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The Candiolo Cancer Institute
The Candiolo Cancer Institute is a private non-profit institution founded and supported by the Fondazione Piemontese per la Ricerca sul Cancro-Onlus (FPRC) and operated by the Fondazione del Piemonte per l’Oncologia (FPO: co-founded by FPRC and the Regione Piemonte). The Institute is a recognized IRCCS (Istituto di Ricovero e Cura a Carattere Scientifico) by the Italian Ministry of Health. The Candiolo Cancer Institute works in synergy with the University of Torino Medical School. Its mission is a significant contribution to fight cancer, by understanding the basics, and by providing state-of-the-art diagnostic and therapeutic services. The interface between molecular biology and medicine is at the core of the Institute. FPRC provides enduring fund raising to complete and develop the Institute’s buildings and technologies in order to foster research.
The Candiolo Cancer Institute is a biomedical and clinical research center entirely devoted to the study and the treatment of cancer. Its mission is to transfer experimental preclinical information into clinical practice, through the continuous flow of knowledge from the fields of genetics, molecular and cell biology and pathology. The Institute aims to offer a significant contribution to the defeat of the disease through scientific research and clinical practice of excellence. In order to fulfill these goals, the Institute: (i) capitalizes on knowledge by conducting scientific research in oncology and – at the same time - by promoting fast transfer of knowledge to the clinical practice; (ii) provides assistance in cancer prevention, including the identification of genetic risk factors; (iii) performs diagnostic studies, using state-of-the-art instrumentation and technology; (iv) provides a full cycle of treatment on the premises, employing besides the best conventional therapies - protocols for novel targeted therapies and clinical trials for ‘precision medicine’.

Focus
Clinical and basic research makes the Institute of Candiolo a center of excellence focussed on the study, prevention, and treatment of the dreadful complication of cancer, metastasis.

Molecular diagnosis and “precision” medicine
The success of targeted therapies is based on the rationale that the target molecule is ‘druggable’ - as a consequence of the genetic anomaly - in the tumor but not in healthy tissues. Therefore the target molecule deactivation has consequences restricted to the neoplastic mass, with a minimum of ‘off-target’ consequences, leading to generic organ damage. This notion has two important clinical outcomes: first of all, before treating patients with a given targeted therapy, it is necessary to verify the presence of the genetic lesion “predictive” of sensitivity to the drug. Second - in the perspective of targeted therapies - tumors will be classified not only according to their site of origin and/or morphological features, but also by the molecular lesion(s) which earmark them and, at the same time, make them vulnerable to a targeted treatment. Therefore, new therapies are not only “targeted” but also “personalized”. This new approach is called “precision medicine”.

Clinical Research
At the Institute, oncologists, surgeons and radiotherapists cooperate with scientists to design clinical trials based on molecular data, intended to verify hypotheses and generate novel ones. In order to make this cooperation productive, the Institute manages a daily net of interactions involving formal aspects (seminars and meetings), training (refresher courses and lessons), and operational efforts (contacts with pharmaceutical companies, and management of regulatory instruments). Clinical Research is the last and more direct haven to improve the assistance to cancer patients, providing them with the most appropriate, novel, safe and effective therapeutic approach, in accordance with the genetic profile of their own tumor, as an ultimate means of increasing their life expectancy.
Investigational Clinical Oncology (INCO)
Cancer is a complex disease, tied to genetic lesions that increase in number over time, as a consequence of genetic instability and exposure to environmental carcinogens. Thus, a cell clone proliferates and invades the adjacent tissues without control. Cancer cells show several genetic anomalies that tend to increase as time passes. Cancer’s heterogeneity makes it a hard-to-attack disease because of its multiple and continuously changing target. The molecular lesions that cause and sustain most tumors are, however, finite in number (the bona fide oncogenes). These findings have driven clinical pharmacology to commit to an epochal effort to create drugs, called “targeted”, able to contrast the function of specific oncogenes. The strategy of the Institute at Candiolo intends to make significant contributions to the field of cancer targeted therapy by: (i) identifying pathologies and recruiting patients sensitive to the therapies currently in use; (ii) planning and performing, in international networks, the related clinical trials; (iii) developing translational and preclinical research aimed at designing new targeted therapies.

Translational Research (ECMO: Experimental Clinical Molecular Oncology)
Translational research is the “heart” of the Institute, bridging the gap between basic and clinical research. Based on recent technological advances, genomic analysis makes possible—in a significant percentage of cases—the identification of genetic alteration(s) with a “driver” role in tumor development. However, the contribution of each lesion to the transformed phenotype remains elusive. Moreover, there is still insufficient knowledge of the mechanisms that control the lack of response to targeted therapies, even in the presence of the molecular target (primary resistance), as well as of the mechanisms that lead to a progressive attenuation of the response after prolonged treatment (acquired resistance). ECMO research aims at the fulfillment of some ambitious goals to integrate the traditional prognostic and diagnostic factors with a detailed characterization of the genetic and functional alterations of the tumor; to identify new malfunctioning regulatory pathways in cancer; to isolate and study cancer stem cells; to develop new preclinical platforms that can reliably disclose and understand in detail—the prospective results of clinical practice. The generation of this kind of knowledge is necessary to design clinical trials that will no longer be based on empirical observations but on a strong rationale.

Basic Research
The current knowledge of the mechanisms of cancer onset and progression is provided by basic disciplines, such as genetics, cell and developmental biology. Thanks to these studies, it is possible to classify tumors not only according to the basis of their site of origin and histopathological features, but, notably, according to the identification of the genetic lesion(s) that support their growth. Basic Research tasks at the Institute are aimed at understanding the mechanisms that control normal cell functions responsible for proliferation, and at analyzing how these mechanisms are corrupted during neoplastic transformation. The topics include signal transduction, DNA duplication, cell division, differentiation, senescence, apoptosis and cell motility. Recent studies suggest that anti-neoplastic therapy is really effective not only when it hits the appropriate molecular target (vide infra), but especially when it hits the cells feeding the tumor mass. Indeed, most of the cells of the neoplastic mass are quite innocuous and can be attacked with classical therapies, while only a small fraction of them are resistant to treatment and able to regenerate the tumor. This small subpopulation includes the “cancer stem cells”—strictly related to normal “stem cells”—which control the development of our organism during embryonic life and allow us to renew worn parts in adult life. The up-to-date conceptualization of Cancer defines it as a “somatic, genetic disease of the stem cells”.

Paolo M. Comoglio MD
(Scientific Director)
TWENTY YEARS OF RESEARCH AT THE INSTITUTE
A story of courage and success

In the fall of 1996, even before the official opening, the most popular Turin daily newspaper dedicated a series of full-page ads to the brand-new Candiolo Cancer Institute. It was a truly innovative advertising campaign in terms of communication: big photos taking up the entire page and a short catch phrase at the bottom. One of the most incisive pages recited something like, “In Candiolo, the light never goes out” and the photo captured the bright windows on the fourth floor of the west wing, on an ordinary night, rather late. That lab on the fourth floor had been installed in June of that year and was directed by a professor in his 50s, with a solid and successful research experience behind him and ambitious ideas for the future. He could count on the work of a dozen young researchers, as ambitious as he was.

Twenty years have passed since then. The lab on the fourth floor does no longer exist: in the summer of 2015, it was relocated to the three large floors of the new research tower; the director of the small lab has become the scientific director of the Institute; the young researchers have reached their 50s (some more, some less) and have forged their ways within the international scientific community with brilliant discoveries and successes. Meanwhile, new research labs were opened up and new scientists were recruited (often from abroad), giving their precious contribution to the image and the visibility of the Institute with their own discoveries and successes. Young generations have been formed and are now about to take flight. Over the years, serial installments of state-of-the-art technologies have rapidly grown old – as it often happens with this kind of instruments – but have been promptly replaced with the latest models available. Research has developed and modified year after year, answering and following the needs of the scientific community: the study of the invasion processes and metastases, and of the mechanisms that generate new tumor vessels, first evolved into the exploration of the molecular bases underlying response to non-chemotherapy targeted drugs, and then into the analysis of how the tumors that initially respond to therapy become resistant after a certain period of treatment.

At the same time, the Institute has grown in terms of clinical assistance: in rapid sequence in-patient bed areas, the ORs, the Outpatient Surgery and Oncology Units, and the Pathology and Clinical Biochemistry Divisions were opened; the patients were ever increasing; the most advanced instrumentation (and often the only one available in the whole Italian landscape) for diagnosis and therapy was set up. Over time, researchers and clinicians started talking each other and working together, finding a common language to identify shared goals. On the one hand, researchers acknowledged the unmet medical needs that had to be addressed by experimental approaches. On the other hand, clinicians recognized the value of molecular analysis to inform diagnostic and therapeutic approaches. As a reward for all these efforts, in 2013, along came the acknowledgment of the Institute as a Comprehensive Research Center (IRCCS). Candiolo is now one of the few centers of excellence meticulously evaluated by the Italian Ministry of Health, which monitors the fulfilment of extremely high standards of scientific production and healthcare.

The lights of Candiolo are still on when it’s dark outside. Sometimes they faded, when the night was falling, to honor the rest of those who lost the battle; but over these twenty years, they have shone on the all-watched nights of the researchers performing their long experiments and on the night shifts of doctors and nurses, and have brightened the way home for the many who made it through.

Livio Trusolino MD
1996, The Pioneers
RESEARCH SYNOPSIS

Topic 1: The mechanisms of onset and progression of cancer
1.1 Role of Semaphorins in invasion, metastasis and angiogenesis
1.2 The plasma membrane in cell migration and invasiveness
1.3 Tumor angiogenesis

Topic 2: From Molecular Biology to ‘Precision Medicine’
2.1 Oncogenes and growth factors
2.2 Genetics of the response to anti-cancer therapy
2.3 Preclinical models of ‘personalized’ therapies
2.4 Resistance to targeted therapy

Topic 3: Investigative clinical research: rationale for the planning of clinical trials
3.1 HERACLES and ARES: ‘Precision medicine’ trials for metastatic colon carcinoma
3.2 HERLAP: ‘Personalized’ treatment of mammary carcinoma
3.3 AGNOSTOS: Therapy of metastatic cancers of unknown primary origin

Topic 4: Applied clinical research
4.1 Colorectal cancer: onset and progression
4.2 Implementation of current therapeutic strategies
4.3 Novel approaches to surgical oncology
4.4 Radiotherapy, imaging and laboratory medicine
The cell ‘scattering’: a complex biological phenomenon resulting from activation of a cascade of events triggered by the MET oncogene
Research Topic 1: 
Mechanisms of cancer onset and progression

The current knowledge of mechanisms of cancer onset and progression has been provided by basic disciplines, such as cell biology, developmental biology and genetics. The tasks of Research Topic 1 are aimed at understanding the mechanisms controlling the normal cell functions responsible for the regulation of cell proliferation and at analyzing how these mechanisms are corrupted during neoplastic transformation. The topics include signal transduction, DNA duplication and cell division processes, differentiation, senescence, apoptosis and cell invasion. Special attention is given to the microenvironment and to the relations of the neoplastic cell with adjacent cells. The angiogenic process and the vascularization of the tumor mass have been studied in detail in the past.

Research Topic 1 is focussed on:
1.1 The role of Semaphorins in invasiveness, metastases and angiogenesis
1.2 The plasma membrane in metastasis dissemination
1.3 Tumor angiogenesis
Semaphorins: structure & functions

The elucidation of the functional role and molecular mechanisms through which Semaphorin genes control the invasiveness of tumor cells and the formation of metastasis will prompt the development of innovative diagnostic and therapeutic approaches

Team
Michael Rehman, Sabrina Rizzolio, Lorena Capparuccia, Chiara Battistini, Gabriella Cagnoni, Gurrapu Sreeharsha, Massimo Accardo

Research topic:
Semaphorins and Semaphorin Receptors in Cancer Progression.

Background:
Semaphorins and their receptors (Neuropilins and Plexins), beyond their role in embryo development and morphogenesis, are relevant players in cancer progression and emerging targets for innovative therapeutic approaches. Semaphorins form a conserved family of over 20 members in vertebrates, including transmembrane molecules and secreted members. Secreted semaphorins often act to restrict cell migration; for instance, they can negatively regulate angiogenesis, preventing its pathological role in cancer. Certain semaphorins can play diverse functional roles in different cell populations, due to the involvement of distinctive receptor complexes and signaling cascades. Our lab demonstrated that Sema3A and Sema3E are relevant targets for innovative therapeutic approaches, as they can regulate cancer cells and stromal cells in concomitant manner in the tumor microenvironment. In addition to their role as semaphorin and VEGF receptors, we and others found that neuropilins represent receptor hubs on the cell surface, controlling the function of multiple signaling cascades that support tumor growth, such as EGFR and integrins.

Research achievements:
The laboratory has been working on several aspects of the research topic. We studied the function of Plexin-D1 and Neuropilin-1 receptors in cancer cells and in the tumor microenvironment, and found that they can play multiple roles in cancer progression. For instance, oncogene addicted tumor cells use Nrp1 to uphold survival signaling cascades in response to targeted therapies. Moreover, Nrp1 mediates Sema3A-dependent recruitment and localization of tumor associated macrophages in hypoxic tumor areas, where they can deploy their pro-angiogenic and immune-suppressor activity. Beside secreted family members, transmembrane semaphorins are also currently investigated in the lab, such as Sema4C and Sema6A. We accomplished novel and exciting results about their role in cancer cells, regulating their intrinsic metastatic potential and therapeutic response to targeted therapies. The lab is also a leader in semaphorin research at international level, contributing expert know-how and reagents to frontline collaborative studies.

Conclusions and perspectives:
Semaphorins and their receptors are novel and exciting players in cancer progression. By understanding the specific role of different family members in human cancers, we could validate novel predictive markers of progression or response to innovative targeted therapies. Moreover, certain semaphorins (and receptors) have already been put forward as potential targets for functional interference approaches, aimed at regulating angiogenesis, cancer cell invasiveness, and metastatic progression.
Biographical Sketch:
Luca Tamagnone, MD, PhD started his research activity with Paolo Comoglio. During his training in the lab of Kari Alitalo, he discovered new genes encoding tyrosine kinases, such as RYK and BMX (published in Oncogene, 1993 and 1994). Returning to Italy, Dr. Tamagnone started working on a new family of receptors, the Plexins (described in PNAS-USA, 1996). Three years later he published the identification of plexin ligands, the Semaphorins (in Cell, 1999). As tenured Associate Professor at the University of Torino, and Young Investigator of the European Molecular Biology Organization, in 2001, Dr. Tamagnone started his own lab in the Institute for Cancer Research of Candiolo-Torino (IRCC). He focused his attention on semaphorin/plexin activities in cancer cells, identifying fundamental signaling mechanisms and functional properties in the regulation of tumor progression (e.g. Neuron, 2001; Nat Cell Biol, 2002; Nature, 2003; FASEB J, 2004; J Cell Sci, 2005). By exploiting experimental models in vivo, he found that semaphorin signals regulate multiple steps of tumor progression: from tumor growth, to angiogenesis, to the recruitment of tumor-associated leucocytes, to the formation of distant metastases (e.g. J Exp Med, 2008; J Clin Invest, 2010; EMBO Mol Med, 2012), validating their relevance as targets for molecular therapy. Moreover, recent findings have revealed novel functions of the semaphorin co-receptor Neuropilin-1 in the regulation of EGFR oncogene signalling (Cancer Res, 2012), as well as in the recruitment of tumor associated macrophages (Cancer Cell, 2013).

Selected references


Serini G, Tamagnone L. Bad vessels beware! Semaphorins will sort you out! EMBO Mol Med. 2015 26;7:1251

Dynamic control of cell adhesion: ‘normalization’ of tumor blood vessels

The pharmacological modulation of integrin function can be therapeutically exploited to improve the biodistribution of anti-neoplastic drugs and counteract the hypoxia-driven metastatic dissemination of cancer cells

Team
Donatella Valdembri, Chiara Camillo, Noemi Gioelli, Giulia Mana, Chiara Sandri, Giulia Villari

Research topic:
Grasping the molecular basis of cell adhesion dynamics to design effective ‘vascular normalizing’ drugs for anti-cancer therapy.

Background:
Tumor blood vessels are structurally and functionally aberrant, thus they hamper the delivery of anti-cancer drugs and cause hypoxia-driven metastatic dissemination. Normalizing the vasculature of tumors could hence sizably improve anti-cancer therapy. The binding of vascular endothelial cells (ECs) to the extracellular matrix (ECM) is mediated by integrins, a class of adhesive receptors that can assume active or inactive conformations, respectively characterized by high or low affinity binding to ECM ligands. Blood fluid shear stress is the main determinant of normal vascular architecture and function, its foremost outcomes being that of triggering integrin activation and fostering EC-to-ECM adhesion. As a direct consequence, ECs modify their adhesive interactions and reciprocal positions in space, finally giving rise to a remodeled, mature, and functional vascular tree. In this framework, inhibitors of integrin function should warrant a swift responsivity of EC-to-ECM adhesion to variations in blood flow-elicited forces. Therefore, pharmacological modulation of integrin function might be therapeutically exploited to ‘normalize’ the tumor vasculature.

Research achievements:
We have previously shown how: (i) the chemorepulsive guidance cue semaphorin 3A (Sema3A) and its receptors plexins and neuropilin-1 (Nrp1) allow correct vascular morphogenesis by negatively modulating the conformational activation of integrins and inducing active/ECM bound integrin endocytosis; (ii) the abrogation of Sema3A-dependent integrin-inhibitory signals underpins the alterations that characterize tumor blood vessels; (iii) somatic gene transfer of Sema3A restores the physiological inhibition of endothelial integrins and effectively prevents cancer vascular abnormalities, thus improving the penetration of anti-neoplastic drugs and impairing metastases. In collaboration with the Laboratories of Transgenic Mouse Models and Cancer Cell Biology of our Institute, we generated a parenterally-deliverable mutant Sema3A* protein that, thanks to its ability to bind with high affinity the signaling receptor plexin A4, is much more active than its wild type counterpart both on cultured ECs and in mouse models of pancreatic cancer. More recently, we identified: i) a novel Sema that is endowed with an unpredictable in vivo pro-angiogenic activity ii) a novel signaling pathway that, by coordinating the endocytosis of active integrins and the exocytosis of freshly synthesized ECM, controls blood vessel formation and patterning both in vitro and in vivo.

Conclusions and perspectives:
Our data provide evidence of how the negative pharmacological modulation of endothelial integrins by Sema3A can be therapeutically exploited to improve the biodistribution of anti-neoplastic drugs and counteract the hypoxia-driven metastatic dissemination of cancer cells. It will therefore be crucial to further characterize the mechanisms and molecular determinants responsible for: (i) the inhibition of integrin-dependent EC adhesion by Sema3A; (ii) the selective Nrp1-dependent control of active integrin traffic in ECs.
Selected references


Biographical Sketch:
Over the last two decades, Guido Serini has investigated the role of integrin-mediated cell adhesion in the control of tumor progression and angiogenesis. He firstly reported (J. Natl. Cancer Inst. 1996) how the neo-expression of alpha6-beta4 integrin, now well-known for its pro-metastatic activity, as a hallmark of the transition of human benign adenomas into malignant carcinomas. He also demonstrated (J. Cell Biol. 1998) how the generation of pro-invasive and pro-angiogenic cancer associated fibroblasts requires integrin-triggered signals. He then focused on the mechanisms that, by tuning the conformational activation of integrins, control physiological angiogenesis and are disrupted in cancer, finally resulting in hypoxia-driven metastatization. After reporting that autocrine VEGF is critical for integrin activation and vascular network formation (EMBO J., 2003), he showed for the first time how endothelial class 3 semaphorins (SEMA3) signal through plexin receptors to inhibit integrins and allow vascular morphogenesis (Nature 2003). Next, together with Dr. Giraudo, he discovered that SEMA3A is present in endothelial cells of pre-malignant lesions, is lost during tumor progression, and, when therapeutically reintroduced, is able to normalize the vasculature, to inhibit tumor growth, and to extend survival (J. Clin. Invest., 2009). He recently unveiled how vascular morphogenesis relies not only on integrin activation, but also on dedicated signaling pathways that control active integrin traffic back and forth from adhesion sites (PloS Biol. 2009; Cell Res. 2012; Curr. Opin. Cell Biol. 2012).
New targets for anti-angiogenic therapy

The better understanding of the anti-metastatic and normalizing effects of Sema3s on tumor blood vessels, will allow to design new strategies in order to overcome the resistance to anti-angiogenic therapy

Team
Federica Maione, Yaqu Qiu, Marco Soster, Alessia Visentin

Research topic:
Semaphorins: new tools to “normalize” the tumor microenvironment and to halt metastasis formation.

Background:
Angiogenesis is required for invasive tumor growth and metastasis. It is well described that tumor vessel normalization represents a remarkably advantageous anti-cancer strategy, reducing tumor hypoxia and also being able to favor chemotherapy delivery and response to radiotherapy. It is critical therefore to identify new “pro-normalizing” modulators to define new anti-angiogenic combinatory regimens to block tumor growth. In these years, several studies have showed that class3 semaphorins (Sema3s) - that act via receptor complexes binding neuropilins 1 and 2 (Nrp1/2) and transducing the signal by plexins (Plxns) - represent new targets to inhibit tumor angiogenesis and cancer growth.

Research achievements:
Sema3A has been uncovered as a new vessel normalizing and anti-metastatic agent in mouse models of spontaneous pancreatic neuroendocrine tumors (RIP-Tag2) of cervical carcinomas (HPV16/E2), of pancreatic adenocarcinoma (PDAC) and of breast cancer (4T1). Interestingly, the treatment of mice with adeno-associate virus AAV-8-Sema3A counteracted the resistance to the anti-angiogenic therapies by normalizing the tumor vasculature, inhibiting hypoxia and several hypoxia-induced pro-metastatic signaling pathways. Sema3A was able to recruit into tumors a sub-population of CD11b+, Nrp-1+, GR-1- and Tie-2- monocyte (NEMs) inhibiting tumor progression and to normalize the tumor vasculature. Notably, Sema3A acted directly on tumor cells. In fact, inhibited HGF-induced Met phosphorylation and impaired the chemo-invasion of several met-addicted tumor cell lines, by interfering with Met recycling and internalization. Remarkably, Met phosphorylation was strongly inhibited both in vessels and cancer cells in Sema3A-treated PDAC mouse model. In collaboration with the Laboratories of Cell Adhesion Dynamics of our Institute, we generated a furin-resistant mutant Sema3A* protein that, binding with high affinity with plexin A4, efficiently inhibited metastasis formation and normalized the tumor vasculature in mouse models of pancreatic cancer. We uncovered together a novel Sema that is endowed with an unpredictable in vivo pro-angiogenic activity.

Conclusions and perspectives:
Our findings provide evidences that Sema3A, by acting on several tumor cell types and activating the immune-system, blocks tumor growth and metastasis spreading in different tumor types. Based on these findings the main focus of research are the following: (i) investigation of the molecular mechanisms by which Sema3A turns off the HGF/Met pathway in different stroma cell types and cancer cells; (ii) evaluation of new therapeutic combinatory strategies to enhance with Sema3A (and other Semas) the anti-tumor immune response in several mouse models of cancers; (iii) evaluation of the anti-metastatic and pro-vessel normalizing effects of mutant Sema3A* protein in different human mouse models of pancreatic, breast, cervical, and colon cancer.
Biographical Sketch:
Enrico Giraudo has contributed with a high quality track record of publications to the research field of tumor angiogenesis. Since the beginning, he has contributed to the identification of the molecular mechanisms that regulate the motility and differentiation of angiogenic endothelial cells (J. Biol. Chem, 1998; Nature Med. 1996; J Biol Chem. 2003). His main focus has been the study of the mechanisms regulating tumor angiogenesis and cancer progression in transgenic mouse models of tumorigenesis. He described the molecular zip-code deciphering blood and lymphatic heterogeneity during tumorigenesis (Cancer Cell. 2003). Then, he uncovered an important role of matrix metallo-proteases (MMP)-9 and macrophages on tumor angiogenesis in a transgenic model of cervical cancer, by employing an amino-bisphosphonate Zoledronic acid able to efficiently inhibit cancer growth (J Clin Invest. 2004). More recently he demonstrated that the axon guidance cue Semaphorin 3A is an endogenous angiogenic inhibitor and that its therapeutically re-expression in cancers impairs angiogenesis, tumor growth and induced vessel normalization in mouse models of cancer (J Clin. Invest. 2009). On these bases, he showed that Sema3A was able to overcome resistance to anti-angiogenic therapies, by normalizing tumor vessels and inhibiting hypoxia (J. Clin. Invest. 2012).

Selected references


Role of membrane receptor endocytosis in the dissemination of metastatic cells

Knowing the mechanisms that control the downregulation of receptors involved in metastatic cell dissemination provides the rationale for the generation of innovative anti-neoplastic drugs

Team
Daniele Avanzato, Emanuela Pupo, Nadia Ducano

Research topic:
Role of endocytosis in the regulation of signaling by oncogenic tyrosine kinase receptors.

Background:
The increased activity of receptor tyrosine kinases (RTKs) is recognized as one of the most common alteration in cancer. This is mainly achieved by the occurrence of activating mutations, or by gene amplification. Beside these mutational events, other mechanisms may occur to increase the RTKs activity. Among them endocytosis and receptor degradation play a relevant function. Although mutations in endocytic genes have crucial roles in the manifestation of the neoplastic phenotype, the possibility to exploit endocytic proteins as targets of pharmacological intervention, alone or in combination with other treatments, are still poorly investigated. Research in our unit addresses the role of key endocytic players, the small GTPase Rab5 and its negative regulator RN-tre, as well as the effects of endocytosis inhibition, in cancer cell proliferation and invasion.

Research achievements:
We found that withdrawal of tyrosine kinase inhibitors can exert rebound cancer cell proliferation and in vivo tumor growth. Cancer cells bearing the amplification of the MET receptor depend on MET for growth and survival. Treatment with a specific kinase inhibitor halts their proliferation, but, upon inhibitor discontinuation, MET phosphorylation and downstream activation resume with higher levels if compared to untreated cells. This generates a “rebound” effect pushing quiescent cancer cells back into the cell cycle both in vitro and experimental tumor models in vivo. We investigated the function of RN-tre in breast cancer cells. We found that RN-tre silencing reduces AKT phosphorylation, while its overexpression in a normal mammary cell line promotes 3D overgrowth of acinar-like sphere.

Conclusions and perspectives:
Database analyses has revealed that RN-tre is amplified/overexpressed in 16% of breast tumors. Based on its function in receptor signaling and cell adhesion, we hypothesize that RN-tre overexpression in breast cancer cells might confer proliferative advantages by upregulating oncogenic tyrosine kinase receptors, and/or promoting anchorage-independent growth and cell survival. Based on our findings on MET kinase inhibition, we plan to address the effects of derailed endocytosis in tumor recurrence. We will study the rebound effects on proliferation of cancer cells that were initially subjected to ATP-competitive RTK inhibitors and then released from the inhibition, trying to mimic what might happen in patients treated with similar drugs in a time window. We will address whether alternate or metronomic treatment with RTKs inhibitors and therapeutic antibodies might counteract this harmful effect in vivo.
Biographical Sketch:
Letizia Lanzetti completed her training at the Department of Medical Genetic in Torino where she participated in the isolation of a novel gene family: the endophilins. Next, she joined the European Institute of Oncology in Milan to study the impact of endocytosis on the signal transduction cascade. Her work pioneered the existence of crosstalk between these processes as described in two of her major publications (Nature, 2000; Nature, 2004). In 2008 she established her laboratory at the Candiolo Cancer Institute. Here, she revealed a novel function for endocytic proteins in cancer cell division (PNAS 2011), and in cell migration and invasion (Curr. Biol. 2013).

Her work has led to the identification of molecular pathways that control signaling and trafficking of receptors involved in cell transformation and invasion. Currently, she is widening her research looking at the feasibility of targeting endocytic molecules, in combination with other treatments, in cancer therapy.

Selected references


The mechanisms of cancer onset and progression | 21
From neuronal biology to neoplastic progression

The identification of ‘molecular keys’ that mediate the relationship between tumor cells, blood vessels and nerves will contribute to the definition of a new class of therapeutic targets

Team
Laura Bizzozero, Margherita Pergolizzi, Elena Riccitelli

Research topic:
Neuroligin: a novel modulator of cell transformation and cancer diffusion through nerves.

Background:
Neuroligin (NLGN) is a neuronal and tumoral adhesion protein. Tumor-nerve interactions are a clinically significant, but often underestimated way of cancer diffusion, including colorectal cancer (CRC). Tumor and nerve relations occur through Perineural Invasion (PNI), namely the invasion of nearby nerves by cancer cells, and neo-neurogenesis, or the stimulation of neurite outgrowth by cancer through soluble signals. These aspects represent different conditions of a wide range of molecular interactions that are nevertheless poorly defined. There is no specific therapy able to target tumor nerve interactions. Therefore, new molecular players and therapies are needed.

Research achievements:
This laboratory has been working on the extra-neuronal activities of NLGN for the last ten years. Since 2013 we have focused on the following questions on the role of tumoral/nervous NLGN (mainly in CRC): (a) How does it impact tumor “autonomous” cell behaviour? (b) Does it modulate PNI and neo-neurogenesis? The results obtained to date show that NLGN inhibits tumor cell proliferation, anchorage-independent growth and in vivo tumor growth but, conversely, promotes PNI.

Conclusions and perspectives:
If broadly confirmed, our data reveal NLGN as a double-faced cue (i.e. growth suppressing but pro-invasive). We now want to exploit the knowledge coming from the neuronal field, the large amount of reagents accumulated in this laboratory, and both in vitro and in vivo experimental settings (mouse xeno- and ortho-transplants) in order to fully answer the questions stated above.
Biographical Sketch:
Marco Arese has dedicated his early research activity to the study of various mediators of intercellular communication that involve cancer and vascular cells (J Clin Invest. 1995). In the laboratory of Prof. Daniel B. Rifkin, at the New York University Medical Center (USA), he examined the mechanisms of action of two growth factors: Fibroblast Growth Factor-2 (Mol Biol Cell. 1999) and transforming growth factor-beta (TGF-beta). For the last fifteen years, he has devoted himself to studying the “molecular parallels” between the nervous system and vascular and tumoral tissues, with the aim of finding new targets that drive the cooperation between the tumor and its “micro-environment” (specifically, infiltrating vessels and nerves). In turn, this cooperation is known to fuel disease progression. Initially, he discovered that two synaptic proteins, Neurexin and Neuroligin, have widespread functions in the vascular system, including tumor angiogenesis (Proc Natl Acad Sci U S A. 2009; Arterioscler Thromb Vasc Biol, 2012). He is currently focused on the role these two nervous proteins - still widely underestimated - play in the tumor/nerves connections.

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Samarelli AV, et all. Neuroligin 1 induces blood vessel maturation by cooperating with the α6 integrin. The Journal of biological chemistry. 2014, 289:19466-76


Cellular and molecular mechanisms sustaining tumor angiogenesis

All target therapies, including antiangiogenic therapies, are far from achieving the wished results. However, antiangiogenic regimens are an important tool for solid tumor treatment. Our studies are aimed at understanding the complexity of angiogenic processes in physiology.

Team
Serena Marchiò, Elena Astanina, Valentina Comunanza, Gabriella Doronzo, Lucia Napione, Alessio Noghero, Davide Corà, Maria Alvaro, Anna Gualandris, Stefania Rosano, Emanuele Middonti, Emanuela Doronzo

Research topic:
Identification of early biomarkers of pancreatic ductal carcinoma (PDAC).

Background:
Elegant tracking studies of the genetic evolution of PDACs indicate at least 15 years between the occurrence of the initiating mutation and the acquisition of metastatic ability. This result demonstrates a broad time window of opportunity for early detection to prevent deaths from metastatic disease. We reasoned that an early biomarker had to play a necessary role in the onset of the disease and it had to be preferentially released from the cells. Therefore we performed a whole transcriptomic analysis of the early in situ carcinomas (PanIN) isolated by laser capture microdissection from a genetically engineered mouse model characterized by the expression of KRASG12V in acinar cells through the use of the elastase promoter (Elas-K-RasG12V).

Research achievements:
RNAs highly expressed in PanIN encode proteins involved in axon guidance cues and synaptogenesis that have been recently demonstrated to be altered in PDAC. We focused on neuregulins (Nlg), a family of adhesive molecules regulating synaptic activity. Anti-pan-Nlgs antibody showed a faint membrane signal of acinar and ductal cells whereas PanIN cells displayed a stronger staining that dropped in PDAC. Similar results were obtained in human lesions. By analysing the expression of the 5 Nlgs we visualized a minute population of acinar cells carrying KRasG12V and expressing Nlg-2 associated with stemness markers (DCLK1, CD24, CD44, and CXCR4). The current hypothesis of the pathogenesis of PDAC relies on the differentiation of acinar precursors carrying KRAS mutation into ductal cells (acinar-ductal metaplasia). We demonstrated that KRasG12V acinar cells isolated from Elas-KRasG12V underwent in vitro acinar ductal metaplasia with an increased expression of Nlg-2. An isogenic cell line carrying KRasG12V overexpressed Nlg-2 if compared with wild-type cells. Loss-of-function experiments showed that Nlg-2 ablation halted acinar-ductal metaplasia, supporting the idea that Nlg-2 is necessary in the early phase of PDAC. Then, we considered the possibility that Nlg-2 was shed by the cells, supported by the presence of a Disintegrin And Metalloproteinase (ADAM) proteolytic cleavage site in the extracellular domain. Nlg-2 accumulated in the serum-free supernatant of pancreatic PT45 human cell line overexpressing Nlg-2. This phenomenon is blocked by ADAM inhibitors TAPE-2 and GM 6001.

Conclusions and perspectives:
We reported that Nlg2 (i) has an early role in PDAC by promoting the acinar-ductal metaplasia of precursors; (ii) is shed by an ADAM-dependent mechanism. To further support the role of Nlg2 as an early biomarker in PDAC, we will continue the following activities: (i) demonstration of the role of Nlg2 in stemness features of acinar precursors undergoing acinar-ductal metaplasia; (ii) analysis of PDAC progression in Elas-K-RasG12V model crossed-back with Nlg-2/- mice (iii) generation of mAb anti-Nlg2 to set-up an ELISA for Nlg2 quantification in biological fluids; (iv) increase of human samples to analyse Nlg2 expression and activities.
Biographical Sketch:
Federico Bussolino began his scientific career by investigating the cross-talks between the mediators of inflammation and vascular cells with the goal of understanding the cellular traffic across vascular walls. Then (1988), he was captured by the discovery of colony stimulating factors and their strict specificity during hematopoiesis. He has gone beyond this concept, showing that these molecules activate a pro-survival and pro-angiogenic program in endothelial cells (Nature, 1989). After 20 years, the proangiogenic roles of these molecules have clarified their fundamental role in the bone marrow vascular niche, which is fundamental in the maturation of blood cells. Then he moved to tumor angiogenesis showing the role of the MET receptor in cancer-associated vascularization (J Cell Biol, 1992). This seminal observation supported the concept that MET is pivotal in regulating cancer invasive growth. In 1996, he began to work on VEGFs showing that HIV-1 Tat activates the VEGF pathway. This result emphasizes the ability of the virus to directly support the progression of tumors appearing in AIDS (Nat Med., 1996; Nat Med, 2002). He firstly demonstrated (Embo J., 1999) the existence of a dynamic platform constituted by adhesive and tyrosine-kinase receptors. In 2003, he funded a successful interdisciplinary PhD program on complexity in biology that has anticipated the concepts of Systems Biology. In 2003, he contributed to open a frontier in angiogenesis, by connecting the 3D architecture of the vascular bed with the mechanisms that wire neurons. (Nature, 2003). Recently, he has added another piece of information about the cues shared by the brain and vascular system by demonstrating that two synaptic proteins, neurexin and neuroligin, regulate the assembly of vascular units.

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The mechanisms of cancer onset and progression | 25
Cell migration in tumor angiogenesis and invasion

The identification of molecular mechanisms involved in tumor cell migration will allow the prevention of metastatic dissemination by pharmacological inhibition

Team
Laura Di Blasio, Alberto Puliafito, Giulia Chiaverina, Paolo Gagliardi, Desiana Somale

Research topic:
Molecular mechanisms that differentiate invasive cancer cells from their non-motile counterparts, and the relationship between tumor and non-tumor cells during cell migration.

Background:
Cancer cells spread from the initial site of tumor growth acquiring an invasive phenotype characterized by both the loss of cell-cell interactions and increased cell motility. Cells undergoing this epithelial to mesenchymal transition are then able to move and spread throughout the entire body as isolated and highly motile cells. Recent evidence shows that tumor cells also move as groups both in normal development and in cancer models, in a process named “collective migration”. Even vascular endothelial cells collectively migrate into tumor mass forming new vessels in a process called “tumor angiogenesis”.

Research achievements:
We are developing new three-dimensional (3D) culture systems that in combination with conventional cell biology approaches, allow the study of collective and directional cell migration. A new model for in vitro tumor angiogenesis studies has been described and cell spheroid cultures from breast and prostate cell lines have been developed. With these three-dimensional models we have been able to discover new mechanisms of PI3K regulation of collective migration and the role of the endothelial podosome in tumor angiogenesis.

Conclusions and perspectives:
We believe that spheroids/organoids 3D culture from cells and tissues could overcome limitations of established cell lines, such as adaptation to 2D growth, providing better models to study collective migration, epithelial-to-mesenchymal transition and tumor vascularization, and the identification of molecular mechanisms involved in these processes. Moreover, we are establishing 3D cell cultures from patient-derived xenograft (PDX) of colon cancer with a high percentage of in vitro engraftment. By combining our know-how in 3D cultures with the PDX platform developed in the Institute, we will implement an unprecedented in vitro platform for genetic functional and drug screening purposes.
Biographical Sketch:
Luca Primo has a remarkable experience in the field of vascular cell biology. He started his research experience characterizing the thrombospondin receptor CD36. He showed that this membrane protein was involved in the pathogenesis of cerebral malaria (Blood, 1993; J Immunol, 2001) and angiogenesis (Faseb J., 2005). During his post-doc he contributed to unveil the molecular mechanisms of several angiogenic growth factors, such as VEGF-A (J Biol Chem, 1998) and GM-CSF (Blood, 1997). He then moved to the Candiolo Cancer Institute where he focused his research efforts on the PI3K signalling pathway in angiogenesis and cell motility (Oncogene, 2000). During a research experience at the Tuft University of Boston, he started to study the protein kinase PDK1, a downstream effector of PI3K. He discovered the central role of PDK1 in cell directional motility (J Cell Biol, 2007) and he elucidated the function PDK1 in breast cancer growth (Neoplasia, 2012) and invasion (J Cell Biol, 2014). By gene expression analysis in endothelial cells he discovered the pro-angiogenic role of integrin α6 (Cancer Res, 2010) and the effects of miR126 on Angiopoietin1 signaling (BBA-MolCellRes, 2012). Moreover, he developed a new model for the three-dimensional study of tumor angiogenesis, the Human Arterial Ring assay, which can be exploited for drug screening and gene function analysis on human vessels (Blood, 2013). More recently, he described regulatory mechanisms of integrin endocytosis (Traffic, 2010; J Cell Sci, 2015) and the role of endothelial podosomes in angiogenesis (Nat Cell Biol, 2014). Luca Primo is Associate Professor of Biochemistry and group leader of the Laboratory of Cell Migration.

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The mechanisms of cancer onset and progression | 27
Exploiting glucose ‘addiction’ of KRAS-mutant colorectal tumors for cancer therapy

The development of novel approaches such as anti-metabolic therapy can open new paths to the treatment of aggressive forms of colorectal cancer

Team
Manuela Cazzanti, Alessia Mira, Virginia Morello

Research topic
Enhanced glucose uptake and increased glycolysis are hallmarks of tumor cells. Building on key preliminary results generated in the past years, the Laboratory of Cancer Metabolism is studying a strategy for exploiting these peculiar metabolic characteristics for cancer therapy.

Background
KRAS-mutant colorectal tumors display an exceptionally high glycolysis rate, which makes them ‘addicted’ to glucose. This dependency suggests a potential therapeutic approach based on anti-glycolytic agents, including drugs used for treating diabetes. However, preliminary experiments conducted in our lab suggest that Hepatocyte Growth Factor (HGF), a cytokine that is abundant in the tumor micro-environment, can rescue KRAS-mutant CRC cells from starvation-induced apoptosis. Based on this evidence, we hypothesized that inhibition of HGF or its receptor MET would render KRAS-mutant tumors more sensitive to anti-metabolic therapy.

Achievements
We selected a panel of metabolism-targeting drugs that affected glucose metabolism by different mechanisms and at different levels, including metformin and phenformin (both inhibitors of mitochondrial complex I), dichloroacetate (an inhibitor of pyruvate dehydrogenase kinase), and WZB-117 (an inhibitor of the facilitative glucose transporter GLUT1). Pharmacological analysis of these drugs in isogenic KRAS CRC cell lines revealed that CRC cell clones expressing mutant KRAS are dramatically more sensitive than their wild-type KRAS counterpart to glucose deprivation or to treatment with metformin, phenformin, dichloroacetate or WZB-117. Apoptosis analysis showed that all of these anti-metabolic agents were causing a pronounced increase in the percentage of apoptotic cells, which could be prevented or mitigated by recombinant HGF. In an orthotopic mouse model of KRAS-mutant CRC using human HGF knock-in SCID mice, metformin inhibited tumor growth and inhibited hepatic and pulmonary metastasis. Moreover, anti-metabolic drugs cooperated with the HGF-neutralizing antibody ficlatuzumab in inhibiting tumor growth and in suppressing metastasis.

Conclusions and perspectives
These preliminary data strongly suggest that KRAS-mutant CRC cell metabolic vulnerability can be exploited therapeutically by interfering with glucose metabolism. They also point at micro-environment-derived HGF as a major source of resistance to any kind of therapy that causes metabolic stress to KRAS-mutant CRC cells. Research in the Laboratory of Cancer Metabolism focuses on how to transfer this knowledge into clinical practice.
Biographical Sketch:
Paolo Michieli graduated in Biological Sciences at the University of Milano in 1990. After a few years of training at the Mario Negri Institute for Pharmacological Research and the Istituto Nazionale dei Tumori, both in Milano, he carried out his post-doc in the Laboratory of Cellular and Molecular Biology at the National Cancer Institute in Bethesda, Maryland. During this period he contributed to several scientific publications in the field of oncogenes, growth factors and cell cycle. In 1997 he moved back to Italy to the newly founded Candiolo Cancer Institute in Candiolo, Torino. Since then he has been Group Leader of a small team committed to experimenting novel therapeutic strategies in oncology, with particular reference to the molecular targeting of growth factors and their receptors. In 2001 he specialized in Clinical Pathology at the University of Torino Medical School. In 2008 he received a Ph.D. in Cellular Sciences and Technologies from the same University, and in December 2011 he was appointed tenured Assistant Professor at the Department of Oncology.

Selected references

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Research topic 2
From Molecular Biology to “Precision Medicine”

Research topic 2 is the core scientific basis for most of the Institute's research projects.

Advances in genome sequencing and expression analysis have made it possible to quickly identify genetic alterations with a potential “driver” role in tumor development, with unprecedented informative power. Thanks to these studies, today it is possible to classify tumors, not only according to their site of origin and histopathological features, but also – and especially – according to the identification of the genetic lesions supporting their growth. Genetic lesions' identification is crucial to kill tumors by using targeted therapies. However, the contribution of each lesion to the transformed phenotype remains elusive. Moreover, there is still insufficient knowledge of the mechanisms that control the lack of response to targeted therapies, even in the presence of a molecular target (primary resistance), as well as of the mechanisms that lead to progressive attenuation of the response after prolonged treatment (acquired resistance). Translational Research is a multidisciplinary task based on the expertise of a new generation of scientists, specifically trained to translate molecular information into more effective treatments. The goals are ambitious: to integrate the traditional prognostic and diagnostic factors with a very detailed characterization of the genetic and functional alterations of the tumor; to identify new malfunctioning regulatory pathways in cancer; to isolate and study cancer stem cells; to develop new preclinical platforms that can reliably disclose – and understand in detail – the prospective results of clinical practice. The generation of this kind of knowledge is instrumental to instructing clinical trials that will be no longer based on empirical information but on a strong rational connotation.

Research Topic 2 concerns:
2.1. Oncogenes and growth factors
2.2. Genes responsible for response to antineoplastic drugs
2.3. Preclinical models of “personalized” therapies
2.4. Resistance to targeted therapies
Oncogenes involved in invasive growth

Genetics and molecular biology underlying the metastatic process are largely unknown. It is mandatory to identify and to validate therapeutic targets to develop long-sought (and still missing) effective therapies.

Team
Anna Cristina Basilico, Silvia Benvenuti, Alessandra Gentile, Federica Verginelli, Maria Rita Virzi, Chiara Modica, Melissa Milan

Research topic:
Oncogenes involved in invasive growth

Background:
Growing evidence prompted us to hypothesize that the metastatic process involves members of the gene ‘super-family’ of tyrosine kinase receptors for ‘scatter factor’, homologues to the Met oncogene (Ron, Axl, Mer and Tyro). Recently, other poorly characterized genes of the family have been identified, Ror1 and Ror2, that can possibly interfere with the response to drugs directed against Met (entered in the clinic), generating resistance.

Research achievements:
In the past we have shown that the Met oncogene encodes the receptor for the ‘scatter factor’ HGF. A series of studies - performed for two decades in this and other laboratories - revealed the involvement and the importance of this oncogene in human cancers, demonstrating its key role in the control of ‘invasive growth’, a genetic program driving the metastatic process. We contributed to the development of drugs that inactivate the Met tyrosine kinase: either chemical inhibitors or monoclonal antibodies. These drugs inactivate cancer stem cells in preclinical models of glioblastoma, colorectal and gastric carcinomas, and provide promising results in clinical trials.

Conclusions and perspectives:
We are now working on the characterization of tyrosine kinase receptors of the Met oncogene family (Ron, Axl, Mer and Tyro, Ror1-2) at genetic, biochemical and functional levels. In particular, we are studying their possible interference - positive or negative - with the intracellular signaling triggered by Met during execution of the invasive growth program. Emphasis is given to the study of a hyper-metastatic syndrome, the so-called CUP, metastatic cancer from unknown primary origin.
Biographical Sketch

Paolo Comoglio has a long and distinguished record in the field of research on tyrosine kinase receptors and related oncogenes. He developed the first anti-phosphotyrosine antibody (EMBO J., 1984); through this tool he identified the tyrosine kinase encoded by the rearranged Bcr-Abl oncogene, responsible for the onset of Chronic Myeloid Leukemia (Mol Cell Biol. 1986).

He focused his research efforts on the full mechanistic insight of the genetics, biochemistry and biology of the Met receptor, with a special emphasis on the role of this oncogene in human cancers. After a long standing career he is now acknowledged as a leader in the field. He identified the tyrosine kinase receptor encoded by the Met oncogene (Nature, 1989), discovered that Hepatocyte Growth Factor (HGF) is the cognate ligand (Oncogene, 1991) and cloned the Plexin genes (Cell, 1999), recognizing their role as receptors for Semaphorins, signals for cell-cell repelling clues. He identified and elucidated the functions of two tyrosine kinase receptors, encoded by genes structurally related to Met: Ron, (EMBO J, 1994), and an homologous kinase-dead receptors, Ror (Cancer Res, 2011).

On the translational front he generated the anti-Met antibody DN30, and its monovalent counterpart, to be used in cancer therapy, either conventional or by ‘gene transfer’ (Cancer Res, 2008).

Paolo Comoglio expounded and introduced a number of insights that are now largely accepted and widespread, notably the concept of ‘invasive growth’, a genetic program otherwise ‘physiological’ but ‘usurped’ by cancer cells to progress toward metastasis (Nature Rev. Cancer, 2002).

Selected references


Preclinical models of oncogene ‘addiction’

We aim to identify ‘dominant’ genetic aberrations that drive tumorigenesis and put them in the context of concurrent ‘recessive’ alterations that may act as modifiers of response to targeted therapies

Team
Andrea Bertotti, Barbara Lupo, Francesco Galimi, Francesco Sassi, Giorgia Migliardi, Francesca Cottino, Simonetta Maria Leto, Eugenia Zanella

Research topic:
Translational models of cancer precision medicine.

Background:
Advances in technologies have allowed the attainment of powerful insights into the molecular determinants of human tumours, and in many cases inactivation of individual oncogenic drivers results in tumor regression. However, this knowledge has been translated into effective treatments very slowly, due to difficulty in predicting how the complex mutational background and the adaptive resilience of cancer cells can influence the activity of the dominant oncogene and modify response to therapies.

Research achievements:
Our projects aim at exploring the mechanisms of tumor dependency on oncogenic drivers and at understanding how such dependency is affected by genomic or functional modifiers, with an emphasis on colorectal cancer (CRC). Our experimental pipeline entails the deployment of multi-dimensional data for discovery and hypothesis validation, followed by cell-based mechanistic investigation and preclinical validation in patient-derived tumorgrafts – ‘xenopatients’. By this approach, we have contributed to finding that hyperactivation of the HER2 and MET oncogenes correlate with resistance to anti-EGFR therapies (cetuximab and panitumumab). To examine the global effects of somatic genetic changes in CRC on sensitivity to EGFR-targeted therapies, we performed complete exome sequence and copy number analyses of 129 tumors and analyzed their response to anti-EGFR blockade in xenopatients. We identified several alterations as potential mechanisms of resistance to this therapy as well as genetic lesions segregating in tumors with increased sensitivity to anti-EGFR therapy. Resistance to EGFR blockade could be overcome in xenopatients through combinatorial therapies targeting actionable genes. A typical response of CRC patients sensitive to EGFR neutralization is stable disease rather than massive regression. Mining of candidate gene expression outliers – coupled with retrospective analysis of patient material and in vitro functional studies – identified IGF2 as a predictor of poor sensitivity to cetuximab and as a new target. One limitation of xenopatients is that human tumour stroma is substituted by host (murine) components. However, this drawback can be exploited analytically to dissect the representation of human (cancer-cell specific) versus mouse (stromal-cell specific) traits in CRCs. By doing so, we contributed to re-categorising the transcriptional classification of CRC by demonstrating that a subtype displaying features of epithelial-to-mesenchymal transition and poor prognosis is not an expression of aggressive cancer cell dedifferentiation (as originally argued) but rather a read-out of abundant stromal content.

Conclusions and perspectives:
Our studies provide a systematic functional approach to evaluate response to targeted therapies in human cancer, highlight new mechanisms of responsiveness to anti-EGFR therapies, and provide a new vocabulary for the molecular management of colorectal cancer with immediate clinical implications.
Biographical Sketch:
Since 2006, Livio Trusolino has been Associate Professor of Histology at the University of Turin Medical School, Italy. He received his M.D. (1993) from the University of Turin and a Ph.D. in Human Oncology (1997) from the San Raffaele Scientific Institute, Milan, Italy.
Livio initially studied the cross-talk between growth factor receptors and adhesion molecules in tumour transformation and cancer progression. Later, his work concentrated on the characterisation of the molecular mechanisms underlying responsiveness to anti-cancer targeted therapies, using innovative preclinical models. The results of Livio’s research have been published in leading journals such as Cell, Nature Genetics, Science Translational Medicine, and Cancer Discovery. He has also written review articles for Nature Reviews Drug Discovery, Nature Reviews Cancer, and Nature Reviews Molecular and Cellular Biology.

Selected references


Histological section of a colorectal tumor. Nuclei of actively proliferating cells are stained in brown
The puzzling oncosuppressive function of oncogenes

*Identification of novel therapeutic targets and predictive biomarkers responsive to chemotherapy or targeted drugs in ovarian cancer*

Team
Annalisa Lorenzato, Martina Olivero, Jessica Erriquez, John David Konda, Erika Torchiaro

Research topic:
Unleashing the intrinsic tumor suppressive properties of oncogenes to kill cancer cells.

Background:
Oncogenic tyrosine kinases (TKs) might harbor intrinsic tumor suppressive functions. They sit at the apex of multiple downstream signaling pathways that exert various biological effects depending upon cell type and context. While some of these pathways are mitogenic and pro-survival, others might restrain the oncogenic potential by promoting, for example, apoptosis and senescence. Obviously, the latter programs do not confer a selective advantage and thus are hidden in cancer cells. However, intrinsic tumor suppression of TKs might be unleashed to kill cancer cells. This Laboratory had demonstrated that activation of the MET TK by its ligand Hepatocyte Growth Factor (HGF) results in the commitment to death of ovarian cancer cells. This model has been exploited to identify actionable molecules, that could be targeted to treat ovarian cancer and to sensitize ovarian cancer to standard platinum and taxane based treatment, once they have developed resistance.

Research achievements:
Among the actionable molecules this laboratory has identified (i) CDT2, (ii) the small heat shock protein of 27 Kda (HSP27), (iii) the PIM 2 kinase and (iv) the 90 kDa ribosomal S6 kinases (RSKs). In detail, using transcriptomic and small scale proteomics we found that MET activation in ovarian cancer cells resulted in the down regulation of the anti-apoptotic small heat shock protein of 27 KDa (HSP27, HSPB1) while MET inhibition in addicted cancer cell lines resulted in the increase of HSP27 and cell protection from death. Likewise, in human cancer cells susceptible to EGFR inhibition, EGFR inhibitors induced HSP27 expression and were strengthened by HSP27 suppression. Therefore, in cancer therapies targeting the MET or the EGFR pathways, the induction of HSP27 might limit the efficacy of targeted agents, alone or in combination with chemotherapy. A SILAC-based approach allowed the identification of PIM2 as a kinase whose phosphorylation is regulated by DNA damaging agents in ovarian cancer cell lines. Targeting PIM2 kinase sensitized ovarian cancer cells to drug-induced apoptosis, while PIM2 over-expression resulted in cell resistance to DNA damaging agents. The study of the RSKs has clarified their role not only in sustaining the metastatic phenotype of ovarian cancer cells but also in counteracting standard chemotherapy.

Conclusions and perspectives:
Altogether data showed that pro-apoptotic pathways can be unveiled in cancer cells by studying the unexpected pro-death outcome of MET activation. This allowed the identification of actionable molecules for which inhibitors are available and may find clinical application as an alternative or an adjunct to standard chemotherapy.
Biographical Sketch:
Maria Flavia Di Renzo got an MD degree at the University of Torino Medical School in 1977 and then the residency in Neurology in 1981. Besides short periods spent working in another University and abroad, she has been working in the same University as professor since 1981 and as full professor of Histology since 1994 to date. In 1997 she became head of the Laboratory of Cancer Genetics of the Candiolo Cancer Institute. Since 1981 she has been involved in the study of oncogenes and tumor suppressor genes in human cancer with a particular focus on tyrosine kinase oncogenes, demonstrating for the first time the increased expression of the MET oncogene in human cancer (Oncogene, 1991). Moreover, she identified mutations of the MET oncogene in lymph node metastases of head and neck squamous cell carcinomas, reinforcing the concept of a genetic basis of the cancer progression towards metastasis.

In 2004 her major research interest turned in the study of human ovarian cancer with the aim of identifying suitable targets for molecular therapy. For this purpose the group led by Maria Flavia Di Renzo has used functional (Cancer Res 2004; Clin. Cancer Res 2007), genomic (Mol. Cancer Ther., 2006; FASEB J 2012) and proteomic approaches (FASEB J and J Proteome Res 2014), that have led to the identification of possibly actionable molecules.

Selected references


Cancer stem cells and resistance to standard and targeted therapies

To set up therapies targeted at ‘cancer stem cells’ that constitute the ‘roots of tumors’. These specific cells may resist chemo- and radio-therapy, and, after these conventional treatments, can cause tumor relapse.

Team
Francesca De Bacco, Paolo Luraghi, Federica Verginelli, Viola Bigatto, Antonio D’Ambrosio, Francesca Orzan, Raffaella Albano, Elena Casanova, Elia Cipriano, Roberta Neggia, Gigliola Reato

Research topic:
Cancer stem cells and resistance to standard and targeted therapies

Background:
Like normal tissues, tumors such as colorectal cancer or glioblastoma are structured according to a hierarchy that includes two main components. At the hierarchy’s apex there is a (small) subpopulation of ‘cancer stem cells’ (CSC) able to self-renew, namely to endlessly propagate, and support tumor histogenesis, regeneration, and dissemination. At the hierarchy’s base there is an (ample) subpopulation of cells that, unlike CSC, have limited self-propagation ability, and tend to aberrantly differentiate and die. This model implies that, to cure the tumor, CSC must be eradicated. This goal is challenging, as CSC are often inherently resistant to therapies that so far have proven effective against the hierarchical basis of tumor cells, therapies that also include the most innovative agents, targeted at the genetic alterations driving cancer pathogenesis. To improve therapeutic outcomes, it is mandatory to identify and attack the molecular and genetic mechanisms underlying CSC resistance to conventional chemo- and radio-therapy, or to agents targeted at molecular lesions.

Research achievements:
In CSC isolated from glioblastoma (GBM-CSC), we have shown that the wild-type MET oncogene (the HGF receptor) is a functional marker of a specific subset of CSC, it activates a signalling pathway that sustains the DNA damage response, and counteracts cell death induced by ionizing radiation. As result, MET inhibition sensitizes GBM-CSC to radiotherapy in vitro and in vivo, and promotes regression of tumors obtained by GBM-CSC transplantation through eradication of the radioresistant GBM-CSC subpopulation. In CSC isolated from metastatic colorectal cancer (CRC-CSC), we’ve shown that several growth factors, including HGF and members of the EGF family, counteract the proliferative blockade imposed by EGFR inhibitors, currently used to treat metastatic CRC with significant but incomplete success. In mouse models obtained by CRC-CSC transplantation, we’ve shown that the therapeutic efficacy of standard EGFR inhibition is greatly enhanced by concomitant inhibition of MET and multiple members of the EGFR family.

Conclusions and perspectives:
The MET oncogene and EGFR, are both essential to sustain CSC viability and long-term propagation in tumors such as GBM and CRC. In GBM, where MET and EGFR are often expressed in a mutually exclusive manner, we’ve provided preclinical evidence that MET targeting impairs CSC properties, and increases the effectiveness of current therapies aimed at affecting DNA integrity and cell survival (e.g. radiotherapy). In CRC, we dissected the relative contribution of the four members of EGFR family and MET to CSC propagation and tumorigenicity. These results can instruct clinical trials that associate MET or EGFR family inhibitors with standard therapeutic protocols. Current work is aimed at identifying (i) the genetic and molecular context that modulates the CSC response to MET and EGFR inhibitors; (ii) the intratumoral CSC genetic and phenotypic heterogeneity, which must be recognized to refine the targeted treatment of each individual tumor.
Biographical Sketch:
From the beginning of her career, Carla Boccaccio has contributed to elucidate the signalling and transcriptional mechanisms by which the MET oncogene controls invasive growth, a physiological program of stem and progenitor cells, inappropriately activated during tumor onset and progression (nature Rev Cancer, 2006). She discovered that MET regulates a genetic program concomitantly driving a procoagulant and a neoplastic phenotype (Nature, 2005), thus helping to unravel the pathogenesis of Trousseau's syndrome, the association of cancer and thrombosis frequently observed, but often unexplained, in patients (J.Clin.Oncol, 2009). More recently, she discovered that MET is a functional marker of cancer stem cells isolated from brain and colorectal tumors (Cancer Res, 2012). In these cells, MET sustains self-renewal, invasion, and resistance against DNA damaging treatments, such as ionizing radiation, and biological agents targeting other tyrosine kinase receptors such as EGFR. She is now engaged in understanding the interactions between MET activity, the genetic lesions occurring in cancer stem cells, and the cellular response to standard and targeted treatments, in order to identify the clinical context where MET inhibition can be effective (EMBO Mol.Med., 2015).

Selected references


From Molecular Biology to “Precision Medicine” | 39
Integrative genomics of cancer progression and resistance to treatments

The integration of molecular profiles and clinical information enables accurate determination of cancer aggressiveness and response to treatment and to individualize therapeutic approaches (precision medicine)

Team
Claudio Isella, Consalvo Petti, Gabriele Picco, Sara Erika Bellomo, Andrea Mignone, Federica Invrea

Research Topic:
Integrative genomics of cancer progression and resistance to treatment.

Background:
Cancer progression involves acquisition by neoplastic cells of several molecular alterations, conferring metastatic ability and resistance to anticancer treatments. Integrative genomics evaluates systematically, in cohorts of tumor samples, multiple layers of molecular information, at the DNA, mRNA and microRNA level. The same analyses are conducted on large series of preclinical models, such as cell lines and patient-derived xenografts (PDXs), to consolidate regulatory network alterations in individual cancers and cancer subtypes, to identify key lesions driving cancer progression and possibly to determine sensitivity to targeted treatments. Extensive validation in the preclinical models allows exploiting the genes as therapeutic targets, and molecular signatures as diagnostic tools for precision medicine.

Research achievements:
The Laboratory of Oncogenomics has been focusing on colorectal cancer (CRC) and on the generation and analysis of multi-layer molecular and pharmacological data on large sets of CRC samples, patient-derived xenografts (PDXs) and cell lines. Such analysis was then followed by functional validation of identified candidates and mechanisms of neoplastic progression. The main achievements are here summarized: (i) species-specific genomics of CRC PDXs to distinguish the stromal transcriptome, contributed by murine cells, from the human epithelial cancer transcriptome, with implications in CRC aggressiveness and resistance to treatment (Nature Genetics, 2015); (ii) identification of mixed mRNA/microRNA networks associated to CRC subtypes (Nature Commun., 2015) and of multi-dimensional networks to uncover cancer drivers (Scientific Reports, 2015); (iii) Integrative pharmacogenomic profiling of a large collection of CRC cell lines (n=151), validating molecular subtypes and providing new actionable expression outliers in specific cases (Nature Commun., 2015) (iv) Development of a procedure for high efficiency in vivo transduction of PDXs with lentiviral vectors (Scientific Reports, 2015); (viii) Identification of NEDD8 pathway inhibition as an effective therapeutic option in KRAS- and BRAF-mutant CRC, and in vivo validation of a molecular response predictor (JNCI, manuscript submitted). Sharing the genomic expertise of the Lab with other groups inside and outside the Institute also led to publication of several collaborative works.

Conclusions and Perspectives:
The Laboratory will continue exploiting integrative genomics for better molecular stratification and for highlighting actionable therapeutic targets in CRC and other neoplastic diseases.
Biographical Sketch:
Enzo Medico graduated in Medicine at the University of Torino in 1989 and got a PhD in Cell and Developmental Biology in 1994, University “La Sapienza” of Rome. Since 2005 he is associate professor at the University of Torino. Dr. Medico has a long-standing activity in the field of tyrosine kinase receptor signaling and genomics. In particular, he has worked on the MET family of tyrosine kinase receptors, showing their role in the control of epithelial morphogenesis and invasive growth. Since 1998, Dr. Medico has re-directed his research in the field of functional genomics. In collaboration with the Fred Hutchinson Cancer Research Center, Seattle, he developed a “gene trapping” approach for genome-wide exploration of transcriptional regulation. Dr. Medico has been among the first Italian users of DNA microarrays technology, exploiting it since 1999 to study alterations in transcriptional regulation, gene expression and function associated with cancer aggressiveness and resistance to treatment. In 2004 he became director of the Laboratory of Oncogenomics at the Candiolo Cancer Institute. Over the last ten years, he has been managing and coordinating functional oncogenomics projects led by the Institute and funded, among others, by the Italian Ministry of Health, by AIRC and by the European Commission.

Selected references


Cantini L, Isella C, Petti C, Picco G, Chiola S, Ficarra E, Caselle M, Medico E

Medico E, et al. The molecular landscape of colorectal cancer cell lines unveils clinically actionable kinase targets. Nat Commun. 2015, 6:7002
Pharmacogenomics of colorectal cancer

*The knowledge of specific molecular alterations in the tumor DNA repair pathway could define a novel context of susceptibility for alkylating agents*

Team
Ludovic Barault, Daniele Oddo

Research topic:
Biomarkers for personalized colorectal cancer therapy

Background:
Colorectal cancer patients often show variable responses to treatment. This variation is the result of the molecular heterogeneity underlying the complex biology of cancer cells. Our main interest is therefore focused in understanding the changes that occur in tumor cells on exposure to anti-cancer agents. Our current programme concentrates on projects aimed at determining biomarkers of response or resistance to anti-cancer treatments with the ultimate goal of designing and testing sequences of drugs rationally designed on the modifications induced by the first agent on the tumor molecular landscape.

Research achievements:
As a model, we focused on BRAF mutant colorectal cells with acquired resistance to target therapies combinations. Genotyping of resistant cells identified heterogeneous molecular mechanisms of acquired drug resistance that converge on reactivating MAPK signaling. Drug resistance could be overcome by ERK inhibitor-based or other targeted therapy combinations, based upon the selective target(s) identified in resistant cells. Of translational relevance, we demonstrated how the combined data obtained by tumor molecular profiling and functional experiments in cell lines informed physician’s choice of administering a specific sequential regimen, which induced clinical benefit in an individual BRAF mutant colorectal cancer patient.

Conclusions and perspectives:
This approach led us to define sequential strategies suitable for clinical testing in BRAF mutant colorectal cancer patients. As a future perspective, we intend to study how tumor preconditioning with epigenetic drugs could influence the response of BRAF mutant malignancies to subsequent anti-cancer therapies.
Biographical Sketch:
Federica Di Nicolantonio holds a PharmD degree (1999) and gained a PhD (Medicine) from University College London, UK, in 2004. Her research focuses on the molecular mechanisms underlying cancer cells’ response to antineoplastic drugs. She identified KRAS mutations and KRAS amplification as biomarkers of resistance to the mTOR inhibitor everolimus (J Clin Invest. 2010) and EGFR monoclonal antibodies (Int J Cancer 2013), respectively. She first described BRAF mutations as a biomarker of adverse prognosis and of resistance to EGFR targeted therapies in colorectal cancer patients (J Clin Oncol. 2008) and discovered that lack of efficacy of BRAF inhibitors could be mediated by EGFR (Nature 2012). This preclinical work has provided the rationale for testing BRAF and EGFR inhibitors in colorectal cancer patients.

Selected references


Personalized therapy and non-invasive molecular diagnostics of colorectal cancer

To define the genetic and molecular characteristics of patients in order to lead and check the treatment of colorectal cancer

Team
Sabrina Arena, Giovanni Crisafulli, Giovanni Germano, Sebastian Hobor, Simona Lamba, Luca Lazzari, Sandra Misale, Mariangela Russo, Beth Van Emburgh, Giulia Siravegna, Carlotta Cancelliere, Benedetta Mussolin, Luca Novara, Giuseppe Rospo

Research topic:
Clonal evolution and drug resistance: from cancer avatars to liquid biopsies.

Background:
A central paradigm of modern oncology is that tumor-specific molecular alterations underlie ‘functional’ dependencies that can be therapeutically exploited. Remarkable results have been obtained by applying this paradigm in the clinic, and several targeted drugs have been approved to treat, among others, melanomas, lung and colorectal cancers. Unfortunately, when metastatic cancers are challenged with targeted agents almost invariably a subset of cells insensitive to the drug emerges. As a result, in most instances, targeted therapies are only transiently effective in patients. Arguably, the rapid and apparently unavoidable emergence of resistance is the major limitation to further progress in the application of targeted therapies in oncology. How can we overcome the near-certainty of disease recurrence following treatment with targeted agents?

Research achievements:
We have used colorectal cancer (CRC) as a model system to test the hypothesis that the emergence of drug resistance can be controlled. To this goal, we studied how clonal evolution impacts the development of resistance in patients’ avatars (cell lines and tumor xenografts). In parallel, we analysed clonal evolution dynamics and drug resistance in the blood of patients (liquid biopsies) who received targeted therapies. Our results indicate that acquired resistance is -for the most part- a ‘fait accompli’. Mathematical models based on our data suggest that each (traceable) metastatic lesion contains hundreds of cells with mutations to virtually any targeted agents. According to this model, the time to relapse is simply the interval required for mutant or otherwise altered subclones to re-populate the lesion. On the positive side, we found that resistance can be prevented when drug combinations are rationally used.

Conclusions and perspectives:
The awareness that in nearly all patients targeted drugs are only transiently effective due to the rapid emergence of resistance poses a formidable challenge. Although the overall picture is looming, our data and the concepts they underlie offer several opportunities for intervention. Genetic tracking of cancer cell populations can be used to study how the development of resistance can be restrained in vitro and in vivo. Furthermore, liquid biopsies can be exploited to monitor clonal evolution and to intercept the emergence of resistant clones non-invasively in patients. When applied together cancer avatars and liquid biopsies may identify, monitor, and potentially overcome resistance to targeted therapies.
Biographical Sketch:
A. Bardelli received a PhD in Molecular Biology from the UCL (University College London, UK). As a post doc at the Johns Hopkins University (Baltimore, USA, supervisor Dr. Bert Vogelstein) he developed the first comprehensive mutational profile of kinases (Science 2003). As an independent investigator, A. Bardelli has then translated these findings into clinical practice (Lancet Oncology 2005; JCO 2008). Recently, he has defined mechanisms of acquired resistance to anti EGFR therapies in colorectal cancer patients thus providing insights into new therapies aimed at overcoming resistance (Nature 2012; Cancer Discovery 2014). These studies involve an innovative methodology - named liquid biopsy - which allows the use of circulating tumor DNA to monitor patient’s response using a blood draw (Science Translational Medicine 2014).

Selected references


Targeted cancer immunotherapy

*Development of novel therapeutic strategies to generate, produce, and deliver monoclonal anti-cancer antibodies*

Team
Simona Cignetto, Cristina Chiriaco, Lara Fontani

Research topic:
Development and validation of cancer immunotherapy strategies based on MET antibody and MET antibody-derived molecules.

Background:
Clinical evidence indicates that the MET oncogene plays a role in progression of cancer toward metastasis and/or resistance to targeted therapies. While mutations are rare, the common mechanism of MET activation is overexpression, either by gene amplification ('addiction') or transcriptional activation ('expedience'). In both instances ligand-independent kinase activation plays the major role in sustaining the transformed phenotype. Currently available MET antibodies are directed against the receptor binding site, behaving essentially as a ligand (HGF) antagonist, and are ineffective in ligand-independent activation.

Research achievements:
The monovalent chimeric MvDN30 antibody, delivered as a purified protein, binds the extracellular domain and induces the proteolytic cleavage of MET, dramatically inhibiting downstream signaling pathways, in both the absence or presence of ligand. MvDN30 has the limit of poor in vivo stability. To address this issue MvDN30 was delivered by ‘gene therapy’, to induce a stable and constant in vivo production that counterbalances the high rate of clearance. As alternatives, the molecule has also been chemically modified (i.e. Pegylated) or engineered, including two repetitions of the constant domain (DCD). All the explored strategies led to the improvement of the MvDN30 potency. By the gene therapy approach, direct Mv-DN30 gene transfer in nude mice, intra-tumor or systemic, was followed by a therapeutic response of MET addicted human glioblastomas and lung carcinomas. Upon Pegylation the antibody overcame the resistance to EGFR targeted therapy in a MET-amplified patient-derived colorectal tumor (xenopatient). The DCD approach was successfully applied to inhibit the peritoneal carcinomatosis of a MET-addicted gastric tumor.

Conclusions and perspectives:
We developed strategies suitable to improve the potency of MvDN30, rendering it an attractive candidate for clinical applications. To further enlarge the panel of anti-MET immunotherapy routes we aim to explore a strategy of re-directed adoptive immune response by a Chimeric Antigen Receptor anti-Met.
Biographical Sketch:
Elisa Vigna was involved in the identification of HGF/SF as the Met ligand (Oncogene, 1991; EMBO J., 1991) and in the characterization of the biological activation of HGF by urokinase (EMBO J., 1992; J Biol Chem, 1995). She studied the role of the Met juxtamembrane domain in the regulation of Met activity (Oncogene). In the gene therapy field she acquired experience collaborating with Prof. Naldini, who conceived and developed the Lentiviral Vectors. She performed pionieristic ‘vectorology’ studies optimizing lentiviral vectors for multiple gene transfer and exogenously regulated expression (Mol. Therapy, 2002; Mol. Therapy, 2005; Nature Biotech, 2005). She characterized the anti-Met antibody DN30 and generated its monovalent non-agonistic counterpart. She provided proof of concept for either conventional or ‘gene transfer based’ protocols for DN30 application (Cancer Res, 2008; J Biol Chem, 2010; J Mol Med 2014).

Selected references

Vigna E, Comoglio PM. Targeting the oncogenic met receptor by antibodies and gene therapy. Oncogene. 2015, 34:1883-1889.


From Molecular Biology to “Precision Medicine” | 47
Mechanisms of resistance to tyrosine kinase receptor inhibitors

Design of strategies bypassing resistance to targeted therapies

Team
Simona Corso, Elena Ghiso, Valentina Martin, Silvia Menegon, Cristina Migliore, Annalisa Petrelli, Maria Apicella, Marilisa Cargnelutti, Elena Morando

Research topic
Altered regulation of tyrosine kinase receptors is frequent in solid tumors and it is often associated with the acquisition of an aggressive phenotype. Thus, therapies targeting these receptors have been proposed as molecular approaches to treat human cancers. The main problem with these therapies is the onset of resistance, which leads to treatment failure. It is therefore important to understand the mechanisms of resistance to molecular targeted therapies, in an effort to optimize the outcome of the treatments.

Background:
The goal of delivering the right drug to the right cancer patient (precision medicine) requires a detailed understanding of how genomic alterations are linked to drug response. Hence, the clinical efficacy of target therapy depends on: i) the validation of the true biological relevance of putative targets in the context of a specific tumor type; ii) the context-specific identification of the active drug(s) and of additional molecular alterations affecting the responsiveness to treatment.

Research achievements:
Our group aims to study the molecular mechanisms that allow tumor cells to become insensitive to molecular targeted therapies. We focused on the study of gastric cancer that is the world’s third leading cause of cancer mortality. We generated a platform of more than 70 patient-derived xenografts (PDXs) that recapitulate the heterogeneity of this disease. We aim to genetically dissect the gastric tumor landscape in order to investigate mechanisms involved in sensitivity/resistance to targeted therapies against receptor tyrosine kinases.

We performed the genomic analysis of the PDX tumors and we identified several alterations of tyrosine kinases such as HER2, EGFR, MET, FGFR2 and of KRAS. We exploited a MET-amplified PDX model to optimize anti-MET therapeutic strategies in gastric cancer. We report that despite the high MET amplification level (26 copies), tumors showed only partial and transient sensitivity to anti-MET therapy while dual MET/EGFR inhibition led to complete regression and prevented resistance onset. The finding that combined anti-MET/EGFR therapy even in the absence of EGFR genetic alterations induced complete and durable response, represents a proof of concept and guarantees further investigations, opening a new perspective of treatment for these patients. We also performed pre-clinical trials on several HER2-amplified gastric xenopatiens to identify the best anti-HER2 therapeutic approach and investigate the mechanisms of resistance to treatment.

Conclusions and perspectives:
By exploiting the gastric cancer PDX platform we have generated, we aim to unravel genetic vulnerabilities in this pathology and to identify the best therapeutic strategies to target them. Since gastric cancer is a very common disease worldwide, the identification of novel 'druggable' and validated targets would be extremely important from a clinical point of view, even if their prevalence is very low. As a whole, the results of this project will provide a scientific basis for future clinical applications and guide the rational design of molecularly-oriented clinical trials for gastric cancer.
Biographical Sketch:
Silvia Giordano has a long experience in the field of research on tyrosine kinase receptors and related onco-
genesis. She has focused her research on the study of the tyrosine kinase receptor encoded by the MET gene. She identified this receptor (Nature, 1989) and contributed to the understanding of the signal transduction mechanisms and of the biological role in physiological as well as in pathological conditions. In particular, she has critically contributed to the understanding of the mechanisms controlling MET down-regulation (Nature, 2002) and HGF-independent activation (Nature Cell Biol., 2002). Moreover, she showed that cancer cells may be dependent (“addicted”) on the constitutive activation of this oncogene (Oncogene, 2008). Recently, she has been deeply involved in the study of the mechanisms of resistance to targeted therapies (Cancer Res., 2010; Cancer Discovery, 2013). She has also performed studies of the role of microRNAs in cancer progression (Cancer Res., 2008; Clin. Cancer Res., 2012; Hepatology, 2014). On the translational front she has identified a MET monoclonal antibody (DN30) able to impair Met activation and tumorigenicity (Proc Natl Acad Sci U S A., 2006).

Selected references


Experimental cancer immunotherapy with Cytokine-induced killer cells

Adoptive immunotherapy against solid tumors

Team
Loretta Gammaitoni, Lidia Giraudo Diego, Giulia Mesiano, Valeria Leuci

Research topic:
Immunotherapy with Cytokine-Induced Killer (CIK) cells for the treatment of solid tumors refractory to conventional treatments.

Background:
Adoptive immunotherapy holds promise for the treatment of solid tumors. Disease relapse and drug-resistance are sustained by a subset of putative cancer stem cells (CSC), poorly responsive to conventional treatments. Immunotherapy is a promising approach capable of targeting putative CSC. Our group has focused over the last years on a subset of T lymphocytes known as Cytokine-induced Killer (CIK) cells. CIK cells are ex vivo expanded T lymphocytes generated from circulating mononuclear cells, endowed with MHC-independent antitumor activity. The aim of our research is to explore adoptive immunotherapy with CIK cells for sarcomas and melanomas.

Research achievements:
We demonstrated that immunotherapy with CIK cells is active against autologous sarcomas and melanomas, including putative CSC. Our main findings are summarized as follows. (i) Generation of CIK cells: clinically relevant rates of CIK cells were successfully expanded from patients with sarcomas and melanomas; (ii) tumor killing activity: patient-derived CIK cells were highly active against autologous sarcomas and melanomas pre-treated with conventional chemotherapy; (iii) activity against CSC: we developed a gene-transfer strategy capable of visualizing putative CSC based on their ability to re-activate stem gene Oct4. Sarcoma and melanoma CSC were relatively resistant to conventional chemotherapy but susceptible to immunotherapy with CIK cells; iv) In preliminary experiments we demonstrated the possibility to redirect CIK cells with a chimeric-antigen receptor (CAR) against a precise target expressed in soft tissue sarcomas.

Conclusions and perspectives:
Adoptive immunotherapy with CIK cells is potentially active against solid tumors and capable of eradicating chemoresistant CSC. We plan to enhance the antitumor activity of CIK cells by their genetic redirection against tumor-specific targets. Furthermore we will explore synergism of immunotherapy with molecular targeted approaches.
Biographical Sketch:
Dario Sangiolo is a physician scientist with research interest in the field of transplant immunology developed at the Fred Hutchinson Cancer Research Center (Seattle, WA). He contributed to the development and characterization of preclinical models based on Cytokine-Induced Killer (CIK) cells (Int Immunol. 2008; J Immunother. 2012). In preclinical studies he demonstrated the activity of CIK cells against melanomas and sarcomas (Clin Cancer Res. 2013; Cancer Res. 2014). He explored strategies to genetically engineer T lymphocytes to enhance antitumor activities and decrease the risk of GVHD across HLA barriers (Hum Gene Ther. 2009; Gene Ther. 2007). In the field of clinical research he investigated the efficacy and toxicity of Hematopoietic Cell Transplant in hematologic and solid tumors (Biol Blood Marrow Transplant. 2010; Blood. 2006 May).

Selected references


The Heracles Clinical Trial

Investigators: Alberto Bardelli, Silvia Marsoni, Salvatore Siena, Silvia Giordano, Carla Boccaccio, Paolo Michieli, Livio Trusolino, Paolo M.Comoglio - Scientific Advisors: Marc Ladanyi (Memorial Sloan Kettering Cancer Center, New York, US), Manuel Hidalgo (Spanish National Cancer Research Centre, Madrid, ES), David Ransohoff (UNC Lineberger Comprehensive Cancer Center, Chapel Hill, US)
Over the past few years, the improved knowledge on the biological, genetic and molecular heterogeneity of tumors, together with the development of pharmacological technologies, has allowed the identification of molecular targets for novel therapeutic strategies. This fast process has led to the overall reconsideration of classical approaches to clinical oncology, traditionally oriented toward design of cancer treatments irrespective of the biological and genetic peculiarities that can make each tumor a pathology on its own. The identification of patients likely to respond to specific treatments according to the presence of relevant molecular targets (personalized medicine) and based on the expression of potential markers of sensitivity or resistance, needs clinical studies that result from a constant and productive interaction among the professionals with a significant background in the various disciplines. The goal of the “Investigational Clinical Oncology” team is to increase the therapeutic index of molecular targeted drugs, by the identification and clinical validation of biomarkers of sensitivity/resistance. This goal is pursued through the development of research programs aimed at designing hypothesis-driven clinical trials that directly derive from the Institute’s biological, genetic and molecular research. In order for these tasks to be implemented, an ad-hoc organizational structure favours the interchange and the synergy among preclinical researchers, oncologists and pharmaceutical companies – which are the potential providers of new drugs for clinical trials. Another goal of INCO will be the promotion of a clinical research culture based on the knowledge of molecular biology and tumor genetics, and particularly of the mechanisms of sensitivity or resistance to molecular targeted drugs. This organization is instrumental to the referral of patients for admission into top-priority clinical trials.

Research Topic 3 is focused on:
3.0 Rational design of clinical trials for targeted drugs
HERACLES and ARES: trials for the ‘Precision Medicine’ of Cancer

Shaping the clinical validation path of new putative targets found in the Institute’s preclinical programs

Team
Antonella Balsamo, Cosimo Martino, Marili Vitiello, Mario Spione

Research topic:
Design, implementation and conduction of ‘hypothesis-driven’, precision medicine trials based on the translational discoveries of the FPO-IRCC research laboratories

Background:
Precision medicine brings a new set of challenges to clinical application. To hasten the adoption of new therapies we need to achieve a deeper molecular understanding of cancers and reliably translate results from representative preclinical models into clinical trials enriched with suitably identified patients.

Research achievements:
At Candiolo we have started to confront precision medicine hurdles by creating a clinical platform integrating, under the same virtual roof, the efforts of surgeons, pathologists, radiologists, and medical oncologists from the IRCC clinical network (25 centers around Italy), in order to assemble the timely collection of high quality, clinically annotated biological samples at meaningful time points, to feed research projects and precision medicine trials. Five translational protocols are now feeding the translational Labs of the Institute (PROFILING, AGNOSTOS-Profiling, GEA, FUNNEL and CORKUCOPIA). Together with the translational scientists, we are also building a “cancer knowledge network” to store the resulting molecular and medical data in digital form and to deliver them, in comprehensible ways, to scientists, clinicians, and patients (Projects LAS and PROBUS). Three hypothesis driven clinical trials have been designed and implemented (HERACLES and ARES in colorectal cancer, AGNOSTOS in cancer of unknown origin). HERACLES has been the Institute first precision trial designed upon the discovery, based on PDX studies, that HER2 amplification in metastatic colorectal cancer (mCRC) associates with resistance to anti-EGFR therapy, and predicts response to the combination of lapatinib and trastuzumab for a dual blockade of the HER2 pathway. Accordingly, we conducted a proof-of-concept trial testing the combination in HER2 amplified mCRC patients failing standard therapies. The trial has proven the efficacy of the combination with 8 long lasting responders out of 23 patients treated, proving HER2 as the first bona fide actionable target in mCRC. Genotype/response correlation has shown that only patients with truly ‘HER2 addicted’ tumors benefit from this therapy. The tumor genomic landscape of non-responders and relapsing cases is under scrutiny to define the determinants of primary and secondary resistance.

Conclusions and perspectives:
The obstacles encountered in HERACLES will be addressed: unexplained primary drug resistance, inadequacy of current criteria for monitoring tumor response and recurrence, limited knowledge of genomic heterogeneity of mCRC. We will also extend the dynamic research infrastructure which we have created for mCRC to gastric cancer and cancers of unknown origin. Two new resource-dense, hypothesis-driven trials deriving their rationale from the results of the Institute’s translational labs, will be embedded and nurtured by this infrastructure.
Biographical Sketch:
Silvia Marsoni has a distinguished career as a clinical trial methodologist, which started in the '80 at the NCI-USA. Since then, she has focused her research efforts on the rationalization of oncology trials. In the nineties at the Mario Negri Institute she founded her first clinical network and designed pivotal phase 3 trials establishing the standard of care in the adjuvant setting of colon cancer. Currently, she still designs standard-defining trials for genotype selected populations. In 2000 she founded SENDO, a non-profit organization for the early development of new cancer drugs in Italy and Spain. SENDO, firstly in Italy, developed targeted drugs in biomarker enriched populations. While there she also developed trabectedin in ovarian cancer. Since 2011 she directs the Clinical Trials Unit, and has built the clinical platform for genomic-driven trials serving the translational labs of the Institute. The first of these trials, HERACLES, is targeting HER2 in colorectal cancer with promising preliminary results.

Selected references


Cancer of Unknown Primary (CUP): the AGNOSTOS PROJECT

A forgotten pathology and an unmet therapeutic goal

Team
Giulia Maria Stella, Elena Geuna, Andrea Milani, Annamaria Nuzzo, Giorgia Zucchini, Marilisa Cargnelutti, Alice Maria Balderacchi

Research topic:
Cancer of Unknown Primary (CUP): the AGNOSTOS PROJECT

Background:
Cancer of Unknown Primary (CUP) is a heterogeneous clinical and pathological syndrome characterized by early tumor metastatic spread without an identifiable, site-specific primary tumor of origin. Despite accounting for 3-5% of all human cancers, CUP represents a still unsolved clinical problem, with a not fully understood biology and paucity of effective therapies. Currently, patients with CUP are given systemic chemotherapy, based on a semi-agnostic approach that considers general histological features rather than organ-derivation. With 25-30% response rate and overall survival ranging from 6 to 16 months, current treatment of CUPs is considered largely suboptimal. Furthermore, because of their innate ability to spread at distant organs early in their natural course, CUPs may represent a valuable clinical and translational model to study the molecular basis of tumor metastatization.

Research Achievements:
We have designed and implemented the AGNOSTOS program, that is aimed at identifying new potential treatments and at conducting systematic translational studies on this rare entity. The AGNOSTOS program includes two prospective clinical studies.

• The AGNOSTOS 1 study is a multicenter, randomized phase 2 trial with a ‘Pick the Winner’ design to assess the efficacy of nab-paclitaxel-based doublet as first line therapy in treatment-naïve CUP patients. In particular we will explore the activity of nab-paclitaxel in combination with the two most active drugs (carboplatin or gemcitabine). The trial is ethically approved and currently ongoing in 10 Italian Institutions under the coordination of the Candiolo Cancer Institute. We plan to enroll 60 patients in each arm of the trial.
• The AGNOSTOS Companion Translational study is aimed at the collection of vital tissues from CUP patients to establish patient-derived-xenografts (PDX) and cancer stem cells (in vitro). Furthermore we aim to collect blood/plasma from CUP patients to obtain free tumor DNA from plasma to follow tumor burden and clonal evolution in CUPs patients (liquid biopsy), and circulating cancer stem cells.

Conclusions and perspectives:
• AGNOSTOS trial will establish whether nab-paclitaxel, a more effective taxane analogue, can be safely and effectively combined to gemcitabine or carboplatin, the two most used CUP drugs, to improve disease control and outcome of these patients.
• AGNOSTOS translational study will deploy the extensive genomic work-up of enrolled patients to pinpoint common denominators of the hypermetastatic phenotype and it will exploit patient-derived-xenograft for an in-depth molecular characterization of these tumors, with the potential to unravel the molecular link between cancer stemness and the hypermetastatic phenotype.
Biographical Sketch:
Dr. Filippo Montemurro is the Director of the Investigative Clinical Oncology Unit (INCO) at the Candiolo Cancer Institute - FPO IRCCS. This recently created Unit is at the intersection between basic, translational, and clinical research and it is strongly committed to designing clinical trials of cancer precision medicine.
During his fellowship and his professional career, Dr. Montemurro has focused on breast cancer and biologically targeted therapies for the HER2-positive subset, where he has acquired competence and international visibility. In particular, he has pioneered reductive approaches aimed at sparing chemotherapy to patients with HER2-positive metastatic breast cancer whose disease showed molecular features of clinical addiction to the HER2 oncogene.
Currently, he is Principal Investigator and steering committee member of a number of Phase II and III, multicentric international Clinical Trials in breast cancer. Furthermore, in the context of the activities of the INCO Unit, he co-chairs a special program for patients with metastatic Cancer of Unknown Primary origin (CUP), which includes clinical and molecular investigations in this rare, but meaningful disease subset.

**Selected references**


Modern pathology: from morphology to molecular genetics
The ultimate task of the Institute is the development of clinical research integrated with health assistance, also through nationally- and internationally-controlled clinical trials. With the introduction of molecular therapies, it has been understood that a specific disease can have different characteristics that need different therapies. Treatments are therefore oriented toward personalized therapy, which needs a very sophisticated molecular diagnostics armamentarium that the Institute is able to provide. Ongoing clinical research integrates the Divisions, the Laboratories and the Facilities, in order to allow synergy among different highly-specialized technologies and complementary, diagnostic, and therapeutic know-how. The research goal is the progress of science, but the figure and the well-being of the patient are an absolute priority. Participation in experimental protocols is voluntary and is suggested to patients according to the biological and clinical characteristics of the disease. The Ethics Committee of IRCCS-FPO guarantees that patients receive only treatments having solid scientific bases and that they are monitored with the most serious attention. In compliance with the Declaration of Helsinki, patients who do not wish to participate in a trial receive the best “standard” therapy. Oncologists, surgeons, radiologists and radiotherapists cooperate with researchers to design clinical trials based on molecular data, intended to verify and generate hypotheses. In order to make this cooperation productive, the Institute manages a daily net of interactions involving formal aspects (seminars and meetings), training (refresher courses and lessons) and operational efforts (contacts with pharmaceutical companies, regulatory and management instruments).

Clinical Research is the last and most important haven to improve the assistance to cancer patients, to increase their life expectancy and to provide them with the most appropriate, safe and effective therapeutic approach in accordance with the genetic profile of their own tumor. The controlled clinical Protocol is the final product of the overall work of a multidisciplinary group composed of basic researchers, oncologists, surgeons, pathologists, pharmacologists, radiologists and nuclear medicine doctors, biostatistics and bioinformatics scientists, research healthcare assistants, data managers, and legal experts. The main projects of the Applied Clinical Research are listed below, followed by the table of Controlled Clinical Trials.

Research Topic 4 concerns:
4.1. Colorectal cancer: onset and progression
4.2. Development of new therapeutic strategies
4.3. New approaches to surgical oncology
4.4. Laboratory Medicine, Imaging and Radiotherapy
Personalized therapies in Medical Oncology

A new avenue of cancer therapy

Team
Marco Fizzotti, Lucia Garetto, Cinzia Ortega, Veronica Prati, Delia Rota Scalabrini, Giorgio Valabrega, Fiorella Ruatta, Celeste Cagnazzo, Caterina Aversa, Rosa La Face, Sofia Genta, Rossella Martinello, Gloria Mittica, Sonia Capellero, Luca Crotto, Marta Fenoglio

Research topic:
Integrating chemotherapy, target therapy and immunotherapy for treatment of advanced cancer.

Background:
The treatment of advanced cancer depends on the combination of chemotherapy, target therapy and recently added immunotherapy. The knowledge of the tumor genotype and phenotype is essential for the planning of the treatment at diagnosis. Unfortunately resistance to treatment eventually occurs: its early detection allows rapid changes of therapy with an improved prognosis. We have set up continuous monitoring of tumor genotype to determine molecular changes induced by the treatment.

Research achievements:
Our units have been focusing mainly on the treatment of sarcomas, ovary, genitourinary and digestive tract carcinomas, becoming a referral center for these diseases. The results achieved derive from a continuous interaction between the laboratory (both the unit’s lab and the other labs of the Institute) and the clinic. This made possible the implementation of clinical trials designed and conducted on the basis of hypotheses generated at the bench and translated to the bedside.

Conclusions and perspectives:
To continue our effort to design clinical trials based on a thorough analysis of the tumor and of its interaction with the host. The secondary projects will detail the ongoing projects coordinated by our Unit. Moreover, strict cooperation is ongoing with other leading clinical oncological institutions in Italy and in Europe.
Biographical Sketch:
Massimo Aglietta started his scientific career working in preclinical models of human tumors with the aim of developing rational chemotherapeutic schedules based on tumor cell kinetics (Cancer Res, 1979). A logical pursuit of these studies has been the characterization of the growth pattern of normal and neoplastic hemopoietic progenitor cells (Cancer Res, 1980; Blood, 1986). These preclinical studies led to the development of phase I/II clinical protocols that contribute to a rational scheduling of hemopoietic growth factors to allow dose dense chemotherapy regimens in solid tumors (J Clin Invest, 1989; Blood, 1993; Cancer, 1993; Eur J Cancer, 1995). This background was also behind the phase II protocols that explored the potential of stem cell transplantation outside hemopoietic malignancies like colon cancer and sarcomas (J Clin Oncol, 2002; Blood, 2006; Nature Clin Practice Oncol (Review), 2008). Serving as Director of the Medical Oncology Unit he has continued to pursue the objective of building clinical trials based on strong preclinical backgrounds. Of relevance: 1) the studies on the development of resistance to anti HER-2 therapy in breast cancer (Review in: Ann Oncol, 2007; Ann Oncol, 2013); 2) the preclinical studies in bilo-pancreatic cancer that led to the development of phase II clinical trials to evaluate the antitumor activity of treatments that combine chemotherapy with monoclonal antibodies directed anti EGFr or anti CTL-4 (Ann Oncol, 2014; Review in: Crit Rev Oncol Hematol, 2013; Crit Rev Oncol hematol, 2014); 3) Preclinical studies in sarcoma and melanoma revealed molecular alterations involved in tumor progression. Molecularly driven clinical trials have been successfully conducted (Cancer, 2009; Cancer, 2011; Ann Oncol, 2012, Lancet, 2012; Clin Cancer Res, 2013, Lancet Oncol, 2015). We are now continuing to translate into the clinic the discoveries of basic research (Clin Cancer Res., 2013; Cancer Res., 2014). We are engaged in clinical trials exploring the best way to integrate immunotherapy with chemotherapy and molecular based therapy.

Selected references


Unravelling Triple Negative Breast Cancer (TNBC) diagnosis
To subtype TNBC using a bottom-up approach from cell lines to formalin fixed paraffin embedded (FFPE) tissues

Team
Laura Casorzo, Carmine Dell’Aglio, Ivana Sarotto, Elena Frangipane, Alberto Pisacane

Research Topic:
To subtype TNBC using a bottom-up approach from cell lines to formalin fixed paraffin embedded (FFPE) tissues

Background:
The standard of care of TNBC is still chemotherapy. However, according to gene expression profiling TNBC can be divided into 6 intrinsic subtypes, which may respond to different therapeutic protocols. In addition, TNBC show a low frequency of oncogenic driver mutations but a high heterogeneity of gene copy number. This molecular alteration may follow to hypomethylation and activation of Long Interspersed Nuclear Elements (LINE-1). LINE-1 may reverse transcribe their own mRNAs and insert their DNA copies into new sites within the genome and in turn impair oncosuppressor genes and activate oncogenes.

Research achievements:
We are setting up panels of biomarkers to classify cell lines, profiled for the 6 molecular variants, within each intrinsic subtype of TNBC. The clinical significance of these classifiers will be validated in a retrospective cohort of FFPE TNBC by correlating their expression with patient follow up. In addition, they will serve for annotating TNBC prospectively collected in order to create a biobank of fresh tissues and organoids. Finally, we are setting specific procedures to test the methylation status of LINE-1 sequences to explore new mechanisms of chromosome and gene instability in TNBC subtypes.

Conclusions and perspectives:
Our study on TNBC may provide specific biomarkers to be used for patient selection in clinical trials. The cell lines and organoids representing TNBC subtypes will provide key models for preclinical studies with targeted agents. Epigenetic analysis will shed light on the activation of complex molecular pathway and on the possibility to suppress the tumor growth.
Biographical Sketch:
Anna Sapino is director of the Division of Pathology. She is full Professor of Pathology at the Medical School University of Torino, and Professor of the European School of Pathology. She has been Vice-Director for Research of the Dept. of Medical Sciences (2013 - 2015). In 2013 she has been appointed chair of the Working Group for Breast Pathology of the European Society of Pathology and in 2014 Member of the Board of Governors of Health (Consiglio Superiore della Sanità) of the Italian Ministry of Health. She started as a diagnostic pathologist with a vivid curiosity towards breast cancer biology (Cancer Res. 1986). In 1987 she accrued experience in experimental studies on pre-neoplastic breast lesions through the sabbatical period spent in the USA. Upon her return to Italy she set up her own cell biology lab working on effects of hormones on breast cancer cells and mouse mammary gland development on whole gland organ cultures. These studies provided the first data suggesting myoepithelial cell involvement in the development of pathological entities occurring in the human breast (Cancer Res. 1992) and the possible involvement of oxytocin in the proliferation and differentiation of breast stem cells in myoepithelial cells (Endocrinology. 1993). Currently her scientific activity has two key missions: (i) to improve the quality of diagnostic tests and innovate breast cancer diagnosis by working on pre-analytical phase in surgical pathology, and to guarantee the optimal quality for any downstream analysis (PLoS One. 2011); (ii) to sort out the main drawbacks of oncologists by searching and validating biomarkers that may guide towards a more personalized therapeutic approach (J Pathol. 2009; J Natl Cancer Inst Monogr. 2015)

Selected references


Identification of potential targets in cancer of the biliary tract

Development of new cancer therapies to improve the prognosis of biliary carcinomas, a group of highly lethal malignances

Team
Renato Ferraris, Federica Colombi, Ilaria Depretis, Elisabetta Fenocchio, Roberto Filippi, Donatella Marino, Giuliana Cavalloni, Caterina Peraldo Neia

Research topic:
New strategies in advanced biliary cancer treatment

Background:
Biliary tract cancer (BTC) is a rare and lethal disease with very few therapeutic options. Previous studies suggest that Epithelial Growth Factor Receptor (EGFR) pathway activation could be involved in BTC pathogenesis, envisaging a potential role of anti-EGFR monoclonal antibodies.

Research achievements:
Despite encouraging early evidence of a major involvement of EGFR pathway in BTC, targeted anti-EGFR therapy has proven disappointing in the clinic, and resistance seems to involve mechanisms other than RAS mutations. Our group has coordinated a multicenter randomized Phase II clinical trial (Vecti-BIL study) to evaluate the effectiveness of Panitumumab (P) in combination with chemotherapy in patients with advanced BTC selected for the absence of KRAS mutations on exon 2. Patients were randomized to receive the combination of P with gemcitabine and oxaliplatin (GEMOX) or GEMOX alone. The addition of P did not result in a statistically significant improvement in progression-free survival (PFS) (5.3 months vs 4.4 months of GEMOX alone; log-rank test, p= .27) and had no impact on overall survival (OS, 9.9 months with P-GEMOX vs 10.2 months with GEMOX; p= .42). Even restricting survival analyses to all RAS and BRAF wild-type patients, no statistically significant differences in PFS or OS were found between treatment arms. Resistance to chemo/targeted treatments may be explained through alternative pathway activation or the presence of a stem cell compartment. Comprehensive molecular profiling and genetic analyses of the last 10 years have provided considerable advances in the identification of druggable targets in BTC. In our preclinical experience, we explored alternative molecules such as ET-743 and the Src inhibitor Saracatinib. Moreover, we demonstrated that 9% of patients harbor the FIGROS1 rearrangement, in a case series of Italian patients with BTC, suggesting that inhibition of ROS1 may have important clinical implications. To test new anticancer treatments in BTC we established a human intrahepatic cholangiocarcinoma cell line derived from an Italian patient and a patient-derived intrahepatic cholangiocarcinoma xenograft model.

Conclusions and perspectives:
Molecular profiling has outlined complex oncogenic pathway alterations in BTC including ERBB2, RAS-RAF-MEK, PI3K-AKT-mTOR, JAK/STAT, and IDH1/2. Gene fusions involving ROS1 and FGFR2 represent timely druggable alterations. These findings are expected to provide important implications for developing future therapies in BTC.
Biographical Sketch:
Francesco Leone is Assistant Professor at the University of Turin, Department of Oncology. Since 2006 he has been coordinator of the activities of the multidisciplinary team for the treatment of digestive cancers at the Candiolo Cancer Institute and coordinator of translational research projects in this field between the Medical Oncology Division and the Oncology Laboratory of the Institute. In colorectal cancer, his major area of interest is represented by “conversion therapy” to maximize the possibility to offer surgery to metastatic patients (Crit Rev Oncol Hematol. 2014, Cancer 2013). He realized a significant synergy also for the multidisciplinary treatment of locally advanced pancreatic cancer (Cancer 2013) and, in the metastatic setting, he participated in the pioneering of modern immunotherapy concepts (Ann Oncol. 2014). In recent years, his main area of research has been the study of biliary tumors. He contributed to exploring the feasibility of targeted therapies for these highly malignant cancers (Crit Rev Oncol Hematol. 2013, Clin Cancer Res. 2006) and is currently engaged in the final analysis of a multicenter randomized clinical trial of panitumumab in combination with chemotherapy for advanced biliary tumors, coordinated by the Candiolo Cancer Institute (ASCO GI Proceedings 2015). Given the unsatisfactory results of the anti-EGFR based strategy, he developed insights in new directions such as the evaluation of new chemotherapy agents (BMC Cancer 2014) and the identification, through extensive molecular analysis, of specific genetic targets to be explored in future clinical trials (Genes Chromosomes Cancer 2014).

Selected references


Cell therapy for metastatic tumors

Application of new cell-therapy based strategies, to synergize with molecular targeted therapies

Team
Daniela Caravelli, Valentina Coha, Paolo Becco, Susanna Gallo

Research topic:
Hematopoietic Cell Transplantation (HCT): toward an immunological platform for cell and molecular therapy in the treatment of metastatic refractory cancers.

Background:
Allogeneic HCT, coupling to high dose chemotherapy a powerful donor immunological anti-tumoral effect, is a curative therapy for many hematological malignancies. However in the past years its broader application has been limited by two relevant obstacles: the first being represented by Graft Versus Host Disease (GVHD) - the second being represented by the incapability of generating a specific Graft Versus Tumor effect (GVT). Only when it will be possible to achieve a complete control of GVHD along with the generation of a specific GVT, allogeneic HCT will accomplish its extraordinary curative potential. Our research is focused on the clinical application of a strategy able to fully control GVHD and that transforms HCT into a safe platform for adoptive cell therapy.

Research achievements:
In regard to GVHD our group contributed to the study of conventional treatment and classification of this complication and is currently running an interventional study of post-transplant cyclophosphamide (PT-CY) as a safe strategy for GVHD control. In this context in the first 40 patients treated we reported an extremely low rate of acute GVHD and of chronic GVHD that translated in a sharp reduction of transplant related mortality (2%).
In regard to GVT (i) we at first revealed that following HCT it was possible to generate donor T cells specifically directed against patient-tumor antigens. (ii) We contributed to demonstrating that allogeneic HCT is an effective cell-therapy in the treatment of Multiple Myeloma. (iii) We have been involved in pre-clinical studies regarding a specific form of cell-therapy derived from the generation and infusion of Cytokine Induced-Killer cells (CIK).
In regard to Cell therapy regulatory issues and Law: cell therapy clinical application will be allowed only in Clinical centers that are JACIE-FACT accredited. Our Transplant center as part of the Turin metropolitan transplant network was accredited in July 2013.

Conclusions and perspectives:
The introduction in the clinical scenario of a safe strategy for GVHD control, as the on-going results HCT with PT-CY suggest, represents the ideal basis for future application of tumor-specific T-Cell-therapy. We plan to design and conduct clinical trials of cell-therapy (CIK and tumor specific T-cells) in the context of the Turin metropolitan transplant network both in solid tumors (melanomas and sarcomas) and in hematological malignancies.
Biographical Sketch:
Fabrizio Carnevale Schianca has been working in the field of hematopoietic stem cell transplantation (HCT) since the time of fellowship (1997) accumulating robust clinical knowledge both in the biological rationale and clinical management of this strategy. As an oncology fellow he contributed to the start-up of the clinical unit of Medical Oncology at the Candiolo Institute (1998). At the end of his fellowship he spent 18 months at Fred Hutchinson Cancer Research Center mentored by Rainer Storb. In Seattle he worked in the clinic, gaining deep experience of allogeneic HCT; in clinical research he has carried out studies on GVHD treatment and classification (Biol Blood Marrow Transplant 2000, Bone Marrow Transplant 2009, Biol Blood Marrow Transplant 2009). Once back in Candiolo he led the opening of the Medical Oncology Stem Cell Transplant Unit (2001); this unit is now part of a large metropolitan network that was JACIE-FACT accredited in 2013.
In clinical research, since his return, he has dedicated his efforts to mechanistically demonstrating that allogeneic HCT can generate a specific, long lasting graft versus tumor effect in hematological and solid tumors (Blood 2006, NEJM 2007, Blood 2009, Biol Blood Marrow Transplant 2009, Blood 2011). In the last years his group efforts have been aimed at exploring strategies for controlling GVHD and transforming HCT into a safe platform for specific anti-tumoral cell-therapies. In this regard he is collaborating with immunologists (Int Immunol 2008, J. immunother 2012). Since 2010 he and his group have been involved in the constitution of a melanoma-unit (BJC 2014, Cancer Invest, 2014). In metastatic melanoma, taking advantage of his experience in cellular therapies, he is collaborating with immunologists to project clinical trials aimed at combining targeted therapies and check-point modulators to adoptive cell therapy (Clin Cancer Res 2013, Expert Opinion Biol Ther 2014).

Selected references


Integrated therapies for the treatment of sarcomas

Cooperative trials for new therapeutic approaches

Team
Ymera Pignochino, Sandra Aliberti, Lorenzo D’Ambrosio, Danilo Galizia, Federica Capozzi, Paola Boccone, Sara Miano, Erica Palesandro, Marta Canta, Maja Todorovic

Research topic:
Integrated therapies for the treatment of sarcomas

Background:
Bone and soft tissue sarcomas (B-STS) are a rare and heterogeneous group of tumors. As of today, a multidisciplinary treatment encompassing complete surgical removal of the tumor +/- chemotherapy and radiotherapy cures roughly 50% of patients. Unfortunately, the most active chemotherapies may eradicate micro-metastatic disease, but do not cure non-resectable disease. Several second- and further-line treatments have been tested showing marginal activity at most. Therefore, innovative therapeutic strategies are urgently needed.

Research achievements:
We focused on the identification of key-pathways that can be exploited with innovative therapeutic tools. In osteosarcoma we identified the role of the mTOR pathway as a mechanism of resistance to sorafenib and showed the possibility of hitting this pathway with an innovative target combination therapy with sorafenib and everolimus. In this context we ran and completed a phase II trial. We identified on several B-STS the expression of specific antigens that can elicit a MHC-unrestricted T-cell immune-response. Thereafter, we demonstrated in B-STS patients that we could sort and ex-vivo expand a specific T-cell sub-population endowed with antitumor activity directed against patients’ tumors. After ongoing AIFA protocol approval, a phase I/II trial will be run within the Italian Sarcoma Group.

Conclusions and perspectives:
The achieved results show the importance of dissecting B-STS heterogeneity to identify histotype specific Achilles’ heel. Moreover, we are developing a form of immunotherapy that can be combined with any of the multidisciplinary therapies that are the real backbone of sarcoma treatment. In perspective we look for an even greater integration with the other immunotherapeutic tools directed to reactivating patient anti-tumor response. Secondly, several new compounds with improved selectivity to targets are in development. We have built a successful partnership with several pharmaceutical industries based on clinical trials designed and conducted by our team. On this ground, we will keep on joining international trials of important drugs, as we did with pazopanib, eribulin, and investigating combination therapies based on our preclinical/translational research activity.
Biographical Sketch:
Giovanni Grignani has been involved in clinical research focusing on the development of prognostic tools in oncology and later in the field of sarcomas. During his residency he worked on the identification of prognostic factors to improve multiple myeloma and lymphoma stratification as well as on several phase II trials exploring innovative chemotherapy combinations. Since he moved to the Candiolo Institute, he has been actively involved in translational research in the field of stem cells and sarcomas. In particular, he focused on the mechanisms of metastatic seeding of osteosarcoma (Clin Cancer Res 2005) identifying the CXCR4/CL12 axis as one of the major determinants of the peculiar pattern of osteosarcoma diffusion. He took part in several pivotal trials of targeted therapies and took advantage of his large experience to develop preclinical models to test targeted therapies in sarcomas other than gastrointestinal stromal tumors (Cancer 2011). His group identified the role of the MAPK pathway (Clin Cancer Res 2013) in osteosarcoma progression and, on this original finding, he demonstrated the activity of sorafenib and mTOR inhibitor in the advanced stages of this tumor (Ann Oncol 2012 and Lancet Oncol 2015).

In the last years, he set up a preclinical collaboration with immunologists in the attempt to overcome the intrinsic limit of target therapies related to tumor heterogeneity. In this setting, he has recently published an original model to expand patient derived lymphocytes showing that so-called cytokine induced killer cells selectively kill autologous sarcoma cells (Cancer Res 2014). In the forthcoming year this experience is going to be translated into the clinic in a phase I/II trial.

Selected references

Pignochino Y, et al., The Combination of Sorafenib and Everolimus Abrogates mTORC1 and mTORC2 upregulation in osteosarcoma preclinical models. Clin Cancer Res 2013, 19:2117-31


Hyperthermic intraperitoneal chemotherapy (HIPEC)

Treatment of peritoneal carcinomatosis

Team
Armando Cinquegrana, Patrizia Marsanic, Andrea Muratore, Demetro Siatis, Alfredo Mellano, Marco Vaira

Research topic:
Treatment of peritoneal carcinomatosis by surgical cytoreduction combined with HIPEC.

Background:
Our clinical experience is focused on the treatment of peritoneal carcinomatosis and tumor recurrence. Since 1995, about 900 operations for peritoneal carcinomatosis have been performed; since January 2013 to date more than 150 patients with peritoneal carcinomatosis have been treated in our Istitute. Our operative unit is specialized in the treatment of this kind of disease by cytoreductive surgery (CRS) combined or not with hyperthermic intraperitoneal chemotherapy (HIPEC) and secondary surgical cytoreduction of advanced cancers.

Research achievements:
In the last 3 years we have performed about 65 CRS + HIPEC (peritoneal mesothelioma = 11; colorectal carcinomatosis = 14; pseudomyxoma peritonei = 33; ovarian carcinomatosis = 7). Through the constant improvement of surgical and perioperative skills, we have achieved promising results in terms of long-term survival (median overall survival of 65 months for peritoneal mesothelioma, 42 months for ovarian cancer, 58 months for colorectal carcinomatosis, 144 months for PMP associated with a 10-yr overall survival of 80%) associated with low perioperative morbidity and mortality rates. We have registered an overall major morbidity rate of 15%, decreased to 6.7% considering only the last three years associated with no perioperative death. Since January 2013 in our Institution we have performed more than 100 surgical cytoreductions, which 65 for ovarian carcinomatosis. The mainstay of treatment is the attempt to achieve complete surgical cytoreduction, identified as the most significant prognostic factor: in this regard we perform a high number of primary or interval debulking surgeries with no macroscopic residual disease (CC-0) in a substantial number of patients. In cooperation with medical oncology, considering that the follow-up is too short, the results in terms of long-term outcome are still being processed. We can assert that in the last two years we registered a major complications rate of 2-3% with no postoperative deaths. All the HIPEC procedures (65 cases) were carried out intraoperatively with an original “semi-closed” technique. During the last years we have redefined our patient selection policy and sought to restrict indications to patients with less advanced or less aggressive disease: the selection process for CRS and HIPEC is critical and prognostic factors are required to identify patients who may most benefit from these treatments. The standardization of the surgical technique borrowed from experience in the treatment of peritoneal carcinomatosis allows us to obtain good long-term results with limited costs and mean hospital stay time.

Conclusions and perspectives:
In order to perform a more careful selection of patients, we are investigating the practice of second look (technique) to identify patients that early present an higher risk of relapse; for that purpose we are, also, testing the use of the liquid biopsy for the detection of circulating DNA. We are also evaluating laparoscopic HIPEC on selected patients (PIPAC), but we only have preliminary results.
Biographical Sketch:
Michele De Simone has always been interested in research of new technologies in Surgical Oncology. (Anticancer Res. 2001). He focused his experience in the field of primary and metastatic tumors taking an active part in many clinical research projects (Rev Recent Clin Trials 2007). He developed research experience in treatment of peritoneal carcinomatosis (J Clin Oncol. 2004).

Selected references


New strategies for breast cancer local-regional control

*Re-definition of local-regional breast cancer treatment according to the risk of relapse*

Team
Franziska Kubatzki, Furio Maggiorotto, Alessandra Magistris, Francesco Marocco, Stefania Renditore, Alessandro Rivolin, Paola Sgandurra, Salvatore Carlucci

Research topic:
Strategies for breast cancer loco-regional control

Background:
The prevention and early detection of local-regional relapses requires a better understanding of the complex relationship between the primary tumor and its metastatic dissemination. For optimal breast cancer care new advancements in surgery and radiotherapy must be integrated into the complex and rapidly evolving armamentarium of targeted systemic therapies.

Research achievements:
The influence of several treatment-related (accelerated partial radiotherapy, width of surgical margins, endocrine therapy with aromatase inhibitors), tumor-related (lobular histology, miR148b expression) and host-related parameters (body mass index, polymorphisms at the CYP19A1 locus) on the likelihood of breast cancer relapse have been examined. The available data and the new hypotheses on the relationship between loco-regional control and survival have been reviewed from a breast surgeon’s standpoint.

Conclusions and perspectives:
The current understanding of breast cancer natural history points towards the existence of a complex interplay between loco-regional and systemic processes. Loco-regional recurrences are associated with decreased overall survival and this may be related to a complex relationship between circulating tumor cells, re-seeding of the primary tumor site and several metabolic effects linked to the act of surgery. A re-definition of local-regional breast cancer treatment according to the risk of relapse based on improved bio-molecular characterization of the tumor is required to tailor the adoption of diagnostic tools, loco-regional and systemic treatments.
Biographical Sketch:
In 1987 and 1988, Dr. Ponzone was employed in the laboratories of the Wistar Institute and Children Hospital of Philadelphia, U.S.A., where he acquired the basics of Molecular Biology and was involved in research projects on hyperphenylalaninemia and pterin metabolism (J Inher Metab Dis. 1989).

After graduation, he was actively involved in the clinical and research activities at the Dept. of Gynecological Oncology, University of Turin, Italy, where he specialized in 1997. Over these years, he co-authored several papers on the prognostic significance of the family of kallikreins in breast and ovarian cancer (Cancer Res 1995).

In 1995 – 1996 he attended as clinical research fellow the Royal Marsden and University College Hospital of London, UK, where he conducted clinical trials on innovative endocrine therapies for breast cancer (Br J Cancer 1999), and developed a personal interest in the clinical issues related to hereditary breast and ovarian cancer (Eur J Cancer 1998). From 1999 to 2009 he was appointed attending Gynaecologist at the Mauriziano Hospital of Turin and at the Institute of Candolo. During these years he focused his research activity on the adjuvant (Ann Oncol 2006) and surgical (J Clin Oncol. 2009) therapy of breast cancer. In 2010 he was appointed Director of the Division of Gynaecological Oncology at the Institute of Candolo.

His current research interests are devoted to tailoring loco-regional breast cancer treatments (Eur J Cancer. 2012) and minimizing their side effects (Ann Surg Oncol. 2012). He has also consolidated his expertise in pelvic surgery, with a particular interest in molecularly-based and innovative treatments of ovarian cancer. In his career, he has performed more than 4500 breast and pelvic surgeries and has authored more than 90 papers published in international peer-reviewed journals, as well as book chapters and national guidelines.

Selected references


Applications of Computer Assisted Diagnosis (CAD) in Radiology

The diagnostic imaging, improved by CAD systems, allows tumor diagnosis to be brought forward to an early asymptomatic phase

Team
Ilaria Bertotto, Delia Campanella, Gabriele Chiara, Andrea Crivellaro, Veronica Deantoni, Valentina Giannini, Antonio Manca, Laura Martincich, Simone Mazzetti, Filippo Russo

Research topic:
Computer Aided Diagnosis (CAD) applications in Radiology

Background:
High resolution images of the human body are generally obtained by Computed Tomography (CT) or Magnetic Resonance (MR) imaging. CT Colonography is an example of such detailed imaging in which data from a CT scanner are processed by dedicated software to obtain a 3D representation of the colon from inside the intestinal lumen in a similar way to a conventional endoscope view. On the other side, MR imaging has shown promise in localizing cancers, because of its intrinsic high soft-tissue resolution, and combining two or more image modalities can improve the sensitivity of MR tests. However the more variables are introduced the more difficult it is to integrate all available information into one reliable final report, even for an experienced reader. To deal with these complex problems, computer aided diagnosis systems (CAD) have been introduced to help radiologists in diagnosing disease.

Research achievements:
The research group developed a CAD able to produce quantitative maps of the prostate, providing malignancy probability information with useful data that might be difficult to assess visually from MR scans. Regarding the CAD colon, diagnostic performance of CT colonography in individuals at increased risk of colorectal cancer was investigated, and further studies are being conducted using the innovative dual energy technology, to generate so-called virtual non-contrast images. Finally, within the CAD-breast project, we developed a fully automatic method to compute the distance between the Nipple-Area Complex (NAC) and the tumor to preoperatively evaluate the likelihood of NAC involvement to help select candidates to nipple sparing mastectomy.

Conclusions and perspectives:
Regarding the prostate CAD system, it has been demonstrated useful in automatically detecting prostate cancer using MR images, showing a per-patient sensitivity of 97% - i.e. at least one PCa lesion was detected in 54 of the 56 patients - and a median number of FP per exam equal to 3 (1st-3rd quartile; 1-4). A multireader study is being conducted to evaluate if CAD system could help to improve sensitivity and reduce reading time. A new branch of research is now focusing on assessing the role of CAD in supporting prostatic MR-guided biopsy, and on estimating tumor aggressiveness by integrating the Gleason Score with imaging, in order to better stratify patients and personalize treatments. Moreover, we are performing a study, whose aim is to compare PCa detection rate of in bore MR-targeted biopsy with the detection rate of TRUS-guided prostate biopsy in patients with high PSA values and at least one suspicious region identified by the radiologist at mp-MR imaging.
Biographical Sketch:
Dr. Regge is the Director of the Radiology Unit at the Candiolo Cancer Institute and he supervises a multidisciplinary team, which investigates advanced medical imaging technologies. The group seeks to enhance cancer diagnosis by means of Computer Aided Diagnosis (CAD) systems. His major scientific accomplishments are the following: (i) He is internationally recognized for his contributions to the CT-colonography field, being involved in clinical trials and coordinating the largest european multicentre study. This study, together with the ACRIN-6664, lead to the introduction of CT-colonography into the guidelines of the American Cancer Society as a screening test for colorectal cancer. (ii) He is PI of the first large world mass screening trial with CT-colonography conducted in Piemonte and in the province of Verona (http://clinicaltrials.gov/show/NCT01739608); (iii) He is active in the development and clinical validation of CAD systems for diagnosis of colorectal, breast and prostate cancer. (iv) He has given important contributions to the field of multiparametric-MRI, which helped change the diagnostic pipeline of prostate cancer. He is the author of more than 100 peer-reviewed publications and more than 300 scientific contributions in national and international congresses, and he has been moderator or invited speaker at more than 250 conferences worldwide. Dr. Regge has been PI of several supported research projects, and he is part of the faculty of the European School of Radiology, founding Member and president elect of the ESOI, board Member and Secretary of the European Society of EuSoMII and President of the Italian Chapter of Imaging Informatics.

Selected references


“Personalized” radiation-therapy based on genetic, biological, and “theragnostic” parameters

Adapting radiotherapy treatment to the modern concept of “personalized medicine” (“Precision radiotherapy”)

Team
Gaetano Belli, Gabriella Cattari, Marco Gatti, Gabriele Petrilli, Antonia Salatino, Antonella Suma, Domenico Gabriele

Research topic:
“Personalized” Radiation-therapy based on genetic, biological, statistical and “theragnostic” parameters.

Background:
The improved IMRT techniques (Intensity Modulated Radiation Therapy) allow the use of high doses, perfectly “sculpted” on the areas that are going to be treated. A further improvement of radiation treatment is provided by “daily image control” based on the IGRT technique (Image Guided Radiotherapy). Genomic information and “theragnostic” data allow the defining models to optimize the use of radiotherapy, in order to personalize treatment.

Research achievements:
(i) We have proved - in collaboration with the cancer stem cell laboratory - that ionizing radiation (IR) leads to the expression of an oncogene (Met) involved in invasion and metastasis control; this data contributes to the ‘vexata questio’ debate about the possible radiotherapy side effects on metastasis sprouting. (ii) We performed a feasibility study on large fields, using Tomotherapy. (iii) A controlled clinical trial on IMRT-IGRT treatment protocols for head and neck tumors and recurrences, and three studies on prostate cancers are in progress. (iv) We are contributing to an international multicenter randomized phase III clinical trial on locally advanced rectal cancer. (v) We are leading the european project “Computational Horizons in Cancer” to create a predictive nomogram of prognosis.

Conclusions and perspectives:
We (i) are evaluating the possible impact of genetics and molecular biology on the radiotherapy of Glioblastoma Multiforme (GBM). The preclinical research is implemented by “xenopatients” (immunocompromised mice transplanted with GBM of single patients, see above); (ii) we are developing personalized radiotherapy treatment for pancreatic cancer, for clivus cordoma, for pleural mesothelioma and for gynecological tumors; (iii) we are implementing treatment protocols for microwaves and radiofrequencies and for radiochemo-hyperthermia.
Biographical Sketch:

Selected references


Personalized medicine driven by nuclear molecular imaging in cancer patients

Providing personalized molecular imaging strategies in cancer patients

Team
Manuela Racca, Paola Scapoli, Valeria Pirro

Research topic:
Providing Personalized Medicine with Nuclear Molecular Imaging in Cancer Patients - PET/CT in early evaluation of cancer treatment response and targeting resistance to molecular therapies.

Background:
Early differentiation between responders and non-responders is a very important issue for the management of cancer patients. Wide evidence exists that FDG-PET can predict outcomes for many tumor types very soon after conventional systemic chemotherapy is started. In particular, compared to cytotoxic chemotherapy, many novel targeted therapies result in disease stabilization rather than tumor shrinkage. The activity of these novel agents might, therefore, be better reflected by changes in molecular features of the tumor rather than reduction in size or volume. Recent prospective studies show that a change in tumor glucose activity (measured with FDG PET standard uptake value-SUV) is a significantly more accurate parameter than a change in size in assessing histopathological response to neoadjuvant therapy in patients with GIST and high grade soft tissue sarcoma.

Research achievements:
The PET imaging facility has been qualified by CoreLab Partners, Inc. for the submission of Nuclear Medicine and PET images of the patients who are enrolled in the CAUY922A2109 study. The aim of this study is to evaluate the efficacy of AUY922 in combination with Trastuzumab in patients with locally advanced or metastatic HER2-positive breast cancer that has progressed after or during at least one Trastuzumab-containing regimen.

Conclusions and perspectives:
Research goals will be to assess the value of metabolic imaging with PET in predicting response to therapy, targeting resistance to molecular therapies and improving personalized therapy and prognosis.
Biographical Sketch:
Teresio Varetto has more than 25 years’ experience in all diagnostic and therapeutic activities in Nuclear Medicine. During his career he has been a pioneer in the introduction of new diagnostic radiopharmaceutical methodologies in Cardiology (J Am Coll Cardiol, 1993 – Q J Nucl Med Mol Imaging., 2005) and in Oncology (Eur J Nucl Med Mol Imaging 2005). Since 1998 he is Professor at Turin University Postgraduate Specialization School in Nuclear Medicine and Postgraduate Specialization School in Hospital Pharmacology. As Senior Expert in Nuclear Medicine at International Atomic Energy Agency he accomplished Technical Cooperation Expert Missions in several African Countries collaborating on the creation and dissemination of Nuclear Medicine Units and giving lectures and counseling at the local University Hospitals.

As chairman of the Continuing Medical Education Committee of the UEMS European Board of Nuclear Medicine he is in charge of accreditation of International educational activities. (Eur J Nucl Med Mol Imaging 2013; Eur J Nucl Med Mol Imaging 2014; Eur J Nucl Med Mol Imaging 2014). Member of the Executive Board of the Italian Association of Nuclear Medicine and Molecular Imaging since 2000, he is Chairperson for CME in Nuclear Medicine at the National level. Since 2008, as Director of the Nuclear Medicine Department at the Candiolo Institute for Cancer Research, he has been involved in research projects aimed at providing personalized medicine to cancer patients as the use of PET/CT in early evaluation of cancer treatment response and targeting resistance to molecular therapies.

Selected references


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A mouse ‘xenopatient bearing a human Gliobastoma Multiforme (IVIS scan)
Core Facilities

The ‘Xenopatients’ Platform for Experimental Oncology.
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Immortalized cancer cells exhibit a genetic drift, a biological compliance and phenotypic features different from original cancers in patients. Another drawback of the use of continuous cell lines is that the catalogue of currently available models is inevitably finite, and possibly poor for some tumour subtypes. Therefore, experiments with cell lines cannot recapitulate the wide heterogeneity of human malignancy that occurs among individuals on a population basis. One way to tackle this issue is to perform population-based in vivo studies by using large series of human cancer specimens directly transplanted into mice. This setting can be exploited as a bridgehead to unravel the genetic and biological complexity of cancer, as a prelude for the identification of novel therapeutic targets and unanticipated pharmacological approaches. XPeRö provides researchers with a collection of liver metastases from colorectal cancer that have been systematically transplanted in immunocompromised mice to obtain stable tumor lines (xenopatients), which are available for any kind of in vivo/ex vivo study. This resource builds on three main cornerstones: (i) The viable biobank of colorectal cancer liver metastases. An institutional effort has led to the collection and implantation of more than 500 samples of metastatic colorectal carcinoma. This resource allows us to select the appropriate candidates among more than 300 experimental models, based on clinical, biological, genetic or gene expression traits. This, in turn, is instrumental for achieving the appropriate statistical power for any kind of translational study, even if focused on relatively small tumour subpopulations. (ii) The ‘Xenopatients’ experimental platform for in vivo drug screening. We have implemented a dedicated experimental pipeline that required the recruitment of ad-hoc personnel and the development of a specific workflow. The possibility to exploit trained staff and to apply robust standard operating procedures (SOPs) to experimental work confers high added value to research projects, in terms of both cost effectiveness and efficiency of the resources used. (iii) The Laboratory Assistant Suite package for data management and analysis. We have developed a web-based bioinformatics platform that assists biomedical researchers in multiple activities, which range from tracking data generation and SOPs execution to the management of multidimensional molecular profiles and complex data integration (The Laboratory Assistant Suite or LAS, http://devircc.polito.it/wordpress/). This kind of informatic support provides invaluable benefit by optimising quality and reproducibility of the experiments and by accelerating data mining and analysis.

The Oncogenomics Center (OGC)
Genetic alterations in tumors are predictors of response or resistance to targeted therapies, and their identification is mandatory for molecular diagnosis and therapeutic decisions. Technological advances in experimental and informatics methodologies over the past 10 years have made possible the characterization of cancer genomes. OGC is the supportive infrastructure for all genomic studies, including transcriptional, mutational and gene copy number analysis of cells, tissues and liquid biopsies. Dedicated personnel and instrumentation are devoted to provide services for qRT-PCR studies, Sanger and Next Generation Sequencing experiments, Gene Expression Array analyses and BEAMing tests.
The Bioinformatics Center (BIC)
Modern research in cancer biology implies the collection of extensive data from experimental models concerning specific genetic lesions that drive cancer initiation and progression. Such data will include, for example, large sets of expression transcript profiling, comparative genomic hybridization profiling, whole genome sequencing, immunohistochemical data, and morphologic data that will be peculiar to each specific tumor. Thus, a bioinformatics platform for integrated data tracking and normalization is critical to the successful realization of this endeavor. BIC comprises a web-based bioinformatics platform (Laboratory Assistant Suite, LAS; http://devircc.polito.it/wordpress/) that assists biomedical researchers in multiple activities, which range from tracking data generation and execution of standard operating procedures (SOPs) to management of multidimensional molecular profiles and complex data analysis and integration, by managing multiple independent databases that are linked together in an interconnected network (‘oncogrid’).

The Oncology Imaging Center (OIC)
Basic research in disciplines such as cell biology, molecular genetics and developmental biology has provided invaluable insights into the regulatory circuits that govern cancer onset and progression. Within this context, we postulate that imaging studies in cell topics and tissues will parallel genomic analyses and in vivo experimentation, constituting an integrative platform for rapid testing of emerging research directions. OIC technologies include comprehensive microscopic imaging systems, such as confocal microscopes, live-cell devices for real-time monitoring of cellular behaviors, and high-throughput platforms for functional screening.

The Flow Cytometry Center (FLOCC)
Increasing evidence shows that tumors are structured in a hierarchical form, with a majority of cells undergoing aberrant differentiation but retaining a proliferative capacity limited over time, and a tiny fraction of cancer stem cells (CSCs) or cancer-initiating cells (CICs) that are able to self-renew and continuously regenerate the tumor. FLOCC enables researchers to take advantage of state-of-the-art FACS (fluorescence-activated cell sorter) technologies and dedicated personnel with highly specialized technical skills in order to tackle these issues, by allowing analysis and prospective isolation of individual cancer cells within highly heterogeneous populations.
Clinical Services

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The head of the department of clinical services participates in the strategic planning process and contributes, with the formulation of proposals and opinions, to hospital management. He directs health services and is responsible for hygiene and prevention (hospital infection control and environmental hygiene). The clinical service office monitors the appropriateness of admissions, the hospital stay lengths, the average weight of the so called ‘case mix’ and supervises the waiting list. He coordinates with the Operation Department the proper use of spaces and the timely flow of goods and services. The clinical service office:
(i) is in charge of the control of the fulfillement of results obtained within the assigned objectives; (ii) coordinates the facilities of the hospital including the provision of appropriate protocols; (iii) defines strategies and guidelines for extraordinary and/or urgent intervention; (iv) advises on building planning regarding further hospital expansion; (v) supervises the ‘conventional’ agreements and the related relations with the University.

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This Unit is mainly devoted to patient diagnosis and treatment. Nevertheless, a strong effort is made in performing analysis supporting clinical trials as well as translational research. The laboratory is also engaged in its own research activity aimed at developing and testing novel diagnostic procedures. A special emphasis is placed on the analysis of prostate cancer markers.

Felicino De Bernardi, MD
Cancer Pain
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Cancer pain significantly affects the physical, psychological and emotional components and quality of life of the individual. Our goal is the optimisation of pain relief through a patient-centred approach with particular attention to quality of life, personalized care and application of the newest drugs and treatments.

Giovanni Succo, MD
Otolaryngology
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The Otolaryngology Unit has many related clinical functions focused on with the diagnosis and treatment of disorders and diseases of the head and neck. These specifically includes illness or injury of the ear, nose, and throat, as well as medical or surgical treatments for diseases of the skull base.
Giovanni Galatola, MD
Gastroenterology
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The Gastroenterology Unit at IRCC is a dedicated provider of a wide range of diagnostic and therapeutic endoscopic procedures for the upper and lower gastrointestinal tracts, and is involved in the clinical care of patients with chronic viral hepatitis and those with gastrointestinal disorders in the medical oncology setting.

Alessandro Bonzano, MD
Cardiology
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The Cardiology Unit at the IRCCS is engaged in the prevention, diagnosis and treatment of cardiotoxicity caused by old chemotherapy drugs (anthracyclines, 5-fluorouracil) and new agents (trastuzumab). Doppler echocardiography and biomarkers as troponin are used to identify early damage of the heart during chemotherapy. A growing experience in the field of Cardioncology has lead to tailored treatment for cancer patients with cardiovascular complications.

Franca Goffredo, MD
Pharmacy
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The Pharmacy Unit manages drug/medication related activities and issues for all IRCCS wards (drug lifecycle). Some of these activities include supplying, storage, distribution, drug information, therapeutic policy/formulary. Preparation of chemotherapy and supportive care medications for in and out patients, together with investigational drug management and research support (including regulatory issues) are the main and distinguishing activities, enhanced by integrated IT and automation. The staff is an active partner of the University in the pharmacy education and training programs.

Michele Stasi, M.D.
Medical Physics
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The Medical Physics Unit is focused on patients’ tumor segmentation based on multi-modal imaging, sophisticated patient specific Quality Assurance and treatment options such as Helical Tomotherapy (HT), Intensity Modulated Radiation Therapy (IMRT), Image Guided Radiation Therapy (IGRT), and Adaptive Radiotherapy. The use of sophisticated imaging tools (MRI, PET/CT) and IMRT, IGRT may help to achieve better conformal radiation dose to target with respect to 3D standard conformal radiotherapy.
Educational Activities: the Chiron international program

The Institute provides teaching and training programs in the area of basic and clinical cancer research. These are listed in order of increasing complexity as follows:
(a) Courses.
(b) Seminars and Workshops. (i) “Lezioni Magistrali”: one in basic research and one in clinical research, each held by eminent scientists. (ii) Formal seminars: throughout the year, divided among basic and clinical research, held by external invited speakers. (iii) Progress reports held by members of the Institute, once a week for 48 weeks of the year. (iv) International Workshops, on either basic or clinical research. These workshops are usually organised within the framework program of the European Molecular Biology Organization (EMBO) and of the European Society for Medical Oncology (ESMO).
(c) Research Doctorates focused on basic and clinical research training.
(d) Master in Molecular Oncology.
(e) Post-doctoral programs.

IRCC Cancer Conferences
• “From Signal Transduction to Cancer Precision Medicine” - June 6, 2015
• “International conference on Cancers of Unknown Primary” - May 20-21, 2017

2015 Seminars
• Regulation of self renewal in Cancer Stem Cells - Prof. Pier Giuseppe Pelicci, December 11, 2015
• p140Cap is a Chromosome 17q12-q21 scaffold protein that limits ErbB2 breast cancer progression - Prof.ssa Paola Defilippi, December 4, 2015
• Every Cell Has A Story - Advances in Single Cell Genomics - Dr. Amy Hamilton, December 2, 2015
• NanoBiT Complementation Reporter Provides Accurate Assessment of Intracellular Protein Interactions under Physiological Conditions - Keith V. Wood, PhD, November 9, 2015
• Data intensive biology and data provenance graphs, ovvero l’importanza di tracciare i dati - Prof. Gianluigi Zanetti, October 12, 2015
• Therapeutic strategies based on cancer stem cell targeting - Prof. Ruggero De Maria, October 2, 2015
• The Network Genomic Medicine: A Platform to implement Personalised Cancer Treatment in Germany - Prof. Reinhard Büttner, October 1, 2015
• Genomics and Therapeutic Opportunities in Gastroesophageal Cancer - Dr. Adam Bass, October 1, 2015
• Neuropilin and Integrin Control of Cancer Stem Cell Fate - Dr. Arthur M.Mercurio, Friday, March 20, 2015
• NK cells: from surface receptors to the cure of high risk leukemias - Prof. Lorenzo Moretta, March 19, 2015
• Colon cancer stem cell resistance to therapy - Dr. Jan Paul Medema, January 26, 2015

PhD Programs
The Institute offers four different PhD programs.
(a) PhD in Molecular Medicine (Cell Sciences and Technology): a four-year course (under the auspices of the University of Torino) open to young graduates in Medicine, Biological Sciences, Biotechnology, Chemistry, and Pharmacology. It is aimed at training for basic and translational research in biomedical sciences.
(b) PhD in Complex Systems and Post Genomic Biology: a three-year course (under the auspices of the University of Torino) aimed at training graduates in the fields at the interface of medicine, life sciences, mathematics and physics.
(c) PhD Program in Biomedical Sciences and Oncology: a four-year course (under the auspices of the University of Torino) open to graduates in Medicine, and Biological Sciences and mainly aimed at training clinical researchers.
(d) PhD program in Oncological Sciences: a three-year course (under the auspices of the Catholic University of Milano-Roma) for exceptionally talented graduate students to be trained in cutting-edge oncological research at the highest level.
Graduate students are provided with an individual tailored guidance program and required to rotate through a number of laboratories to learn different research techniques collaborating with world-class scientists. Career counselors help students to investigate job types, develop curriculum vitae and resumes, and refine interview skills. After completing the PhD program students are encouraged to participate in internship opportunities in both basic or clinical research, technology transfer, science policy, and management. All programs are open to Italian and foreign students. Fellowships are provided on a competitive basis.

Post-doctoral Programs
IRCC International Cancer Research Training Program
This scheme employs scientists of all nationalities to improve scientific exchange between the Institute’s researchers and colleagues who have matured qualifying experience in foreign institutions. The research field is at the interface between molecular biology and medicine, referring in particular to today’s problems in molecular oncology.

The Marie Curie European Training Center
The European Commission has recognized IRCC as a qualified center for training young researchers in the experimental medical science field. Within the framework of the program, IRCC employs in rotation graduate students from member states for periods varying from six months to two years in the research laboratories.

Molecular Oncology Master
The Institute, under an agreement with the University of Torino, also offers a two-year Master in Molecular Oncology aimed at providing clinical oncologists, surgical oncologists, radiologists, radiotherapists, and pathologists with up-to-date knowledge of cancer biology and genetics as well as of the novel diagnostic and therapeutic (e.g. precision medicine) approaches that arose from latest molecular and genomic information.
The Scientific Director’s Office supervises, coordinates and manages the basic, translational and clinical research at the Institute. To this end, the Grant Office provides the scientific support for Institute research activities and acts as a liaison between researchers and funding agencies.

The Grant Office assists researchers in identifying appropriate research funding opportunities, centralizing all information on major national and international, private or public, agencies, foundations, and institutions that support research. Whenever a call for a research grant is issued, it is advertised throughout the Institute by e-mail. The Grant Office provides assistance to researchers throughout the application process, in drawing up budgets – together with the administrative office of the Institute –, in interpreting the regulations of the granting agencies, assuring compliance with the sponsors’ policies and requirements. In case of successful outcome of the proposals, the Grant Office helps in preparing reports, consortium agreements with collaborating institutions, and renewal forms.

The Office of Research Administration (ORA) handles the administration of research funds, assigned to the Fondazione del Piemonte per l’Oncologia (FPO) and to the Fondazione Piemontese per la Ricerca sul Cancro (FPRC). The staff consists of 5 people.

The office manages the registration of every accounting movement:
i) organization of the first cash note; ii) revenues and payments; iii) management of personnel; iv) drafting of the research budget.

With the support of the Grants Office, ORA prepares economic reports on the costs incurred, in accordance with the requirements of the single funding agencies, providing the required supporting documentation.

In 2015, the Office has managed 4347 invoices (2521 by the FPO administration, 1826 by the FPRC administration), has issued 3025 orders (1587/1438) and has drawn 125 employment contracts (94/31).
Electronic Data Processing Unit

The EDP unit develops and maintains the Master Database, a unique tool, developed in-house, which tracks the funds, human resources and research publication output, of the Research Units at the Institute. The EDP unit runs the Candiolo Cancer Institute public web site and the Intranet. The unit also provides IT support to the Research departments and to the Institute’s meetings and conferences.

Ethics Committee

The ethics committee, following the European Directive 2001/20/EC, is an independent body of the Candiolo Cancer Institute, consisting of healthcare professionals and non-medical members, whose responsibility is to protect the rights, safety and well being of human subjects involved in a clinical trial and to provide public assurance of that protection, by, among other things, expressing an opinion on the clinical trial protocol, the suitability of the investigators involved in the trial and the adequacy of facilities, and on the methods and documents to be used to inform trial subjects and obtain their informed consent.


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Errichiello E, Balsamo A, Cerni M, Venesio T. Mitochondrial variants in MT-CO2 and D-loop instability are involved in MUTYH-associated polyposis. Journal of molecular medicine (Berlin, Germany), 2015, 93:1271-81 · IF: 5,107


Gagliardi PA, di Blasio L, Primo L. PDK1: A signaling hub for cell migration and tumor invasion. Biochimica et biophysica acta, 2015, 1856:178-188 · IF: 7,845


Garassino MC, Marsoni S. A lesson from vorinostat in pleural mesothelioma. The lancet oncology, 2015, 16:359-60 · IF: 24,725


Ponzone R. Breast Cancer Prevention: Can Women’s Expectations Be Met? The oncologist, 2015, 21:2-3 · IF: 4,865


Sangiolo D. Redirected T cells in cancer therapy. Expert opinion on biological therapy, 2015, 15:1667-70 · IF: 3,653


Seretti G, Tamagnone L. Bad vessels beware! Semaphorins will sort you out! EMBO molecular medicine, 2015, 7:1251-3 · IF: 8,665


Torchiaro E, Lorenzato A, Olivero M, Valdembri D, Gagliardi PA, Gai M, Erriquez J, Serini G, Di Renzo MF. Peritoneal and hematogenous metastases of ovarian cancer cells are both controlled by the p90RSK through a self-reinforcing cell autonomous mechanism. Oncotarget, 2015, 7:712-28 · IF: 6,359


Vigna E, Chiriaco C, Cignetto S, Fontani L, Basilico C, Petronzelli F, Comoglio PM. Inhibition of ligand-independent constitutive activation of the Met oncogenic receptor by the engineered chemically-modified antibody DN30. Molecular oncology, 2015, 9:1760-72 · IF: 5,331


