dotsinsky I., Mudrov N., Baldi A.
Electrochemotherapy for localized lymphoma: a preliminary study in companion animals.
JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH 2007; 26(3):343-6.

228. Spugnini E., Dotsinsky I., Mudrov N., Cardosi G., Citro G., D'Avino A., Baldi A.
Biphasic pulses enhance bleomycin efficacy in a spontaneous canine perianal tumors model.
JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH 2007; 26(4)::483-7.

229. Spugnini E., Filipponi M., Romani L., Dotsinsky I., Mudrov N., Baroni A., Ruocco E., Laieta M.T., Montesarchio V., Cassandro R., Citro G., Baldi A.
Local control and distant metastasis after electrochemotherapy of a canine anal melanoma.
IN VIVO 2007; 21(5):897-9.

230. Spugnini E., Vincenzi B., Citro G., Santini D., Dotsinsky I., Mudrov N., Montesarchio V., Laieta M.T., Esposito V., Baldi A.
Adjuvant electrochemotherapy for the treatment of incompletely excised spontaneous canine sarcomas.
IN VIVO 2007; 21(5):819-22.

231. Spugnini E., Vincenzi B., Citro G., Tonini G., Dotsinsky I., Mudrov N., Baldi A.
Electrochemotherapy for the treatment of squamous cell carcinoma in cats: A preliminary report.
VETERINARY JOURNAL 2007.

232. Strano S., Blandino G.
YAP1 meets tumor suppression.
MOLECULAR CELL 2007; 27(6):863-4.

233. Strano S., Dell'Orso S., Di Agostino S., Fontemaggi G., Sacchi A., Blandino G.
Mutant p53: an oncogenic transcription factor.
ONCOGENE 2007; 26:2212-9.

234. Strigari L., Sciuto R., D'Andrea M., Pasqualoni R., Benassi B., Maini C.
Radiopharmaceutical therapy of bone metastases with 89SrCl2, 186Re-HEDP and 153Sm-EDTMP: a dosimetric study using Monte Carlo simulation.
EUROPEAN JOURNAL OF NUCLEAR MEDICINE AND MOLECULAR IMAGING 2007; 34:1031-8.

235. Tavani A., Zucchetto A., Dal Maso L., Montella M., Ramazzotti V., Talamini R., Franceschi S., La Vecchia C.
Lifetime physical activity and the risk of renal cell cancer.
INTERNATIONAL JOURNAL OF CANCER 2007; 120(9):1977-80.

236. Telera S., Conte A., Cristalli G., Occhipinti E., Pompili A.
Spontaneous cerebrospinal fluid rhinorrhea as the presenting symptom of sellar pathologies: three demonstrative cases.
NEUROSURGICAL REVIEW 2007; 30:78-82.

237. Tentori L., Lacal P.M., Muzi A., Dorio A.S., Leonetti C., Scarsella M., Ruffini F., Xu W., Min W., Stoppacciaro A., Colarossi C., Wang Z.Q., Zhang J., Graziani G.
Poly(ADP-ribose) polymerase (PARP) inhibition or PARP-1 gene deletion reduces angiogenesis.
EUROPEAN JOURNAL OF CANCER 2007; 43(14):2124-33.

238. Tomaino B., Cappello P., Capello M., Fredolini C., Ponzetto A., Novarino A., Ciuffreda L., Bertetto O., De Angelis C., Gaia E., Salacone P., Milella M., Nisticò P., Alessio M., Chiarle R., Giuffrida M.G., Giovarelli M., Novelli F.
Autoantibody signature in human ductal pancreatic adenocarcinoma.
JOURNAL OF PROTEOME RESEARCH 2007; 6(10):4025-31.

239. Tortora G., Bianco R., Daniele G., Ciardiello F., McCubrey J.A., Ricciardi M.R., Ciuffreda L., Cognetti F., Tafuri A., Milella M.
Overcoming resistance to molecularly targeted anticancer therapies: Rational drug combinations based on EGFR and MAPK inhibition for solid tumors and haematologic malignancies.
DRUG RESISTANCE UPDATES 2007; 10(3):81-100.

240. Turco M.C., Lamberti A., Bisogni R., Romano M.F., Petrella A., Ammirante M., Rosati A., Avenia M.D., Arra C., Spugnini E., Venuta S.
Modulation of cell apoptosis by AIR.
LEUKEMIA 2007; 218912):2557-9.

241. Vennarecci G., Ettorre M., Anastasi S., Antonini M., Santoro R., Perracchio L., Visco G., Santoro E.
Liver transplantation in HIV-positive patients.
TRANSPLANTATION PROCEEDINGS 2007; 39:1936-8.

242. Vennarecci G., Ettorre M., Antonini M., Santoro R., Maritti M., Tacconi G., Spoletini D., Tessitore L., Perracchio L., Visco G., Puoti C., Santoro E.
First-line liver resection and salvage liver transplantation are increasing therapeutic strategies for patients with hepatocellular carcinoma and child a cirrhosis.
TRANSPLANTATION PROCEEDINGS 2007; 39:1857-60.

243. Vidiri A., Ruscito P., Pichi B., Pellini R., Covello R., Sperduti I., Di Giovanni S., Spriano G., Crecco M.
Oral cavity and base of the tongue tumors. Correlation between clinical, MRI and pathological staging of primary tumor.
JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH 2007; 26(4):575-82.

244. Villaret A.B., Piazza C., Peretti, Calabrese L., Ansarin M., Chiesa F., Pellini R., Spriano G., Nicolai P.
Multicentric prospective study on the prevalence of sublevel IIb metastases in head and neck cancer.
ARCHIVES OF OTOLARYNGOLOGY-HEAD & NECK SURGERY 2007; 133(9):897-903.

245. Viola G., Sergi D., Conti F., Lopez M.
Neoadjuvant endocrine therapy for locally advanced breast cancer.
CLIN. TER. 2007; 158(5):441-52.

246. Virgili G., Gatta G., Ciccolallo L., Capocaccia R., Biggeri A., Crocetti E., Lutz J.M., Paci E., EUROCARE Working Group. (Cercato C., Ramazzotti V.).
Incidence of uveal melanoma in Europe.
OPHTHALMOLOGY 2007; 114(12):2309-15.

247. Wahlqvist P., Guyatt G.H., Armstrong D., Degl'Innocenti A., Heels-Ansdell D., El-Dika S., Wiklund I., Fallone C.A., Tanser L., Veldhuyzen Van Zanten S., Austin P., Barkun A.N., Chiba N., Schünemann H.
The Work Productivity and Activity Impairment Questionnaire for Patients with Gastroesophageal Reflux Disease (WPAI-GERD): responsiveness to change and English language validation.
PHARMACOECONOMICS 2007; 25(5):385-96.

248. Weisz L., Damalas A., Liontos M., Karakaidos, Fontemaggi G., Maor-Aloni R., Kalis M., Levrero M., Strano S., Gorgoulis V.G., Rotter V., Blandino G., Oren M.
Mutant p53 enhances nuclear factor kappaB activation by tumor necrosis factor alpha in cancer cells.
CANCER RESEARCH 2007; 67:2396-401.

249. Zucchetto A., Dal Maso L., Tavani A., Montella M., Ramazzotti V., Talamini R., Canzonieri V., Garbeglio A., Negri E., Franceschi S., La Vecchia C.
History of treated hypertension and diabetes mellitus and risk of renal cell cancer.
ANNALS OF ONCOLOGY 2007; 18(3):596-600.

Publications in Referenced Journals 2008*

1. Akl E.A., Barba M., Rohilla S., Terrenato I., Sperati F., Muti P., Schünemann H.J.
Low-molecular-weight heparins are superior to vitamin K antagonists for the long term treatment of venous thromboembolism in patients with cancer: a cochrane systematic review.
J EXP CLIN CANCER RES. 2008 JUL 18;27:21.

2. Akl E.A., Barba M., Rohilla S., Terrenato I., Sperati F., Muti P., Schünemann H.J.
Anticoagulation for the long term treatment of venous thromboembolism in patients with cancer.
COCHRANE DATABASE SYST REV. 2008 APR 16;(2).

3. Akl E.A., Kamath G., Yosuico V., Kim S.Y., Barba M., Sperati F., Cook D.J., Schünemann H.J.
Thromboprophylaxis for patients with cancer and central venous catheters: a systematic review and a meta-analysis.
CANCER. 2008 JUN;112(11):2483-92.

4. Akl E.A., Maroun N., Major S., Afif C., Abdo A., Choucair J., Sakr M., Li C.K., Grant B.J., Schünemann H.J.
Post-graduation migration intentions of students of Lebanese medical schools: a survey study.
BMC PUBLIC HEALTH. 2008 JUN 2;8:191.

5. Akl E.A., Muti P., Schünemann H.J.
Anticoagulation in patients with cancer: an overview of reviews.
POL ARCH MED WEWN. 2008 APR;118(4):183-93. REVIEW.

6. Akl E.A., Rohilla S., Barba M., Sperati F., Terrenato I., Muti P., Bdair F., Schünemann H.J.
Anticoagulation for the initial treatment of venous thromboembolism in patients with cancer: a systematic review.
CANCER. 2008 OCT 1;113(7):1685-94.

7. Akl E.A., Rohilla S., Barba M., Sperati F., Terrenato I., Muti P., Schünemann H.J.
Anticoagulation for the initial treatment of venous thromboembolism in patients with cancer.
COCHRANE DATABASE SYST REV. 2008 JAN 23;(1).

8. Akl E.A., Sackett K., Pretorius R., Erdley S., Bhoopathi P.S., Mustafa R., Schünemann H.J.
Educational games for health professionals.
COCHRANE DATABASE SYST REV. 2008 JAN 23;(1).

9. Akl E.A., Terrenato I., Barba M., Sperati F., Sempos E.V., Muti P., Cook D.J., Schünemann H.J.
Low-molecular-weight heparin vs unfractionated heparin for perioperative thromboprophylaxis in patients with cancer: a systematic review and meta-analysis.
ARCH INTERN MED. 2008 JUN 23;168(12):1261-9.

* January - October 2008

10. Akl E.A., van Doormaal F.F., Barba M., Kamath G., Kim S.Y., Kuipers S., Middeldorp S., Yosuico V., Dickinson H.O., Schünemann H.J.
Parenteral anticoagulation may prolong the survival of patients with limited small cell lung cancer: a Cochrane systematic review.
J EXP CLIN CANCER RES. 2008 MAY 15;27:4.

11. Annovazzi A., Viceconte G., Romano L., Sciuto R., Maini C.L.
Detection of a suspected bronchobiliary fistula by hepatobiliaryscintigraphy.
ANN NUCL MED. 2008 AUG;22(7):641-3.

13. Arcangeli S., Strigari L., Soete G., De Meerleer G., Gomellini S., Fonteyne V., Storme G., Arcangeli G.
Clinical and Dosimetric Predictors of Acute Toxicity after a 4-Week Hypofractionated External Beam Radiotherapy Regimen for Prostate Cancer: Results from a Multicentric Prospective Trial.
INT J RADIAT ONCOL BIOL PHYS. 2008 JUN 4.

14. Arcuri E.
Beginning again from the end.
PALLIAT MED. 2008 SEP;22(6):685-6.

15. Bagnato A., Rosanò L.
The endothelin axis in cancer.
INT J BIOCHEM CELL BIOL. 2008;40(8):1443-51.

16. Baldelli R., De Marinis L., Bianchi A., Pivonello R., Gasco V, Auriemma R., Pasimeni G., Cimino V., Appetecchia M., Maccario M., Lombardi G., Pontecorvi A., Colao A., Grottoli S.
Microalbuminuria in insulin sensitivity in patients with growth hormone-secreting pituitary tumor.
J CLIN ENDOCRINOL METAB. 2008 MAR;93(3):710-4. EPUB 2007 DEC 26.

17. Baldi A., Mottolese M., Vincenzi B., Campioni M., Mellone P., Di Marino M., Di Crescenzo V.G., Visca P., Menegozzo S., Spugnini E.P., Citro G., Ceribelli A., Mirri A., Chien J., Shridhar V.., Ehrmann M., Santini M., Facciolo F.
The serine protease HtrA1 is a novel prognostic factor for human mesothelioma.
PHARMACOGENOMICS. 2008 AUG;9(8):1069-77.

18. Barba M., Terrenato I., Schünemann H.J., Fuhrman B., Sperati F., Teter B., Gallucci M., D'Amato A., Muti P.
Indicators of sexual and somatic development and adolescent body size in relation to prostate cancer risk: results from a case-control study.
UROLOGY. 2008 JUL;72(1):183-7.

19. Begnozzi L., Benassi M., Bertanelli M., Bonini A., Cionini L., Conte L., Fiorino C., Gabriele P., Gardani G., Giani A., Magri S., Morelli M., Morrica B., Olmi P., Orecchia R., Penduzzu G., Raffaele L., Rosi A., Tabocchini M.A., Valdagni R., Viti V.
Quality assurance of 3D-CRT: Indications and difficulties in their applications.
CRIT REV ONCOL HEMATOL. 2008 SEP 1.

20. Bellacchio E., Paggi M.G.
Protease-mediated arsenic prodrug strategy in cancer and infectious diseases: a hypothesis for targeted activation.
J CELL PHYSIOL. 2008 MAR;214(3):681-6.

21. Benevolo M., Mottolese M., Marandino F., Carosi M., Diodoro M.G., Sentinelli S., Visca P., Rollo F., Mariani L., Vocaturo G., Sindico R., Terrenato I., Donnorso R.P., Vocaturo A.
HPV prevalence among healthy Italian male sexual partners of women with cervical HPV infection.
J MED VIROL. 2008 JUL;80(7):1275-81.

22. Benevolo M., Vocaturo A., Mottolese M., Mariani L., Vocaturo G., Marandino F., Sperduti I., Rollo F., Antoniani B., Donnorso R.P.
Clinical role of p16INK4a expression in liquid-based cervical cytology: correlation with HPV testing and histologic diagnosis.
AM J CLIN PATHOL. 2008 APR;129(4):606-12.

23. Biocca S., Filesi I., Mango R., Maggiore L., Baldini F., Vecchione L., Viola A., Citro G., Federici G., Romeo F., Novelli G.
The splice variant LOXIN inhibits LOX-1 receptor function through hetero-oligomerization.
J MOL CELL CARDIOL. 2008 MAR;44(3):561-70. EPUB 2007 DEC 7.

24. Bossi G., Marampon F., Maor-Aloni R., Zani B., Rotter V., Oren M., Strano S., Blandino G., Sacchi A.
Conditional RNA interference *in vivo* to study mutant p53 oncogenic gain of function on tumor malignancy.
CELL CYCLE. 2008 JUN;7(12):1870-9.

25. Bousquet J., Bieber T., Fokkens W., Humbert M., Kowalski M.L., Niggemann B., Simon H.U., Schünemann H.
Consensus statements, evidence-based medicine and guidelines in allergic diseases.
ALLERGY. 2008 JAN;63(1):1-4.

26. Bousquet J., Fokkens W., Burney P., Durham S.R., Bachert C., Akdis C.A., Canonica G.W., Dahlen S.E., Zuberbier T., Bieber T., Bonini S., Bousquet P.J., Brozek J.L., Cardell L.O., Crameri R., Custovic A., Demoly P., van Wijk R.G., Gjomarkaj M., Holland C., Howarth P., Humbert M., Johnston S.L., Kauffmann F., Kowalski M.L., Lambrecht B., Lehmann S., Leynaert B., Lodrup-Carlsen K., Mullol J., Niggemann B., Nizankowska-Mogilnicka E., Papadopoulos N., Passalacqua G., Schünemann H.J., Simon H.U., Todo-Bom A., Toskala E., Valenta R., Wickman M., Zock J.P.
Important research questions in allergy and related diseases: nonallergic rhinitis: a GA2LEN paper.
ALLERGY. 2008 JUL;63(7):842-53. REVIEW.

27. Bria E., Cuppone F., Fornier M., Nisticò C., Carlini P., Milella M,. Sperduti I., Terzoli E., Cognetti F., Giannarelli D.
Cardiotoxicity and incidence of brain metastases after adjuvant trastuzumab for early breast cancer: the dark side of the moon? A meta-analysis of the randomized trials.
BREAST CANCER RES TREAT. 2008 MAY;109(2):231-9. EPUB 2007 JUL 19. REVIEW.

28. Bria E., Gralla R.J., Raftopoulos H., Cuppone F., Milella M., Sperduti I., Carlini P., Terzoli E., Cognetti F., Giannarelli D.
Magnitude of benefit of adjuvant chemotherapy for non-small cell lung cancer: Meta-analysis of randomized clinical trials.
LUNG CANCER. 2008 JUN 17.

29. Bria E., Visca P., Novelli F., Casini B., Diodoro M.G., Perrone-Donnorso R., Botti C., Sperduti I., Facciolo F., Milella M., Cecere F.L., Cognetti F., Mottolese M.
Nuclear and cytoplasmic cellular distribution of survivin as survival predictor in resected non-small-cell lung cancer.
EUR J SURG ONCOL. 2008 MAY;34(5):593-8. EPUB 2007 AUG 10.

30. Bro_ek J.L., Guyatt G.H., Heels-Ansdell D., Degl'Inocenti A., Armstrong D., Fallone C.A., Wiklund I., Veldhuyzen van Zanten S., Chiba N., Barkun A.N., Akl E.A., Schünemann H.J.
Specific HRQL instruments and symptom scores were more responsive than preference-based generic instruments in patients with GERD.
J CLIN EPIDEMIOL. 2008 JUL 9.

31. Brozek J.L., Baena-Cagnani C.E., Bonini S., Canonica G.W., Rasi G., van Wijk R.G., Zuberbier T., Guyatt G., Bousquet J., Schünemann H.J.
Methodology for development of the Allergic Rhinitis and its Impact on Asthma guideline 2008 update.
ALLERGY. 2008 JAN;63(1):38-46. REVIEW.

32. Campioni M., Ambrogi V., Pompeo E., Citro G., Castelli M., Spugnini E.P., Gatti A., Cardelli P., Lorenzon L., Baldi A., Mineo T.C.
Identification of genes down-regulated during lung cancer progression: a cDNA array study.
J EXP CLIN CANCER RES. 2008 SEP 15;27(1):38.

33. Capurso G., Schünemann H.J., Delle Fave G.
Passive smoking and the use of noncigarette tobacco products in association with risk for pancreatic cancer: a case-control study.
CANCER. 2008 FEB 1;112(3):671-2; AUTHOR REPLY 672.

34. Caruso R.A., Fedele F., Consolo P., Luigiano C., Venuti A., Cavallari V.
Abnormal nuclear structures (micronuclei, nucleoplasmic bridges, and nuclear buds) in a pleomorphic giant cell carcinoma of the stomach.
ULTRASTRUCT PATHOL. 2008 JAN-FEB;32(1):11-5.

35. Castelli M.
A new era for Journal of Experimental & Clinical Cancer Research.
J EXP CLIN CANCER RES. 2008 MAY 15;27(1):1

36. Cercato M.C., Nagore E., Ramazzotti V., Guillén C., Terrenato I., Villena J., Lomuscio M., Natali P.G., Schünemann H.J.
Self and parent-assessed skin cancer risk factors in school-age children.
PREV MED. 2008 JUL;47(1):133-5.

37. Corrado G., Baiocco E., Carosi M., Vizza E.
Progression of conservatively treated endometrial complex atypical hyperplasia in a young woman: a case report.
FERTIL STERIL. 2008 AUG 8.

38. Costantini A.S., Benvenuti A., Vineis P., Kriebel D., Tumino R., Ramazzotti V., Rodella S., Stagnaro E., Crosignani P., Amadori D., Mirabelli D., Sommani L., Belletti I., Troschel L., Romeo L., Miceli G., Tozzi G.A., Mendico I., Maltoni S.A., Miligi L.
Risk of leukemia and multiple myeloma associated with exposure to benzene and other organic solvents: Evidence from the Italian Multicenter Case-control study.
AM J IND MED. 2008 JUL 23.

39. Cuppone F., Bria E., Carlini P., Milella M., Felici A., Sperduti I., Nisticò C., Terzoli E., Cognetti F., Giannarelli D.
Taxanes as primary chemotherapy for early breast cancer: meta-analysis of randomized trials.
CANCER. 2008 JUL 15;113(2):238-46.

40. Cuppone F., Bria E., Verma S., Pritchard K.I., Gandhi S., Carlini P., Milella M., Nisticò C., Terzoli E., Cognetti F., Giannarelli D.
Do adjuvant aromatase inhibitors increase the cardiovascular risk in postmenopausal women with early breast cancer? Meta-analysis of randomized trials.
CANCER. 2008 JAN 15;112(2):260-7.

41. De Luca A., De Falco M., Manente L., Dattilo D., Lucariello A., Esposito V., Gnarini M., Citro G., Baldi A., Tufano M.A., Iaquinto G.
Helicobacter pylori heat shock protein B (HspB) localizes *in vivo* in the gastric mucosa and MALT lymphoma.
J CELL PHYSIOL. 2008 JUL;216(1):78-82.

42. Di Costanzo F., Gasperoni S., Manzione L., Bisagni G., Labianca R., Bravi S., Cortesi E., Carlini P., Bracci R., Tomao S., Messerini L., Arcangeli A., Torri V., Bilancia D., Floriani I., Tonato M., Italian Oncology Group for Cancer Research, Dinota A., Strafiuso G., Corgna E., Porrozzi S., Boni C., Rondini E., Giunta A., Monzio Compagnoni B., Biagioni F., Cesari M., Fornarini G., Nelli F., Carboni M., Cognetti F., Enzo M.R., Piga A., Romiti A., Olivetti A., Masoni L., De Stefanis M., Dalla Mola A., Camera S., Recchia F., De Filippis S., Scipioni L,. Zironi S., Luppi G., Italia M., Banducci S., Pisani Leretti A., Massidda B., Ionta M.T., Nicolosi A., Canaletti R., Biscottini B., Grigniani F., Di Costanzo F., Rovei R., Croce E., Carroccio R., Gilli G., Cavalli C., Olgiati A., Pandolfi U., Rossetti R., Natalini G., Foa P., Oldani S., Bruno L., Cascinu S., Catalano G., Catalano V., Lungarotti F., Farris A., Sarobba M.G., Trignano M., Muscogiuri A., Francavilla F., Figoli F., Leoni M., Papiani G., Orselli G., Antimi M., Bellini V., Cabassi A., Contu A., Pazzola A., Frignano M., Lastraioli E., Saggese M., Bianchini D., Antonuzzo L., Mela M., Camisa R.
Adjuvant chemotherapy in completely resected gastric cancer: a randomized phase III trial conducted by GOIRC.
J NATL CANCER INST. 2008 MAR 19;100(6):388-98.

43. Di Maio M., Bria E., Rossi A., Perrone F.
Progression-free survival as primary end point in advanced non-small-cell lung cancer: does the size matter?
ANN ONCOL. 2008 FEB;19(2):402-3.

44. Di Nallo A.M., Strigari L., Giliberti C., Bedini A., Palomba R., Benassi M.
Monitoring of people and workers exposure to the electric, magnetic and electromagnetic fields in an Italian National Cancer Institute.
J EXP CLIN CANCER RES. 2008 JUL 3;27:16.

45. Di Palma S., Collins N., Bilous M., Sapino A., Mottolese M., Kapranos N., Schmitt F., Isola J.
A quality assurance exercise to evaluate the accuracy and reproducibility of chromogenic in situ hybridisation for HER2 analysis in breast cancer.
J CLIN PATHOL. 2008 JUN;61(6):757-60.

46. Fabi A., Ciccarese M., Metro G., Savarese A., Giannarelli D., Nuzzo C.M., Russillo M., Sperduti I., Carbone I., Bria E., Cognetti F.
Oral ondansetron is highly active as rescue antiemetic treatment for moderately emetogenic chemotherapy: results of a randomized phase II study.
SUPPORT CARE CANCER. 2008 MAY 14.

47. Fabi A., Metro G., Ferretti G., Giannarelli D., Di Cosimo S., Papaldo P., Mottolese M., Carlini P., Felici A., Russillo M., Cognetti F.
Do HER-2 positive metastatic breast cancer patients benefit from the use of trastuzumab beyond disease progression? A mono-institutional experience and systematic review of observational studies.
BREAST. 2008 APR 29.

48. Fabi A., Mirri A., Felici A., Vidiri A., Pace A., Occhipinti E., Cognetti F., Arcangeli G., Iandolo B., Carosi M.A., Metro G., Carapella C.M.
Fixed dose-rate gemcitabine as radiosensitizer for newly diagnosed glioblastoma: a dose-finding study.
J NEUROONCOL. 2008 MAR;87(1):79-84. EPUB 2007 NOV 7.

49. Falck-Ytter Y., Kunz R., Guyatt G.H., Schünemann H.J.
How strong is the evidence?
AM J GASTROENTEROL. 2008 JUN;103(6):1334-8. REVIEW. AVAILABLE.

50. Fattoruso S.I., Di Lauro L., Conti F., Amodio A., Lopez M.
Target selectivity of anticancer drugs.
CLIN TER. 2008 MAY-JUN;159(3):189-206. ITALIAN.

51. Ferrandina G., Ludovisi M., Lorusso D., Pignata S., Breda E., Savarese A., Del Medico P., Scaltriti L., Katsaros D., Priolo D., Scambia G.
Phase III trial of gemcitabine compared with pegylated liposomal doxorubicin in progressive or recurrent ovarian cancer.
J CLIN ONCOL. 2008 FEB 20;26(6):890-6.

52. Ferrandina G., Petrillo M., Carbone A., Zannoni G., Martinelli E., Prisco M., Pignata S., Breda E., Savarese A., Scambia G.
Prognostic role of topoisomerase-IIalpha in advanced ovarian cancer patients.
BR J CANCER. 2008 JUN 17;98(12):1910-5.

53. Ferraresi V., Ciccarese M., Cercato M.C., Nuzzo C., Zeuli M., Di Filippo F., Giannarelli D., Cognetti F.
Gemcitabine at fixed dose-rate in patients with advanced soft tissue sarcomas: a mono-institutional phase II study.
CANCER CHEMOTHER PHARMACOL. 2008 MAR 20.

54. Ferrari A., Buccini P., Covello R., De Simone P., Silipo V., Mariani G., Eibenschutz L., Mariani L., Catricalà C.
The ringlike pattern in vulvar melanosis: a new dermoscopic clue for diagnosis.
ARCH DERMATOL. 2008 AUG;144(8):1030-4.

55. Ferretti G., Felici A., Cognetti F.
HER2 and response to paclitaxel in node-positive breast cancer.
N ENGL J MED. 2008 JAN 10;358(2):197; AUTHOR REPLY 198.

56. Folgiero V., Avetrani P., Bon G., Di Carlo S.E., Fabi A., Nisticò C., Vici P., Melucci E., Buglioni S., Perracchio L., Sperduti I., Rosanò L., Sacchi A., Mottolese M., Falcioni R.
Induction of ErbB-3 expression by alpha6beta4 integrin contributes to tamoxifen resistance in ERbeta1-negative breast carcinomas.
PLOS ONE. 2008 FEB 13;3(2):E1592.

57. Fontana L., Fiori M.E., Albini S., Cifaldi L., Giovinazzi S., Forloni M., Boldrini R., Donfrancesco A., Federici V., Giacomini P., Peschle C., Fruci D.
Antagomir-17-5p abolishes the growth of therapy-resistant neuroblastoma through p21 and BIM.
PLOS ONE. 2008 MAY 21;3(5):E2236.

58. Frasci G., Crispo A., D'Aiuto G., Barba M., Rinaldo M., De Marco M., Grimaldi M., Capasso I., Botti G., Di Bonito M., Montella M.
Outcome analysis of breast cancer patients receiving breast-conserving surgery in Southern Italy.
J CHEMOTHER. 2008 APR;20(2):269-77.

59. Fruci D., Giacomini P., Nicotra M.R., Forloni M., Fraioli R., Saveanu L., Van Endert P., Natali P.G.
Altered expression of endoplasmic reticulum aminopeptidases ERAP1 and ERAP2 in transformed non-lymphoid human tissues.
J CELL PHYSIOL. 2008 SEP;216(3):742-9.

60. Fuhrman B.J., Teter B.E., Barba M., Byrne C., Cavalleri A., Grant B.J., Horvath P.J., Morelli D., Venturelli E., Muti P.C.
Equol status modifies the association of soy intake and mammographic density in a sample of postmenopausal women.
CANCER EPIDEMIOL BIOMARKERS PREV. 2008 JAN;17(1):33-42.

61. Gabellini C., Castellini L., Trisciuoglio D., Kracht M., Zupi G., Del Bufalo D.
Involvement of Nuclear Factor-kappa B in bcl-xL-induced interleukin 8 expression in glioblastoma.
J NEUROCHEM. 2008 SEP 11.

62. Gaikwad N.W., Yang L., Muti P., Meza J.L., Pruthi S., Ingle J.N., Rogan E.G,. Cavalieri E.L.
The molecular etiology of breast cancer: evidence from biomarkers of risk.
INT J CANCER. 2008 MAY 1;122(9):1949-57.

63. Gallucci M., Merola R., Leonardo C., De Carli P., Farsetti A., Sentinelli S., Sperduti I., Mottolese M., Carlini P., Vico E., Simone G., Cianciulli A.
Genetic profile identification in clinically localized prostate carcinoma.
UROL ONCOL. 2008 JUN 3.

64. Gallucci M., Vico E., Sentinelli S., Leonardo C., Visca P., Orlandi G., Cianciulli A.
Familial papillary renal carcinoma: cytogenetic characterization in normal and tumor tissues in two brothers. A case report.
UROL ONCOL. 2008 MAY-JUN;26(3):295-8.

65. Gamucci T., Nelli F., Cianci G., Grassi G., Moscetti L., Sperduti I., Zeuli M., Cortesi E., D'Auria G., Pollera C.F.
A phase II study of cetuximab/irinotecan in patients with heavily pretreated metastatic colorectal cancer: predictive value of early specific toxicities.
CLIN COLORECTAL CANCER. 2008 JUL;7(4):273-9.

66. Garavello W., Foschi R., Talamini R., La Vecchia C., Rossi M., Dal Maso L., Tavani A., Levi F., Barzan L., Ramazzotti V., Franceschi S., Negri E.
Family history and the risk of oral and pharyngeal cancer.
INT J CANCER. 2008 APR 15;122(8):1827-31.

67. Gelibter A.J., Di Segni S., Zeuli M., Cognetti F.
Bevacizumab: one treatment for all the seasons?
J CLIN ONCOL. 2008 JAN 20;26(3):511; AUTHOR REPLY 511-2. ERRATUM IN: J CLIN ONCOL 2008 APR 10;26(11) 1911.

68. Giorgi C., Di Bonito P., Grasso F., Mochi S., Accardi L., Donà M.G., Branca M., Costa S., Mariani L., Agarossi A., Ciotti M., Syrjänen K., the HPV-PathogenISS group.
Clinical and epidemiological correlates of antibody response to human papillomaviruses (HPVs) as measured by a novel ELISA based on denatured recombinant HPV16 late (L) and early (E) antigens.
INFECT AGENT CANCER. 2008 JUN 26;3(1):9.

69. Grasselli A., Nanni S., Colussi C., Aiello A., Benvenuti V., Ragone G., Moretti F., Sacchi A., Bacchetti S., Gaetano C., Capogrossi M.C., Pontecorvi A., Farsetti A.
Estrogen receptor-alpha and endothelial nitric oxide synthase nuclear complex regulates transcription of human telomerase.
CIRC RES. 2008 JUL 3;103(1):34-42.

70. Guastafierro T., Cecchinelli B., Zampieri M., Reale A., Riggio G., Sthandier O., Zupi G., Calabrese L., Caiafa P.
CCCTC-binding factor activates PARP-1 affecting DNA methylation machinery.
J BIOL CHEM. 2008 AUG 8;283(32):21873-80.

71. Gurtner A., Fuschi P., Magi F., Colussi C., Gaetano C., Dobbelstein M., Sacchi A., Piaggio G.
NF-Y dependent epigenetic modifications discriminate between proliferating and postmitotic tissue.
PLOS ONE. 2008 APR 23;3(4):E2047.

72. Guyatt G.H., Cook D.J., Jaeschke R., Pauker S.G., Schünemann H.J.
Grades of recommendation for antithrombotic agents: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition).
CHEST. 2008 JUN;133(6 SUPPL):123S-131S. ERRATUM IN: CHEST. 2008 AUG;134(2):473.

73. Guyatt G.H., Oxman A.D., Kunz R., Falck-Ytter Y., Vist G.E., Liberati A., Schünemann H.J.; GRADE Working Group.
Going from evidence to recommendations.
BMJ. 2008 MAY 10;336(7652):1049-51. ERRATUM IN: BMJ. 2008 JUN 21;336(7658).

74. Guyatt G.H., Oxman A.D., Kunz R., Jaeschke R., Helfand M., Liberati A., Vist G.E., Schünemann H.J.; GRADE Working Group.
Incorporating considerations of resources use into grading recommendations.
BMJ. 2008 MAY 24;336(7654):1170-3. REVIEW. ERRATUM IN: BMJ.2008 JUN 21;336(7658).

75. Guyatt G.H., Oxman A.D., Kunz R., Vist G.E., Falck-Ytter Y., Schünemann H.J.; GRADE Working Group.
What is "quality of evidence" and why is it important to clinicians?
BMJ. 2008 MAY 3;336(7651):995-8. REVIEW.

76. Guyatt G.H., Oxman A.D., Vist G.E., Kunz R., Falck-Ytter Y., Alonso-Coello P., Schünemann H.J.; GRADE Working Group.
GRADE: an emerging consensus on rating quality of evidence and strength of recommendations.
BMJ. 2008 APR 26;336(7650):924-6.

77. Hirsh J., Guyatt G., Albers G.W., Harrington R., Schünemann H.J., American College of Chest Physician.
Antithrombotic and thrombolytic therapy: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition).
CHEST. 2008 JUN;133(6 SUPPL):110S-112S. ERRATUM IN: CHEST. 2008 AUG;134(2):473.

78. Hagman J.H., Ginebri A., Piemonte P., Frascione P.
Acute papulopustularRosacea-like eruption from oral parabens.
ACTA DERM VENEREOL. 2008;88(3):272-3

79. Hirsh J., Guyatt G., Albers G.W., Harrington R., Schünemann H.J.; American College of Chest Physicians.
Executive summary: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition).
CHEST. 2008 JUN;133(6 SUPPL):71S-109S.

80. Jaeschke R., Guyatt G.H., Dellinger P., Schünemann H., Levy M.M., Kunz R., Norris S., Bion J.; GRADE Working Group.
Use of GRADE grid to reach decisions on clinical practice guidelines when consensus is elusive.
BMJ. 2008 JUL 31;337:A744.

81. Jaeschke R., O'Byrne P.M., Mejza F., Nair P., Lesniak W., Brozek J., Thabane L., Cheng J., Schünemann H.J., Sears M.R., Guyatt G.
The Safety of Long Acting Beta Agonists Among Patients with Asthma Using Inhaled Corticosteroids.
AM J RESPIR CRIT CARE MED. 2008 SEP 5.

82. Karanicolas P..J, Montori V.M., Devereaux P.J., Schünemann H., Guyatt G.H.
A new "Mechanistic-Practical" Framework for designing and interpreting randomized trials.
J CLIN EPIDEMIOL. 2008 MAY 9.

83. Kunz R., Burnand B., Schünemann H..J; Grading of Recommendations, Assessment, Development and Evaluation (GRADE) Working Group.
The GRADE System. An international approach to standardize the graduation of evidence and recommendations in guidelines
INTERNIST (BERL). 2008 JUN;49(6):673-80. GERMAN.

84. Kunz R., Djulbegovic B., Schünemann H.J., Stanulla M., Muti, P., Guyatt G.
Misconceptions, challenges, uncertainty, and progress in guideline recommendations.
SEMIN HEMATOL. 2008 JUL;45(3):167-75.

85. Kurzik-Dumke U., Hörner M., Czaja J., Nicotra M.R., Simiantonaki N., Koslowski M., Natali P.G.
Progression of colorectal cancers correlates with overexpression and loss of polarization of expression of the htid-1 tumor suppressor.
INT J MOL MED. 2008 JAN;21(1):19-31.

86. Lim W., Holinski P., Devereaux P.J., Tkaczyk A., McDonald E., Clarke F., Qushmaq I., Terrenato I., Schünemann H., Crowther M., Cook D.
Detecting myocardial infarction in critical illness using screening troponin measurements and ECG recordings.
CRIT CARE. 2008;12(2):R36.

87. Manente L., Perna A., Buommino E., Altucci L., Lucariello A., Citro G., Baldi A., Iaquinto G., Tufano M.A., De Luca A.
The Helicobacter pylori's protein VacA has direct effects on the regulation of cell cycle and apoptosis in gastric epithelial cells.
J CELL PHYSIOL. 2008 MAR;214(3):582-7.

88. Manni I., Caretti G., Artuso S., Gurtner A., Emiliozzi V., Sacchi A., Mantovani R., Piaggio G.
Posttranslational Regulation of NF-YA Modulates NF-Y Transcriptional Activity.
MOL BIOL CELL. 2008 SEP 24.

89. Maresca V., Flori E., Briganti S., Mastrofrancesco A., Fabbri C., Mileo A.M., Paggi M.G., Picardo M.
Correlation between melanogenic and catalase activity in *in vitro* human melanocytes: a synergic strategy against oxidative stress.
PIGMENT CELL MELANOMA RES. 2008 APR;21(2):200-5.

90. Mariani L., Morrone A., Preti M., Sbiroli C., Tomao F., Tomao S.
Migrant women and cervical cancer: background of a prevention study.
EUR J GYNAECOL ONCOL. 2008;29(1):52-6.

91. Martayan A., Sibilio L., Setini A., Monaco E.L., Tremante E., Fruci D., Colonna M., Giacomini P.
N-linked glycosylation selectively regulates the generic folding of HLA-Cw1.
J BIOL CHEM. 2008 JUN 13;283(24):16469-76. .

92. Marucci L., Pichi B., Iaccarino G., Ruscito P., Spriano G., Arcangeli G.
Intraoperative radiation therapy as an "early boost" in locally advanced head and neck cancer: preliminary results of a feasibility study.
HEAD NECK. 2008 JUN;30(6):701-8.

93. Maschio M., Albani F., Jandolo B., Zarabla A., Contin M., Dinapoli L., Fabi A., Pace A., Baruzzi A.
Temozolomide treatment does not affect topiramate and oxcarbazepine plasma concentrations in chronically treated patients with brain tumor-related epilepsy.
J NEUROONCOL. 2008 JUL 9.

94. Maschio M., Dinapoli L., Zarabla A., Jandolo B.
In reference to Bosma et al. The course of neurocognitive functioning in high-grade glioma patients.
NEURO ONCOL. 2008 FEB;10(1):106-7.

95. Maschio M., Dinapoli L., Zarabla A., Pompili A., Carapella C.M., Pace A., Giannarelli D., Occhipinti E., Jandolo B.
Outcome and tolerability of topiramate in brain tumor associated epilepsy.
J NEUROONCOL. 2008 JAN;86(1):61-70. EPUB 2007 JUN 28.

96. Massa S., Simeone P., Muller A., Benvenuto E., Venuti A., Franconi R.
Antitumor activity of DNA vaccines based on the human papillomavirus-16 E7 protein genetically fused to a plant virus coat protein.
HUM GENE THER. 2008 APR;19(4):354-64.

97. McCubrey J.A., Milella M., Tafuri A., Martelli A.M., Lunghi P., Bonati A., Cervello M., Lee J.T., Steelman L.S.
Targeting the Raf/MEK/ERK pathway with small-molecule inhibitors.
CURR OPIN INVESTIG DRUGS. 2008 JUN;9(6):614-30.

98. McCubrey J.A., Sokolosky M.L., Lehmann B.D., Taylor J.R., Navolanic P.M., Chappell W.H., Abrams S.L., Stadelman K.M., Wong E.W., Misaghian N., Horn S., Bäsecke J., Libra M., Stivala F., Ligresti G., Tafuri A., Milella M., Zarzycki M., Dzugaj A., Chiarini F., Evangelisti C., Martelli A.M., Terrian D.M., Franklin R.A., Steelman L.S.
Alteration of Akt activity increases chemotherapeutic drug and hormonal resistance in breast cancer yet confers an achilles heel by sensitization to targeted therapy.
ADV ENZYME REGUL. 2008 FEB 21.

99. McCubrey J.A., Steelman L.S., Abrams S.L., Bertrand F.E., Ludwig D.E., Bäsecke J., Libra M., Stivala F., Milella M., Tafuri A., Lunghi P., Bonati A., Martelli A.M.
Targeting survival cascades induced by activation of Ras/Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways for effective leukemia therapy.
LEUKEMIA. 2008 APR;22(4):708-22.

100. Ménard S., Balsari A., Tagliabue E., Camerini T., Casalini P., Bufalino R., Castiglioni F., Carcangiu ML., Gloghini A., Scalone S., Querzoli P., Lunardi M., Molino A., Mandarà M., Mottolese M., Marandino F., Venturini M., Bighin C., Cancello G., Montagna E., Perrone F., De Matteis A., Sapino A., Donadio M., Battelli N., Santinelli A., Pavesi L., Lanza A., Zito F.A., Labriola A., Aiello R.A., Caruso M., Zanconati F., Mustacchi G., Barbareschi M., Frisinghelli M., Russo R., Carrillo G.; On the behalf of the OMERO group.
Biology, prognosis and response to therapy of breast carcinomas according to HER2 score.
ANN ONCOL. 2008 JUN 9.

101. Metro G., Fabi A., Russillo M., Papaldo P., De Laurentiis M., Ferretti G., Pellegrini D., Nuzzo C., Graziano V., Vici P., Introna M., Felici A., Cognetti F., Carlini P.
Taxanes and gemcitabine doublets in the management of HER-2 negative metastatic breast cancer: towards optimization of association and schedule.
ANTICANCER RES. 2008 MAR-APR;28(2B):1245-58. REVIEW.

102. Miccadei S., Di Venere D., Cardinali A., Romano F., Durazzo A., Foddai M.S., Fraioli R., Mobarhan S., Maiani G.
Antioxidative and apoptotic properties of polyphenolic extracts from edible part of artichoke (Cynara scolymus L.) on cultured rat hepatocytes and on human hepatoma cells.
NUTR CANCER. 2008 MAR-APR;60(2):276-83.

103. Milella M., Metro G., Gelibter A., Pino S.M., Cognetti F., Fabi A.
COX-2 targeting in cancer: a new beginning?
ANN ONCOL. 2008 JUN;19(6):1209-10.

104. Molica S., Digiesi G., Mauro F., Mirabelli R., Cutrona G., Vitelli G., Morabito F., Iuliano F., Foà R., Ferrarini M.
Increased serum BAFF (B-cell activating factor of the TNF family) level is a peculiar feature associated with familial chronic lymphocytic leukemia.
LEUK RES. 2008 JUN 13.

105. Molica S., Vitelli G., Cutrona G., Todoerti K., Mirabelli R., Digiesi G., Morabito F., Neri A., Ferrarini M.
Serum thrombopoietin compared with ZAP-70 and immunoglobulin heavy-chain gene mutation status as a predictor of time to first treatment in early chronic lymphocytic leukemia.
LEUK LYMPHOMA. 2008 JAN;49(1):62-7.

106. Molica S., Vitelli G., Cutrona G., Todoerti K., Mirabelli R., Digiesi G., Giannarelli D., Sperduti I., Molica M., Gentile M., Morabito F., Neri A., Ferrarini M.
Prognostic relevance of serum levels and cellular expression of adiponectin in B-cell chronic lymphocytic leukemia.
INT J HEMATOL. 2008 SEP 27.

107. Novelli F., Milella M., Melucci E., Di Benedetto A., Sperduti I., Perrone-Donnorso R., Perracchio L., Venturo I., Nisticò C., Fabi A., Buglioni S., Natali P.G., Mottolese M.
A divergent role for estrogen receptor-beta in node-positive and node-negative breast cancer classified according to molecular subtypes: an observational prospective study.
BREAST CANCER RES. 2008 SEP 4;10(5):R74.

108. Nuzzo C., Zeuli M., Ferraresi V., Ciccarese M., Pelligrini D., Cognetti F.
Unexpected clinical outcome in a patient with liver and brain metastasis from melanoma.
ANTICANCER RES. 2008 MAR-APR;28(2B):1429-31.

109. Pace A., Lorenzo C.D., Guariglia L., Jandolo B., Carapella C.M., Pompili A.
End of life issues in brain tumor patients.
J NEUROONCOL. 2008 AUG 14.

110. Pasciuti K., Iaccarino G., Soriani A., Bruzzaniti V., Marzi S., Gomellini S., Arcangeli S., Benassi M., Landoni V.
DVHs evaluation in brain metastases stereotactic radiotherapy treatment plans.
RADIOTHER ONCOL. 2008 APR;87(1):110-5.

111. Pasimeni G., Caroli F., Spriano G., Antonini M., Baldelli R., Appetecchia M.
Refractory thyrotoxicosis induced by iodinated contrast agents treated with therapeutic plasma exchange. A case report.
J CLIN APHER. 2008;23(2):92-5.

112. Pellegrino A., Vizza E., Fruscio R., Villa A., Corrado G., Villa M., Dell'Anna T., Vitobello D.
Total laparoscopic radical hysterectomy and pelvic lymphadenectomy in patients with Ib1 stage cervical cancer: Analysis of surgical and oncological outcome.
EUR J SURG ONCOL. 2008 AUG 27.

113. Pellini R., Pichi B., Ruscito P., Ceroni A.R., Caliceti U., Rizzotto G., Pazzaia A., Laudadio P., Piazza C., Peretti G., Giannarelli D., Spriano G.
Supracricoid partial laryngectomies after radiation failure: a multi-institutional series.
HEAD NECK. 2008 MAR;30(3):372-9.

114. Pentenero M., Cistaro A., Brusa M., Ferraris M.M., Pezzuto C., Carnino R., Colombini E., Valentini M.C., Giovanella L., Spriano G., Gandolfo S.
Accuracy of 18F-FDG-PET/CT for staging of oral squamous cell carcinoma.
HEAD NECK. 2008 SEP 2.

115. Pichi B., Manciocco V., Marchesi P., Pellini R., Ruscito P., Vidiri A., Covello R., Spriano G.
Rhabdomyoma of the parapharyngeal space presenting with dysphagia.
DYSPHAGIA. 2008 JUN;23(2):202-4. EPUB 2007 OCT 27.

116. Pino M.S., Balsamo M., Di Modugno F., Mottolese M., Alessio M., Melucci E., Milella M., McConkey D.J., Philippar U., Gertler F.B., Natali P.G., Nisticò P.
Human Mena+11a isoform serves as a marker of epithelial phenotype and sensitivity to epidermal growth factor receptor inhibition in human pancreatic cancer cell lines.
CLIN CANCER RES. 2008 AUG 1;14(15):4943-50.

117. Pino M.S., Gamucci T., Mansueto G., Trapasso T., Narducci F., Giampaolo M.A., Fariello A.M., Sperduti I., Ceribelli A., Cognetti F.
A phase II study of biweekly paclitaxel (P) and gemcitabine (G), followed by maintenance weekly paclitaxel in elderly patients with advanced non-small cell lung cancer (NSCLC).
LUNG CANCER. 2008 JUN;60(3):381-6. EPUB 2007 DEC 19.

118. Pisani F., Marino M., Sentinelli S., Petti M.C.
Follicular dendritic cell sarcoma of the neck: report of a case treated by surgical excision and COP plus (PEG)-liposomal doxorubicin.
J EXP CLIN CANCER RES. 2008 SEP 2;27(1):33.

119. Pompili A., Carapella C.M., Cattani F., Fabi A., Giannarelli D., Giovannetti M., Mirri A., Occhipinti E., Telera S., Vidiri A., Pace A.
Metastases to the cerebellum. Results and prognostic factors in a consecutive series of 44 operated patients.
J NEUROONCOL. 2008 JUL;88(3):331-7.

120. Prodosmo A., Giglio S., Moretti S., Mancini F., Barbi F., Avenia N., Di Conza G., Schünemann H.J., Pistola L., Ludovini V., Sacchi A., Pontecorvi A., Puxeddu E., Moretti F.
Analysis of human MDM4 variants in papillary thyroid carcinomas reveals new potential markers of cancer properties.
J MOL MED. 2008 MAY;86(5):585-96.

121. Puca R., Nardinocchi L., Pistritto G., D'Orazi G.
Overexpression of HIPK2 circumvents the blockade of apoptosis in chemoresistant ovarian cancer cells.
GYNECOL ONCOL. 2008 JUN;109(3):403-10.

122. Puca R., Nardinocchi L., Gal H., Rechavi G., Amariglio N., Domany E., Notterman D.A., Scarsella M., Leonetti C., Sacchi A., Blandino G., Givol D., D'Orazi G.
Reversible dysfunction of wild-type p53 following homeodomain-interacting protein kinase-2 knockdown.
CANCER RES. 2008 MAY 15;68(10):3707-14.

123. Puhan M.A., Frey M., Büchi S., Schünemann H.J.
The minimal important difference of the hospital anxiety and depression scale in patients with chronic obstructive pulmonary disease.
HEALTH QUAL LIFE OUTCOMES. 2008 JUL 2;6:46.

124. Puhan M.A., Mador M.J., Held U., Goldstein R., Guyatt G.H., Schünemann H.J.
Interpretation of treatment changes in 6-minute walk distance in patients with COPD.
EUR RESPIR J. 2008 SEP;32(3):637-43.

125. Puhan M.A,. Schünemann H.J., Buesching G., VanOort E., Spaar A., Frey M.
COPD patients' ability to follow exercise influences short-term outcomes of rehabilitation.
EUR RESPIR J. 2008 FEB;31(2):304-10. EPUB 2007 NOV 7.

126. Quick S.K., Shields P.G., Nie J., Platek M.E., McCann S.E., Hutson A.D., Trevisan M., Vito D., Modali R., Lehman T.A., Seddon M., Edge S.B., Marian C., Muti P., Freudenheim J.L.
Effect modification by catalase genotype suggests a role for oxidative stress in the association of hormone replacement therapy with postmenopausal breast cancer risk.
CANCER EPIDEMIOL BIOMARKERS PREV. 2008 MAY;17(5):1082-7.

127. Rinaldo C., Siepi F., Prodosmo A., Soddu S.
HIPKs: Jack of all trades in basic nuclear activities.
BIOCHIM BIOPHYS ACTA. 2008 JUN 18.

128. Rossi A., Bria E., Maione P., Palazzolo G., Falanga M., Gridelli C.
The role of cetuximab and other epidermal growth factor receptor monoclonal antibodies in the treatment of advanced non-small cell lung cancer.
REV RECENT CLIN TRIALS. 2008 SEP;3(3):217-27.

129. Rossi A., Gridelli C., Bria E.
Thalidomide in small-cell lung cancer: is it a tombstone?
J CLIN ONCOL. 2008 JAN 1;26(1):160; AUTHOR REPLY 160.

130. Sant M., Allemani C., De Angelis R., Carbone A., de Sanjosè S., Gianni A.M., Giraldo P., Marchesi F., Marcos-Gragera R., Martos-Jiménez C., Maynadié M., Raphael M, Berrino F; EUROCARE-3 Working Group.
Influence of morphology on survival for non-Hodgkin lymphoma in Europe and the United States.
EUR J CANCER. 2008 MAR;44(4):579-87.

131. Saracino B., Gallucci M., De Carli P., Soriani A., Papalia R., Marzi S., Landoni V., Petrongari M.G., Arcangeli S., Forastiere E., Sentinelli S., Arcangeli G.
Phase I-II study of intraoperative radiation therapy (IORT) after radical prostatectomy for prostate cancer.
INT J RADIAT ONCOL BIOL PHYS. 2008 JUL 15;71(4):1049-56.

132. Schernhammer E.S., Berrino F., Krogh V., Secreto G., Micheli A., Venturelli E., Sieri S., Sempos C.T., Cavalleri A., Schünemann H.J., Strano S., Muti P.
Urinary 6-sulfatoxymelatonin levels and risk of breast cancer in postmenopausal women.
J NATL CANCER INST. 2008 JUN 18;100(12):898-905.

133. Schünemann H.J., Cook D., Guyatt G.; American College of Chest Physicians.
Methodology for antithrombotic and thrombolytic therapy guideline development: American College of Chest Physicians Evidence-based Clinical Practice Guidelines (8th Edition).
CHEST. 2008 JUN;133(6 SUPPL):113S-122S. ERRATUM IN: CHEST. 2008 AUG;134(2):473.

134. Schünemann H.J., Oxman A.D., Brozek J., Glasziou P., Jaeschke R., Vist G.E., Williams J.W. Jr, Kunz R., Craig J., Montori V.M., Bossuyt P., Guyatt G.H.; GRADE Working Group.
Grading quality of evidence and strength of recommendations for diagnostic tests and strategies.
BMJ. 2008 MAY 17;336(7653):1106-10.

135. Schünemann H.J.
COPD dashboard: about official documents, authors, science, health status and BOARDs.
EUR RESPIR J. 2008 FEB;31(2):238-40.

136. Sciahbasi A., Fischetti D., Picciolo A., Patrizi R., Sperduti I., Colonna G., Summaria F., Montinaro A., Lioy E.
Transradial access compared with femoral puncture closure devices in percutaneous coronary procedures.
INT J CARDIOL. 2008 AUG 6.

137. Sibilio L., Martayan A., Setini A., Lo Monaco E., Tremante E., Butler R.H., Giacomini P.
A single bottleneck in HLA-C assembly.
J BIOL CHEM. 2008 JAN 18;283(3):1267-74. EPUB 2007 OCT 23.

138. Sigalotti L., Covre A., Zabierowski S., Himes B., Colizzi F., Natali P.G., Herlyn M., Maio M.
Cancer testis antigens in human melanoma stem cells: expression, distribution, and methylation status.
J CELL PHYSIOL. 2008 MAY;215(2):287-91.

139. Simone G., Guaglianone S., Gallucci M.
Re: Günter Janetschek. Laparoscopic partial nephrectomy for RCC: how can we avoid ischemic damage of the renal parenchyma? Eur Urol 2007;52:1303-5.
EUR UROL. 2008 JUN;53(6):1302-3; AUTHOR REPLY 1303-4. EPUB 2008 JAN 17.

140. Simone G., Leonardo C., Gallucci M.
To the Editor: Re: Agrawal et al.: The Safety and Efficacy of Different Doses of Bacillus Calmette-Guérin in Superficial Bladder Transitional Cell Carcinoma. Urology 2007; 70:1075-1078
UROLOGY. 2008 SEP 6.

141. Simone G., Leonardo C., Papalia R., Guaglianone S., Gallucci M.
Laparoscopic Ureterolysis and Omental Wrapping.
UROLOGY. 2008 JUL 30.

142. Simone G., Papalia R., Gallucci M.
Re: Maurizio A. Brausi, Mirko Gavioli, Giuseppe De Luca et al. Retroperitoneal Lymph Node Dissection (RPLD) in Conjunction with Nephroureterectomy in the Treatment of Infiltrative Transitional Cell Carcinoma (TCC) of the Upper Urinary Tract: Impact on Survival. Eur Urol 2007;52:1414-20.
EUR UROL. 2008 AUG;54(2):467.

143. Simone G., Papalia R., Leonardo C., Gallucci M.
Re: Alexander Tsivian, Shalva Benjamin, A. Ami Sidi. A sealed laparoscopic nephroureterectomy: a new technique. Eur urol 2007;52:1015-9.
EUR UROL. 2008 APR;53(4):861; AUTHOR REPLY 862. EPUB 2007 NOV 19.

144. Sinuff T., Patel R.V., Adhikari N.K., Meade M.O., Schünemann H.J., Cook D.J.
Quality of professional society guidelines and consensus conference statements in critical care.
CRIT CARE MED. 2008 APR;36(4):1049-58.

145. Spugnini E.P., Citro G., D'Avino A., Baldi A.
Potential role of electrochemotherapy for the treatment of soft tissue sarcoma: first insights from preclinical studies in animals.
INT J BIOCHEM CELL BIOL. 2008;40(2):159-63. EPUB 2007 OCT 9.

146. Spugnini E.P., Crispi S., Scarabello A., Caruso G., Citro G., Baldi A.
Piroxicam and intracavitary platinum-based chemotherapy for the treatment of advanced mesothelioma in pets: preliminary observations.
J EXP CLIN CANCER RES. 2008 MAY 19;27(1):6.

147. Spugnini E.P., Dotsinsky I., Mudrov N., Bufalini M., Giannini G., Citro G., Feroce F., Baldi A.
Adjuvant electrochemotherapy for incompletely excised anal sac carcinoma in a dog.
IN VIVO. 2008 JAN-FEB;22(1):47-9.

148. Spugnini E.P., Dotsinsky I., Mudrov N., De Luca A., Codini C., Citro G., D'Avino A., Baldi A.
Successful rescue of an apocrine gland carcinoma metastatic to the cervical lymph nodes by mitoxantrone coupled with trains of permeabilizing electrical pulses (electrochemotherapy).
IN VIVO. 2008 JAN-FEB;22(1):51-3.

149. Spugnini E.P., Pagotto A., Zazzera F., D'Avino A., Caruso G., Citro G., Baldi A.
Cutaneous T-cell lymphoma in an African hedgehog (Atelerix albiventris).
IN VIVO. 2008 JAN-FEB;22(1):43-5.

150. Spugnini E.P., Vincenzi B., Betti G., Cordahi F., Dotsinsky I., Mudrov N., Citro G., Baldi A.
Surgery and electrochemotherapy of a high-grade soft tissue sarcoma in a dog.
VET REC. 2008 FEB 9;162(6):186-8.

151. Spugnini E.P., Vincenzi B., Caruso G., Baldi A., Citro G., Santini D., Tonini G.
Zoledronic acid for the treatment of appendicular osteosarcoma in a dog.
J SMALL ANIM PRACT. 2008 SEP 12.

152. Steelman L.S., Abrams S.L., Whelan J., Bertrand F.E., Ludwig D.E., Bäsecke J., Libra M., Stivala F., Milella M., Tafuri A., Lunghi P., Bonati A., Martelli A.M., McCubrey J.A.
Contributions of the Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways to leukemia.
LEUKEMIA. 2008 APR;22(4):686-707.

153. Stigliano V., Assisi D., Cosimelli M., Palmirotta R., Giannarelli D., Mottolese M., Sanchez Mete L., Mancini R., Casale V.
Survival of hereditary non-polyposis colorectal cancer patients compared with sporadic colorectal cancer patients.
J EXP CLIN CANCER RES. 2008 SEP 19;27(1):39.

154. Strigari L., Benassi M., De Felice P., D'Andrea M., Fazio A., Nocentini S., D'Angelo A., Ceccatelli A.
Comparison of methods to determine accurate dose calibrator activity measurements.
J EXP CLIN CANCER RES. 2008 JUL 1;27:14.

155. Strigari L., Orlandini L.C., Andriani I., D'Angelo A., Stefanacci M., Di Nallo A.M., Benassi M.
A mathematical approach for evaluating the influence of dose heterogeneity on TCP for prostate cancer brachytherapy treatment.
PHYS MED BIOL. 2008 SEP 21;53(18):5045-59.

156. Suriano F., Gallucci M., Flammia G.P., Musco S., Alcini A., Imbalzano G., Dicuonzo G.
Bacteriuria in patients with an orthotopic ileal neobladder: urinary tract infection or asymptomatic bacteriuria?
BJU INT. 2008 JUN;101(12):1576-9. EPUB 2007 DEC 5.

157. Swiglo B.A., Murad M.H., Schünemann H.J., Kunz R., Vigersky R.A., Guyatt G.H., Montori V.M.
A case for clarity, consistency, and helpfulness: state-of-the-art clinical practice guidelines in endocrinology using the grading of recommendations, assessment, development, and evaluation system.
J CLIN ENDOCRINOL METAB. 2008 MAR;93(3):666-73.

158. Telera S., Carapella C., Covello R., Cristalli G., Carosi M.A., Pichi B., Spriano G.
Malignant peripheral nerve sheath tumors of the lateral skull base.
J CRANIOFAC SURG. 2008 MAY;19(3):805-12.

159. Torsello A., Garufi C., Cosimelli M., Diodoro M.G., Zeuli M., Vanni B., Campanella C., D'Angelo C., Sperduti I., Perrone Donnorso R., Cognetti F., Terzoli E., Mottolese M.; Colorectal Disease Management Team, Regina Elena Cancer Institute, Rome, Italy.
P53 and bcl-2 in colorectal cancer arising in patients under 40 years of age: distribution and prognostic relevance.
EUR J CANCER. 2008 JUN;44(9):1217-22.

160. Verdina A., Cardillo I., Nebbioso A., Galati R., Menegozzo S., Altucci L., Sacchi A., Baldi A.
Molecular analysis of the effects of Piroxicam and Cisplatin on mesothelioma cells growth and viability.
J TRANSL MED. 2008 MAY 22;6:27.

161. Yang L., Gaikwad N.W., Meza J., Cavalieri E.L., Muti P., Trock B., Rogan E.G.
Novel biomarkers for risk of prostate cancer: Results from a case-control study.
PROSTATE. 2008 SEP 24.

162. Widesott L., Strigari L., Pressello M.C., Benassi M., Landoni V.
Role of the parameters involved in the plan optimization based on the generalized equivalent uniform dose and radiobiological implications.
PHYS MED BIOL. 2008 MAR 21;53(6):1665-75..

163. Zucchetto A., Talamini R., Dal Maso L., Negri E., Polesel J., Ramazzotti V., Montella M., Canzonieri V., Serraino D., La Vecchia C., Franceschi S.
Reproductive, menstrual, and other hormone-related factors and risk of renal cell cancer.
INT J CANCER. 2008 NOV 1;123(9):2213-6.

Intramural Seminars C.R.S. 2007

13 February — DOMENICO DELIA
DNA damage-induced cell cycle regulation and function of novel chk2 phosphoresidues

21 February — LIVIO TRUSOLINO
Proto-oncogÈni e servo-oncogÈni: líintegrina b4 amplifica i segnali della tirosina-cinasi Met e ne slatentizza la capacità tumorigenica

7 March — GIULIA BON
Ruolo dell'integrina a6b4 nella progressione tumorale: implicazioni per la sopravvivenza cellulare in risposta a terapia ormonale

21 March — LAURA ROSANÒ
Signaling pathways activated by endothelin-1 to induce epithelial to mesenchymal transition in human ovarian carcinoma cells

29 March — CHARLES STREULI
The central role of integrin-mediated adhesion in the development and function of breast epithelium

4 April — LUDOVICA CIUFFREDA
Growth-inhibitory and anti-angiogenic effects of the novel MEK inhibitor PD0325901 in preclinical models of human malignant melanoma

5 April — MAURO PIACENTINI
The novel gene Ambra-1 regulates autophagy during development in mammals

18 April — LUIGI FRANCUCCI
DPC - Dispositivi di Protezione Collettiva - La scelta ed il corretto utilizzo

2 May — LUIGI FRANCUCCI
DPI - Dispositivi di Protezione Individuale - Guida per la scelta ed il corretto utilizzo

16 May — FABIOLA MORETTI
MDM4 è un determinante della risposta apoptotica al danno al DNA e un nuovo potenziale marker di sensibilità alle terapie genotossiche.

30 May — FABIO DI DOMENICO
Modulazione delle funzioni melanosomali in cellule di melanoma l'espressione dell'oncogene E5 di HPV16 ripristina l'attività della tirosinasi

13 June — FEDERICO DE MARCO
Oxidative stress and viral oncogenes implication for cell survival and malignant progression

26 September PATRIZIO GIACOMINI
Conformeri HLA effimeri

10 October ELISA LO MONACO
HLA-E: espressione e funzione in cellule normali e neoplastiche

24 October DANIELA TRISCIUOGLIO
Inhibition of Hypoxia inducible factor-1: an attractive strategy for cancer therapy

7 November TIZIANA BRUNO
Ruolo di che-1 nella risposta al danno al DNA

21 November MARCO PAGGI
L'oncoproteina E7 di HPV-16 interagisce con la glutatione S-transferasi P1-1

Breakfast Meetings 2007

22 February Monitoraggio AIFA dei nuovi farmaci oncologici e appropiatezza prescrittiva: procedure e normativa tra sperimentazione, clinica e farmacovigilanza

8 March Il Governo Clinico nel sistema sanitario

13 March Il trattamento neoadiuvante del cancro della cervice e possibile significato prognostico nella persistenza virale

15 March Diagnosi e trattamento delle menigiti neoplastiche

22 March Terapie con trastuzumab nel tumore della mammella avanzato: risultati clinici e fattori predittivi di resistenza

29 March Correlazione tra imaging clinico funzionale e stadiazione patologica nella patologia metastatica laterocervicale

5 April Progressi nell'individuazione del trattamento medico dei sarcomi dei tessuti molli dell'adulto

12 April Gli esiti dei trattamenti per il Carcinoma Mammario. Aspetti psicologici e riabilitativi

19 April Nuove strategie terapeutiche nelle metastasi vertebrali

3 May La gestione del "Rischio Clinico" nelle strutture sanitarie. Un approccio integrato

7 May Variabili psicosociali, CAD, spirometria nello screening del cancro del polmone con TC spirale low-dose

10 May Neoplasie surrenaliche. Diagnosi e terapia

14 May Efficacia della sorveglianza clinico-radiologica e chirurgia profilattica nel counselling genetico

17 May Carcinomi della mammella "Tripli negativi": una nuova entità clinico-patologica

21 May Nuovi ruoli del controllo ormonale in oncologia

24 May Il trattamento delle metastasi in transit da melanoma degli arti

31 May Lo screening del cancro del polmone con TC spirale

28 June Il linfonodo sentinella nel trattamento del melanoma primitivo a rischio (spessore >1 mm)

25 October	Management del rischio clinico in cardiologia
8 November	Trattamento del carcinoma mammario in età geriatria
15 November	Farmaci a bersaglio molecolare nei tumori del rene
22 November	Il trattamento endoscopico delle stenosi neo-plastiche gastro-duodenali
29 November	Il trattamento integrato del carcinoma dello stomaco localmente avanzato
6 December	Il ruolo della linfoadenectomia mediastinica nel tumore del polmone

Research Projects

Funds (2007/2008)

2007

ITALIAN ASSOCIATION FOR CANCER RESEARCH (A.I.R.C.)

Endothelin a receptor pathways and related factors in ovarian cancer progression: Therapeutic implication
RESPONSABILE: ANNA BAGNATO (€ 60.000,00)

Study of telomere maintenance on melanoma apoptosis and senescence to identify new therapeutic strategies
RESPONSABILE: ANNAMARIA BIROCCIO (€ 80.000,00)

Exploring transcriptional activity f gain of function Mutant p53 proteins
RESPONSABILE: GIOVANNI BLANDINO (€ 90.000,00)

Interplay between bcl-2 and hypoxia: implication for cancer therapy
RESPONSABILE: DONATELLA DEL BUFALO (€ 80.000,00)

Role of alpha6beta4integrin in breast cancer progression
RESPONSABILE: RITA FALCIONI (€ 40.000,00)

Characterization of Che-1 activation in response to DNA damage
RESPONSABILE: MAURIZIO FANCIULLI (€ 60.000,00)

Innovative combination of chronotherapy plus cetuximab and Liver resection in colorectal
RESPONSABILE: CARLO GARUFI (€ 40.000,00)

Preclinical development of MEK inhibition-based therapeutic strategies for acute leukemias
RESPONSABILE: MICHELE MILELLA (€ 50.000,00)

Breast Cancer and Estrogen Metabolism: a Pathway of Carcinogenesis for Human Breast Cancer
RESPONSABILE: PAOLA MUTI (€ 40.000,00)

Dissecting NF-Y activity and its role in cell cycle control and apoptosis
RESPONSABILE: GIULIA PIAGGIO (€ 50.000,00)

Novel mechanisms of tumor suppression in human breast cancer
RESPONSABILE: ORESTE SEGATTO (€ 40.000,00)

HIPK2, a potent inductor of apoptosis: characterization of molecular pathways and role in tumorgenesis
RESPONSABILE: SILVIA SODDU (€ 100.000,00)

P53 family interactions as determinants for tumor responses to anti-neoplastic treatment
RESPONSABILE: SABRINA STRANO (€ 60.000,00)

New insights in prostate cancer: implications for innovative preventive, diagnostic and therapeutic strategies
RESPONSABILI: ANNA MARIA CIANCIULLI (€ 35.000,00)
PAOLO CARLINI (€ 20.000,00)

Linking transcriptome to protome: functional oncogenomics for diagnosis and treatment of human cancers
RESPONSABILE: GIOVANNI BLANDINO (€ 480.000,00)

Blockade of multiple molecular pathways as a strategy to improbe the treatment of ovarian carcinoma
RESPONSABILE: LAURA ROSANÒ (€ 26.000,00)

Identification of novel molecular targets in breast cancer. hMena a new SEREX defined antigen overexpressed in preneoplastic breast lesion, as a model for dissecting the spontaneous immune response in breast cancer and its correlation with clinical outcome
RESPONSABILE: PAOLA NISTICÒ (€ 62.000,00)

ITALIAN INSTITUTE OF HEALTH (I.S.S.)

Network nazionale italiano Tumori Eredo-Familiari (in TEF): creazione di strumenti operativi condivisi per l'assistenza e la ricerca
RESPONSABILE: VITTORIA STIGLIANO (€ 30.000,00)

Rete nazionale telepatologia (TESEO)
RESPONSABILE: RAFFAELE PERRONE DONNORSO (€ 40.000,00)

Rete nazionale delle biobanche per l'oncologia
RESPONSABILE: MARCELLA MOTTOLESE (€ 30.000,00)

Malignant Thymoma: a rare disease representing a clinically unresolved problem. A phenotypical and proteomic analysis with clinical implications
RESPONSABILE: MIRELLA MARINO (€ 90.000,00)

Potenziamento banca sieri ed analisi oncoproteomica nel cancro dell'ovaio. Farmacogenomica Oncologica
RESPONSABILE: GENNARO CITRO (€ 250.000,00)

Rete nazionale sui modelli sperimentali e "Facilities" animali
RESPONSABILE: GENNARO CITRO (€ 105.000,00)

Applicazione della chemioterapia alla rimodulazione della risposta immune antitumorale: studio dei meccanismi e "Proof of concept" nell'uomo
RESPONSABILE: PIER GIORGIO NATALI (€ 130.000,00)

Rete nazionale per gli studi clinici di strutture GMP per le bioterapie dei tumori
RESPONSABILE: PAOLA NISTICÒ (€ 50.339,02)

Applicazione della chemioterapia alla rimodulazione della risposta immune antitumorale: studio dei meccanismi e "Proof of concept" nell'uomo
RESPONSABILE: PAOLA NISTICÒ (€ 560.000,00)

Rete nazionale bioinformatica in oncologia (RNBBIO)
RESPONSABILE: GIULIA PIAGGIO (€ 59.958,86)

Identification of sirma of p53 family members to develop new antineoplastic drugs
RESPONSABILE: ADA SACCHI (€ 110.000,00)

Molecular and functional characterization of the newly identified interaction between the rett syndrome-associated factor Mecp 2 and the pro-apoptotic factor HPK2
RESPONSABILE: SILVIA SODDU (€ 90.000,00)

Le basi metodologiche per una chemioterapia antitumorale mirata: il saggio dell'edr nel carcinoma ovario ed in altre neoplasie
RESPONSABILE: FRANCESCO COGNETTI (€ 100.000,00)

Programma Italia-USA Farmacogenomica Oncologica – Sieroproteomica (borse di studio)
RESPONSABILE: FRANCESCO COGNETTI (€ 60.000,00)

MINISTRY OF HEALTH

Oncologia: Riabilitazione in oncologia: dalla diagnosi alle cure palliative, integrazione tra istituzioni e volontariato nella ricerca dei percorsi adeguati e appropriati (H.O.C.U.R.A.)
RESPONSABILI: ALBERTO PIETRANGELI - ANDREA PACE (€ 100.000,00)

FIRB

Studio del ruolo fisiopatologico dei recettori chemiotattici CXCR1-2 E CXCR4 e dei loro agonisti naturali nello sviluppo e nella progressione di patologie neoplastiche
RESPONSABILE: GABRIELLA ZUPI (€ 144.000,00)

ITALIAN CANCER LEAGUE

Prevenzione primaria e secondaria in soggetti a rischio per cancro del polmone: studio multicentrico
RESPONSABILE: SALVATORE GIUNTA (€ 51.000,00)

Screening clinico e molecolare in soggetti ad alto rischio per cancro del colon retto CCR
RESPONSABILE: VITTORIA STIGLIANO (€ 25.000,00)

Migliorare le abilità comunicative del medico e ridurre il di stress del paziente: uno studio clinico randomizzato
UNITÀ OPERATIVA: ANITA CARUSO (€ 28.500,00)

Nuovi approcci metodologici per l'identificazione di marcatori molecolari di rischio di trasformazione e diagnosi precoce del carcinoma della mammella
RESPONSABILE: PAOLA NISTICÒ (€ 55.000,00)

E.C.

Grading the quality of evidence and strength of recommendation for health care recommendations (GRADE)
RESPONSABILE: HOLGER SCHÜNEMANN (€ 80.000,00)

2008

ITALIAN INSTITUTE OF HEALTH (I.S.S.)

Methods and Advanced Equipment for Simulation and Treatment in Radio-Oncology (MAESTRO)
RESPONSABILE: ANNA DI NALLO (€ 28.000,00)

Farmaci cellulari, vaccini e bioterapie innovative dei tumori
RESPONSABILE: FRANCESCO COGNETTI (€ 60.000,00)

Studio dell'impatto di un programma formativo sugli skills comunicativi dei medici per ridurre il destress dei pazienti
RESPONSABILE: ANITA CARUSO (€ 80.000,00)

Network nazionale italiano tumori eredo-familiari (in TEF): creazione di strumenti operativi condivisi per l'assistenza e la ricerca
RESPONSABILE: VITTORIA STIGLIANO (€ 30.000,00)

Farmaci cellulari, vaccini e bioterapie innovative dei tumori -
Linea ricerca: Nuove strategie terapeutiche: studio clinico di fase I
RESPONSABILE: PIER GIORGIO NATALI (€ 230.000,00)
(NISTICÒ - FERRARESI - CATRICALÀ)

MINISTRY OF HEALTH

Meccanismo d'azione ed efficacia di molecole biologiche e farmaci citotossici di ultima generazione e loro interazione
RESPONSABILE: GABRIELLA ZUPI (€ 1.900.000,00)
(BIROCCIO - BAGNATO - PIAGGIO - MILELLA)

p53 family interaction network as a target of antitumoral peptide therapy
RESPONSABILE: GIOVANNI BLANDINO (CITRO - FELICI) (€ 350.000,00)

Definizione di un modello di percorso riabilitativo multidisciplinare e costituzione di un osservatorio nazionale di riabilitazione oncologica
RESPONSABILI: PATRIZIA PUGLIESE - ANDREA PACE (€ 350.000,00)

Analytical and clinical validation of biomarkers for non-invasive early diagnosis of virus associated cancer
RESPONSABILE: AMINA VOCATURO (€ 245.000,00)
(BENEVOLO - MARIANI - DE MARCO - VENUTI)

Tumor radioresistance and predictive markers of the response to radiotherapy
RESPONSABILE: MAURIZIO FANCIULLI (€ 46.000,00)

Tumor radioresistance and predictive markers of the response to radiotherapy
RESPONSABILE: SILVIA SODDU (€ 46.000,00)

Antivascular therapy in melanoma: from preclinical to clinical studies
RESPONSABILE: DONATELLA DEL BUFALO (€ 113.400,00)

Detection of the oncologic disease at early stage
RESPONSABILI: RITA FALCIONI - SILVIA SODDU (€ 60.000,00)

Centrosomes and centrosome-associated regulatory proteins in menome maintenance and in the DNA damage response
RESPONSABILE: SILVIA SODDU (€ 400.000,00) (FANCIULLI - PAGGI - NISTICÒ)

Melanoma and integrated chip technologies: identification of novel prognosticators and (immuno)therapeutic protocols
RESPONSABILE: PATRIZIO GIACOMINI (€ 500.000,00) (DI FILIPPO - BIROCCIO)

Progetto oncologico di medicina molecolare: i tumori femminili
RESPONSABILE: PAOLA MUTI (€ 1.000.000,00)

Effetto della Metformina, un agent insulino-sensibilizzante, nella prevenzione primaria dei tumori alla mammella: il trial randomizzato e controllato PLOTINA
RESPONSABILE: PAOLA MUTI (€ 2.000.000,00)

ITALIAN CANCER LEAGUE

Definizione e validazione di biomarcatori per la chemioprevenzione e la diagnosi precoce del carcinoma mammario e della cervice uterina
RESPONSABILE: CECILIA NISTICÒ (€ 110.000,00)

FIRB

Identificazione di antitumorali innovativi: dalla genomica alla terapia
RESPONSABILE: GENNARO CITRO (€ 144.900,00)

ITALIAN CANCER ALLIANCE

Nuove molecole e peptidi quali farmaci regolatori del ciclo cellulare e della risposta a chemioterapici nei tumori epiteliali e cutanei
RESPONSABILE: GIOVANNI BLANDINO (€ 90.000,00)

Piattaforme per la produzione bioindustriale di anticorpi ricombinati anti erbB-2 per uso oncologico (ErbAric)
RESPONSABILE: PATRIZIO GIACOMINI (€ 100.000,00)

OTHER PROJECTS

National Institutes of Health - Endogenous hormones and premenopausal breast cancer risk
RESPONSABILE: PAOLA MUTI (€ 378.839,00)

Food intake and quality of life-developing a quality of life instrument related to food intake
RESPONSABILE: HOLGER SCHÜNEMANN (€ 105.759,60)

Combined ZD4054 and gefitinib treatment represents a novel strategy for overcoming resistance to EGFR inhibitors in ovarian cancer
RESPONSABILE: ANNA BAGNATO (€ 52.000,00)

Centro per la Biotecnologia Molecolare Franhofer USA - Vaccini terapeutici contro i papillomavirus umani HPV
RESPONSABILE: ALDO VENUTI ($ 50.000,00)

Accordo con Pharminox per esecuzione studi di biologia, farmacologia e farmacocinetica di prodotti oncologici
RESPONSABILI: CARLO LEONETTI - ANNA BIROCCIO (€ 21.700,00)

Protocols

STATE A = OPEN
C = CLOSED
AP = APPROVED BY ETHICAL COMMITTEE

PATHOLOGY	STATE	STUDY TITLE	RESPONSIBLE	DIVISION
Colon	C	Trial clinico di fase II: trattamento delle metastasi epatiche da carcinoma colo-rettale, non responsive alla chemioterapia endovenosa standard, mediante la somministrazione intra-arteriosa epatica di SIR-Spheres	COSIMELLI	CHB
	A	Ruolo di Erbitux più una combinazione di CPT-11/5-fluorouracile/leucovorin/oxaliplatino come chemioterapia neoadiuvante in pazienti con metastasi epatiche da cancro del colon-retto	GARUFI	OMC
	A	Studio di fase II/III, in doppio cieco, ranodmizzato, multicentrico per confrontare l'efficacia di AZD2171 in combinazione con 5-fluorouracile, leucovorina e oxaliplatino (FOLFOX) e l'efficacia di bevacizumab in combinazione con FOLFOX in pazienti affetti da cancro del colon-retto metastatico non trattato precedentemente	GARUFI	OMC
	A	Uso del Pregabalin nella prevenzione e nel trattamento della neuropatia periferica in pazienti affetti da carcinoma del colon-retto e candidati a chemioterapia adiuvante: studio clinico randomizzato di fase III (placebo-controlled)	GARUFI	OMC
	A	Trattamento adiuvante nella neoplasia completamente resecata del colon in stadio 3 volto a confrontare folfox4 versus folfox4 plus cetuximab	LOPEZ	OMB
	A	Ruolo di Erbitux più una combinazione di CPT-11/5-fluorouracile/leucovorin/oxaliplatino come chemioterapia neoadiuvante in pazienti con metastasi epatiche da cancro del colon-retto	PAOLETTI	OMB
	A	Valutazione del rischio del cancro del colon in pazienti operate per cancro della mammella	STIGLIANO	Gastro
	A	Consulenza genetica gastroenterologica per i tumori familiari ereditari del colon	STIGLIANO	Gastro
	A	Studio di fase II/III, in doppio cieco, ranodmizzato, multicentrico per confrontare l'efficacia di AZD2171 in combinazione con 5-fluorouracile, leucovorina e oxaliplatino (FOLFOX) e l'efficacia di bevacizumab in combinazione con FOLFOX in pazienti affetti da cancro del colon-retto metastatico non trattato precedentemente	ZEULI	OMA
	C	Studio clinico in aperto, multicentrico, a braccio singolo, per determinare la sicurezza di una terapia continuata con ABX-EGF in soggetti con tumore del colon metastatico	ZEULI	OMA
	A	Uno studio di Fase III randomizzato e in aperto per valutare l'efficacia e la sicurezza di bevacizumab in combinazione con capecitabina come trattamento di prima linea in pazienti anziani con cancro colorettale metastatico	ZEULI	OMA
	A	Ruolo di Erbitux più una combinazione di CPT-11/5-fluorouracile/leucovorin/oxaliplatino come chemioterapia neoadiuvante in pazienti con metastasi epatiche da cancro del colon-retto	ZEULI	OMA
Cordoma				
	A	Programma di uso terapeutico di medicinale sottoposto a sperimentazione clinica: Imatinib mesilato (Glivec) nei cordomi in fase avanzata	FERRARESI	OMA
Endometrio				
	A	Valutazione degli indici di efficienza di test diagnostici in popolazioni a rischio per il carcinoma dell'endometrio	VOCATURO	Gine
Fegato				
	C	Studio multicentrico sulla prevenzione primaria e secondaria dell'epatocarcinoma (HCC). Analisi su di un campione di popolazione a rischio	ETTORRE	CHB
	C	Protocollo per la selezione, per il trattamento immunosoppressivo e antiretrovirale ed il monitoraggio post-trapianto. Trapianto di fegato nei soggetti con infezione da HIV: valutazione osservazionale di fattibilità	SANTORO/ VENNARECCI	CHB
	AP	Studio randomizzato, multicentrico, di fase II, a gruppi paralleli di vandetanib in monoterapia o vandetanib associato a gemcitabina verso placebo piu' gemcitabina in soggetti con cancro avanzato delle vie biliari (cancro della colecisti, cancro del dotto biliare extraepatico, colangiocarcinoma intraepatico e carcinoma ampollare)	TERZOLI	OMC
Gastro				
	A	Studio clinico di Fase III con Celecoxib controllato con placebo in soggetti genotipo-positivi per poliposi adenomatosa familiare	STIGLIANO	Gastro
GIST				
	A	Registro osservazionale globale di raccolta dei dati longitudinali di pazienti con GIST in stadio avanzato (Gold reGISTry)	AMODIO	OMB
	C	Tumori stromali gastrointestinali (GIST) con espressione del recettore KIT, a rischio intermedio-elevato, in fase localizzata, resecati in maniera completa: studio controllato randomizzato su Imatinib mesilato (Glivec) a scopo adiuvante versus nessuna terapia ulteriore dopo chirurgia completa	CARPANO	OMB

PATHOLOGY	STATE	STUDY TITLE	RESPONSIBLE	DIVISION
GIST	C	I tumori gastroenterici (GIST): studio retrospettivo di incidenza e di sopravvivenza	COVELLO	AnPat
	A	Registro osservazionale globale di raccolta dei dati longitudinali di pazienti con GIST in stadio avanzato (Gold reGISTry)	FERRARESI	OMA
	A	Studio in aperto, controllato, randomizzato, di fase IIIB, del sunitinib malato (SUTENT) 37.5mg giornalieri vs imatinib mesilato 800mg giornalieri nel trattamento di pazienti con Tumori Stromali Gastrointestinali (GIST) che hanno avuto progressione di malattia durante la terapia con imatinib 400mg giornalieri	FERRARESI	OMA
	C	Tumori stromali gastrointestinali (GIST) con espressione del recettore KIT, a rischio intermedio-elevato, in fase localizzata, resecati in maniera completa: studio controllato randomizzato su Imatinib mesilato (Glivec) a scopo adiuvante versus nessuna terapia ulteriore dopo chirurgia completa	FERRARESI	OMA
HPV				
	A	Rischio di carcinoma squamoso cutaneo associato alla presenza di HPV	VENUTI	CRS
Leucemia				
	C	Programma di uso compassionevole Dasatinib BMS-354825 somministrato in pazienti con leucemia mieloide cronica o leucemia linfoblastica acuta cromosoma Philadelphia positiva, che risultano resistenti o intolleranti ad Imatinib mesilato	PETTI	Emat
	A	Studio pilota di fase II per la valutazione di una strategia terapeutica diversificata sulla base del profilo biologico in pazienti con Leucemia Linfatica Cronica (LLC) in stadio avanzato e/o progressiva di età = 60 anni	PETTI	Emat
	A	Studio randomizzato di fase III che compara il triossido di arsenico (ATO) associato a ATRA verso chemioterapia a base di antracicline (AIDA) in pazienti all'esordio e non ad alto rischio affetti da leucemia promielocitica	PETTI	Emat
	A	Studio esplorativo di fase II sul trattamento intermittente con Imatinib (IM) in pazienti anziani con leucemia mieloide cronica (CML) che hanno ottenuto una risposta citogenetica completa (CCgR) con dosi standard di Imatinib	PISANI	Emat
	C	Il valore delle alte dosi standard di ARA-C durante l'induzione e dell'IL-2 dopo consolidamento intensivo/trapianto autologo di cellule staminali in pazienti (età 15-60 anni) con leucemia mieloide acuta	ROMANO	Emat
	C	Gemtuzumab ozogamicin (GO) in associazione con chemioterapia intensiva standard verso solo chemioterapia intensiva standard come induzione/consolidamento per pazienti di età 64-75 anni addetti da leucemia mieloide acuta all'esordio: studio randomizzato di fase III (AML17) dell'EORTC-LG	ROMANO	Emat

PATHOLOGY	STATE	STUDY TITLE	RESPONSIBLE	DIVISION
Leucemia	A	Confronto tra la sola terapia con Gemtuzumab Ozogamicin (GO) e la terapia di supporto standard in pazienti anziani affetti da Leucemia Mieloide Acuta non eleggibili per chemioterapia intensive: studio randomizzato di fase II/III dell'EORTC-LG e il GIMEMA ALWP	ROMANO	Emat
	C	Studio randomizzato di fase II con Infliximab (Remicade) in pazienti con mielodisplasia a basso rischio di evoluzione leucemica	SPADEA	Emat
	A	Intensificazione della terapia post-remissoriale nella leucemia acuta linfoide dell'adulto ad alto rischio di recidiva e monitoraggio della malattia minima residua	SPADEA	Emat
	A	GLIVEC (imatinib mesilato già noto come STI 571) nel trattamento della Leucemia Linfoide Acuta cromosoma Philadephia e/o BCR/ABL positiva	SPADEA	Emat
Linfoma				
	C	Terapia con ciclofosfamide-fludarabina-rituximab verso il miglior trattamento convenzionale in pazienti con Linfoma non-Hodgkin follicolare ricaduti: valutazione della risposta clinica e della predittività dei test di chemio-sensibilità	PETTI	Emat
	C	Studio prospettico randomizzato nei linfonomi non-Hodgkin (LNH) aggressivi - NHLCSG: 1) VACOP-B e high dose sequential therapy (HDS) nei LNH in stadio avanzato; 2) anti-CD 20 nei linfonomi b/CD 20 + in prima remissione completa	PETTI	Emat
	A	Studio di fase II, in aperto, prospettico e multicentrico per valutare l'efficacia e la sicurezza del trattamento con Zevalin (Ibritumomab Tiuxetan) in pazienti anziani affetti da linfoma diffuso a grandi cellule B all'esordio dopo 4 cicli di CHOP21-Rituximab (CHOP21-R)	PETTI	Emat
	A	Studio I.I.L. di fase III, multicentrico, randomizzato a tre bracci (R-CVP vs R-CHOP vs RFM), per il trattamento di prima linea di pazienti con Linfoma Follicolare in stadio II-IV	PETTI	Emat
	A	Studio randomizzato di fase III, multicentrico per il trattamento di pazienti giovani con Linfoma Diffuso a Grandi Cellule B a prognosi sfavorevole (IPI 2-3). Chemioterapia dose-dense + Rituximab +/- chemioimmunoterapia intensiva e ad alte dosi con supporto di cellule staminali periferiche autologhe	PETTI	Emat
	A	Studio di fase II per il trattamento di pazienti affetti da linfoma mantellare con lo schema R-HyperCVAD	PETTI	Emat
	AP	Studio collaborativo H10 EORTC/GELA sul trattamento adattato in base al risultato della valutazione precoce tramite PET verso un trattamento standard combinato di chemioterapia e radioterapia in pazienti con linfoma di Hodgkin in stadio I/II sopradiaframmatico	PETTI	Emat

PATHOLOGY	STATE	STUDY TITLE	RESPONSIBLE	DIVISION
Mammella	C	Prevenzione della menopausa chemio-indotta attraverso soppressione ovarica temporanea con Triptorelin verso controllo in pazienti giovani affette da carcinoma della mammella. Studio multicentrico randomizzato di fase III	CARLINI	OMA
	A	Studio randomizzato di fase II per valutare l'efficacia, la sicurezza e la tollerabilità del farmaco ZK 230211 a due dosaggi (100 verso 25 mg) come seconda linea di terapia ormonale nelle donne in post-menopausa con carcinoma metastatico della mammella e positività ai recettori ormonali	CARLINI	OMA
	A	Studio sulla durata del trattamento con Letrozolo come terapia adiuvante per le donne in post-menopausa con carcinoma della mammella: trattamento lungo verso trattamento breve	CARLINI	OMA
	A	Terapia adiuvante con Letrozolo dopo Tamoxifene. Studio di correlazione tra il gene CYP19 e l'efficacia di Letrozolo in pazienti in postmenopausa con tumore della mammella	CARLINI	OMA
	A	Studio randomizzato di fase III su Sunitinib in associazione con Capecitabina rispetto a Capecitabina in pazienti affette da carcinoma mammario precedentemente trattato	COGNETTI	OMA
	A	Studio di fase III di confronto tra anastrazolo, letrozolo ed exemestane e tra strategia sequenziale (2 anni di terapia con tamoxifen seguiti da 3 anni di terapia con inibitori delle aromatasi) verso strategia up-front (5 anni di terapia con inibitori delle aromatasi) nel trattamento adiuvante del carcinoma mammario ormono-responsivo	COGNETTI	OMA
	A	Studio di fase II sul dosaggio ripetuto dell'anti-HER-2/neu monoclonale x anticorpo anti-CD3 ertumaxomab in pazienti con HER-2/neu 1+ o 2+/FISH negativo che evidenziano cancro alla mammella avanzato o metastatico (fase IIIb/IV) in progressione dopo trattamento endocrino	COGNETTI	OMA
	A	Studio prospettico osservazionale del tumore della mammella in stadio T4, infiammatorio e non (R.I.T.MA 4)	COGNETTI	OMA
	AP	Trattamento adiuvante con herceptin per 3 mesi verso 12 mesi, in associazione con 2 differenti regimi di chemioterapia, nella pazienti con carcinoma mammario HER2 positive	COGNETTI	OMA
	AP	Studio randomizzato con disegno fattoriale che confronta Fulvestrant ± Lapatinib ± Inibitori dell'Aromatasi in pazienti con carcinoma della mammella in progressione dopo terapia con Inibitori dell'Aromatasi	COGNETTI	OMA
	AP	Studio di fattibilità con FEC intensificato con supporto di G-CSF seguito da Ixabepilone intensificato con supporto di G-CSF come chemioterapia neoadiuvante nel carcinoma mammario con recettore per l'estrogeno assente	COGNETTI	OMA

PATHOLOGY	STATE	STUDY TITLE	RESPONSIBLE	DIVISION
Mammella	A	Studio clinico randomizzato di fase III di terapia sequenziale con Epidoxorubicina e Ciclofosfamide (EC) seguito da Docetaxel versus la combinazione 5-Fluorouracile, Epidoxorubicina e Ciclofosfamide (FEC) come trattamento adiuvante nelle pazienti con carcinoma della mammella con lindonodi negativi	DI LAURO	OMB
	C	SPARTACO (Survey on PAln, Renal funcTionality And treatment options in metastatiC breast tumOr)	FABI	OMA
	A	Studio di fase III, randomizzato, multicentrico, in aperto con lapatinib in neo-adiuvante, trastuzumab e le loro combinazioni più paclitaxel, in donne con carcinoma mammario primario HER2/ErbB2 positivo	FABI	OMA
	C	Studio in doppio cieco per confrontare la efficacia di Palonosetron associato o meno a Desametasone nei giorni 2 e 3, nella prevenzione di nausea e vomito indotti da terapia Chemioterapia moderatamente Emetogena (MEC) somministrata a pazienti femmine con tumore alla mammella	FABI	OMA
	A	Gemcitabina in infusione costante prolungata in combinazione con paclitaxel nel carcinoma mammario metastatizzato pretrattato con antracicline	FABI	OMA
	A	Studio di fase II, non randomizzato, in aperto, multicentrico per la valutazione della doxorubicina pegilata liposomiale (Caelix) in associazione a Trastuzumab (Herceptin) e Docetaxel (Taxotere) come prima linea di terapia nel trattamento del carcinoma mammario in stadio metastatico	FABI	OMA
	A	Studio randomizzato di fase III gemcitabina e docetaxel versus gemcitabina e paclitaxel in pazienti con carcinoma mammario metastatico: confronto di due differenti schedule	FABI	OMA
	C	Studio in aperto con bevacizumab (Avastin) in combinazione con un tassano, somministrato in monoterapia o in combinazione, per il trattamento di prima linea di pazienti con carcinoma mammario metastatico o con ricaduta loco-regionale	FABI	OMA
	A	Studio Randomizzato di Fase II con Ixabepilone più Trastuzumab verso Docetaxel più Trastuzumab in Donne affette da Tumore della Mammella Her2+ Localmente Avanzato e/o Metastatico	FABI	OMA
	A	Studio randomizzato di fase II in doppio cieco: Fulvestrant-Enzastaurin verso Fulvestrant-Placebo, in pazienti affette da carcinoma mammario metastatico resistente a trattamento con Inibitori delle Aromatasi	FABI	OMA
	A	Studio di farmacocinetica del Trastuzumab (Herceptin®) in pazienti con neoplasia della mammella	FABI	OMA

PATHOLOGY	STATE	STUDY TITLE	RESPONSIBLE	DIVISION
Mammella	A	Doxorubicina Liposomiale Peghilata nel trattamento delle metastasi cerebrali da neoplasia della mammella: studio di dose-finding, farmacocinetica ed efficacia terapeutica	FABI	OMA
	A	Studio osservazionale GHEA - Gruppo Herceptin in Adiuvante	FABI	OMA
	A	Docetaxel e gemcitabina verso docetaxel e capecitabina nel trattamento di I linea del carcinoma mammario metastatizzato. Studio multicentrico randomizzato di fase II (GOIM 2305)	LOPEZ	OMB
	A	Epirubicina e vinorelbina verso doxorubicina liposomiale pegilata e vinorelbina nel trattamento di I linea del carcinoma mammario metastatizzato. Studio multicentrico randomizzato di fase II (GOIM 2304)	LOPEZ	OMB
	A	Studio di fase III randomizzato con Docetaxel in associazione a Sunitinib versus Docetaxel nella prima linea di pazienti con carcinoma mammario in fase avanzata	LOPEZ	OMB
	AP	FACT: Anastrozolo in monoterapia verso blocco estrogenico completo con Anastrozolo e Fulvestrant in combinazione; studio di fase III, randomizzato, in aperto, multicentrico, in donne in post-menopausa con tumore della mammella, con recettori ormonali positivi, in prima recidiva successiva al trattamento primario per il tumore localizzato	LOPEZ	OMB
	A	Studio di fase III di vinflunina in associazione a gemcitabina verso paclitaxel in associazione e gemcitabina in pazienti affette da carcinoma della mammella localmente ricorrente o metastatico, non operabile, dopo una chemioterapia adiuvante a base di antracicline	LOPEZ	OMB
	A	Studio prospettico osservazionale del tumore della mammella in stadio T4, infiammatorio e non (R.I.T.MA 4)	LOPEZ	OMB
	C	Studio esplorativo di fase II con Capecitabina e Celecoxib in pazienti affetti da neoplasie avanzate del pancreas/vie biliari (CapCel-01), del distretto cervico-facciale (CapCel-02), del colon-retto (CapCel-03) e della mammella (CapCel-04).	MILELLA	OMA
	C	Studio di fase II con SKI-606 in soggetti con tumore avanzato o metastatico della mammella	MILELLA	OMA
	A	Glucomad: Metabolismo del Glucosio e Densità Mammografica. Implicazioni Eziologiche della Ralazione in Studio nel Cancro della mammella	MUTI	EPI
	A	Protocollo per uno studio pilota per la valutazione dell'impatto della metformina sul testosterone sierico in donne in stato menopausale	MUTI	EPI
	C	La proteomica nella ricerca clinica: definizione e caratterizzazione dei fattori determinanti differenti profili proteomici in individui sani ed in modelli animali	MUTI/BARBA	EPI

PATHOLOGY	STATE	STUDY TITLE	RESPONSIBLE	DIVISION
Mammella	C	Prevenzione della menopausa chemio-indotta attraverso soppressione ovarica temporanea con Triptorelin verso controllo in pazienti giovani affette da carcinoma della mammella. Studio multicentrico randomizzato di fase III	NISTICÒ	OMC
	A	Studio clinico randomizzato di fase III di terapia sequenziale con Epidoxorubicina e Ciclofosfamide (EC) seguito da Docetaxel versus la combinazione 5-Fluorouracile, Epidoxorubicina e Ciclofosfamide (FEC) come trattamento adiuvante nelle pazienti con carcinoma della mammella con lindonodi negativi	NISTICÒ	OMC
	A	Studio randomizzato di fase II, con valutazione dei biomarcatori in neoadiuvante, del trattamento sequenziale AC seguito Ixabepilone in rapporto al trattamento AC seguito da Paclitaxel in donne affette da tumore della mammella in stadio precoce, con negatività di HER2 e dei recettori per l'estrogeno	NISTICÒ	OMC
	A	Nuovi approcci metodologici per l'identificazione di marcatori molecolari di rischio di trasformazione e diagnosi precoce nel carcinoma della mammella	NISTICÒ	CRS
	A	Studio sulla durata del trattamento con Letrozolo come terapia adiuvante per le donne in postmenopausa con carcinoma della mammella: trattamento lungo verso trattamento breve	NISTICÒ	OMC
	A	Terapia adiuvante con Letrozolo dopo Tamoxifene. Studio di correlazione tra il gene CYP19 e l'efficacia di Letrozolo in pazienti in postmenopausa con tumore della mammella	NISTICÒ	OMC
	A	Studio clinico randomizzato di fase III di terapia sequenziale con Epidoxorubicina e Ciclofosfamide (EC) seguito da Docetaxel versus la combinazione 5-Fluorouracile, Epidoxorubicina e Ciclofosfamide (FEC) come trattamento adiuvante nelle pazienti con carcinoma della mammella con lindonodi negativi	PAPALDO	OMA
	A	Uno studio osservazionale sugli eventi cardiaci in pazienti con carcinoma mammario in fase precoce HER2 positivo trattate con herceptin	PAPALDO	OMA
	A	Studio in aperto, di accesso allargato, con lapatinib e capecitabina nel trattamento del carcinoma mammario localmente avanzato o metastatico con iperespressione di ErbB2	PAPALDO	OMA
	A	Studio di fase I-II e farmacocinetica con Vinorelbina orale a basse dosi continuative in pazienti con tumore della mammella in fase avanzata	PAPALDO	OMA
	A	BEATRICE: Studio multicentrico, internazionale, in aperto, a due bracci, di fase III sul trattamento coadiuvante con bevacizumab in carcinoma mammario triplo negativo	PAPALDO	OMA

PATHOLOGY	STATE	STUDY TITLE	RESPONSIBLE	DIVISION
Mammella	A	Studio di fase II di comparazione tra dose standard e dose da carico di Fulvestrant in pazienti in postmenopausa con carcinoma mammario avanzato ormonoresponsivo dopo una prima linea di trattamento	PAPALDO	OMA
	A	Qualità di vita e linfedema nelle pazienti con neoplasia mammaria operata. Utilità di un intervento integrato medico-psicologo	PIETRANGELI/ PUGLIESE	Neuro
	A	Studio prospettico e randomizzato di confronto tra quadrantectomia seguita da radioterapia esterna complementare e quadrantectomia associata a radioterapia intraoperatoria o a irradiazione parziale della mammella dall'esterno in un'unica frazione in pazienti affette da carcinoma mammario di piccole dimensioni e di età > a 48 anni in postmenopausa	PINNARÒ	Radio
	A	Studio di fattibilità di un regime di radioterapia ipofrazionata nelle pazienti sottoposte a chirurgia conservativa per cancro della mammella	PINNARÒ	Radio
	A	Studio di fattibilità di una radioterapia complementare di quadrante somministrata in un'unica frazione in pazienti sottoposte a chirurgia conservativa per carcinoma della mammella I-II stadio	PINNARÒ	Radio
	A	Consulenza integrata oncologica-genetica-psicooncologica (counselling genetico) nelle neoplasie mammarie ad incidenza familiare . Programma di sorveglianza per la identificazione e prevenzione dei tumori mammari in soggetti ad alto rischio genetico	SAVARESE	OMA
	A	Studio GHERB: Glicemia a digiuno come Marker predittivo dell' espressione di HER2/neu e del Tempo di Sviluppo di resistenza al Trastuzumab, in pazienti affetti da Cancro della Mammella	SCHÜNEMANN	EPI
	C	Studio in aperto con bevacizumab (Avastin) in combinazione con un tassano, somministrato in monoterapia o in combinazione, per il trattamento di prima linea di pazienti con carcinoma mammario metastatico o con ricaduta loco-regionale	TERZOLI	OMC
	A	BEATRICE: Studio multicentrico, internazionale, in aperto, a due bracci, di fase III sul trattamento coadiuvante con bevacizumab in carcinoma mammario triplo negativo	TERZOLI	OMC
	A	Studio prospettico osservazionale del tumore della mammella in stadio T4, infiammatorio e non (R.I.T.MA 4)	TERZOLI	OMC
	AP	Studio randomizzato con disegno fattoriale che confronta Fulvestrant ± Lapatinib ± Inibitori dell'Aromatasi in pazienti con carcinoma della mammella in progressione dopo terapia con Inibitori dell'Aromatasi	TERZOLI	OMC

PATHOLOGY	STATE	STUDY TITLE	RESPONSIBLE	DIVISION
Mammella	A	Gemcitabina in infusione costante prolungata in combinazione con paclitaxel nel carcinoma mammario metastatizzato pretrattato con antracicline	VICI	OMB
Melanoma				
	C	Post-operative adjuvant ganglioside GM2-KLH/QS-21 vaccinacion treatment vs observation after resection of primary cutaneous melanoma (AJCC Stage II, T3-T4N0M0)	FERRARESI	OMA
	C	Studio multicentrico randomizzato in doppio cieco a due bracci di Fase III su pazienti affetti da melanoma non trattato di stadio III (non operabile) o IV, trattati con dacarbazina più 10mg/kg di ipilimumab (MDX-010) verso dacarbazina con placebo	FERRARESI	OMA
	C	Uno studio di fase 3, aperto, randomizzato, comparato su Ticilimumab e Dacarbazina o Temozolomide in pazienti con melanoma in stadio avanzato	FERRARESI	OMA
Mesotelioma				
	A	Studio di fase II neoadiuvante di ALIMTA più Cisplatino seguiti da chirurgia e radiazione nel trattamento del mesotelioma pleurico	FACCIOLO	CHTor
Mieloma multiplo				
	C	Studio multicentrico, randomizzato, in doppio cieco con denosumab in confronto all'acido zoledronico (Zometa) nel trattamento delle metastasi ossee in soggetti affetti da carcinoma avanzato (esclusi il carcinoma mammario e prostatico) o mieloma multiplo	PETTI	Emat
	C	Programma multicentrico di accesso allargato (Expanded Access Programm - EAP) a lenalidomide in combinazione con desametasone in pazienti con diagnosi di mieloma multiplo precedentemente trattato	PETTI	Emat
	A	Studio multicentrico, in aperto di Bortezomib, Talidomide e Desametasone come terapia di consolidamento in pazienti affetti da mieloma multiplo di nuova diagnosi, che abbiano raggiunto almeno una buona remissione parziale di malattia dopo trapianto autologo	PISANI	Emat
	C	Studio di fase II di Melphalan 100 mg/m2 (mel 100) come trapianto, Revlimid e Prednisone (RP) come terapia di consolidamento e Revlimid da solo come mantenimento in pazienti anziani con nuova diagnosi di mieloma multiplo	PISANI	Emat
	C	Studio prospettico randomizzato di fase III con talidomide + desametasone vs velcade + talidomide + desametasone per pazienti con mieloma multiplo di nuova diagnosi candidati a ricevere un doppio trapianto autologo di cellule staminali	PISANI	Emat

PATHOLOGY	STATE	STUDY TITLE	RESPONSIBLE	DIVISION
Mieloma multiplo	C	Studio multicentrico, di fase II di Bortezomib, Doxorubicina Liposomiale Pegihilata, Desametasone (PAD) come terapia di induzione, Melphalan 100 mg/m2 (MEL100) come trapianto, in pazienti anziani con nuova diagnosi di mieloma multiplo	PISANI	Emat
	A	Studio di fase III, multicentrico, randomizzato, in aperto di Velcade, Melphalan, Prednisone e Talidomide (V-MPT) versus Velcade, Melphalan, Prednisone (V-MP) in pazienti anziani con mieloma multiplo di nuova diagnosi	PISANI	Emat
	A	Studio multicentrico, controllato, randomizzato di fase III per determinare l'efficacia e la sicurezza dell'associazione di Lenalidomide + Melphan + Prednisone (MPR) vs Melphan (200mg/mq) e trapianto autologo di cellule staminali in pazienti affetti da mieloma multiplo di nuova diagnosi	PISANI	Emat
	AP	Studio di Fase III, randomizzato, in aperto, a 3 bracci, per valutare l'efficacia e la sicurezza di Lenalidomide (Revlimid) più desametazone a basse dosi somministrati fino a progressione di malattia o per 18 cicli di quattro settimane verso l'associazione di melfalan, prednisone e talidomide somministrati per 12 cicli di sei settimane in pazienti affetti da mieloma multiplo non precedentemente trattato di età pari o superiore a 65 anni o che non siano candidati al trapianto di cellule staminali (IFM 07-01)	PISANI	Emat
Morbo Celiaco				
	A	Studio multidisciplinare sull'associazione tra Morbo Celiaco e Malattie autoimmuni endocrinologiche e dermatologiche	STIGLIANO	Gastro
Neuroendocrino NET				
	A	Progetto NET Management: Protocollo epidemiologicoretrospettivo sui tumori neuroendocrini del torace e del tratto gastro-entero-pancreatico. Protocollo epidemiologico retrospettivo sui tumori neuroendocrini non funzionanti	APPETECCHIA	Endo
	A	Studio aperto multicentrico sulla efficacia diagnostica dell'111In pentetreotide nella visualizzazione dei tumori GEP non funzionanti	SCIUTO	Med.Nucl
Ovaio				
	A	Confronto tra chemioterapia standard e chemioterapia Estreme Drug Resistance-test selezionata dopo chirurgia citoriduttiva di prima istanza nel carcinoma ovarico avanzato: studio randomizzato di fase III	FERRETTI	OMA
	C	Carboplatino/Paclitaxel vs Carboplatino/doxorubicina liposomiale stealth in pazienti con carcinoma ovarico: studio multicentrico randomizzato	SAVARESE	OMA
	C	Studio Randomizzato di Fase III di comparazione fra Gemcitabina, Topotecan e Doxorubicina Liposomiale nel trattamento del carcinoma ovarico recidivante	SAVARESE	OMA

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Ovaio	A	Studio di efficacia multicentrico, randomizzato, in doppio cieco, di fase III, con comparazione del fenoxodiol (in forma di dosaggio orale) in combinazione con il carboplatino, rispetto al carboplatino con placebo, in pazienti affette da carcinoma epiteliale dell'ovaio, tubarico o peritoneale primario in stadio avanzato platino-resistente o platino refrattario, dopo almeno una terapia di seconda linea a base di platino	SAVARESE	OMA
Pancreas				
	C	Gemcitabina vs gemcitabina + cisplatino nel trattamento del carcinoma del pancreas avanzato	CARLINI	OMA
	A	Infusione Prolungata di Gemcitabina (Dose-Rate Fisso: 10 mg/m2/min) nei Tumori Pancreatici e delle Vie Biliari Localmente Avanzati (Inoperabili) e/o Metastatici: Studio Osservazionale.	MILELLA	OMA
	A	Studio multicentrico di fase II con chemioterapia (Gemcitabine + Oxaliplatino) seguita da Gemcitabine + RT nei Carcinomi del pancreas localmente avanzati, non resecabili chirurgicamente	MILELLA	OMA
	C	Studio esplorativo di fase II con Capecitabina e Celecoxib in pazienti affetti da neoplasie avanzate del pancreas/vie biliari (CapCel-01), del distretto cervico-facciale (CapCel-02), del colon-retto (CapCel-03) e della mammella (CapCel-04).	MILELLA	OMA
	A	Studio multicentrico di fase II sulla combinazione di Erlotinib (Tarceva®) e Gemcitabina (Gemzar®) infusa a "dose-rate" costante come trattamento di I linea in pazienti affetti da carcinoma pancreatico avanzato non resecabile	MILELLA	OMA
	C	Gemcitabina vs gemcitabina + cisplatino nel trattamento del carcinoma del pancreeas avanzato	PAOLETTI	OMB
	A	Studio multicentrico di fase II sulla combinazione di Erlotinib (Tarceva®) e Gemcitabina (Gemzar®) infusa a "dose-rate" costante come trattamento di I linea in pazienti affetti da carcinoma pancreatico avanzato non resecabile	TERZOLI	OMC
Più patologie				
	C	Lo screening del distress psicologico nei pazienti oncologici in follow-up: uno studio di fattibilità	CARUSO	Psico
	A	Migliorare le abilità comunicative del medico e ridurre il distress del paziente: uno studio clinico randomizzato	CARUSO	Psico
	C	Variabili che influenzano la decisione di sottoporsi al test genetico per i tumori della mammella e/o ovaio	CARUSO	Psico

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Più patologie	AP	TETimaX: studio clinico di fase II, monitorato, per la valutazione dell'efficacia del trattamento, con Imatinib Mesylte, in pazienti refrattari affetti da Tumore Epiteliale del Timo (TET) o Istioitosi X (LCH)	CERIBELLI	OMA
	A	Siero e fosfoproteomica per l'identificazione di marcatori tumore specifici per la diagnosi precoce e la terapia mirata dei tumori solidi-Progetto Italia-USA	CITRO	CRS
	A	OBSERVE: Studio osservazionale sulla modalità d'uso dei fattori di crescita granulocitari nei tumori solidi	COGNETTI	OMA
	A	Valutazione degli effetti immunologici ed antitumorali dell'acido zoledronico in pazienti con tumore della mammella o della prostata con metastasi ossee	FABI	OMA
	A	Il profilo sierico di fattori angiogenetici, citochine e metalloproteinasi durante l'utilizzo di acido zoledronico in pazienti con tumore della mammella o della prostata con metastasi ossee	FABI/FERRETTI	OMA
	A	Valutazione degli effetti immunologici ed antitumorali dell'acido zoledronico in pazienti con tumore della mammella o della prostata con metastasi ossee	IZZO	OMC
	A	Trapianto allogenico di cellule staminali emopoietiche basato su un regime di condizionamento mieloablativo e sulla fotoferesi extracorporea	MENGARELLI	Emat
	C	Studio di farmacocinetica in pazienti in trattamento con gemcitabina 1000mg/m2 in infusione di 10 mg/m2/min con normale o alterata funzionalità epatica o renale	MILELLA	OMA
	A	Studio osservazionale, in aperto, multicentrico e prospettico per valutare gli effetti di alcune strategie terapeutiche sul dolore nel paziente con cancro	TERZOLI	OMC
Polmone				
	A	Studio di fase I/II riguardante l'impiego della radioterapia ipofrazionata conformata in pazienti con tumori polmonari primitivi o secondari di piccole dimensioni	ARCANGELI	Radio
	A	Ruolo del Transforming Growth Factor ß (TGF-ß) plasmatico come fattore predittivo di tossicita' polmonare in pazienti affetti da tumore polmonare trattati con radioterapia. Studio osservazionale.	ARCANGELI	Radio
	A	Trattamento concomitante di Gemcitabina e Radioterapia dopo chemioterapia di induzione nel NSCLC inoperabile in stadio IIIA-IIIB. Studio multicentrico di fase II	CERIBELLI	OMA

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Polmone	A	Studio randomizzato di fase II di cetuximab in combinazione con gemcitabina o gemcitabina seguita da cetuximab in pazienti con carcinoma polmonare non a piccole cellule avanzato, non candidati a terapia contenente un derivato del platino	CERIBELLI	OMA
	C	Studio internazionale, randomizzato, in aperto di fase III con Gemcitabina/Cisplatino in aggiunta a PF-3512676 verso Gemcitabina/Cisplatino come trattamento in prima linea di pazienti con carcinoma del polmone non a piccole cellule (NSCLC)	CERIBELLI	OMA
	A	Studio prospettico, multicentrico, randomizzato, di fase III per valutare l'efficacia di acido zoldronico nel prevenire o nel ritardare le metastasi ossee in pazienti con carcinoma del polmone non a piccole cellule di stadio III	CERIBELLI	OMA
	A	Studio internazionale multicentrico randomizzato di fase III che valuta Erlotinib in I linea di terapia seguito da una II linea con Cisplatino + Gemcitabina versus Cistplatino + Gemcitabina in I linea seguita da una II linea con Erlotinib nel tumore del polmone non a piccole cellule in stadio avanzato	CERIBELLI	OMA
	A	Studio in aperto con bevacizumab (Avastin) in combinazione con un regime chemioterapico contenente platino per il trattamento di prima linea in pazienti con carcinoma polmonare non a piccole cellule (NSCLC), ad istotipo non-squamoso, in stadio avanzato o in ricaduta	COGNETTI	OMA
	A	Studio di fase II/III in doppio cieco sulla combinazione di Paclitaxel e Carboplatino con Vorinostat (MK-0863) o Placebo in pazienti con carcinoma Polmonre a cellule non piccole (NSCLC) di stadio IIIB (con diffusione pleurica) o stadio IV	COGNETTI	OMA
	A	Studio di fase II randomizzato di sorafenib più gemcitabina o sorafenib più erlotinib in pazienti affetti da tumore del polmone non a piccole cellule avanzato (NSCLC) anziani o con PS 2	COGNETTI	OMA
	A	Studio di fase III, randomizzato, in doppio cieco, controllato con placebo, di confronto dell'efficacia di gemcitabina, cisplatino e sorafenib vs quella di gemcitabina, cisplatino e placebo nel trattamento di prima linea di pazienti con carcinoma polmonare non a piccole cellule in stadio IIIb con versamento e in stadio IV (NSCLC)	COGNETTI	OMA
	A	Studio aperto, randomizzato, prospettico, multicentrico a gruppi paralleli sul confronto tra l'efficacia e la sicurezza di TachoSil® ed il trattamento chirurgico standard in pazienti sottoposti a lobectomia polmonare a causa di tumore polmonare maligno e che richiedono trattamento per dispersione d'aria	FACCIOLO	CHTor
	A	La TAC spirale "low-dose" nella diagnosi precoce del cancro del polmone nei soggetti a rischio	GIUNTA	RX

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Polmone	A	Prevenzione primaria e secondaria in soggetti a rischio per cancro del polmone: studio multicentrico	GIUNTA	RX
	A	Studio in aperto con bevacizumab (Avastin) in combinazione con un regime chemioterapico contenente platino per il trattamento di prima linea in pazienti con carcinoma polmonare non a piccole cellule (NSCLC), ad istotipo non-squamoso, in stadio avanzato o in ricaduta	LOPEZ	OMB
	C	Studio di fase III randomizzato di chemioterapia con Cisplatino e Gemcitabina preoperatoria versus postoperatoria in pazienti con tumore del polmone non a piccole cellule stadio IB-IIIA	MILELLA	OMA
	C	Studio di fase II a tre coorti di BMS-275183 somministrato oralmente due volte a settimana in pazienti con tumore del polmone Non a Piccole Cellule localmente avanzato o metastatico (NSCLC) già precedentemente trattati	MILELLA	OMA
	C	Studio di fase II, non randomizzato, multicentrico, mirato a valuatare la tollerabilità e l'efficacia di Pazopanib (GW786034) come terapia prechirurgica in soggetti che non abbiano mai assunto alcun trattamento, con diagnosi di carcinoma polmonare non a piccole cellule (NSCLC) operabile, di stadio IA o IB	MILELLA	OMA
	A	Studio aperto randomizzato multicentrico di fase III di confronto tra il trattamento con Erlotinib (Tarceva ®),e la chemioterapia nei pazienti affetti da carcinoma non a piccole cellule del polmone in stadio avanzato che presentano mutazioni nel dominio della tirosin-chinasi (TK) del recettore del fattore di crescita epidermica (EGFR)	MILELLA	OMA
	A	Studio internazionale multicentrico randomizzato di fase III che valuta Erlotinib in I linea di terapia seguito da una II linea con Cisplatino + Gemcitabina versus Cistplatino + Gemcitabina in I linea seguita da una II linea con Erlotinib nel tumore del polmone non a piccole cellule in stadio avanzato	TERZOLI	OMC
	A	Studio di fase II/III in doppio cieco sulla combinazione di Paclitaxel e Carboplatino con Vorinostat (MK-0863) o Placebo in pazienti con carcinoma Polmonre a cellule non piccole (NSCLC) di stadio IIIB (con diffusione pleurica) o stadio IV	TERZOLI	OMC
Polmone micro				
	AP	Studio multicentrico randomizzato di fase 3 di confronto tra dosaggio fisso e dosaggio modificato sulla base della tossicità della chemioterapia standard con cisplatino ed etoposide in pazienti affetti da microcitoma polmonare avanzato	CERIBELLI	OMA
Prostata				
	C	Studi paralleli di fase II con assegnazione randomizzata tra frazionamento convenzionale standard e ipofrazionamento nell'irradiamento del carcinoma prostatico a prognosi sfavorevole, in associazione a soppressione androgenica totale	ARCANGELI	Radio

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Prostata	AP	STudio Osservazionale Prostata (ST.O.P.): L'importanza del monitoraggio del paziente con carcinoma prostatico in terapia ormonale	ARCANGELI	Radio
	A	Studio Multicentrico Prospettico Randomizzato di fase III di Radioterapia Ipofrazionata in associazione a Deprivazione Androgenica Totale in pazienti con tumore prostatico a prognosi sfavorevole	ARCANGELI	Radio
	A	Trattamento convenzionale versus trattamento con inibitore delle COX-2 delle complicanze della radioterapia per carcinoma della prostata	ARCANGELI/ TESSITORE	Radio
	A	Docetaxel neoadiuvante e concomitante alla radioterapia in pazienti affetti da carcinoma della prostata localmente avanzato ad alto rischio di recidiva	CARLINI/ ARCANGELI	OMA
	A	Docetaxel settimanale in combinazione con prednisone e ciclofosfamide a basse dosi giornaliere nel trattamento del carcinoma prostatico ormonorefrattario: studio di fasel/II	FERRETTI	OMA
	A	Siero e fosfoproteomica per l'identificazione di marcatori tumore-specifici per la diagnosi precoce e la terapia mirata del tumore della prostata	GALLUCCI	URO
	A	Studio Osservazionale Retrospettivo sulla Sequenzialità dell'Endocrinoterapia nei pazienti addetti da Carinoma della Prostata (CaP)	GALLUCCI/ APPETECCHIA	URO
	A	Effetti degli analoghi della somatostatina (lanreotide) sui livelli circolanti di cromogranina a nel carcinoma prostatico ormono-refrattario	SARACINO	Radio
	C	Studio randomizzato: prostatectomia radicale esclusiva verso prostatectomia radicale + radioterapia intraoperatoria (IORT) nei pazienti con adenocarcinoma prostatico a rischio intermedio	SARACINO/ PETRONGARI	Radio
	A	Studio pilota sulla fattibilità dell'incremento della dose con Radioterapia a Modulazione di Intensità (IMRT) nel carcinoma prostatico a prognosi intermedia		
Psoriasi				
	A	Studio multicentrico in aprto di fase IIIb/IV con efalizumab somministrato per via sottocutanea in pazienti adulti affetti da psoriasi cronica a placche da moderata a severa, che non rispondono o per i quali vi è una controindicazione o che sono intolleranti ad altre terapie sistemiche che includono ciclosporina, metotressato e puva	FRASCIONE	Derm
Rene				
	A	Sunitinib precedente e successivo o solo siccessovo a nefrectomia citoriduttiva, studio di fase II che coinvolge pazienti affetti da carcinoma renale metastatico	COGNETTI	OMA

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Rene	C	Protocollo di “accesso allargato” per la terapia sistemica di pazienti con adenocarcinoma renale (RCC) metastatico che sono ineleggibili a partecipare ad altri protocolli con SU011248 ma che potrebbero averne un beneficio clinico	RUGGERI	OMA
	A	Protocollo per l'uso terapeutico del Temsirolimus nel carcinoma a cellule renali avanzato	RUGGERI	OMA
	A	Studio Randomizzato per valutare Temsirolimus e Sorafenib quale terapia di seconda linea in pazienti con carcinoma a cellule renali che hanno fallito la terapia di prima linea con Sunitinib	RUGGERI	OMA
	AP	Studio Randomizzato, in Aperto, di Fase 3b per Valutare Bevacizumab (Avastin®) + Temsirolimus (Torisel®) vs Bevacizumab (Avastin®) + Interferon-alfa (Roferon®) come Trattamento di Prima Linea in Soggetti con Carcinoma a Cellule Renali in Fase Avanzata	RUGGERI	OMA
Retto				
	A	Studio di fase II con panitumumab, 5-fluorouracile e oxaliplatino in associazione con radioterapia pelvica nel trattamento neoadiuvante del carcinoma del retto resecabile e localmente avanzato	GARUFI	OMC
	A	Studio di fase II con panitumumab, 5-fluorouracile e oxaliplatino in associazione con radioterapia pelvica nel trattamento neoadiuvante del carcinoma del retto resecabile e localmente avanzato	ZEULI	OMA
S.N.C. Sist. Nervoso Centrale				
	A	Radioterapia ipofrazionata in pazienti affetti da tumori cerebrali di alto grado con fattori prognostici sfavorevoli. Studio di fase I/II	ARCANGELI	Radio
	C	Studio multicentrico di fase III sulla terapia intratumorale/interstiziale con TransMID in confronto alla migliore terapia disponibile in pazienti affetti da glioblastoma multiforme progressivo e/o ricorrente non asportabile chirurgicamente	CARAPELLA	NCH
	A	Radioterapia concomitante ad infusione prolungata di gemcitabine nel trattamento del glioblastoma multiforme: studio di fase I-II	CARAPELLA/FABI	NCH
	A	Trattamento primario con Temozolomide versus radioterapia in pazienti con glioma a basso grado suddivisi per la presenza della delezione del gene 1p: studio di fase III	CARAPELLA/PACE	NCH
	A	Studio osservazionale del trattamento chirurgico di ablazione e ricostruzione con stabilizzazione, dei tumori primitivi vertebrali	CAROLI	NCH
	A	Studio osservazionale di pazienti sottoposti a somatectomia e vertebrectomia, effettuate in un solo tempo, per il trattamento di asportazione e ricostruzione, con stabilizzazione, delle metastasi vertebrali	CAROLI	NCH

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S.N.C. Sist. Nervoso Centrale	A	Studio pilota in aperto per la valutazione della qualità della vita, del controllo delle crisi e della tollerabilità di oxcarbazepina in monoterapia in pazienti con tumore cerebrale ed epilessia	MASCHIO	Neuro
	C	Studio pilota per la valutazione della qualità di vita, del controllo delle crisi e degli effetti collaterali in pazienti con tumori cerebrali ed epilessia trattati con levetiracetam in monoterapia	MASCHIO	Neuro
	C	Studio osservazionale per valutare la scelta terapeutica nell'epilessia refrattaria alla monoterapia. Studio THEOREM	MASCHIO	Neuro
	C	Protocollo per lo studio delle cinetiche di oxcarbazepina (Tolep) e topiramato (Topamax) in associazione con temozolomide (Temodal), nel trattamento di pazienti con epilessia sintomatica e neoplasia cerebrale primitiva	MASCHIO	Neuro
	AP	Valutazione del controllo delle crisi e della qualità della vita in pazienti con epilessia secondaria a tumore cerebrale in trattamento con pregabalin in add-on: studio pilota	MASCHIO	Neuro
	A	Progetto di assistenza continuativa integrata e neuroriabilitazione a domicilio per pazienti affetti da tumori cerebrali	PACE	Neuro
	A	Ruolo della vitamina E nella neuroprotezione della neurotossicità e della ototossicità indotte da cisplatino	PACE	Neuro
	A	Chemioterapia di prima linea con Temozolomide a bassa dose continuativa nei gliomi di basso grado (grado II WHO)	PACE	Neuro
	A	Chemioterapia primaria con temozolomide in somministrazione continuativa e prolungata (a settimane alterne) per tumori oligodendrogliali a basso grado di malignità in recidiva o progressione dopo la chirurgia: uno studio di fase II	PACE	Neuro
	A	Studio traslazionale-osservazionale: valore prognostico di alcune alterazioni gentiche (1p, 19q, 10q) nei gliomi di basso grado	PACE/CIANCIULLI	Neuro
	A	Terapia di seconda linea con Fotemustina in pazienti affetti da gliomi maligni in progressione. Valutazione di efficacia e tossicità di una schedula di trattamento a dose ridotta	PACE/FABI	Neuro/OMA
	A	Approccio mininvasivo unilaterale per la rimozione delle neoplasie spinali intradurali. Studio ossevazionale	POMPILI	NCH
	A	Approccio mininvasivo sopraorbitario per l'asportazione delle neoplasie della regione sellare	POMPILI	NCH
	A	Metastasi cerebrali: chirurgia tradizionale, approcci innovativi, utilizzazione di nuove tecnologie di sala operatoria	POMPILI	NCH

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S.N.C. Sist. Nervoso Centrale	A	Asportazione degli adenomi ipofisari per via transfenoidale microneurochirurgica-endoscopio assistita. Studio osservazionale e di valutazione precoce del risultato con RM	POMPILI	NCH
	A	Studio osservazionale sull'utilizzo delle più avanzate tecniche e tecnologie chirurgiche nel campo delle neoplasie spinali	RAUS	NCH
	A	Valutazione con CT-Perfusion delle metastasi cerebrali prima e dopo trattamento radioterapico. Studio osservazionale	VIDIRI	RX
	A	Valutazione con CT-Perfusion delle neoplasie cerebrali nella diagnosi e nel follow-up. Studio osservazionale	VIDIRI	RX
Sarcoma				
	A	Studio di fase II sull'utilizzo di Imatinib mesilato nel trattamento del tumore desmoide e del condrosarcoma	CARPANO	OMB
	A	Studio di fase II sul trattamento neoadiuvante dei sarcomi dei tessuti molli degli arti e superficiali del tronco ad alto rischio	CARPANO	OMB
	C	Gemcitabina in infusione prolungata di 100' (rate costante 10/mg/m2/min) in pazienti con sarcomi dei tessuti molli avanzati: studio di fase II	FERRARESI	OMA
	A	Ifosfamide ad alte dosi in infusione continua prolungata mediante sistema infusorio portatile nei sarcomi dei tessuti molli tipici dell'adulto in fase avanzata in seconda/ulteriore linea chemioterapica	FERRARESI	OMA
	A	Studio europeo per il trattamento dei sarcomi ossei in pazienti con età superiore a 40 anni (A European treatment protocol for bone-sarcoma in patients older than 40 years)	FERRARESI	OMA
	C	Studio prospettico randomizzato per il trattamento dell' osteosarcoma non metastatico delle estremita'	FERRARESI	OMA
	A	Studio di fase II sull'utilizzo di Imatinib mesilato nel trattamento del tumore desmoide e del condrosarcoma	FERRARESI	OMA
	A	Studio clinico osservazionale per il trattamento dell' osteosarcoma non metastatico delle estremita'	FERRARESI	OMA
	A	Studio chiave (pivotal) per stabilire efficacia e sicurezza AP23573 somministrato come terapia di mantenimento nei pazienti con sarcoma metastatico dei tessuti molli o sarcoma metastatico osseo	FERRARESI	OMA
Sclerosi multipla				
	A	Studio osservazionale prospettico mulricentrico sulla valutazione dell aderenza alla terapia con immunomodulanti in pazienti con diagnosi di Sclerosi Multipla	GALIÈ	Neuro

PATHOLOGY	STATE	STUDY TITLE	RESPONSIBLE	DIVISION
Sclerosi multipla	AP	BEACON - Studio prospettico sull'aderenza al trattamento,il modo di affrontare le difficoltà ed il supporto dell'infermiere in pazienti in trattamento con Betaferon	KOUDRIAVTSEVA	Neuro
Stomaco				
	A	Gastrectomia D2 e chemioipertermia intraperitoneale intraoperatoria nell'adenocarcinoma gastrico ad alto rischio per recidiva peritoneale	GAROFALO	CHB
Testa-collo				
	A	Studio osservazionale di rilevazione e monitorizzazione della xerostomia nei pazienti con tumori del cavo orale e del faringe sottoposti a radioterapia ad intensità modulata (IMRT)	ARCANGELI/ MARUCCI	Radio
	A	Studio sulla fattibilità dell'uso integrato della chirurgia di salvataggio, della radioterapia intraoperatoria (IORT) e della radioterapia a fasci esterni (EBRT) nei tumori del distretto cervico-cefalico recidivi dopo trattameno radiante	ARCANGELI/ MARUCCI	Radio
	A	Studio del profilo di espressione di microRNA in carcinomi squamosi della testa e del collo e nei tessuti autologhi peri-tumorale e lontano	BLANDINO	R.O.C.
	A	Effetti ototossici in corso di radioterapia nei distretti testa-collo	CRISTALLI	ORL
	A	Studio pilota sulla fattibilità dell'uso della radioterapia intraoperatoria (IORT) come "boost anticipato" nei tumori localmente avanzati del distretto cervico-cefalico	MARUCCI	Radio
	C	Studio longitudinale prospettico controllato multicentrico sui fattori favorenti la comparsa di micosi faringea nei pazienti candidati a trattamento radiante esclusivo o associato a chemioterapia per carcinoma del capo-collo (studio MIR)	MARUCCI	Radio
	A	Studi paralleli di Fase II sull'impiego della Radioterapia 3D con Amifostina o della RadioTerapia a Modulazione di Intensità (IMRT) per valutare la riduzione della xerostomia nei pazienti con tumori del distretto cervico-cefalico trattati in modo definitivo o adiuvante	MARUCCI	Radio
	A	Studio osservazionale sul ruolo della Imaging morfo-funzionale nella programmazione della chirurgia pianificata del collo (planned neck dissection) dopo trattamento radiochemioterapico dei carcinomi del distretto faringeo	PELLINI	ORL
	A	Studio randomizzato di fase 3 sulla chemioterapia con o senza Panitumumab nel trattamento del carcinoma a cellule squamose della testa e del collo metastatico e/o ricorrente	RUGGERI	OMA

PATHOLOGY	STATE	STUDY TITLE	RESPONSIBLE	DIVISION
Testa-collo	C	Studio randomizzato di radioterapia postoperatoria associata a chemioterapia a basse dosi con cisplatino vs radioterapia postoperatoria e polichemioterapia con cisplatino e fluorouracile nei pazienti trattati con chirurgia per carcinomi spinocellulari localmente avanzati del distretto cervico-facciale, ad alto rischio per ripresa di malattia	RUGGERI	OMA
	A	Studio randomizzato, di fase II sulla chemioterapia con o senza Panitumumab nel carcinoma squamocellulare di testa e collo localmente avanzato non resecato	RUGGERI	OMA
	A	Chemioterapia neoadiuvante a base di docetaxel, cisplatino e 5-fluorouracile (TPF) seguita da radioterapia e chemioterapia concomitante o cetuximab a confronto con radioterapia e chemioterapia concomitante o cetuximab in pazienti con carcinoma a cellule squamose della testa-collo localmente avanzato. Studio randomizzato, di fase III con disegno fattoriale	RUGGERI	OMA
	A	Studio osservazionale sull'utilizzo della terapia fotodinamica mediante temoporfina (Foscan®) come trattamento palliativo nei carcinomi recidivanti della testa e del collo in pazienti non suscettibili di ulteriori trattamenti standard	SPRIANO	ORL
Tiroide				
	A	Studio pilota sulla fattibilità dell'uso della Chirurgia Endoscopica Video-assistita Mininvasiva (MIVA) nel trattamento dei noduli tiroidei di piccole dimensioni	RUSCITO	ORL
Utero				
	A	Studio pilota sull'utilizzo di una chirurgia radicale modulata associata alla radioterapia intraoperatoria (IORT) nel carcinoma della portio allo stadio figo Ib1 e linfonodi pelvici negativi: impatto sulle complicanze e sulla qualità della vita. Studio CA.P.R.I.	VIZZA	Gine
Utero-cervice				
	AP	Studio di fattibilità di una radioterapia neoadiuvante a chirurgia conservativa e sequenziale a chemioterapia secondo schema TIP per carcinoma della cervice uterina stadio FIGO IB-IIB monolaterale	PINZI	Radio
Varie				
	A	Ricerca osservazionale sul controllo e le complicanze della terapia sostitutiva con ormone della crescita in pazienti ipopituitarici (HypoCCS)	APPETECCHIA	Endo
	AP	Studio multicentrico di sorveglianza post-marketing della terapia con SOMAVERT in pazienti affetti da acromegalia negli Stati Uniti ed in Europa	APPETECCHIA	Endo
	C	Studio randomizzato in doppio cieco verso placebo sul tempo di comparsa di un significatico sollievo dal dolore in soggetti con nevralgia posterpetica (PHN) trattati con Pregabalin (150 - 600 mg/giorno a dose flessibile ottimizzata od a dose fissa 300 mg/giorno) o Placebo	ARCURI	Rian

PATHOLOGY	STATE	STUDY TITLE	RESPONSIBLE	DIVISION
Varie	A	Studio clinico di fase iib, in doppio cieco, randomizzato, controllato, a dosi multiple, a gruppi paralleli per stabilire la dose minima efficace e studiare il programma di trattamento con Tetrodotoxin nel dolore da cancro, da moderato a grave, non controllato adeguatamente	ARCURI	Rian
	A	Studio osservazionale, in aperto, multicentrico e prospettico per valutare gli effetti di alcune strategie terapeutiche sul dolore nel paziente con cancro	ARCURI	Rian
	AP	Studio di Estensione in Aperto per Valutare la Sicurezza di un Dosaggio Fisso Sottocutaneo di Metilnaltrexone in Soggetti Con Malattie in Fase Avanzata e Stipsi Indotta da Oppioidi	ARCURI	Rian
	AP	Studio Randomizzato, Doppio Cieco, Controllato Verso Placebo Condotto Con un Dosaggio Fisso Sottocutaneo di Metilnaltrexone in Soggetti Adulti Con Malattie in Fase Avanzata e Stipsi Indotta da Oppioidi: Valutazione dell'Efficacia, della Sicurezza e di Risultati Aggiuntivi Relativi allo Stato di Salute	ARCURI	Rian
	AP	Studio esplorativo, in doppio cieco, randomizzato, controllato verso placebo, a gruppi paralleli, di valutazione della dose sull'utilizzo di Sativex per il trattamento del dolore in pazienti affetti da neoplasie in fase avanzata, che non hanno una sufficiente analgesia con una terapia cronica ottimizzata con oppioidi	ARCURI	Rian
	C	Approccio non convenzionale per un intervento nutrizionale con lactobacillus GG in pazienti sottoposti a radioterapia pelvica	ASSISI	Gastro
	C	Alimentazione e qualità della vita: sviluppo di strumenti atti alla valutazione dell'impatto dell'introito di cibo sulla qualità della vita	BARBA	EPI
	A	Prevalenza dei disturbi dell'umore in pazienti oncologici. Efficacia, sicurezza e modulazione immunologica del trattamento farmacologico in pazienti oncologici con disturbi dell'umore e depressione sotto-soglia	CANTELMI	Psichi
	A	La compliance alla dieta priva di glutine in pazienti affetti da malattia celiaca: strategie di coping e qualità di vita	CARUSO	Psico
	A	Studio GENebl (Gruppo Educazionale per i Nebulizzatori e gli Inalatori). Studio multicentrico osservazionale del Gruppo Attività Educazionale Associazione Italiana Pneumologi Ospedalieri (AIPO) per valutare la tecnica di uso degli inalatori e/o nebulizzatori abitualmente usati a domicilio	CILENTI	Fisio
	C	Studio osservazionale sull'emesi ritardata	COLELLA	OMC

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Varie	C	Studio multicentrico, randomizzato, controllato che confronta palonosetron e desametasone somministrati prima della chemioterapia e lo stesso regime con desametasone somministrato anche nei giorni 2 e 3 nella prevenzione della nausea e del vomito in pazienti con neoplasie solide trattati con chemioterapia moderatamente emetogena	FABI	OMA
	C	Prevenzione del tromboembolismo venoso ed arterioso con l'eparina a basso peso molecolare nadroparina calcica in pazieni in trattamento chemioterapico. Studio randomizzato, placebo-controllato, in doppio-cieco, multicentrico di fase III	FERRETTI	OMA
	C	OSHES (Observational Study on Hemostatis in Surgery): Studio osservazionale sull'emostasti: uno studio non interventistico sull'emostasi in chirurgia	GALLUCCI	URO
	A	Studio clinico osservazionale sullo stato di ipercoaguabilità e sulla efficacia della profilassi antitrombotica di breve durata e di lunga durata con eparine a basso peso molecolare in pazienti oncologici portatori di catetere venoso centrale a permanenza (CVC)	LAURENZI	Rian
	A	Studio di standardizzazione delle misure di outcome della Neuropatia Periferica Indotta da Chemioterapici (CI-Perinoms)	PACE	Neuro
	AP	Registro nazionale sulla radioimmunoterapia	PISANI	Emat
	C	Progetto globale per l'identificazione ed il miglioramento della Qualità di vita nei pazienti oncologici a lunga aspettativa di vita	PUGLIESE/FABI	Psico
	A	SUN: Survey on the lung cancer management	RINALDI	OMB
	A	Studio dell'acquisto di funzione della proteina mutata p53 mediante silenziamento con piccoli RNA (shRNA)	SACCHI	CRS
	A	Studio prospettico, randomizzato, multicentrico, controllato, sulla terapia antibiotica empirica della febbre nel paziente oncoematologico neutropenico: piperacillina/tazobactam più tigeciclina vs una monoterapia con piperacillina/tazobactam	SPADEA	Emat
	C	Prevenzione del tromboembolismo venoso ed arterioso con l'eparina a basso peso molecolare nadroparina calcica in pazieni in trattamento chemioterapico. Studio randomizzato, placebo-controllato, in doppio-cieco, multicentrico di fase III	TERZOLI/GARUFI	OMC
	C	Studio di coorte per la valutazione del rischio degli eventi cardiovascolari nei pazienti che si sottopongono a chirurgia non cardiaca: studio pilota	VIZZA	Gine

PATHOLOGY	STATE	STUDY TITLE	RESPONSIBLE	DIVISION
Varie	A	Studio di coorte per la valutazione del rischio degli eventi vascolari nei pazienti che si sottopongono a chirurgia non cardiaca	VIZZA	Gine
Vescica	C	Chemioterapia adiuvante con Cisplatino/Gemcitabine in pz. con carcinoma transizionale della vescica ad elevato rischio di recidiva dopo cistectomia radicale vs chemioterapia differita alla ripresa di malattia	RUGGERI	OMA