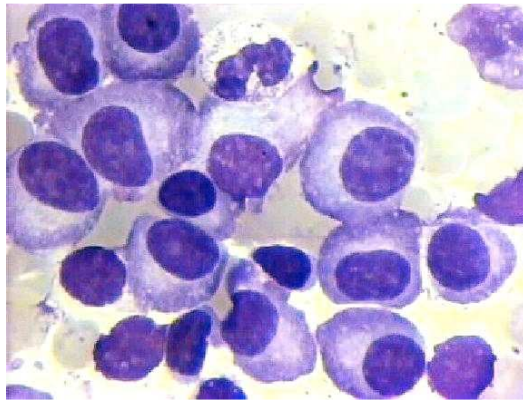




**FIFTH ANNUAL
SCIENTIFIC CONFERENCE**



**ISTITUTO TOSCANO TUMORI
(ITT)**

Thursday, July 1, 2010

*Il Borro
San Giustino Valdarno (AR)*

ABSTRACT BOOK

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Il Borro
San Giustino Valdarno (AR)
Thursday, July 1, 2010

Programme

- 8.30 *Welcome and registration*
- 8.45 *Official opening*
Presidente della Facoltà di Medicina e Chirurgia dell'Università di Siena
Gian Maria Rossolini
Direttore Generale Azienda USL 8
Enrico Desideri
Direttore Operativo Istituto Toscano Tumori
Gianni Amunni
- 9.15 **COLON CANCER**
Chair: **Andrea Ardizzoni** (Parma), **Luca Boni** (CCSC, CRL)

Genetic, epigenetic and environmental factors
Lucia Migliore (Pisa)

Immunophenotypic characterisation of sino-nasal intestinal-type adenocarcinoma
Marco Santucci (Firenze)

Role of bevacizumab in the management of colon cancer: an update
Alfredo Falcone (Pisa)

Chairpersons' comments; and question period
- 10.15 **CANCER PREVENTION**
Chair: **Paolo Vineis** (London), **Adele Seniori Costantini** (Firenze)

Primary prevention of cancer through reduction of inappropriate diagnostic tests
Eugenio Picano (Firenze)

Smoking legislation and smoking patterns
Giuseppe Gorini (Firenze)

Chairpersons' comments; and question period
- 11.00 *Coffee break*
- 11.30 **BREAST CANCER**
Chair: **Luigi Cataliotti** (Firenze), **Sergio Crispino** (Siena)

BRCA1, genomic instability and carcinogenesis
Alvaro Galli (Pisa)

Efficacy and cost-effectiveness of screening for breast cancer
Marco Zappa (Firenze)

Strategies to identify biological tools with predictive value for the activity of DNA damaging cytotoxics in breast cancer patients
Angelo Di Leo (Prato)

Chairpersons' comments; and question period

12.30 ANTI-CANCER PEPTIDES

Chair: *Pier Paolo Pandolfi (Boston), Mario Chiariello (CRL)*

Small molecule mimicking the Spa310 peptide from the spacer region of pRB2/p130 as potential anticancer agents

Antonio Giordano (Siena)

Branched peptides for selective tumor targeting

Luisa Bracci (Siena)

Chairpersons' comments; and question period

13.15 *Lunch break*

14.00 MELANOMA

Chair: *Nicola Pimpinelli (Firenze), Barbara Stecca (CRL)*

Role of calpains in melanoma

Emilia Maellaro (Siena)

Melanoma markers in plasma

Claudio Orlando (Firenze)

New approaches to *in vivo* imaging of melanoma

Alberto Pupi (Firenze)

Immunotherapy clinical trials

Michele Maio (Siena)

Chairpersons' comments; and question period

15.15 ANGIOGENESIS AND CANCER

Chair: *Gianni Del Sal (Trieste), Salvatore Oliviero (Siena)*

Angiogenesis in cancer development

Mario Del Rosso (Firenze)

Hypoxia, tumor growth and angiogenesis

Antonella Naldini (Siena)

Chemotherapy and anti-angiogenesis drugs

Guido Bocci (Pisa)

Anti-angiogenesis trials in various types of (non-colon) cancer

Editta Baldini (Lucca)

Chairpersons' comments; and question period

16.30 *Tea break*

17.00 PROSTATE

Chair: *Francesco Francesca (Pisa), Rosario Notaro (CRL)*

New findings on risk factors for prostate cancer

Domenico Palli (Firenze)

Role of Micro RNAs in the pathogenesis of prostate cancer

Laura Poliseno (New York)

Mechanism of spread of prostate cancer

Paola Chiarugi (Firenze)

New trials for patients with prostate cancer

Sergio Bracarda (Arezzo)

Chairpersons' comments; and question period

18.15 GUEST LECTURE

Chair: *Luca Cionini (Pisa)*

Radiation therapy: an ageing centenarian or a healthy phoenix?

Jean-Claude Horiot (Genolier)

19.00 Adjournment

Anti-angiogenesis trials in non-colon cancer

Edi Editta Baldini

Medical Oncology Division, Campo di Marte Hospital, Lucca

In the recent past several trials have investigated the use of anti-angiogenesis drugs (bevacizumab, sunitinib, sorafenib) alone or in combination with chemotherapy for the treatment of advanced breast, lung and renal cancer.

The first phase III trial, carried out in advanced breast cancer, evaluated the role of bevacizumab in combination with capecitabine vs capecitabine alone in 462 patients previously treated with anthracyclines or taxanes: although the addition of bevacizumab did not improve PFS (progression-free survival) there was an absolute increase of approximately 11% in ORR (objective response rate) (20 vs 9%; $p=0.001$) [1]. In the E2100 trial the addition of bevacizumab to first-line weekly paclitaxel resulted in doubling ORR (36.9 vs 21.2%; $p<0.0001$) and median PFS (11.8 vs 5.9 months; $p<0.0001$), compared to paclitaxel alone in more than 700 HER2 negative MBC (metastatic breast cancer) patients; unfortunately, there was no impact on OS (overall survival) [2]. These findings were corroborated by more complete analyses for regulatory purpose, and were confirmed by IRF (independent review faculty). The AVADO phase III trial compared the addition of bevacizumab to docetaxel vs docetaxel alone as first-line therapy in patients with HER2 negative locally-relapsed or metastatic breast cancer; this study showed a significant improvement in PFS in favour of the combination (8.8 vs 8 months; $p<0.0001$) [3]. These results have led to the development of several phase III trials testing bevacizumab in combination with other antineoplastics or trastuzumab in first-line MBC. To date no predictive response biomarkers have been identified for bevacizumab; several ongoing trials incorporate molecular studies aimed at targeting the correct subset of patients (e.g. triple negative patients) for bevacizumab therapy. Clinical trials are underway to evaluate the use of bevacizumab in adjuvant and neoadjuvant settings; several nonrandomised studies indicate a good safety profile.

The phase III E4599 trial, performed in untreated patients with advanced adenocarcinoma of the lung, demonstrated significant improvement in median OS (12.3 vs 4.5 mos; $p=0.003$), median PFS and RR for the addition of the Mab to carboplatin-paclitaxel in comparison with chemotherapy alone [4]. The phase III BO17704 (AVAiL) trial was designed to determine efficacy and safety of two doses of bevacizumab in combination with cisplatin-gemcitabine vs chemotherapy alone in advanced adenocarcinoma patients: PFS was significantly longer with bevacizumab 7.5 mg/kg plus chemotherapy compared with chemotherapy alone (6.7 vs 6.1 mos; $p=0.003$) and an objective response rate of 34.1% was achieved with the triple combination compared with 20.1% for chemotherapy alone ($p<0.0001$).

Avastin and Roferon in the Renal Cell Carcinoma (AVOREN) trial compared bevacizumab plus IFN with placebo plus IFN in 649 patients with MRCC and demonstrated that the addition of bevacizumab led to a significantly longer PFS interval (10.2 months, versus 5.4 months; HR, 0.63; $p.0001$) [5]. The overall response rate in the bevacizumab plus IFN group was 31%, versus 13% in the placebo plus IFN one ($p .0001$). In the final data analysis, no benefit in OS was observed.

The CALGB 90206 trial compared bevacizumab plus IFN (same doses as in the AVOREN study) with IFN monotherapy in 732 patients. The PFS interval with bevacizumab was 8.5 months, compared with 5.2 months with IFN alone ($p=.0001$). There was also a higher ORR with bevacizumab ($p.0001$). Unfortunately, neither trial included a bevacizumab-only arm, so that the relative contribution of IFN to the regimen remains an open question.

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Chemotherapy and anti-angiogenic drugs

Guido Bocci

*Centro Interdipartimentale di Ricerche di Farmacologia, Clinica e Terapia Sperimentale,
Università di Pisa*

The clinical experience has shown that acquired resistance to antiangiogenic therapeutic strategies is possible since many patients, whose tumors initially respond to drugs such as bevacizumab, tyrosine kinase inhibitors targeting VEGF receptors or metronomic chemotherapy, become nonresponsive. The studies undertaken on antiangiogenic resistance mainly involved mechanisms directly related to the antiangiogenic drugs alone and as such lead one to ask whether the acquired resistance to antiangiogenic therapies might also be mediated by chemotherapy usually associated (at least into the clinic) with these compounds. Could the intrinsic or acquired resistance to antiangiogenic therapy be modulated by the choice of the associated chemotherapeutic drug? Is it possible to identify pharmacodynamic markers associated only to the resistance to the antiangiogenic drugs but not to the combination? Indeed, the role of antineoplastic chemotherapy in antiangiogenic resistance seems to be ignored by the previous studies and the real part played by these drugs has to be written yet. Our preclinical and clinical studies would like to start a rational approach to answer at various questions regarding the role of chemotherapeutic drugs in the antiangiogenic resistance both in a preclinical and clinical setting of gastrointestinal cancers.

New trials for patients with prostate cancer

Sergio Bracarda

U.O.C. of Medical Oncology, Department of Oncology, USL-8, Arezzo

Prostate cancer is one of the leading causes of death for cancer in the male. Treatment of choice for advanced or inoperable disease is androgen ablation which can be achieved by surgical (orchidectomy) or medical (LH-RH analogues) castration and is active in about 85% of the patients. Unfortunately, despite this initial elevated tumour response eventually all patients progress to an androgen-independent status within 12-18 months. A possible survival advantage achievable by adding a peripheral anti-androgen to medical or surgical castration (maximum androgen blockade or MAB), was recently denied in a published meta-analysis on 8275 men by the Prostate Cancer Trialist Group (The Lancet, 2000), data of steroidal vs non-steroidal anti-androgens are, however, under reevaluation because of significant differences.

Patients who had progressed to an androgen-independent status are candidates to second line hormonal manipulations and thereafter to chemotherapy (HRPC). Prognosis of HRPC is extremely poor, even if, recently, the final data of the two largest randomised trial in HRPC treatment (TAX-327, 1.006 cases and SWOG 99-16, 770 cases) were published and for the first time a survival advantage was achieved. Patients were treated with Docetaxel plus prednisone (TAX-327) or estramustine phosphate (SWOG) vs Mitoxantrone and prednisone. The amount of the survival advantage was about 3 months in both trials.

Recently, the HRPC definition was changed in CRPC (Castration-Resistant Prostate Cancer). This change underlying a significant modification in the molecular understanding of the AR active pathways and its persisting activation in this clinical situation. New treatment options under recent evaluation consist in further hormonal approaches through the adrenal suppression (i.e. abiraterone acetate) and bone targeting through agent specifically directed against this site (i.e. dasatinib or denosumab). Further approaches are evaluating immunotherapeutic and antiapoptotic agents.

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Branched peptides for selective tumor targeting

Luisa Bracci

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Selective tumor targeting agents, able to ensure both a diagnostic and a therapeutic application (theranostics), would greatly advance the development of personalized cancer therapy. We have studied the use of protease-resistant tetra-branched peptides containing the sequence of the human regulatory peptide neurotensin (NT), as specific tumor targeting agents [1], demonstrating that oligo-branched peptides can be effective novel theranostic molecules in oncology [2]. We set up a general branched scaffold [3], which allows conjugating different functional units to tetra-branched peptides, making them efficient target-selective carriers, either for in vitro or in vivo cell tracing, or for cell therapy. By using tetra-branched NT peptides (NT4) conjugated to fluorophores, we demonstrated that NT4 can efficiently discriminate between tumor and healthy tissue in human surgical samples from colon or pancreas adenocarcinoma in a high number of patients, with very good statistical significance. Moreover, we demonstrated that NT4 can efficiently deliver functional units for cell imaging or killing, to many different human cancer cells. In fact, NT4: 1- bind to cell membrane receptors more efficiently than monomeric homologous sequences [1,2]; 2- are efficiently internalized in different tumor cells; 3- when conjugated to chemotherapy drugs, can induce selective killing of different human cancer cells from colon, pancreas or prostate carcinoma, in vitro [2,3] and in xenografted mice [2].

Different drug-armed NT4 have been synthesized and tested in vitro and in vivo and we demonstrated that they allow killing of tumor cells through a mechanism mediated by peptide receptors, which greatly increase drug selectivity toward receptor-positive cells. The switch to a receptor-selective drug internalization produces three consequences: i) it dramatically reduces drug non-specific cytotoxicity; ii) it greatly increases in vivo activity of the drug; iii) it may induce reverse of innate cell resistances, when these are produced by mechanism of cell internalization or export of the drugs. Drug-armed oligo-branched peptides can combine the high selectivity produced by multimeric binding to membrane receptors over-expressed by cancer cells with the high efficiency of chemotherapy drugs which interfere with different cellular pathways. By increasing selectivity of small molecules towards tumor cells, NT4 act as Trojan horses, which selectively transport chemotherapy drugs into tumor cells.

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Prostate cancer cells: how they use cancer-associated fibroblasts to perform epithelial-mesenchymal transition and to acquire stem cell properties

Paola Chiarugi

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Carcinoma are the most frequent human malignant tumors and several lines of evidence support the notion that the growth and the invasive potential of carcinoma cells are influenced by host stromal cells, collectively called "reactive stroma" (1). The aim of this study is to explore the role of cancer-associated fibroblasts (CAFs) in epithelial mesenchymal transition (EMT) of prostate carcinoma cells. EMT is a key developmental program, leading to achievement of a motile/invasive phenotype of tumor cells, often activated during cancer invasion and metastasis (2, 3). We used CAFs from human patients with prostate benign adenomas or aggressive carcinomas. Analysis of the reciprocal interplay between prostate carcinoma cells and CAFs revealed a key role of tumor cell-derived interleukin-6 (IL-6) in fibroblasts activation. In turn, prostate carcinoma activated fibroblasts, through secretion of metalloprotease (MMP)-2 and -9, elicit a clear EMT in cancer cells, correlated with increased invasion through proteolytic degradation of ECM. The reciprocal interplay between CAFs and tumor cells was also demonstrated *in vivo*: we found that only tumor cells stimulated by CAFs gave tumors and allow lung micrometastases. In agreement with recent findings (4, 5), CAF-induced EMT leads prostate carcinoma cells to enhance expression of stem-cell markers, as well as their ability to form prostaspheres and to self-renew. EMT of prostate carcinoma cells in response to CAFs is mediated by activation of cyclooxygenase-2, nuclear factor- κ B and hypoxia inducible factor-1 and phenotypic knock-out of this pathway blocks *in vivo* tumor growth. In general terms, our findings suggest that the paracrine interplay between CAFs and cancer cells leads to an EMT-driven gain of stem cell properties, thus enhancing their aggressive characters.

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Cancer angiogenesis: control of TGFβ1 switch-dependent angiogenesis by antagonist peptides

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TGFβ1 switches from tumour suppressor (in the premalignant stages of tumorigenesis) to prooncogene at later stages of the disease, leading to metastasis. Progression to metastatic disease is generally accompanied by increased expression of TGFβ1 by tumour cells themselves (TGFβ1-switch). Pro-metastatic effects of TGFβ1 are either autonomous (acting on epithelial-mesenchymal transition, cell survival, invasion/motility), or achieved through effects on tumour stroma, including angiogenesis promotion. The first aim of this project was to develop tumor cell lines able to conditionally express an active form of TGFβ1. We have chosen the tet-off system from Clontech in order to obtain repressible expression of the gene. After doxycycline administration, cells are expected to switch off transgene expression. A synthetic gene of porcine origin was cloned in the plasmid pTRE2PUR under the tet-regulated promoter and the constructs (mock or TGFβ1) were transiently transfected into breast carcinoma (MCF7) and hepatic carcinoma (HepG2) cells. After assessment of the system functionality, that is TGFβ1 over-production upon doxycycline administration, cells and culture medium have been used to study the main parameters of *in vitro* angiogenesis: proliferation, matrigel invasion and capillary morphogenesis of target human microvascular endothelial cells (HMVEC). Medium from TGFβ1 over-producing cells stimulated HMVEC invasion and capillary-like tubes formation, when compared to the medium of mock cells. All the observed effects were reversed by alternate gain/loss of TGFβ1 production and were inhibited by anti-TGFβ1-inhibiting peptides. The Matrigel sponge angiogenesis assay in SCID mice will be used to evaluate the activity of the antagonist peptides in the *in vivo* setting. The preliminary results so far obtained point to a consistent role of TGFβ1 in angiogenesis of cancer cells undergoing the TGFβ1-switch and to the efficacy of TGFβ1 inhibiting peptides in the control of these effects.

Strategies to identify biological tools with predictiv value for the activity of DNA damaging cytotoxics in breast cancer patients

Angelo Di Leo

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Breast cancer comprises an extraordinarily different group of diseases in terms of presentation, morphology, molecular profile and response to therapy. Gene expression analysis has identified four main molecular classes of breast cancer that are biologically and clinically distinct. Between these classes, triple-negative/basal-like breast cancers show an unfavourable prognosis because of their aggressiveness and the lack of effective treatment strategies. Increasing preclinical and early clinical data suggest that DNA repair dysfunction, frequently seen in triple-negative tumors, might represent a treatment target. In particular, patients with a basal-like breast cancer harbouring BRCA 1 dysfunction may be expected to derive substantial benefit from DNA damaging agents, such as platinum compounds, alkylating agents and PARP inhibitors.

For this reason it is of key importance in breast cancer to identify and validate diagnostic tests which can simply and effectively predict the efficacy of DNA damaging agents. Our research is focused on identification of such predictive tools, particularly the alkaline comet assay in early breast cancer patients and by the CellSearch System™ (Veridex) in advanced breast cancer patients.

Alkaline comet assay is a standard method for assessing DNA damage and DNA repair ability in individual cells, providing valuable information about innate DNA characteristic and cell response to various external factors, such as radiation, chemicals and drugs. The assay is based on the principle that in an electric field, cleaved DNA fragments migrate out of the cell, producing a 'comet'-like image, whereas undamaged DNA do not. The technique is rapid, sensitive and requires a small number of cells. It may predict patients with particular sensitivity to DNA damaging therapy.

Another approach which may allow us to test the efficacy of DNA damaging and non-damaging agents is the isolation and bio-characterization of circulating tumour cells (CTC) from peripheral blood samples by the The Cell Search™ system. The main objective of this study is to demonstrate that, specific CTC defined breast cancer phenotypes determine chemotherapy sensitivity in metastatic breast cancer patients, . In particular, we directly stain CTC by immunofluorescent markers (ER, HER-2, CK5/6 and EGFR) in order to classify patients into the different breast cancer molecular subtypes. The identification and enumeration of CTC are performed with the use of the CellTracks® Analyzer II, a semi-automated fluorescence-based microscopy system that permits computer-generated reconstruction of cellular images.

Both studies appear promising in their ability to identify individuals to benefit from DNA damaging therapy. A strength of both studies is their lack of invasiveness.

Role of bevacizumab in the management of colon cancer: an update

Alfredo Falcone

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The inhibition of angiogenesis by the anti-Vascular Endothelial Growth Factor (VEGF) bevacizumab has proved to be an efficacious strategy in the treatment of metastatic colorectal cancer (mCRC) (1). On the other hand, NSABP C-08 trial has recently given a first, long-awaited answer to the pressing question about the role of bevacizumab in the adjuvant setting. No significant advantage in terms of disease free survival (DFS) was provided by the addition of the anti-VEGF to mFOLFOX6. Nevertheless, new questions have raised by the analysis of such results. In particular, the observation of an initial, significant improvement in DFS in the arm treated with bevacizumab (1 year-DFS: HR: 0.6; $p=0.0004$), which gradually decreased and became insignificant at the longer follow-up planned by study design (3 years-DFS: HR: 0.87; $p=0.08$), suggests a potential “transient” effect of bevacizumab, that vanishes when interrupting its administration. Despite such an intriguing hypothesis and waiting for results of phase III AVANT trial, that randomized patients to FOLFOX4 versus (vs) FOLFOX4 plus bevacizumab vs XELOX plus bevacizumab, the anti-VEGF does not currently find place in the adjuvant setting.

In spite of the wide adoption of the anti-angiogenic, combined with conventional cytotoxics, in the treatment of the metastatic disease, a number of items still need to be addressed, in order to optimize its use in the therapeutic algorithm of mCRC patients. Two large observational trials, BEAT and BRiTE trials, including a global amount of 4000 patients who received the anti-angiogenic in the daily practice, have shown that the combination of bevacizumab with any first-line chemotherapy is safe and effective. The phase II FOIB trial, conducted by GONO group in order to assess the safety and activity of GONO-FOLFOXIRI plus bevacizumab as first-line regimen, has provided impressive results in terms of response rate (RR:77%), disease control rate (DCR:100%), progression free survival (median PFS: 13.1 months) and overall survival (median OS: 30.9 months), with an incidence of adverse events absolutely comparable to data of literature (2).

Based on such promising results, a phase III trial (TRIBE trial) is currently randomizing mCRC patients to receive GONO-FOLFOXIRI plus bevacizumab vs FOLFIRI plus bevacizumab as first-line regimen. According to the study design, 450 patients from about 30 Italian oncological units will be randomized. The interim safety analysis, including the first 100 treated patients, has evidenced no significant differences in terms of registered toxicity between arms (3).

Another debated clinical issue involves the opportunity to continue to administer bevacizumab beyond disease progression to patients who received a bevacizumab-containing regimen as first-line treatment. This hypothesis has been formulated on the basis of the results of phase IV BRiTE study, reporting that the exposure to bevacizumab beyond progression in patients who already received the anti-VEGF as part of first-line treatment was associated with an improvement in survival (median OS: 31.8 vs 19.9 months).

In order to better elucidate the role of a prolonged antiangiogenic strategy, a phase III trial (BEBYP trial) is currently randomizing patients already treated with a bevacizumab-containing regimen to second-line chemotherapy with or without bevacizumab. According to the study design, 262 patients from about 30 Italian oncologic units will be enrolled.

A consistent contribution to the optimization of the use of bevacizumab in the daily practice will certainly come from translational research. No molecular markers able to identify patients more likely to benefit from the inhibition of VEGF are currently available. According to *post-hoc* analyses

of phase III AVF2107 trial, nor *KRAS*, *BRAF*, *p53* mutational status, nor VEGF and thrombospondin-1 immunohistochemical expression, nor microvessel density are related with benefit from bevacizumab. Drawn from the well-known role of host tissues in influencing and driving angiogenesis' steps, attention has been focused on VEGF polymorphisms. In particular, a recent retrospective experience has evidenced a significant correlation of VEGF -1498 TT variant of VEGF -1498 C/T single nucleotide polymorphism (SNP) (rs833061) with worse PFS (median PFS: 7.5 vs 11.1 months; HR: 2.13 [1.41-5.10], $p=0.0027$) in a population of mCRC patients treated with FOLFIRI plus bevacizumab as first-line regimen (4). VEGF -1498 C/T SNP retained its significance as predictor of PFS also in the multivariate model (HR: 2.28 [1.16-4.19], $p=0.018$). No association of VEGF -1498 C/T SNP with clinical outcome was observed among an historical cohort of mCRC patients treated with first-line FOLFIRI (Log-rank test: $p=0.662$), thus suggesting a potential predictive, other than prognostic implication of such genetic variants. This suggestion is corroborated by the significance of the interaction test between -1498 C/T variants and treatment effect ($p=0.011$). A prospective trial (Pro.Ve.TT.A trial) is currently ongoing, in order to further investigate the hypothesis generated by this preliminary retrospective experience.

A basic contribution from translational research is awaited not only to clarify mechanisms of intrinsic resistance to the anti-VEGF, but also to disclose mechanisms of acquired resistance. The pharmacodynamic approach may provide useful insights into the modulation of the angiogenic balance during the administration of bevacizumab-containing regimens and at the time of disease progression (PD), thus allowing to shine a light on tumors' escape pathways and on new potential targets to be considered in the therapeutic strategy.

It has been hypothesized that the increase of basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), Placental growth factor (PlGF) and stromal-derived factor-1 (SDF-1), observed before the time of PD in a cohort of mCRC patients treated with first-line FOLFIRI plus bevacizumab might suggest the role of these factors as potential mechanisms of acquired resistance.

A similar experience, conducted on samples collected at different time-points from patients enrolled in phase II FOIB trial has revealed that VEGF levels, as assessed on immunodepleted plasma samples, are significantly reduced by the triplet plus bevacizumab, independently of baseline levels. More interestingly, VEGF levels remained significantly lower than at baseline also at the time of PD (5).

Such exploratory finding, as well as data about the variation of other pro- and anti-angiogenic factors, deserves further investigation in adequately dimensioned trials, i.e. phase III TRIBE and BEBYP trials, with the aim to better characterize plasmatic changes at the time of PD and to adopt appropriate tailored strategies on the basis of each patient's angiogenic profile.

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**BRCA1, genome instability and carcinogenesis:
exploiting yeast genetics to characterize BRCA1 missense variants**

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Individuals carrying germ-line mutations in BRCA1 have a high risk of developing breast and ovarian cancer. A large body of evidence indicates that BRCA1 plays a central role in DNA damage response including DNA repair and cell cycle-control; however through which mechanism BRCA1 exerts its role in DNA repair has to be elucidated. We recently found that expression of several cancer-related BRCA1-missense variants increases HR in yeast. Presumably, investigating the mechanisms and the genetic factor involved in this phenotype may be helpful to elucidate the BRCA1-carcinogenesis. As, the yeast *Saccharomyces cerevisiae* is an excellent model to investigate genetics of the genome stability control, we aimed to investigate the mechanism by which the expression of BRCA1 variants induces HR in yeast. We also found that, within the C- Terminus domain (BRCT) not all the BRCA1 variants induce HR indicating that the mutated proteins interact and/or affect different pathways controlling HR. We think that a more profound knowledge of how BRCA1 missense variants affect genome stability in yeast can help to understand the mechanisms underlying the BRCA1-driven tumorigenesis. We have determined the effect of the BRCA1wt and the mis-sense variants on HR, gene mutation and aneuploidy in yeast strains carrying defects on DNA repair/recombination pathways in order to identify novel genetic factors that predispose to cancer.

Preliminary experiments carried out with yeast strains defective in one HR pathway or mismatch repair indicate that the BRCA1 missense variants may required specific DNA repair pathway to induce HR in yeast.

Small molecules mimicking the Spa310 peptide from the spacer region of pRB2/p130 as potential anticancer agents

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Over the past decades, cancer research has been mainly aimed at identifying the molecular alterations underlying cancer development, in order to design new drugs for targeted therapy. The proteins of the retinoblastoma family, consisting of pRB1/p105, pRB2/p130 and pRBL1/p107, are of great interest because of their tumor suppressor activity. RB proteins are key regulators of the cell cycle and their inactivation underlies most human cancers (1). We focused in particular on pRB2/p130, the expression of which is altered in many cancer (including lung, hepatic, prostate, breast and ovarian cancer), in order to identify strategies that could restore its tumor suppressor function (2).

pRB2/p130 is able to bind the complex formed by Cyclin A and the Cyclin-dependent kinase CDK2, causing the inhibition of its kinase activity, which is necessary for cell cycle progression. pRB2/p130, as well as the other RB family members, consists of two highly conserved regions, A and B, which are separated by a spacer domain that is longer and more conserved between pRBL1/p107 and pRB2/p130 compared with pRB1/p105. The inhibitory activity of pRB2/p130 on the CDK2/Cyclin A complex has been attributed to this spacer region. Consistently, our previous studies revealed that the peptide Spa310, derived from the spacer region of pRB2/p130, was able to bind the CDK2-CyclinA complex and inhibit its kinase activity thereby arresting human lung cancer proliferation in xenotransplanted nude mice (3-5).

Recently, we used a computational chemistry approach to select a pool of small molecules that mimic Spa310 activity. The analysis of the CDK2-CyclinA crystal structure using a docking approach allowed us to select five hypothetical CDK2-CyclinA inhibitors from chemical libraries. We tested the antiproliferative effects of these five small molecules on cell lines of different tumor types (lung and prostate cancer, osteosarcoma, and medulloblastoma) by the MTS cytotoxicity assay. We observed a significant reduction in the growth rate of these tumor cells and we focused our further analyses on the two most effective compounds. In order to rule out the potential cytotoxic effect on normal cells, we tested these molecules also on non-neoplastic cell lines. We found that they have a significant minor effect on normal cells with respect to their tumoral counterpart. Preliminary FACS analyses show that both the selected small molecules can induce apoptosis in lung cancer cell lines. To dissect the molecular mechanisms of these small-molecule-induced apoptosis we are analyzing by western blotting and real-time qRT-PCR the expression of proteins involved in the regulation of cell cycle and apoptosis .

As a future objective, we intend to test these small molecules in mouse tumor xenografts in order to evaluate their ability to inhibit tumor growth also *in vivo*.

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Smoking Legislation and Smoking Patterns

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Smoking prevalence in Italy has substantially declined in the last decades, with a 37-percent reduction from 1980, in part attributable to the development of tobacco control policies (increasing taxes, advertising ban, smoking cessation clinic development, introduction of warning labels on packages and of a nation-wide smoking ban).

After the introduction of the Italian smoking ban (January 10, 2005), second-hand smoke (SHS) exposure dropped in Italian workplaces and hospitality premises. The ban changed social acceptance of smoking in Italy. Which were the effects of the ban on SHS exposure at home and on smoking cessation? What can you learn from this experience? And now, how can we further decrease SHS exposure and increase cessation rates? How can we predict future smoking prevalence, according to the introduction of tobacco control policies recommended by the WHO Framework Convention on Tobacco Control (FCTC), and not yet implemented in Italy?

Aim of this presentation is to present ISPO studies on Tobacco Control: Smoke-free Home Study, SPRINT Study, SIDRIAT Cohort Study, and Prediction of smoking prevalence in Italy, 2010-2050.

The Smoke-free Home Study is a randomized controlled trial (RCT), funded by ITT (Grant Proposal 2007), for evaluating the effectiveness of a counselling intervention for lowering SHS exposure at home. All 200-250 recruited women, in Florence, Prato, Arezzo, and Empoli, receive a booklet on SHS risks. Women in the intervention group also receive a counselling intervention on SHS delivered by a trained nurse. We are recruiting women for the study.

SPRINT Study (Active & Smoke-Free Women) is a RCT for evaluating the effectiveness of a counselling intervention on smoking cessation and physical activity delivered to 1,200 smoking women attending Cervical Cancer Screening Program in four areas in Toscana, Piemonte, Emilia-Romagna and Lombardia. It is funded by the Italian Minister of Health. We are testing whether the smoking cessation counselling intervention delivered to women randomly assigned to the "Intervention A" arm, could increase the one-year cessation rate, in comparison to that recorded in control arm women. Moreover, we are interested in understanding whether the counselling interventions on smoking cessation and physical activity delivered to women assigned to the "Intervention B" arm, could increase the cessation rate, in comparison to that recorded in women of both the control and the "Intervention A" arms. A self-help booklet on physical activity and smoking cessation is provided to all participants. The study is in the follow-up phase.

SIDRIAT Cohort Study is funded by ITT (Grant proposal 2008). We are assembling a cohort of residents in Tuscany from a 2002 cross-sectional survey on a representative sample of about 6,000 children and adolescents, the Italian Study on Respiratory Disorders in Childhood and the Environment -2 (SIDRIA-2), which collected information on smoking, SHS exposure of children, adolescents, and their parents. We estimated to interview about 9,800 persons for the second wave (1,600 adolescents aged 14-15 years in 2010; 1,800 young adults aged 21-22 years, and 6,400 parents).

Main aim of SIDRIAT is to study predictors of quit attempts and cessation among adult smokers, and the effects of household smoking bans on adolescents' initiation, before and after the introduction of the Italian smoking ban. The hypothesis is that smoking ban determined a significance increase in quit attempts and in household smoking bans.

Finally, we are conducting a study on prediction of smoking prevalence in Italy, 2010-2050. The model simulates scenarios that may arise from the introduction of tobacco control policies recommended by the FCTC, and not yet implemented in Italy, such as further tax increases, well-designed mass media campaigns with an evaluation plan, pictorial warnings on packages, enforcement of banning the sales to minors, total reimbursement of tobacco cessation treatments, further development of Quitlines, changing tobacco product regulation (lowering carcinogens, toxicants, and nicotine in tobacco products; increasing availability of nicotine replacement therapy (NRT) in retail tobacco outlets and giving incentives to try NRT).

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Radiation Therapy (RT): an ageing centenarian or a healthy phoenix?

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The ability to perceive the present with an historical perspective is one of the few privileges of old age. The accumulated evidence that scientific truth is volatile is always a lesson of humility. Young oncologists trained in the first decade of the XXIst century often believe that treatment methods largely used since more than a century have constantly strengthened their position over such a long period. This does not reflect the historical reality: Cancer treatment has grown through a complex multidisciplinary process involving long periods of slow changes alternating with shorter periods of significant progress leading to a re-evaluation of the role of each discipline. Such progresses are either simultaneous or occur independently. They are always interactive due to the multidisciplinary nature of optimal management of cancers.

Radiation therapy is almost born with the discovery of X-Rays in 1895 and Radium in 1898: The first cancer cure by X-rays was reported in 1900 and Radium experiments were on-going in 1903. The next major acceleration in the history of RT occurred in the late fifties with megavoltage beam equipment allowing the delivery of tumoricidal doses to deep tumors. Major research contributions of radiation physics, radiation biology and clinical trials were then translated in routine practice. In the mid-seventies, RT and surgery were considered as the only curative tools in most solid tumors.

In the last three decades of the XXth century, three factors challenged RT indications, fueling some dark predictions about the future of radiotherapy: Better knowledge of detrimental late effects of RT, novel active therapies of cancers, chemotherapies, hormonal treatments, molecular targeted therapies, vaccines, without forgetting the surgical progresses. At last, cancer screening and prevention were expected to sharply reduce the incidence of most cancers amenable to curative radiotherapy (e.g. breast, head and neck, cervix cancers). According to these guesses, RT should gradually become a marginal resource for cancer treatment and fall into oblivion at the dawn of the XXIst century...

Unfortunately for these Nostradamus emulators, a brief glance at cancer diagnostic and treatment facts in 2010 does not confirm such predictions: Cancer incidence has never been so high due to the increase of life expectancy and the failure of most cancer prevention programmes. Although progress of surgery and medical oncology have drastically modified the current management of most solid tumors, not only RT still plays a major role but has spread the range of its indications in the curative and palliative management of cancer patients. How this happened is the core of this presentation, stressing the dead-ends, partial failures and successes of this scientific venture.

Role of calpains in melanoma cells

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Calpains are a family of Ca²⁺-dependent neutral cysteine proteases. Several gene products of the family are known in humans, including the conventional μ -calpain (calpain 1) and m-calpain (calpain 2) ubiquitously expressed, and other tissue-specific isoenzymes. Calpains are involved in a number of cellular events, such as cell proliferation and differentiation, gene expression, cytoskeleton reorganization and motility, and, more recently, in apoptosis.

In the last few years, we focused the involvement of calpains in tumor cell biology, in particular in human metastatic melanoma cells. In these cells we have demonstrated a key contribution of conventional calpains in apoptotic cell death induced by cisplatin: calpains are activated both in apoptotic cells and in viable cells, whereas other typical features of apoptosis are still absent, and calpain pharmacological inhibition affords a significant protection from apoptosis. Such a protective effect is correlated with an upstream mechanism, that is the downregulation of cisplatin-induced p53 activation.

More recently, in melanoma cells and in melanocytic lesions we have identified and sequenced two novel splicing variants of muscle-specific calpain 3 (GenBank accession n. EU91850 and EU91851), both endowed with a Nuclear Localization Signal. In cisplatin-treated pre-apoptotic cells an increase of both transcription and (auto)proteolytic cleavage of this variants occurs. Calpain (auto)proteolysis, regarded as an activating process, is prevented when also apoptosis is prevented by calpain 1/2 inhibitors, suggesting that calpain 3 variants (i) possibly cross-talk with calpain 1/2, in a sort of calpain network, and (ii) can play a pro-apoptotic role. Consistently with this view, the expression of these novel variants is found significantly down-regulated, compared to benign nevi and to early stage melanocytic lesions, in the most aggressive ones (i.e. in vertical growth phase melanomas and, even more, in metastases), characterized by invasiveness properties, and usually resistant to apoptosis.

To give further insights on the role played by calpains 1/2 and calpain 3 variants in melanoma cells, our research in progress is doubly aimed.

First, the molecular mechanisms of autophagy, suggested to counteract drug-induced cell demise, have gained our interest. Preliminary results show that inhibition of conventional calpains (by MDL-28170 and calpeptin), along with protection from cisplatin-induced apoptosis, put into motion a remarkable autophagic response (evaluated as ratio LC3II/LC3I), suggesting that calpain activation in cisplatin-treated cells is somehow able to interfere with the pro-survival autophagic cascade; such a mechanism is confirmed by the co-treatment with the autophagy inhibitor 3-methyladenine, which fully reverse the protective effect of calpain inhibitors on apoptotic cell death. The autophagy inducer CCI-779 (rapamycin analogue, mTOR-dependent) dramatically increases cisplatin-mediated apoptosis, while the autophagy inducer trehalose (mTOR-independent) significantly protects from apoptosis; these preliminary observations suggest that the pro-survival autophagy induced by calpain inhibition (and specularly the anti-autophagic role of activated calpains) is an mTOR-independent mechanism.

Interestingly, the autophagic response afforded by trehalose is also able to decrease the cisplatin-induced calpain activation, suggesting that, in the opposite direction, autophagy can exert its pro-survival effect by down-regulating conventional calpains. Second, we are trying to overexpress the calpain 3 variants in melanoma cells: preliminary results obtained in transient transfections of the longer variant suggest that melanoma cells do not tolerate such a protease over a basal expression level since as soon as one day after transfection cells detach and die, likely through apoptosis. Although this unables us to perform experiments with overexpressing cells, such a death response is interesting, being consistent with our previous results showing an increased transcription and activation of calpain 3 variants correlated to cisplatin-induced apoptosis. Specularly, RNA interference of calpain 3 variants is expected to endow melanoma cells with a pro-survival mechanism, worth to be explored.

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Immunotherapy clinical trials

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The management of surgically unresectable metastatic melanoma is a major clinical challenge because of the substantial lack of effective systemic therapies. However, advances in the understanding of the immunobiology of melanoma cells and of T-cell activation are stimulating the design of new therapeutic strategies to generate more effective host immune responses against neoplastic cells. A recent approach involves targeting of cytotoxic T-lymphocyte antigen-4 (CTLA-4), one of the key immune checkpoint molecules (1). Following T-cell activation, CTLA-4 moves from intracellular stores to the site of antigen-presenting cell interactions at the immunological synapse, imparting a negative signal to T cells and suppressing immune activation (1). Thus, blocking cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) has been proposed as a strategy to enhance cell-mediated immune responses to cancer. Along this line, clinical trials in metastatic melanoma, and in other human malignancies, are demonstrating that anti-CTLA-4 therapy can induce durable outcomes (2) that may develop over time with different patterns than cytotoxic chemotherapy (3). Two different fully human anti-CTLA-4 monoclonal antibodies, ipilimumab and tremelimumab, have been recently tested in the clinics within phase II/III trials. Data generated in these studies will be discussed, as well as the detailed experience with metastatic patients who underwent progressive disease after a variety of previous therapies, including prior immunotherapies, and who achieved good outcomes with ipilimumab (4). Additional therapeutic antibodies (e.g., anti-CD137, anti-PD-1, anti-CD40, anti-OX-40) that modulate the activity of distinct immunologic checkpoints are also being evaluated in phase I/II trials in melanoma patients. Comprehensively, immunomodulating antibodies, utilized alone or in combination regimens, represent a new category of highly promising therapeutic agents for the treatment of melanoma patients.

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Genetic, epigenetic and environmental factors in colorectal carcinoma

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Colorectal carcinoma arises as a consequence of the accumulation of genetic alterations (gene mutations or amplifications..) and epigenetic alterations (aberrant DNA methylation, chromatin modifications..) that transform colonic epithelial cells into colon adenocarcinoma cells. We started a project to investigate the interaction of dietary components (folate levels) with genetic susceptibility (polymorphisms of genes involved in the folate metabolic pathway) in influencing the methylation levels of genes critical for colon cancer. Epigenetic factors are responsible for transcriptional variation in healthy cells. To date, the best studied epigenetic alteration is DNA methylation. Epigenetic instability (hypermethylation of tumor suppressor genes and global genome hypomethylation) are the main epigenetic alterations observed in cancer cells (Nystrom M, Mutanen, 2009). Various environmental and dietary agents and lifestyles are suspected to be implicated in the development of a wide range of human cancers throughout epigenetic changes. Higher intakes of vegetables have been reported to be associated with a reduced risk of colorectal cancer. Folate, a water-soluble B vitamin, and one of the major micronutrients in green vegetables, fruits, beans and meat may be partially responsible for this beneficial effect. Folate appears critical for the synthesis and regeneration of S-adenosylmethionine (SAM), the essential methyl donor in the synthesis of DNA. It has been postulated that methyl groups availability could be an underlying mechanism for the beneficial effect of folate (Kim, 2007).

Several genes participate in folate metabolism, some of them required for the synthesis of DNA precursors, others for the production of SAM. Folates are introduced into the cells by means of the reduced folate carrier (RFC1), which is polymorphic. DNA methylation is potentially affected by methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and methionine synthase reductase (MTRR) gene polymorphisms, whereas DNA synthesis is likely to be affected by polymorphisms of the thymidylate synthase (TYMS) gene. Vitamin B12 (or cobalamin) is a vitamin contained in foods of animal origin and acts as an important cofactor of the enzyme methionine synthase (MTR). Cobalamin-dependent methionine synthase catalyzes the transfer of a methyl group from 5-methylTHF to homocysteine, producing tetrahydrofolate and methionine, which is then converted into SAM. Insufficient availability of cobalamin leads to diminished activity of this enzyme. The folate pathway can be thus altered by low dietary folate intake, by the presence of polymorphic variants of metabolic enzymes and/or by combinations of both dietary and genetic factors.

The multistep carcinogenesis model in colon cancer serves as the classical model of genetic alterations in cancer. A potential mechanism underlying colorectal carcinoma progression is epigenetic silencing associated with promoter hypermethylation carcinogenesis. Differential expression of specific genes within defined class of clinically significant genes has been discovered by genome scan studies, by examining the methylation state of cytosine bases throughout the genome. It can be linked to a possible modulation of the methylation of promoters.

Among the genes found to be involved in colorectal carcinoma and object of recent studies for their methylation status in colon cancer development there are: Adenomatous polyposis coli (*APC*), O(6)-methylguanine-DNA methyltransferase (*MGMT*), human MutL homologue (*hMLH1*), P16 Cyclin-dependent kinase N2A (*P16/CDKN2A*) and RAS association family 1A (*RASSF1A*) (Chen SP et al., 2009). The idea on which this study is based, is that dietary components, in concert with the presence of susceptibility genes, can influence the epigenome, altering genetic expression and potentially modifying colorectal cancer risk (Migliore and Coppede', 2009).

The study ongoing is designed on a 3-years period. Overall, at least 200 patients with colorectal neoplastic lesions and their relatives (>200) will be recruited to search for correlations between plasma folate, homocysteine (Hcy) and vitamin B12 levels, polymorphisms in genes of the folate metabolic pathway and the methylation levels of the promoter of genes involved in colorectal cancer. Genotyping of the first group of patients for the presence of the *MTHFR 677C>T*, and *MTHFR 1298A>C*, *MTR 2756A>G*, *MTRR 66A>G*, *RFC1 80G>A*, *TYMS* 28bp repeat and 1494del6 polymorphisms, have been until now carried out according to Coppede' et al. (2007).

High-resolution melting (HRM) analysis is a novel tool for analysis of promoter methylation. In our study, we started to use HRM analysis to detect the methylation levels of O(6)-methylguanine-DNA methyltransferase (*MGMT*) gene, according to Wojdacz and Dobrovic (2007) in 10 colorectal cancers and plan to apply the same approach to the study of other genes critical for the development of the pathology.

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Hypoxia, tumor growth and angiogenesis

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The casual relationship between hypoxia, angiogenesis and tumor growth is widely accepted. Recently, there has been considerable interest in understanding the hypoxic and non-hypoxic upregulation of the hypoxia-inducible factor-1 α (HIF-1) and HIF-1-related gene regulation, since aberrant expression of HIF-1 correlates with angiogenesis and tumor progression. The protease-activated receptor (PAR)-1 belongs to a family of G-protein-coupled receptors and its expression is associated with tumor angiogenesis and invasiveness in breast cancer. We have demonstrated that hypoxia enhances the expression of both HIF-1 and PAR-1 in the highly invasive human breast cancer cell line MDAMB231. Inhibition of HIF-1 by short interfering (si)RNA resulted in a dramatic reduction of PAR-1 expression, suggesting a significant role for HIF-1 in the hypoxia-dependent induction of PAR-1. Furthermore, we found that treatment of hypoxic MDAMB231 cells with a specific PAR-1 agonist peptide resulted in a significant increase of both cell survival and migration. Additionally, since the etiology of breast cancer has been associated with inflammatory processes, we next evaluated whether HIF-1 α and PAR-1 were upregulated by the pro-inflammatory cytokine interleukin -1 β (IL-1) in normoxic MDAMB231 cells. We observed that IL-1 enhanced the accumulation of HIF-1 and PAR-1 expression. Such enhancement was associated with an increased cell migration, which was reversed by HIF-1 siRNA treatment. Our observation that IL-1 induced HIF-1 accumulation *in vitro* was confirmed in tumor cells growing *in vivo* using an experimental approach, mimicking the endogenous release of IL-1 in mice bearing MDAMB231 xenografts. Altogether, the results point to the relevance of the hypoxic and non-hypoxic regulation of HIF-1 in the tumor microenvironment. This may have important implications in the therapeutic targeting of HIF-1-associated molecular pathways and adaptive responses in tumor cells, especially in breast cancer.

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Multimarker approach to detect circulating cancer cells and cancer-derived DNA in blood of melanoma patients

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The primary objective is to exploit molecular biomarkers that will enable the use of less invasive approaches to tumour investigation, reducing patient distress, improving compliance and lowering risks of procedural and therapeutic complications. Analysis of markers in circulating tumour cells (CTCs), as well as circulating tumour DNA/RNA, may provide an opportunity to assess unique molecular biomarkers originating from both primary tumours and metastases.

Cutaneous melanoma has a high tendency to develop metastasis and its incidence is increasing in the Caucasian population. Several studies aimed to identify new molecular biomarkers in blood of melanoma patients, with potential diagnostic and prognostic relevance. Cancer circulating cells (CTC) and free nucleic acids in plasma (CNA) represent two faces of this scenario.

It is now evident that in cutaneous melanomas, a subset of tumor cells detaches from the primary tumor or metastatic tumor sites, entering the peripheral circulation. For the detection of CTC direct and indirect methods can be used. The *isolation by size of epithelial tumor cells* (ISET®) is a direct method for CTC isolation by filtration, due to their large size in comparison with peripheral blood leukocytes. Isolated tumor cells can be subsequently evaluated by immunohistochemistry and/or molecular-genetic analyses.

We investigated the presence of CTC by ISET® in 87 patients with cutaneous melanomas and 53 controls. By this approach CTC were not detected in the controls, but were detectable in 29% of patients with primary invasive melanoma and in 62.5% of metastatic melanoma patients (1). We also successfully applied ISET to the study of patients with uveal melanoma and data were compared to indirect detection of CTC by real-time PCR measurement of tyrosinase mRNA (2).

Cell-free DNA in plasma is a potential cancer biomarkers (3). We tried to optimize a multi-parametric approach for the simultaneous evaluation genetic and epigenetic variants in melanoma patients. In particular we developed real time methods for the quantification and characterization of the following parameters in human plasma: i. total DNA concentration; ii. DNA integrity index; iii. density of RASSF1A methylation; iv. abundance of BRAF mutated alleles (4). Clinical sensitivity and specificity were assessed for each parameter, by comparing their ROC curves. Fixing the specificity value at 100%, the four indicators achieved a diagnostic sensitivity of 65% for APP, 49% for BRAF, 45% for RASSF1A and 25% for the tyrosinase mRNA, with a resulting overall diagnostic sensitivity of 96%. These data suggest that the simultaneous determination of multiple circulating biomarkers significantly increases the diagnostic sensitivity in cutaneous melanoma.

Studies are ongoing to validate this approach in larger groups of melanoma patients and a study group formed by outdoor and indoor workers with different patterns of exposure to UV solar radiations (*ITT grant proposal 2008*).

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New findings on risk factors for prostate cancer

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Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute - ISPO, Firenze

Rates of prostate cancer (PCa) vary widely across the world, being less common in South and East Asia, and more common in Europe and United States; however, incidence and prevalence data depend on the diffusion of prostate specific antigen (PSA) dosage and subsequent biopsies, more widespread in western countries. Because PSA testing has a much greater effect on incidence than on mortality, there is less variation in mortality rates worldwide (10-fold) than is observed for incidence (25-fold), and the numbers of deaths from PCa do not consistently differ in developed and developing regions (Globocan 2008). The increase in PSA-diagnosed cases represents also a problem for etiologic studies. Overall, PCa represents a huge problem of public health, heavily contributing to the overall cancer burden among men and absorbing a great quantity of economical resources.

Many factors, including genetics and diet, have been implicated in the development of prostate cancer, and several observational, prospective studies have examined the influence of environmental and lifestyle factors on PCa risk, providing however mostly inconclusive findings.

Positive associations with PCa risk consistently emerged for smoking (Huncharek 2010), obesity and height (Guh 2009, Zuccolo 2008, Renehan 2008, Pischon 2008) and circulating levels of IGF-1 (Rowlands 2009, Roddam 2008). The positive associations with pesticide-related occupations (Van Maele-Fabry 2003) and serological evidence of HSV-2 infection (Dennis 2009) were suggestive but require confirmation by further studies. Negative associations emerged for a few factors correlated with clinical history, like diabetes (Kasper 2009) and use of aspirin and other NSAIDs (Mahmud 2010), physical activity levels (Johnsen 2009) and diet, in particular soy food consumption and intake of selenium, vitamin E, vitamin K1, glucosinolates and lycopene (Dagnelie 2004, Steinbrecher 2009, Nimptsch 2008, Hwang 2009, Yan 2009), but further studies are needed to confirm and better investigate possible biological mechanisms for cancer risk reduction and prevention. Finally, no associations seem to exist between PCa risk and vitamin D metabolism (Gilbert 2009, Gandini 2010, Yin 2009, Huncharek 2008), fat, fibres, fruit and vegetable consumption (Crowe 2008, Key 2003, Suzuki 2009), plasma levels of folate, vitamin B12 (Johansson 2008), plasma carotenoids (α - and β - carotene, lycopene, lutein, zeaxanthine, β -cryptoxanthin and canthaxanthin), retinol and α - and γ - tocopherols (Key 2007).

In conclusion, epidemiological studies published so far could not provide sufficient evidence to inform public health policies and recommendations for the primary prevention of prostate cancer.

Primary prevention of cancer through reduction of inappropriate diagnostic tests in cardiology

Eugenio Picano

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Background. Medical imaging is the largest controllable source of radiation exposure in the population of industrialized countries – totalling around 150 chest x-rays per head per year. Of these exposures, two-thirds come from cardiovascular testing (cardio-CT, nuclear cardiology and interventional cardiology).

Relevance to cancer: the high level of radiation exposure provides immense benefits when appropriate, but may result in an increased incidence of radiation-induced cancer in the not-too-distant future. With current estimates, about 10% of all cancers may be due to medical radiation exposure. One out of 2 examinations is completely or partially inappropriate (i.e. risk outweighs benefit) and cardiologists are often unaware of the radiological dose of the examination they prescribe or practice.

Aims of the study are: 1) **CLINICAL:** to assess the level of appropriateness of main cardiological ionizing testing examinations in a high-tech tertiary care referral center; 2) **RADIOLOGICAL:** to calculate the patient and population dose from reference doses and actual radiological exposures in the index hospital area; 3) **ECONOMIC:** to develop innovative models of health technology assessment, including long-term cancer risk in the risk-benefit balance; 4) **RADIOPROTECTION-INFORMATIC:** to produce user-friendly software for calculating and communicating radiological risk; 5) **MEDICO-LEGAL:** to develop an informative, transparent template of an informed consent form for radiological examinations, together with communications experts and patients' rights representatives; 6) **ONCOLOGIC:** to estimate the number of avoidable cancers produced by current levels of inappropriate testing.

The overarching aim of the SUIT-HEART (Stop Useless Imaging Testing in HEART disease) study is to promote a better appreciation of radiation risks in the cardiology community and in patients, as now unanimously recommended by official documents of the American College of Radiology 2007, International Atomic Energy Agency 2008 and American Heart Association 2009.

Preliminary data: The preliminary data obtained in our Institution during the last 5 years suggest that: 1) the increase in complexity and radiation dose levels of state-of-the art imaging techniques is not matched by adequate increase in knowledge of users; 2) practitioners and prescribers substantially underestimate the radiological dose (and corresponding cancer risk) of commonly prescribed imaging examinations; 3) our currently accepted risk-benefit analysis in health technology assessment do not include long-term risks and downstream costs due to cancer; 4) better radiological risk communication is needed, with support of user-friendly software; 5) patient's awareness of dose and risk is often absent - an overt violation of patients' rights; 6) inappropriate testing in cardiology is proliferating out of control in spite of the top priority of medical appropriateness strongly recommended by medical associations and political authorities.

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Role of microRNAs in the pathogenesis of prostate cancer

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PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressor that antagonizes signaling through the phosphatidylinositol-3-kinase–Akt pathway [1]. Small decreases in PTEN protein have marked consequences on prostate tumor initiation and progression, as we have previously demonstrated *in vivo* in a Pten hypomorphic allelic series in the mouse [2].

MicroRNAs (miRNAs) are endogenous single stranded RNA molecules that act as posttranscriptional regulators of gene expression. miRNAs play diverse roles in numerous cellular processes; in particular, miRNA abundance is altered during tumorigenesis and miRNAs can act as oncosuppressors or oncogenes [3]. Since cells are ultrasensitive to PTEN dosage, subtle modulators of gene expression, such as miRNAs, may be predicted to have a profound effect on prostate tumorigenesis.

We have used a computational approach to identify miR-22, miR-25 and miR-302 as three *PTEN*-targeting miRNA families found within nine genomic loci. In particular, we have shown that the *miR-106b~25* cluster is composed of 3 *PTEN*-targeting and cooperating miRNAs: miR-93, miR-106b and miR-25. We have found that the miRNAs belonging to this cluster are aberrantly overexpressed in human prostate cancer, and correlate with abundance of the miRNA processing enzyme DICER [4], suggesting that their overexpression might be due to increased maturation. We have also found that these miRNAs potentiate cellular transformation both *in vitro* and in a xenograft model.

The *miR-106b~25* cluster is located within the intron 13 of *MCM7*, a protein widely used as diagnostic and prognostic marker in prostate cancer [5]. Interestingly, we have found that the *miR-106b~25* intronic cluster cooperates with its host gene *MCM7* in cellular transformation *in vitro*. Furthermore, we have demonstrated that the concomitant overexpression of *MCM7* and the miRNA cluster in the prostate of transgenic mice triggers prostatic intraepithelial neoplasia. Therefore, the *MCM7* gene locus delivers two simultaneous oncogenic insults when amplified or overexpressed in human cancer.

Thus, we have uncovered a proto-oncogenic miRNA-dependent network for PTEN regulation and defined the *MCM7* locus as a critical factor in initiating prostate tumorigenesis.

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New approaches to *in vivo* imaging of melanoma

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Melanoma is the most fatal form of skin cancer due to its rapid metastasis and its frequent resistance to current chemotherapy regimens and radiotherapy. During the progression from benign melanocytic disease to metastatic malignant melanoma, melanocytes undergo a series of changes in the expression of cell-surface molecules. One of the up-regulated proteins in association with melanoma progression is the integrin $\alpha_v\beta_3$ (1). Several studies indicate that $\alpha_v\beta_3$ up-regulation correlates with the invasive phase of human melanoma, suggesting that this integrin is crucial in melanoma progression. Also, $\alpha_v\beta_3$ plays a critical role in melanoma cell survival within human skin, and the blockade of $\alpha_v\beta_3$ triggers apoptosis of melanoma cells, which ultimately blocks tumour growth. Therefore, in the last years, great efforts have been made to develop anti-angiogenesis drugs based on $\alpha_v\beta_3$ integrin antagonism. However, the successful introduction of these therapies into the clinical practice requires the development of reliable ways to assess angiogenesis and its modification or inhibition *in vivo*. The ability to non-invasively visualize and quantify integrin $\alpha_v\beta_3$ expression *in vivo* at different tumor growth stages would provide new opportunities to document the tumor receptor expression, more appropriately to select patients considered for antiangiogenesis treatments, and to monitor the treatment efficacy in integrin-positive patients (2). One important recognition site in a ligand for many integrins is the arginine-glycine-aspartic acid (RGD) tripeptide sequence (3), which is found in all peptide-based ligands identified for the vitronectin receptor integrins. Several radiolabeled ligands of the $\alpha_v\beta_3$ integrin adhesion receptor have been developed based on the integrin's recognition of the RGD sequence (4). Recently, in our research group we have synthesized some peptidomimetics (having both peptidic and non-peptidic backbone) which demonstrated high affinity *in vitro* for $\alpha_v\beta_3$ (IC₅₀ in the nanomolar range) (5). Starting from these promising results, we are developing new potential PET radiotracer for imaging the expression levels of $\alpha_v\beta_3$ in melanoma, to be used in oncology for the management of patients. Preliminary studies by *in vivo* imaging in melanoma rodent model (mice) using a Micro(SPECT)PET/CT system demonstrated interesting properties of the radiotracer.

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Immunophenotypic characterisation of sinonasal intestinal-type adenocarcinoma

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Sinonasal intestinal-type adenocarcinoma (ITAC) is an uncommon neoplasm morphologically similar to or indistinguishable from colorectal adenocarcinoma, with a well-recognised association with occupational exposure to wood or leather dusts (1). Relatively little is known about the molecular alterations involved in the development of these tumours, and most studies conducted so far were based on small series of patients, precluding the analysis of the correlations with clinicopathological features (2-4). The aim of our study was to investigate immunohistochemically several proteins known to serve pivotal roles in tumorigenesis, including the products of the tumour suppressor genes p53, p16, deleted in colon cancer (DCC), and retinoblastoma (RB), factors implicated in the Wnt signalling pathway, such as adenomatous polyposis coli (APC) and β -catenin, and factors related to tumour aggressiveness such as E-cadherin and CD10. The results show that aberrant expression of p53 and p16 were the most commonly observed alterations (61.3% and 64.5% of cases, respectively), while loss of DCC expression was detected in 43.5% of cases. Loss of E-cadherin expression occurred in 52% of ITACs examined, while over expression of CD10 was observed in 43.5% of the cases. Alteration of APC and/or nuclear localization of β -catenin, an indirect evidence of deregulated Wnt signalling pathway, were not observed, as it was loss of RB expression. Analysis of the distribution of these alterations according to the histological subtype showed that there were significant differences in their frequencies between mucinous and non-mucinous adenocarcinomas. In particular, p53 overexpression was less frequent in mucinous ITACs (28.3% vs. 71.7%, $p=0.007$), while loss of DCC and E-cadherin were more frequently observed in this subtype (81.3% vs. 30.4%, $p=0.001$, and 81.3% vs 32.6%, $p=0.001$, respectively). No correlation was found between aberrant protein expression and clinical behaviour of the tumour, while mucinous adenocarcinomas presented more frequently in advanced stage ($p=0.001$) and had a significantly worse 5-year survival rate than non-mucinous adenocarcinomas ($p=0.005$). In conclusion, mucinous ITACs appear to follow a distinct molecular pathway(s) from the non-mucinous variants, and pursue an aggressive clinical behaviour.

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Efficacy and cost-effectiveness of screening for breast cancer

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Despite several randomized clinical trials demonstrated the efficacy of mammographic screening in reducing mortality from breast cancer (IARC, 2002), the effectiveness of mammographic programmes has been recently argued (Jorgensen et al, 2010). Several others recent studies carried out in Sweden, Finland, England, the Netherlands and in Italy have reached an opposite conclusion, demonstrating a reduction in mortality similar to that observed in clinical trials. Furthermore recently the occurrence of overdiagnosis as a consequence of screening programme has been pointed out (Biesheuvel et al, 2007) although the estimates of such on overdiagnosis are quite different. First of all excess of incidence, which is necessary consequence of breast cancer screening in order to achieve the wished outcomes, is not overdiagnosis. Furthermore the differences in the overdiagnosis estimates depend on the used methods and measures. In this situation finally the crucial point is to assess, in a real situation, the ratio between the number of saved lives and the number of cases overdiagnosed.

We will present a review of the more updated data (Puliti and Paci, 2009). Furthermore we will present the first result (not yet published) in terms of lives saved and overdiagnosed cases of the cohort of about 51.000 women invited for the first time in 1991-1993 within the screening programme of the city of Florence. We divided the cohort into 3 groups namely frequent attenders (women who complied the first two invitations), never attenders (women who did not complied both the first two invitations) and the irregular attenders (women who complied only one of the first two invitations). These 3 cohorts have been followed for 17 years in terms of incidence and mortality from breast cancers. The problems of comparability will be discussed. The results of mortality reduction, overdiagnosis and ratio between these two outcomes will be presented.

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As additional information on work in progress in Tuscany in the area on cancer research we have received from Dr. Pamela Gabelloni the following abstract:

**New approaches in the treatment of glioblastoma
using a low dosage of matrix-metalloproteinase inhibitors**

Claudia Martini^a, Sara Bendinelli^a, Pamela Gabelloni^a, Eleonora Da Pozzo^a, Barbara Costa^b, Elisa Nuti^c, Francesca Casalini^c, Elisabetta Orlandini^c, Federico Da Settimo^c, Armando Rossello^c, Ettore Novellino^d.

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The most common brain tumour in adults is the glioblastoma multiforme, an highly invasive malignant primary cancer.

Tumour cell-derived gelatinases (MMP-2 and MMP-9) can be considered prime factors in glioma invasiveness, the major problem in glioblastoma aggressivity¹.

New synthetic compounds with high activity on MMP-2 and MMP-9 were developed and tested at very low dosage on glioblastoma cells (U87MG). The use of nanomolar concentration of MMP-2 inhibitors has been already reported in previously published paper².

The usage of MMP inhibitors at low concentrations should spare the inhibition of both protective MMPs (anti-cancer) and MMPs involved in other cell processes, thus probably resulting in reduced toxicity compared to the use of broad-spectrum MMP inhibitors.

First of all, the activity of the compounds was characterized by assays carried out in tube and on natural substrate (gelatin) by zymography. Moreover, the ability of these compounds to inhibit cellular invasiveness was assessed on matrigel. The results showed that these compounds can inhibit the invasiveness without affecting cell viability. Interesting, after treatment with the compounds, an inhibition of MMP-2 expression was demonstrated, showing the use of inhibitors able to modulate the molecular mechanisms underlying MMP-2 expression.

The compounds were used alone or in co-treatment with an alkylating agent such as temozolomide, (TMZ), widely used in chemotherapeutic protocols. Although the compounds did not affect cell viability (no additive effect with TMZ treatment), the compound anti-invasiveness activity was maintained.

The ability of these MMP inhibitors to act at nanomolar concentrations paves the way in developing efficacious clinical therapies without the side effects typically associated with broad spectrum MMP inhibitors. Although clinical trials are always required to confirm every cell model approach, our data underlie the importance of the availability of high affinity and selective compounds able to act on cells that over-express a specific protein, sparing those characterized by a normal expression.

Part of data about activity, invasiveness, viability and expression have been previously published³.

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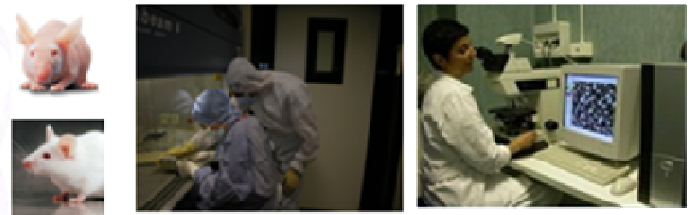
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Services summary:

- .Construct planning
- .Transgenic mouse production
- .Genetargeting: knock-out and knock-in mouse production
- .ES cells isolation and electroporation
- .Mouse embryo recovery
- .Rederivation of mouse strains
- .Genomic DNA isolation and genetic screening of mice
- .Transgenic lines breeding
- .Subcutaneous injection of human neoplastic cells or primary tumor fragments in nu/nu mice
- .Orthotopic implantation of human neoplastic cells in immunodeficient mice
- .Intravenous injection of human leucemic cells in NOD/SCID mice
- .Flowcytometric analysis of peripheral and medullary blood
- .Tossiological test *in vivo*
- .Evaluation of pharmacological efficacy in particular pathological conditions
- .Murine model production for human pathologies
- .Monoclonal and polyclonal antibodies production

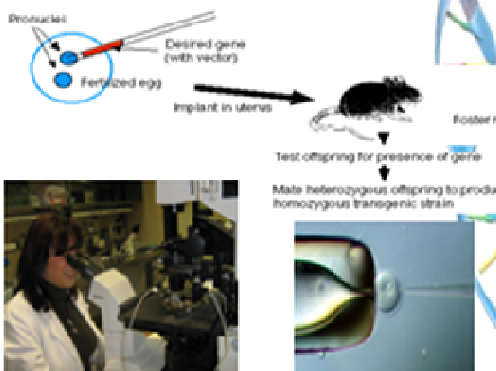
Oncological and farmacological analyses:

Preclinical stage of oncological research needs animal models obtained by subcutaneous, intravenous or orthotopic injection of neoplastic cells or small primary tumor fragments in immunodeficient mice. Such animal models led to evaluate the ability of a human primary tumour to grow and metastatize *in vivo*. These models are also used to test the efficacy of new pharmacological therapy in inhibiting primary tumours and metastases development.



Transgenic mice production:

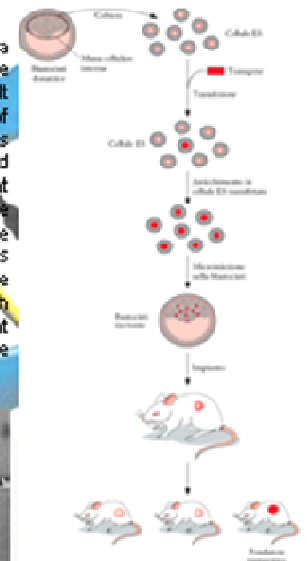
Pronuclear injection is the most commonly used method for the transgenic mice production. It consists of the isolation of fertilized oocytes (at one cell stage of development) and of the DNA construct injection into male pronucleus. The injected oocytes are then implanted into the oviduct of pseudopregnant females. Usually about 10% of progeny has the transgene integrated at random in the genome and, for this reason, each positive animal is a founder for a transgenic line. This technology allows to evaluate the consequences of ectopic expression or overexpression of a gene. L.I.Ge.M.A. provides a transgenic mice production service on the basis of researcher's demand.



Gene-Targeting:

By "loss-of-function" approach is possible to study what happens in absence of a mouse target gene. The technology, named "gene knock-out", is well defined in murine model. Knock-out phenotype analysis can lead to define the function of target gene in physiological and pathological processes.

The most important step when generating a knock-out mouse is represented by the preparation of knock-out ES cell lines. It requires the homologous recombination of the endogenous target gene with an its modified, non functional, copy. The obtained ES cells are then injected in recipient blastocysts, that are then implanted in the uterus of a pseudo pregnant female. The injected ES cells mix with the staminal cells of blastocyst's inner cell mass (ICM). The newborn mice will be chimeras, as both injected and stem cells of recipient blastocyst contribute to the mouse development.



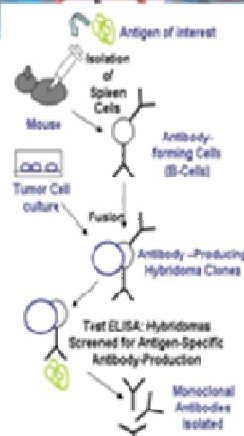
Monoclonal Antibodies

L.I.Ge.M.A. has a very efficient monoclonal antibody production service using BALB/c mice immunized with different kind of antigens provided by the customer.

This service includes the possibility to choose one or more hybridomas after the first screening and to proceed with purification and characterization of antibodies.

Producing monoclonal antibodies normally takes 20-24 weeks, which can be divided into three stages: immunisation and serum tests, fusion and ELISA screening, subcloning and isotyping. We shall report our progresses to customers at each stage so that customers will know exactly what is going on just like they do in their own labs.

At the screening stage, many positive clones may be found. Customers will decide how many cell lines they want to have further subcloned. At the end of each project, we shall provide customers stable subcloned cell lines. On requests by customers, we shall do antibody further purification and characterisation.



Polyclonal Antibodies

L.I.Ge.M.A. provides an efficient service for the production of polyclonal antibodies from rabbits. The service is offered in different formats, based on customer's demand. Producing polyclonal antibodies normally takes 12 weeks. Antibody titre in serum is checked by ELISA.

L.I.Ge.M.A. also offers additional services: Antibody purification by affinity chromatography, Antibody labeling, Immunometric assay. Approx. 2-3 mg of protein antigen are necessary for immunization and analysis.

**FIFTH ANNUAL SCIENTIFIC CONFERENCE
ISTITUTO TOSCANO TUMORI
S. Giustino Valdarno (AR), July 1, 2010**

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Stecca	Barbara	Unit Research CRL - ITT	AOU Careggi	Firenze
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