The Pezcoller Foundation

Journal

Summary

News from the
Pezcoller Foundation World

Year 22 - no 38
June 2012

June 2012

It's with great pleasure that I announce that the recipient of the 2012 Pezcoller Foundation-AACR Award for Cancer Research is Robert A. Weinberg, PhD , professor of Biology, Director of Ludwig Center for Molecular Oncology at MIT in Cambridge, MA and founder of the Whitehead Institute for Biomedical Research in Cambridge.
As alternate has been selected James P. Allison, Ph.D., Chairman, Immunology Program, Director, Ludwig Center for Cancer Immunotherapy, Investigator, Howard Hughes Medical Institute, David H. Koch Chair of Immunologic Sciences, Memorial Sloan-Kettering Cancer Center, New York, NY.
The Selection Committee met in Trento on November 20, 2011 and was chaired by Axel Ullrich, Ph.D., Director Dept. of Molecular Biology Max Planck Inst. of Biochemistry, Martinsried, Germany.
The members of the Committee were: Myles A. Brown, M.D., Director Ctr. for Functional Cancer Epigenetics, Dana-Farber Cancer Inst. Harvard Medical School, Boston, MA - Dean E. Brenner, M.D., professor of Internal Medicine Univ. of Michigan Medical Ctr , Ann Arbor, MI - Vincenzo Bronte, M.D. Professor and Head of Immunology Section, Univ. of Verona, Italy - Lewis C. Cantley, Ph.D., professor Dept. of Systems Bio. Harvard Medical School, Boston, MA - Verena Jendrossek, Ph.D., professor of Molecular Cell Biology Institute of Cell Biology (Cancer Research), Essen, Germany - Stefano Piccolo, Ph.D., professor of Biomedical Sciences Section of Histology and Embryology Dept. of Medical Biotechnologies, Univ. of Padova Italy - Enrico Mihich, M.D. Distinguished Member, Dept. of Medical Oncology Dana-Farber Cancer Inst. Boston, MA
Ex--officio members: Margaret Foti, Ph.D., M.D., CEO American Association for Cancer Research and Gios Bernardi, M.D., President of the Pezcoller Foundation.
The Weinberg laboratory prepared DNA from mouse cells that had been transformed into tumor cells by exposure to a chemical carcinogen. These cellular DNAs were then introduced, via transfection, into normal mouse fibroblasts. Weinberg and coworkers had spent the previous several years optimizing the gene transfer procedure so that it was reproducible and efficient in transferring DNA from a donor cell to a recipient cell. Some of the normal mouse cells into which the tumor cell DNA was transfected then became transformed into tumor cells. This outcome indicated that a transforming principle was present in the tumor cell DNA, and that this transforming principle was likely to be a mutant form of a normal cellular gene. Indeed, when the DNA from normal mouse cells was transfected into other normal mouse cells, no transformed cells appeared.
This insight represents the single most important accomplishment of the Weinberg laboratory. It prepared the foundation for the subsequent work that was done by both the Weinberg group and by three other laboratories that soon thereafter entered this field, which was opened up by the above advance.
Soon after these experiments were completed, they were extended to include DNA from chemically induced rat neuroblastomas as well as DNAs derived from human tumor cells. Transfection of the rat tumor DNA led to the discovery of the neu oncogene and oncoprotein; the human ortholog of this gene was later discovered by others and renamed ErbB2 or HER2. An antibody against the encoded oncoprotein, termed Herceptin, proved 20 years later to be effective in aiding in the treatment of certain human breast cancers.
More importantly, transfection of the human tumor DNAs into mouse fibroblasts led to the discovery of oncogenic DNA present in a human bladder carcinoma cell line named variously EJ or T24. This indicated that the transforming principle, whatever its nature, could work across species and tissue boundaries. By 1982, the Weinberg lab, simultaneously with a second laboratory (that of M. Wigler), had isolated the EJ/T24 bladder carcinoma oncogene by molecular cloning. Later that year, the Weinberg laboratory, simultaneously with a second laboratory (that of G. Cooper), demonstrated that this bladder carcinoma oncogene was related to the Harvey ras oncogene that had been identified by others through its association with a murine retrovirus - Harvey sarcoma virus. In doing so, the Weinberg laboratory demonstrated that a common repertoire of protooncogenes resides in normal vertebrate DNA, and that genes within this repertoire, such as the ras oncogene, can be activated to an oncogenic state either by association with a retrovirus or by somatic mutations that occur during the development of human tumors.
Also in 1982 the Weinberg laboratory, simultaneous with a second laboratory (that of M. Barbacid), was able to determine the genetic origin of the human bladder carcinoma oncogene. Specifically, a point mutation in the reading frame of the bladder carcinoma oncogene caused a glycine-to-valine substitution in the encoded protein, and this subtle change, on its own, sufficed to convert a normal proto-oncogene into an oncogene. The discovery of this point mutation represented the first time that a somatic mutation, defined at the molecular level, could be associated with the genome of a human tumor cell. This work thereby proved, directly and definitively, that human cancer pathogenesis is a process driven by somatic mutations.
In 1983 Weinberg and coworkers demonstrated for the first time that two distinct cellular oncogenes, in this case ras and myc, can collaborate with one another to transform a fully normal cell into a tumor cell. This demonstration of oncogene collaboration created a conceptual foundation for the now-widely accepted model of multi-step tumorigenesis, which postulates that human tumors arise following the accumulation of a sequence of somatic mutations; once formed the resulting mutant alleles collaborate with one another to yield the fully malignant phenotype of cancer cells.
In 1986 the Weinberg laboratory collaborated with a second laboratory (that of T. Dryja) to isolate a molecular clone of a known tumor suppressor gene - the retinoblastoma or RB gene. This gene had been described, albeit indirectly, in research going back to Alfred Knudson's 1971 report on familial and sporadic retinoblastomas. The 1986 report revealed a number of substantial deletions affecting RB in various retinoblastomas and an osteosarcoma - the first time that specific inactivating mutations were described at the molecular level in a tumor suppressor gene.

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In 1999, the Weinberg laboratory reported the first successful transformation of a normal human cell into a cancer cell. While countless research groups had demonstrated the ability to transform normal rodent cells into cancer cells through the introduction of various oncogenes (e.g., see above), such transformation had never succeeded with human cells. This revealed fundamental differences in the biology of rodent versus human cells, demonstrating that at least five distinct regulatory circuits needed to be perturbed within a normal human cell before it will proliferate as a tumor cell. This work created a conceptual framework with which to understand and rationalize the numerous genetic and biochemical changes that have been documented in human tumor cells by many groups over the past two decades.

In ongoing research the Weinberg laboratory has demonstrated the role of the Twist, Goosecoid and FOXC2 transcription factors in inducing an epithelial-mesenchymal transition (EMT) in carcinoma cells, demonstrating that Twist expression by mouse mammary carcinoma cells is critical to their metastatic properties. Most recently, the laboratory has demonstrated that cells forced through an EMT have many of the properties of epithelial stem cells.

Prof. Weinberg was introduced at the 2012 AACR Annual Meeting in Chicago where he delivered to a large audience the Pezcoller Lecture : "Epithelial-Mesenchymal Transition, Cancer Stem Cells and Metastasis".

The Award was given to Weinberg on May 11, 2012 with a solemn ceremony in the prestigious reception hall of the Buonconsiglio Castle in Trento, Italy. In the same week he gave the "Korsmeyer Lecture" in Padova at VIMM (Venetian Institute for Molecular Medicine) to honor the memory of the late Stanley Kosmeyer who received the Pezcoller-AACR Award in 2004.

This issue of the Journal is dedicated to the 24th Pezcoller Symposium entitled "Molecular Basis for Resistance to Targeted Agents" which will be held in Trento from June 14 to June 16, 2012.

The meeting program is being co-organized by Richard Marais, The Institute of Cancer Research, London, UK - William Sellers, Novartis Institute for Biomedical Research, Cambridge, MA - David Livingston, Dana Farber Cancer Institute, Boston, MA and with the collaboration of Enrico Mihich, Dana Farber Cancer Institute, Boston, MA.

The topic will be Cancer escape from Therapy, and the focus of the meeting will be the mechanisms of resistance to new tumor targeted therapies.

The Symposium will be opened by the keynote address of Prof. J. Engelman, Massachusetts General Hospital, Charlestown, MA. There will be 22 speakers divided in five sessions:

I and IV the genetics of this phenotype ;
II clinical advances in overcoming resistance ;
III resistance through perturbed signaling;
V role of the tumor microenvironment in resistance .

Speakers give approximately 25 minute talks followed by a similar amount of time for audience discussion. These meetings have regularly provided a fertile climate for highly revealing and stimulating scientific discussion on topics of extraordinary interest in cancer science.

The speakers are:
James Allison, Memorial Sloan Kettering Cancer Ctr, New York, NY -- Alberto Bardelli, Inst for Cancer Res & Treatment, Candiolo, Torino, Italy - Jose Baselga, Massachusetts General Hospital, Boston, MA Vall D'Hebron Inst of Oncology, Barcelona, Spain - Silvia Buonamici, H3 Biomedicine Inc. Cambridge, MA - Johann De Bono, Institute of Cancer Research, London, UK - Jeffrey Engelman, Massachusetts General Hospital, Charlestown, MA - Levi Garraway Dana Farber Cancer Inst, Boston, MA - Todd Golub, Broad Inst at MIT, Cambridge, MA - Jos Jonkers, Netherlands Cancer Inst, Amsterdam, NL - David Livingston Dana Farber Cancer Inst, Boston, MA - Richard Marais The Inst of Cancer Research, London, UK - Elaine Mardis The Genome Inst at Washington Univ, St. Louis, MO - Enrico Mihich, Dana Farber Cancer Inst, Boston, MA - Thomas O'Hare, Univ of Utah School of Medicine, Salt Lake City, UT - Neal Rosen, Memorial Sloan Kettering Cancer Ctr, New York, NY - William Sellers Novartis Inst for Biomedical Res, Cambridge, MA - Rafaella Sordella, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY - Jeffrey Sosman, Vanderbilt Univ, Nashville, TN - Ben Stanger University of Pennsylvania, Philadelphia, PA - Giulio Superti-Furga CeMM Res Ctr Molecular Medicine, Vienna, Austria - Roman Thomas University Hospital Cologne, Cologne, Germany - David Tuveson Cambridge Research Inst, Cambridge, UK.

The abstracts of this symposium are in the following pages.

Gios Bernardi
Editor and Pezcoller Foundation President Emeritus

Picture on the front page: 2012 Pezcoller Foundation-AACR International Award for Cancer Research ceremony in Trento. From the left: Axell Ullrich, Gios Bernardi, Davide Bassi, Robert H. Weinberg (the winner), Judy Garber, Margaret Foti.

24th Pezcoller Symposium

Molecular basis for resistance to targeted agents

Trento, Italy, June 14-16, 2012

ABSTRACTS OF ORAL PRESENTATIONS

Mechanisms of resistance to EGFR targeted therapies in colorectal cancer

Alberto Bardelli
Inst for Cancer Res & Treatment, Candiolo, Torino, Italy

Personalized cancer medicine based on the genetic milieu of individual colorectal tumors has long been postulated but until recently this concept was not supported by clinical evidence. The advent of the EGFR-targeted monoclonal antibodies cetuximab and panitumumab has paved the way to the individualized treatment of metastatic colorectal cancer (mCRC). There is clear evidence that mCRCs respond differently to EGFR-targeted agents and that the tumor specific response has a genetic basis. From the initial observation that cetuximab or panitumumab as monotherapy are effective only in 10-20%, of mCRCs, knowledge has being gained on the molecular mechanisms underlying primary resistance to these agents. The role of oncogenic activation of EGFR downstream effectors such as KRAS, BRAF, PIK3CA and PTEN on response to therapy will be discussed. The rapid and effective translation of these findings into predictive biomarkers to couple EGFR-targeted antibodies to the patients that benefit from them will be presented as a paradigm of modern clinical oncology. Unresolved questions such as understanding the molecular basis of response as well the mechanisms of secondary resistance will be discussed as the future fundamental goals in this research field.

Identifying the mechanisms of resistance to smoothened inhibitors

Buonamici S, Williams J, Morrissey M, Wang A, Guo R, Vattay A, Hsiao K, Yuan J, Green J, Ospina B, Yu Q, Ostrom L, Fordjour P, Anderson DL, Monahan JE, Kelleher JF, Peukert S, Pan S, Wu X, Maira SM, García-Echeverría C, Briggs KJ, Watkins DN, Yao YM, Lengauer C, Warmuth M, Sellers WR, Dorsch M., H3 Biomedicine, Inc., Cambridge, MA

Hedgehog (Hh) is a developmental pathway linked to tumorigenesis in several cancers. In the resting state, the 12-pass transmembrane protein Patched (Ptch) inhibits Smoothened (Smo), a G-protein coupled receptor (GPCR)-like molecule. When Ptch inhibition is attenuated, Smo signals via a cytosolic complex of proteins leading to activation of the Gli family of transcription factors. Somatic mutations in Ptch and Smo have been shown to lead to constitutive pathway activation and are found in particular in sporadic medulloblastoma (MB) and basal cell carcinoma (BCC). Several groups showed that antagonists of Smo abrogate the tumorigenic phenotype engendered by Ptch inactivation. NVP-LDE225 is a potent and selective orally available Smo antagonist that robustly inhibits Smo-dependent signaling *in vitro* and *in vivo*. NVP-LDE225 showed dose-related anti-tumor activity *in vivo* in several genetically defined MB models that are driven by mutations in Ptch leading to near complete tumor regression and Hh pathway inhibition. However, following long-term continuous dosing

of NVP-LDE225 in medulloblastoma allograft models, evidence of resistance to NVP-LDE225 was observed. Three different mechanisms of resistance were identified using genome-wide DNA- and RNA-profiling and sequencing of resistant tumors that evaded the inhibitory effects of Smo antagonists. Chromosomal amplification of Gli2, a downstream effector of Hh signaling, was identified as one mechanism leading to restoration of pathway signaling despite adequate drug exposure. In a minority of resistant tumors, mutations in smo were detected. Additional mining of the gene expression data for pathway signatures that are selectively deregulated in resistant tumors identified increased phosphatidylinositol-3-kinase (PI3K) signaling as another potential resistance mechanism. Probing the functional relevance of increased PI3K signaling, we showed that the combination of NVP-LDE225 with PI3K/mTor inhibitors markedly delayed the development of resistance. Our findings have important clinical implications for future treatment strategies in medulloblastoma.

Improving outcome from prostate cancer

Johann de Bono, Institute of Cancer Research, London, UK

Prostate cancer is the commonest cancer in men and the second commonest cause of death from cancer in men. Until recently, men with progressing cancer despite chemical or surgical castration were described as having 'hormone refractory' disease. Recent studies with the CYP17 inhibitor abiraterone acetate and the novel androgen receptor signaling inhibitor enzalutamide (MDV3100) have now provided incontrovertible evidence that this disease remains hormone driven. These agents have now been shown to improve overall survival and have changed our understanding of endocrine resistance in this disease.

Tumor microenvironment induces innate RAF-inhibitor resistance via HGF secretion

Todd R. Golub, Dana-Farber Cancer Institute and Broad Institute of MIT and Harvard, Cambridge, MA

Drug resistance remains a vexing problem in the treatment of cancer patients. While many studies have focused on cell autonomous mechanisms of drug resistance, we hypothesized that the tumor microenvironment may confer innate resistance to therapy. Here we developed a co-culture system to systematically assay the ability of 23 stromal cell types to influence the innate resistance of 45 cancer cell lines to 35 anti-cancer drugs. We found that stroma-mediated resistance is surprisingly common - particularly to targeted agents. We further characterized the stroma-mediated resistance of *BRAF*-mutant melanoma to RAF inhibition because most of these patients exhibit some degree of innate resistance. Proteomic analysis showed that stromal secretion of the growth factor hepatocyte growth factor (HGF) resulted in activation of the HGF receptor MET, reactivation of the MAPK and PI3K/AKT pathways, and immediate resistance to RAF inhibition.
Immunohistochemistry confirmed stromal HGF expression in patients with *BRAF*-mutant melanoma and a statistically significant correlation between stromal HGF expression and innate resistance to treatment. Dual inhibition of RAF and MET resulted in reversal of drug resistance, suggesting RAF/MET combination therapy as a potential therapeutic strategy for *BRAF*-mutant melanoma. A similar resistance mechanism was uncovered in a subset of *BRAF*-mutant colorectal and glioblastoma cell lines. More generally, these studies indicate that the systematic dissection of tumor-microenvironment interactions may reveal important mechanisms underlying drug resistance.

Studying therapy response and resistance in mouse models of BRCA-associated breast cancer

Dr. Jos Jonkers, Netherlands Cancer Institute, Amsterdam, The Netherlands

Advancing personalized cancer medicine through development of tailor-made treatments for individual tumors requires detailed knowledge of the mechanisms underlying drug response and acquired resistance. Once these processes are understood in sufficient detail

it may be possible to design (combination) therapies that not only cause complete remissions but also eliminate remnant cells that might elicit recurrent disease. Mouse models of human cancer provide powerful tools to study drug resistance mechanisms in a realistic *in vivo* setting.
We have established genetically engineered mouse models (GEMMs) and patient-derived tumor graft models for BRCA-deficient breast cancer. These mice develop mammary tumors that are characterized by genomic instability and hypersensitivity to DNA-damaging agents, including platinum drugs and PARP inhibitors. We have used these mammary tumor models for preclinical evaluation of therapy response and elucidation of mechanisms of acquired drug resistance. BRCA-deficient mammary tumors are highly sensitive to PARP inhibitors and platinum drugs, but none of these drugs is capable of causing tumor eradication: tumors grow back after drug treatment and eventually become resistant. Using *in vitro* functional genetic screens, *in vivo* genotype-phenotype correlation studies and genomic analysis of therapy-resistant tumors, we found that therapy response and resistance to platinum drugs and the clinical PARP inhibitor olaparib is affected by several factors, including drug efflux transporter activity, type of BRCA1 founder mutation and 53BP1 status. Also BRCA1 re-expression via genetic or epigenetic mechanisms contributes to acquired therapy resistance in patient-derived tumor graft models of BRCA1-deficient triple-negative breast cancer.

BRAF and RAS signalling in cancer: from basic biology to clinical benefit

Richard Marais PhD, The Paterson Institute for Cancer Research, Manchester, UK.

The protein kinase BRAF regulates cell growth and survival through the MEK/ERK signalling pathway. BRAF is mutated in about half of all melanoma cases, where it acts as a driver oncogene that induces tumour progression. Critically, drugs that target BRAF achieve impressive responses in about 60% of melanoma patients whose tumours are driven by oncogenic BRAF, demonstrating that targeting this protein provides a validated approach to treatment of these patients. However, these drugs have the curious side-effect of inducing non-melanoma skin lesions (keratoacanthomas and cutaneous squamous cell carcinomas) in about 30% of patients. This occurs because BRAF inhibitors drive paradoxical activation of the MAP kinase pathways in cells that carry mutations in RAS, the upstream activator of BRAF. The paradoxical activation of the pathway is driven by the induction of BRAF dimerization with a closely related protein called CRAF, and by the induction of BRAF and CRAF homodimers. The induction of these side-effects can be blocked by co-administration of MEK inhibitors. However, we also find that other drugs can drive this paradox. In particular, we find that nilotinib induced RAF dimerization and pathway activation in drug-resistant chronic myeloid leukaemia (CML) cells, but that the combination of nilotinib with a MEK inhibitor induces a synthetic lethality that induces the death of these cells. Our studies demonstrate how understanding of the pathways that drive cell responses to drugs can be used to develop novel therapeutic strategies for cancer patients.

Genomic Characterization of Breast Cancer Response to Aromatase Inhibitors

Elaine R. Mardis[1], Li Ding[1], Dong Shen[1], Jeremy Hoog[2], Malachi Griffith[1], Richard K. Wilson[1] and Matthew J. Ellis[2], School of Medicine, St. Louis, MO
1 The Genome Institute at Washington University School of Medicine
2 Department of Medicine, Division of Oncology, Washington University School of Medicine

Massively parallel sequencing of whole genomes in clinical trial samples provides an outstanding potential to correlate genomic alterations to clinical phenotypes. We have pursued this approach in a clinical trial designed to evaluate response of ER+ breast tumors to aromatase inhibitors (AI), where the trial design collected both pre- and post-treatment biopsies of the breast tumors, as well as matched normal blood. Using whole genome analysis and deep read count data from targeted capture of suspect variant regions of 22 patients with different responses to therapy, we have demonstrated differential impacts on the

heterogeneity of tumor cells for AI-responsive and -resistant patients. Interestingly, the remaining tumor cells in either response class often display known driver mutations that were not detected in the pre-treatment cell population. This phenomenon presents an intriguing possibility for future metastatic tumor development, even in patients who appear to have a strong response to AI therapy.

Compounding the Problem of Clinical Resistance to TKIs in CML

Thomas O'Hare, Univ of Utah School of Medicine, Salt Lake City, UT

Chronic myeloid leukemia (CML) cells are uniquely reliant on the BCR-ABL tyrosine kinase. Doubts about selective tyrosine kinase inhibitor (TKI) design were overcome with the discovery of the flagship ABL TKI, imatinib (kinase profile: ABL, KIT, PDGFR). In the intervening 14 years, patients diagnosed with CML in the chronic phase have literally been granted a new lease on life. Along the way, problems have been encountered and dispatched. For example, a major mechanism of imatinib resistance was traced to point mutations in the BCR-ABL kinase domain. Two new TKIs, nilotinib and dasatinib, rapidly came to prominence as salvage and now first-line CML therapies. These TKIs control most BCR-ABL mutations that confer resistance to imatinib, with partially complementary exceptions. The most problematic point mutation is the clinically frequent BCR-ABL^{T315I} 'gatekeeper' mutation, which is insensitive to all three approved therapies as well as the advanced investigational TKI, bosutinib. Third-generation TKIs with activity against BCR-ABL^{T315I} may provide the first targeted option for relapsed patients harboring this mutation.

Ponatinib is a recently developed, high-affinity BCR-ABL TKI with activity against all known single kinase domain mutants including BCR-ABL^{T315I}. However, ponatinib is vulnerable to certain compound mutants (two or more mutations in the same BCR-ABL molecule) in *in vitro* pre-clinical model systems. We have established comprehensive BCR-ABL compound mutation resistance profiles for six TKIs: imatinib, nilotinib, dasatinib, bosutinib, ponatinib and DCC-2036 (a BCR-ABL^{T315I} TKI in phase 1 evaluation). As ponatinib and other new TKIs become available, this expandable resource will provide guidance for personalized drug selection.

The extent to which BCR-ABL compound mutation-based resistance tempers the effectiveness of ponatinib and other TKIs in the clinical setting is a focus of our research. Ponatinib is in phase 2 evaluation for treatment-refractory CML [Ponatinib Ph+ ALL and CML Evaluation (PACE) trial]. Patients with resistance or intolerance (R/I) to dasatinib or nilotinib or a confirmed BCR-ABL^{T315I} mutation are eligible. Of note, the majority of patients in the R/I cohort lacked any detectable BCR-ABL baseline mutation, suggesting a degree of BCR-ABL independence. Pre-clinical and clinical evidence will be presented in support of the hypothesis that ponatinib resistance can be divided into two main categories: *1)* BCR-ABL compound mutation-mediated resistance in which BCR-ABL remains an appropriate, if challenging, sole target and 2) resistance despite inhibition of BCR-ABL, calling for concurrent inhibition of BCR-ABL and newly identified co-critical targets.
Our overarching goal is to understand the mechanisms of resistance to ponatinib and other TKIs and optimize their use in treatment-refractory CML. Compound mutations and/or alternative pathway activation are emerging as recurrent resistance mechanisms in malignancies treated with advanced TKIs, such as melanoma and non-small cell lung cancer. The presentation will center on ponatinib resistance in CML, and a theme of broader implications for molecularly targeted cancer therapy will be incorporated.

Approaches to understanding therapeutic resistance and the application to drug discovery efforts

William Sellers, Novartis Inst for Biomedical Res, Cambridge, MA

Cancer is a disease driven by the acquisition and selection of gain- and loss-of-function genetic alterations. While the full understanding of the pattern and consequences of such genetic events remains

elusive, it is now clear that therapeutics directed against the genetic underpinnings of cancer have had a marked clinical impact. Highlights of this genetic-therapeutic paradigm include the success of imatinib in BCR-ABL driven CML, erlotinib and gefitinib in EGFR mutated lung cancer and more recently crizotinib in ALK translocated lung cancer as well as vemurafenib in BRAF-mutant melanoma. Indeed, the efficacy of small molecule inhibitors of KIT, PDGFR, HER2, EGFR, BRAF and ALK in a diversity of malignances including GIST, lung cancer and melanoma support the notion that a new generation of efficacious drugs is emerging. Along with this success has come the problem of therapeutic resistance. It is noteworthy, however, that the mechanistic understanding of resistance to targeted therapies is more rapidly advancing when compared to the understanding of resistance to more conventional cytotoxics. Moreover, such mechanistic insights have led to the rapid development of second generation inhibitors of key targets including the development of nilotinib initially in imatinib-resistance CML and then in the first-line therapy of CML. A robust set of pre-clinical model systems can now be deployed to discover the specific mechanisms of resistance to targeted therapy with a strong basis for the translation of such discoveries into the clinic. In this talk the discovery of diverse resistance mechanisms and their implications for new therapeutics and for novel combinations will be discussed in the context of targeting BCR-ABL, the Smoothened receptor, the MET receptor and the ALK kinase.

Selective and adaptive mechanisms of resistance to molecular targeted therapy in lung cancer.

Rafaella Sordella, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

The epidermal growth-factor receptor (EGFR) tyrosine kinase inhibitor erlotinib has been proven to be highly effective in the treatment of nonsmall cell lung cancer (NSCLC) harboring oncogenic EGFR mutations. The majority of patients, however, will eventually develop resistance and succumb to the disease. Recent studies have identified secondary mutations in the EGFR (EGFR T790M) and amplification of the N-Methyl-N'-nitro-N-nitroso-guanidine (MNNG) HOS transforming gene (MET) oncogene as two principal mechanisms of acquired resistance. Although they can account for approximately 50% of acquired resistance cases together, in the remaining 50%, the mechanism remains unknown. In NSCLC-derived cell lines and early-stage tumors before erlotinib treatment, we have uncovered the existence of a subpopulation of cells that are intrinsically resistant to erlotinib and display features suggestive of epithelial-to-mesenchymal transition (EMT). We showed that activation of TGF-ß-mediated signaling was sufficient to induce these phenotypes. In particular, we determined that an increased TGF-ß-dependent IL-6 secretion unleashed previously addicted lung tumor cells from their EGFR dependency. Because IL-6 and TGF-ß are prominently produced during inflammatory response, we used a mouse model system to determine whether inflammation might impair erlotinib sensitivity. Indeed, induction of inflammation not only stimulated IL-6 secretion but was sufficient to decrease the tumor response to erlotinib. Our data, thus, argue that both tumor cell-autonomous mechanisms and/or activation of the tumor microenvironment could contribute to primary and acquired erlotinib resistance, and as such, treatments based on EGFR inhibition may not be sufficient for the effective treatment of lung-cancer patients harboring mutant EGFR.

Clinical Approaches to BRAF inhibitor (BRAFi) resistance in melanoma.

Jeffrey A Sosman, MD and Igor Puzanov, MD, MSCI - Vanderbilt Univ, Nashville, TN

The access to effective BRAFi in melanoma patients with $BRAF^{V600}$ mutated melanoma has revolutionized the treatment of metastatic disease. Most patients treated with BRAFi, vemurafenib or dabrafenib have rapid clinical responses that can improve symptoms within a few days and over 80% of patients have some initial regression in tumor size, while ≈55% proceed onto an objective (RECIST 1.1) confirmed response. However, these responses can usually be measured in months

with a median progression-free survival of 6.8 months. While overall survival is impressively improved (to 13-16 months) for these patients, they will all relapse (presumably) with BRAFi-resistant disease. Clinical strategies have now been formulated to address BRAFi-resistant disease based on the known mechanisms defined at this point. While the number of mechanisms of resistance is daunting they fall into two main categories which may or may not be mutually exclusive; MEK dependent and MEK-independent resistance. The MEK-dependent mechanisms include activating mutations in upstream NRAS or downstream MEK1; overexpression of COT, which activates MEK, and either alternate splicing, or amplification of the mutant *BRAF^{V600}* gene. MEK-independent resistance includes activation of RTKs (PDGFR, IGF1R) or their ligands (HGF for met). We do not know with any degree of certainty how frequent each one of these mechanisms occurs and likely multiple can occur at the same time in the same melanoma.

Prevention of resistance will likely be much more effective than treatment of resistance once it is present clinically. The initial approach taken has been the combination of a BRAFi with a MEK inhibitor (MEKi). Several such trials have been performed and a randomized phase III trial comparing the combination to single agent BRAFi is being launched. We already know that once resistance occurs MEKi alone are ineffective, while the addition of a MEKi to BRAFi appears to re-induce responses in about 20% of patients. There are no reports identifying the mechanism of resistance in the responding patients. Large phase II trials with the combination of BRAFi + MEKi (including a randomized cohort) have completed accrual and will soon be reported. Preliminary results suggest the combination may have a longer progression-free survival than BRAFi alone. It is established that the combination is very effective at diminishing skin toxicities including keratoacanthomas. Other approaches are underway, all targeting one or more mechanism of resistance. These include the combination of a MEKi with an AKTi (AZD6244+MK2206); BRAFi with a PI3K inhibitor (PX-866), BRAFi+HGF/met inhibitor (AMG102, Metmab, AMG337), and an ERK inhibitor (SCH900353). In addition, triple therapy (BRAFi+MEKi+ PI3/AKTi) has been discussed and if tolerable targeting both MEK-dependent and independent BRAFi resistance may be necessary. It will be critical to define the mechanism of BRAF inhibitor resistance in all cases to allow one to ultimately select treatment based on the resistance mechanisms. Finally a very different approach to resistance is the combination of an immune activating agent such as anti-CTLA4, anti-PD1, or Interleukin-2 with a BRAFi. Immune based therapy has always been effective in a small minority of patients, but those responses tend to be durable (years). These approaches will be dependent on schedule to allow maximum melanoma cell death and then most effectively stimulate the anti-tumor immune response. The combination of anti-CTLA4 and vemurafenib is in phase I trials and if tolerable, will rapidly proceed to phase II and III trials.

We have definitely made progress with this disease, but in advanced, unresectable melanoma no one will be cured with BRAFi alone at this time . The future is in the application to earlier stage disease (stage III) or in combination therapy. It is likely that individualization of therapy targeting resistance mechanisms will need to be implemented.

The dynamics of primary tumor spread in pancreatic cancer

Ben Stanger, University of Pennsylvania, Philadelphia, PA

Although most cancer morbidity is caused by metastasis, the cellular and molecular events that underlie tumor spread are far less understood than the events that govern primary tumor formation. We have utilized an autochthonous mouse model of pancreatic cancer to understand the changes that occur during tumor progression in vivo. During stochastic tumor evolution, cells undergo a phenotypic change - epithelial-to-mesenchymal transition, or EMT - that is associated with the acquisition of stem cell-like features. This change occurs quite early, well before frank malignancy, suggesting that EMT enables early spread of pancreatic tumor cells. EMT and invasive behavior is associated with areas of inflammation; induction of pancreatitis is

associated with an increase in the number of circulating pancreatic cells, while an inhibitor of inflammation (dexamethasone) blocks cell entry into the bloodstream. These results indicate that inflammation may participate in tumor progression in part by facilitating cancer cell spread and account for the fact that pancreatic cancer in humans is almost always disseminated at the time of detection.

The Pancreatic Cancer Stroma Influences Therapeutic Response

David Tuveson, CRI/CRUK University of Cambridge, UK

Pancreatic ductal adenocarcinoma (PDAC) is the most lethal common malignancy, with little improvement in patient outcomes over the past 40 years. PDAC frequently harbors somatic mutations in 4 genes (KRAS, P16, Tp53, and SMAD4), and recent exomic and whole genome sequencing efforts have identified a number of less frequent events. Accurate mouse models of PDAC were generated over the last decade, confirming the relevance of these 4 genes and enabling the investigation of fundamental aspects of PDAC tumor biology and therapeutic response. The poor response of pancreatic cancer patients to systemic agents is not predicted by xenografted cancer cell model systems. An evaluation of this discordant behavior revealed that transplanted tumor models have superior tissue perfusion and delivery of the chemotherapeutic gemcitabine compared to primary murine PDAC, and that this inversely correlates with stromal content. Human PDAC was subsequently confirmed to phenocopy the mouse model as it is also profoundly hypovascular and contains a compressed residual vasculature. We have developed two methods that improve the perfusion of mouse PDAC tumors to increase drug delivery and animal survival, and have translated these observations to the clinic. First, we found that smoothened inhibitors such as IPI-926 depleted the PDAC stroma, resulting in transient increases in vascular density and prolonged survival to support a causative role of the hypovascular state in drug responsiveness in PDAC[1]. The clinical translation of this observation in patients with metastatic PDAC was initially promising, although a RP2 trial in the USA was unfortunately negative, and analysis is still underway. We also have initiated a proof-of-concept trial using GDC-0449 monotherapy in patients undergoing surgery. Secondly, we and others found that systemic administration of polymerized hyaluronidase (PEGPH20) resulted in the rapid dilation of the intratumoral vasculature and delivery of chemotherapy to prolong the survival of mice with PDAC. Such an approach in xenografts had previously been shown to decrease intratumoral pressure and this result was therefore expected. Using electron microscopy, we surprisingly found that the PDAC vasculature was quiescent in untreated mice, and exhibited numerous endothelial gaps and fenestrae following treatment. Such fenestrae may contribute to the increased delivery of small anti-neoplastics as well as large molecular therapies. This data formed the clinical rationale for testing in patients in the USA.

Alternative methods that target the tumor stroma may also be beneficial in PDAC, including the use of nab-Paclitaxel (Abraxane), an albumin-Paclitaxel formulation proposed to be sequestered in the intratumoral stroma. Similar to the hedgehog inhibitor, nab-Paclitaxel also elevated the gemcitabine levels in the mouse PDAC tumors. However, the mechanism employed was distinct as nab-Paclitaxel did not deplete the stroma and increase the vascular density. Rather, paclitaxel elicits the liberation of reactive oxygen species (ROS), and ROS destabilize and induce the destruction of cytidine deaminase, the major pathway of gemcitabine inactivation in cells[4]. This novel method of drug synergy was overlooked in the PDX (patient derived xenograft) models, as the chimeric murine stroma is only loosely associated with the human neoplastic cells and they therefore may provide misleading results[5]. Nab-Paclitaxel treatment with gemcitabine is active in early clinical trials, and genetic variants affecting gemcitabine metabolism may modify this outcome.

PDAC is notoriously resistant to VEGF targeted therapeutics despite numerous preclinical experiments that predicted efficacy, and recent approaches in murine PDAC confirmed the prior clinical data[6]. The hypovascular content of PDAC suggests that the intratumoral endothelial cells are poorly responsive to the available VEGF ligand, and therefore additional pathways may play a

more dominant role in vessel proliferation and maintenance.
The notch pathway has previously been implicated in vessel morphogenesis, and can be chemically inhibited with gamma secretase inhibitors (GSI). GSI treatment induced the rapid destabilization of the PDAC vasculature, by promoting the death of intratumoral endothelial cells. These effects were exacerbated by concomitant exposure to gemcitabine, resulting in hypoxic necrosis and death of both endothelial cells and neoplastic cells[7]. A clinical trial has recently begun to evaluate this approach. Although the unique attributes of the PDAC microenvironment participate in the resiliency of PDAC to therapeutics, they also serve as vulnerabilities to exploit for clinical benefit.

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Reverting Endocrine resistance by targeting the mTOR pathway: A new paradigm in the therapy of breast cancer.

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Hormonal therapy represents the mainstay of treatment of patients with metastatic hormone receptor-positive breast cancer. However, resistance to hormonal therapy, either de novo or acquired, is currently a major limitation in the therapy of this disease and new therapeutic strategies are needed to enhance the efficacy of currently available treatment regimens.
The study of resistance to endocrine therapies in hormone receptor positive (HR+) breast cancer has aimed at identifying new therapeutic strategies that would enhance the efficacy of endocrine therapies. An emerging mechanism of endocrine resistance is aberrant signaling via the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway. Recently, we conducted a phase III study comparing the mTOR inhibitor everolimus and exemestane to exemestane and placebo in 724 patients with HR+ breast cancer refractory to nonsteroidal aromatase inhibitors[1]. In this pivotal study, the combination of everolimus and exemestane resulted in marked improvement in progression-free survival as determined by local investigator assessment (6.9 vs. 2.8 months; hazard ratio [HR], 0.43; $P=1.4\times10^{-15}$) and by central assessment (10.6 vs. 4.1 months; HR, 0.36; $P=3.3\times10^{-15}$).The clinical benefit observed in the combination arm also far exceeds the clinical benefit of single-agent everolimus in a similar population of patients.

The striking clinical benefit observed with the combination of an mTOR inhibitor, an agent marginally active in this clinical setting, and an aromatase inhibitor, in a hormone-refractory patient population, suggests a true synergism that requires careful analysis. In preclinical models, activation of PI3K/mTOR is required for the adaptation of ER-positive cells to hormone deprivation, and combined estrogen deprivation and PI3K/AKT/mTOR pathway inhibition causes synthetic lethality in ER+ breast cancer cells. We are currently molecularly characterizing these compensatory pathways. In addition we are studying if the estrogen degrader fulvestrant may be superior to the aromatase inhibitor exemestane. Since both ligand-dependent and ligand-independent ER-signalling can be inhibited with an ER degrader fulvestrant, it is tempting to speculate that this agent will be more efficacious than exemestane when combined with everolimus. The end result of our proposal would be the selection of novel strategies to block mTOR-mediated compensatory pathways and, importantly, to identify the patient population more likely to respond to combined ER-mTOR blockade.

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Effectors and targets of anticancer agents

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Redundancy and multi-functionality are inherent characteristics of biological systems that limit the therapeutic opportunity of single-agent applications. Combinations of drugs that yield a synergistic effect are thought to be the most effective way of countering biological buffering and furthermore allow reduced dosing of each agent while increasing therapeutically relevant selectivity.
Deconvolution of the relevant cellular mechanism underlying a combined treatment with two drugs that yield a synergistic and therefore unpredictable effect are a particular challenge. Occurrence of the BCR-ABLT315I gatekeeper mutation is one of the most pressing challenges in the therapy of chronic myeloid leukemia (CML). Several BCR-ABL inhibitors with clinically tested safety profiles have multiple targets and pleiotropic effects that could be exploited for their synergistic potential. Testing pair-wise combinations of such kinase inhibitors identified a strong synergy between danusertib and bosutinib that exclusively affected CML cells harboring BCR-ABLT315I. We applied a systems-level approach comprising phosphoproteomics, transcriptomics and proteome-wide drug target surveys. Intersecting these orthogonal datasets revealed that both compounds effectively targeted Mapk pathways downstream of BCR-ABL resulting in impaired activity of c-Myc and associated downregulation of c-Myc target genes. Pharmacological validation assessed that the relative contribution of danusertib and bosutinib could be mimicked individually by specific Mapk inhibitors and collectively by downregulation of c-Myc through Brd4 inhibition. Thus integration of genome- and proteome-wide technologies allowed for the elucidation of the complex mechanism by which a novel drug synergy targets the dependency of BCR-ABLT315I CML cells on c-Myc through non-obvious off-targets. We are using human test-tube genetics with haploid cells mutagenised by random retroviral insertion to identify the genes required to effect drug action. Considering also the data obtained with chemical proteomics, the emerging picture is that of several gene products contributing to the drug action. The complexity of the cellular pharmacodynamic parameters are believed to be the rule rather the exception and the arsenal of available tools is allowing an unprecedented depth of insight into cellular drug action. We believe that this strategy of gaining functional understanding the activity of single agents or of a drug synergies may serve as a model for further mode of action studies and for the elucidation of resistance mechanisms.

Identifying genomics-based therapeutic targets in lung cancer

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Cancer is a disease of the genome; genetic lesions (gene mutations, gene copy-number changes, structural genetic changes, etc.) lead to irreversible changes in the intracellular signal transduction pathways that the tumor cells become dependent upon. A new class of cancer therapeutics targeting specific signaling pathways activated by genetic lesions has shown clinical success. Understanding the dependency associated with each genetic alteration is crucial in order

to device specific inhibitory strategies to interfere with the activity of the respective oncogene or the pathways activated downstream of the oncogene. Such preclinical work can help expediting the preclinical-to-clinical transition of novel cancer therapeutics and to make them more effective. Similarly, linking drug response and clinical features of patients to genetic alterations is essential for a continuous re-assessment of the validity of such preclinical predictions.
Our laboratory has created a conceptual framework as well as methodological strategies for approaching these needs.
We have developed an international network for cancer genome analyses and we have developed a platform for functional cell biology analysis of the novel mutations that we discover. We have successfully applied this two-pronged approach to the discovery of cancer genotypes that may be connected with drug response.

Immune Checkpoint Blockade: New Insights and Opportunities

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Tumors have inherent genetic diversity. While many of the genetic and epigenetic changes may not be driver mutations, many genetic changes result in mutations that can be recognized by the immune system. While the field of immunology has been around for many years, recently the field of co-stimulatory blockade was pioneered by Dr. Allison and others, and has brought a resurgence of immunotherapy into the treatment of cancer. CTLA-4, a protein normally serving to restrict T cell responses, was blocked using an antibody. Anti-CTLA-4 blockade was the first drug shown to improve overall survival in a randomized control trial in patients with Stage IV melanoma. While the response rates of the drug are low, the durability of response is years. Recently, the results of another co-stimulatory blockade, anti-PD-1 were reported by Dr. Topalian. The efficacy of PD-1 blockade spanned across many tumor types, with similar if not less, toxicity.

As we look to improve the response rate of immunotherapy, the drugs will be combined with additional therapies. Theoretically any therapy that causes tumor cell death can cause antigen release and be a target for the immune system. Initially we have investigated whether the BRAF inhibitors are immunosuppressive. Like others, we have demonstrated that the drugs are not immunosuppressive , and in fact may have stimulatory effects on vaccine responses in vivo. Further characterization of the targeted therapies, and how they interact with the immune system will have to continue to optimize combination treatments in the future. Results from these studies will have significant implications on the timing and sequence of combination therapies in the future.

Defining the spectrum of resistance to targeted cancer therapeutics

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In recent years, great strides have been made towards effective therapeutic inhibition of several cancer subtypes driven by mutated kinase oncogenes; however, resistance to these therapeutics is nearly ubiquitous in solid tumors. Melanoma represents an instructive example: the clinical development of selective RAF and MEK inhibitors has resulted in prolonged survival of melanoma patients whose tumors harbor BRAF(V600E/K) mutations, but the clinical benefit is limited to ~6-11 months prior to disease relapse. Thus, characterizing mechanisms of resistance to these agents will likely be crucial to the future development of rational therapeutic combinations that may achieve durable control of this genetically defined tumor subtype. Resistance to kinase inhibitors may be grouped into three categories: 1, "target-oriented" resistance mechanisms (e.g., secondary mutation, amplification, or dysregulation of the target oncoprotein); 2, "bypass" mechanisms (engagement of a signaling module that circumvents the target oncoprotein); or 3, alterations in downstream effectors (e.g., key signaling proteins that are activated by the target oncoprotein). Work by our group and others has described mechanisms of resistance in BRAF-mutant melanoma that are pertinent to each of these mechanistic categories. Examples include discovery of

several mechanisms that re-establish RAF signaling (often by activating C-RAF), bypass mechanisms such as the COT kinase or various receptor tyrosine kinases, and activating mutations of MEK1, which signals downstream of mutated B-RAF. In most cases, the resistance mechanisms result in sustained ERK signaling–hence restoring activation of the oncogenic MAP kinase pathway that is dysregulated by mutant BRAF in melanoma.
More recently, we have deployed systematic gain- and loss-of-function screens to achieve a more comprehensive understanding of MAP kinase pathway inhibition in melanoma. The preliminary results of these efforts have identified multiple mechanisms of MAP kinase activation, dysregulation of the oncogenic MAP kinase transcriptional output, and possible ERK-independent mechanisms of resistance. Interestingly, although 150-200 individual resistance effectors have been nominated by these screens, many of these seem to converge upon a much narrower set of key cellular effectors. This raises the possibility that parsimonious therapeutic combinations (e.g., 3-4 drugs) could be developed that impede many individual "upstream" resistance mechanisms. If this knowledge can be combined with a detailed understanding of "steady state" tumor dependencies as well as deep clinical genomic characterization of relapsing tumors, a framework may emerge for prioritizing novel therapeutic combinations worthy of clinical evaluation. Overall, these results have provided new insights into mechanisms of resistance in melanoma while informing a broader translational framework for linking knowledge of resistance to more effective cancer therapeutics.

ABSTRACTS OF POSTERS

VEGF restrains paclitaxel response: molecular analyses of the tumor micro-environment

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A fundamental characteristic of malignant cancers is the ability to change and exploit the host environment to favor local tumor growth, invasion, and metastasis, possibly affecting drug response. Tumor-derived vascular endothelial growth factor (VEGF), a major regulator of tumor microenvironment remodeling, is associated to poor prognosis. The purpose of this study was to assess the role of VEGF provided by the cancer cells in stroma remodeling and tumor response to chemotherapy.
Human ovarian carcinoma cells producing high (1A9-VS1) or low (1A9-VAS3) VEGF [1] were xenotransplanted into nude mice and compared for responsiveness to chemotherapy. Subcutaneously growing 1A9-VS1 tumors were less affected by paclitaxel treatment than 1A9-VAS3; to the contrary, equal sensitivity of the two high/low VEGF cell variants was observed *in vitro*. These findings suggest that the *"in vivo protective effect"* might be mediated by modification of the tumor environment.
Transcriptional differences were evaluated by microdissecting (PALM Microlaser System) 1A9VS1 (N=5) and 1A9VAS3 (N=5) tumors, and by hybridizing the stroma RNA to GeneChip® Mouse Genome 430 2.0 Arrays (Affymetrix). VEGF provided by the 1A9-VS1 cancer cells triggered tumor microenvironment's modification: listed as differentially expressed were genes associated to extracellular matrix remodeling (e.g. collagens and matrix metalloproteinases) and to cell-cell adhesion and interaction (e.g. junction proteins and cadherins), as well as genes expressed by vascular and inflammatory cells (e.g. chemokines and growth factors).
VEGF availability resulted in an abnormal vasculature, characterized by dilated and irregular vessels, increased collagen IV perivascular deposition and expression of endoglin, neuropilin-1 and RGS5 (regulator of G-protein signaling-5) protein, that we showed for the first time to co-localize with the lining endothelium co-expressing CD31 [2].
Toughening the relevance of the findings, we demonstrated RGS5 protein associated to the vasculature of ovarian carcinoma clinical specimens and expressed by the blood vessels of other cancer xenograft models producing high VEGF.
RGS5 protein was not detectable in a panel of healthy mouse tissues including ovaries.
These outcomes altogether put forth evidence that cancer cells provided VEGF alters the tumor microenvironment and support the notion that this might affect the responsiveness to chemotherapy.
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Supported by the Italian Association for Cancer Research (AIRC) and CARIPLO foundation

Analysis of Tyrosine Kinase Receptors Status in Endometrial Stromal Sarcoma

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Endometrial stromal sarcomas (ESS) are rare neoplasms, which are currently classified as low grade-(LG)-ESS, with a tendency for local recurrences and, more rarely, for metastasis,

and also as undifferentiated endometrial sarcomas (UES), formerly described as high grade-(HG)-ESS, with very aggressive behaviour. Current practice has shown that surgery continues to be the treatment of choice for ESS, when effective adjuvant therapies have not yet been established. Tyrosine kinase inhibitors have rarely been applied in ESS therapy, with few reports describing Imatinib responsivity. The aim of this study was to analyse the status of different tyrosine kinase receptors (TKRs) in an ESS series, in order to evaluate their potential role as molecular targets.
The study was performed on a series of 28 ESS, including 23 LG-ESS and 5 UES. Immunohistochemistry was performed for EGFR, c-KIT, PDGFR-alpha, PDGFR-beta and ABL on all ESS. EGFR, PDGFR-alpha and PDGFR-beta gene expression was investigated by qRT-PCR on selected cases. 'Hot-spot' mutations were screened for on EGFR, c-KIT, PDGFR-alpha and PDGFR-beta genes, by sequencing. All analysis was executed from formalin-fixed, paraffin-embedded specimens.
Expression of 2 or more TKRs was observed in 18 out of 28 tumors (64%), with at least 2 receptors expressed simultaneously in 10 cases, 3 receptors in 7 cases, and 4 receptors in a single UES. Only 5 LG-ESS out of 28 tumors were consistently negative for all the antibodies. Gene expression profiles were concordant with immunohistochemical over-expression in only one tumour which displayed both high mRNA levels and specific immunoreactivity for PDGFR-alpha and PDGFR-beta. No activating mutations were found on the tumours included in the study. This study confirms that TKRs expression is frequently observed in ESS. Considering that the responsiveness to tyrosine kinase inhibitors is known to be related to the presence of specific activating mutations or gene over-expression, which are not detectable in ESS, TKRs immunohistochemical over-expression alone should not be considered as a reliable marker for targeted therapies in ESS. Specific post-translational abnormalities, responsible for activation of TKRs, should be further investigated. This would be potentially useful to subsequently select patients who might benefit from current targeted therapeutic options, or to identify new therapeutic targets.

The combination of 13-cis retinoic acid and a flavonol exerts synergistic anticancer activity against neuroblastoma cell line.

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High-risk cases of neuroblastoma do not respond adequately to chemotherapy and are progressive of refractory, highlighting the need of novel therapies. Well-chosen drug combination offers therapeutic promise for hard-to-treated cancer. 13-cis-retinoic acid (13-CRA) is a differentiating agent used in most current neuroblastoma treatment regimens, while naturally occurring polyphenols have been shown to exert anti-proliferative effects in a wide variety of cancer cell lines. The purpose of this study was to identify natural compounds potentiating the effect of 13-CRA against neuroblastoma cells, and investigate the molecular mechanisms behind.
For this purpose, an *in vitro* screening of about 160 natural compounds was performed on CHP134 cells challenged with a sublethal dose of 13-CRA for 48h. Cell viability assays of 13-CRA and a flavonol, identified as a hit from the initial screening, were carried out, by exposing the cells for 24 and 48h to medium alone, single drugs alone or to different concentrations of the combination of the two drug. The analysis of drug combination revealed that the two molecules exerted synergistic inhibition of the growth of neuroblastoma cells both at 24h and 48h treatment. Changes in cell cycle and cell death processes were investigated by flow cytometry and fluorescence microscopy. Microarray gene expression analysis was performed on polysomal RNA isolated form control cells and treated cells for 24h with a single concentration of 13-CRA and the flavonol alone and with their combination. Treatment of 13-CRA plus this flavonol induced enhanced apoptosis and led to coherent perturbations of gene expression, mainly concerning neural differentiation. The experimental data allowed to advance hypotheses on the molecular mechanism of this drug synergism and suggested potential clinically therapeutic capabilities.

Tab2 as a novel mediator of resistance to endocrine therapy in breast cancer cell lines

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ERα status is used to identify breast cancer patients who are likely to respond to tamoxifen (Tam), but resistance nonetheless occurs in 30 to 50% of treated ERα-positive breast cancer patients. It is known that the balance between corepressors and coactivators plays a critical role in defining the threshold of transcriptional repression to activation in response to activated receptors. Therefore, alterations to coactivators and corepressor activity is thought to represent one of the major molecular mechanisms of resistance to nuclear receptor antagonists. In fact, a number of mechanisms have been shown to lead to NcoR/SMRT destabilization and dismissal from ER-responsive genes in selective estrogen receptor modulators (SERM) treated cells.
It was recently shown that the phosphorylation of Tab2, a component of the NCoR complex, is responsible of NCoR dismissal from TAM/ER-bound genes and translocation to the cytoplasm. We observed, in Tam-resistant (TamR) ER-positive breast cancer cells, that Tab2 presents a shift in mobility on SDS-PAGE similar to that seen in MCF7 wt cell line upon treatment with IL-1b, suggesting constitutive activation. In addition, down-regulation of Tab2 by siRNA results in the nuclear re-localization of NCoR, recovering a complete antiproliferative response to Tam.
Tab2-NCoR complexes are specifically recruited to ER through a bivalent interaction of NCoR to the TAM-occupied ligand binding domain and of Tab2 to the short N-terminal domain of ERa. Therefore we synthesized a peptide composed of a 14-aa motif of this domain, capable of competing out ERa/Tab2 interaction in pull-down and co-immunoprecipitation experiments. This peptide was fused to the carrier TAT peptide to permit its internalization. Treatment of TamR cells with this peptide recovered the antiproliferative response to Tam in these cells. Now, we are currently mapping the site of interaction with ERa on Tab2 protein in order to obtain a new tool to interfere with resistance.
Afterwards, we analyzed the changes in gene expression profiling, in a TamR cell line after treatment with Tab2 siRNA, in order to understand which genes are dependent on the activity of this protein and, hence, may relate to endocrine resistance. We found a gene set related to the control of cell cycle and extensively connected to BRCA1 in a functional network. To investigate the prognostic power of this gene set, we analyze a published data set of Tam-treated breast cancer profiles and we found that these genes were able to discern two groups of patients with significantly different disease-free survival. At present, we are investigating the role of the genes involved in Tab2 signalling and in BRCA1 network in breast cancer recurrence.
Comprehensively, our data implicate Tab2 as a mediator of resistance to endocrine therapy and as a potential new target to reverse pharmacological resistance and potentiate antiestrogen action.

Beyond single pathway inhibition: MEK inhibition-based vertical and lateral combination strategies

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Background and Results: The RAF/MEK/ERK pathway is an attractive therapeutic target in hematologic and solid malignancies. However, interference with a single signaling component leads to the activation of inter- and intra-pathway feedback loops and functional resistance, as recently demonstrated by our group in AML models. We have thus devised rational, mechanism-based, *lateral* and *vertical* MEK inhibition-based combination strategies, potentially endowed with synergistic antitumor activity. In wt-*PTEN* cells (including AML, melanoma, breast, lung, and colon cancer), combined MEK/mTOR blockade achieved synergistic effects at suboptimal concentrations, but became frankly antagonistic at a high fraction affected (CI: 1.2->10^4). This led to the identification of a novel crosstalk mechanism by which MEK blockade restores PTEN expression and cross-inhibits the PI3K/AKT/mTOR pathway. In agreement with this model, combined MEK and mTOR blockade resulted in strongly synergistic effects (CI: 0.0005-0.4) in cells lacking PTEN, including two patient-derived lung cancer stem cell lines, with low PTEN protein levels. On the other hand, different tumor models exhibit variable responses to MEK inhibition and MEK blockade may also induce compensatory signaling through upstream pathway elements (RAF). BRAF-selective inhibitors have potent antitumor effects in mutant BRAF(V600E) tumors and are clinically effective in malignant melanoma, but may paradoxically activate the MAPK pathway in wt-*BRAF* cells, potentially fostering tumor growth. Indeed, combined BRAF/MEK inhibition suppressed malignant growth with highly synergistic effects in lung, colon, and pancreatic cancer models, in which selective BRAF inhibition alone induced hyperphosphorylation of CRAF, MEK, ERK, and p90RSK, particularly in *RAS*-mutant cellular contexts. Conversely, in *BRAF*-mutant melanoma and colon carcinoma models, combined BRAF/MEK inhibition was frankly antagonistic. In AML, sorafenib also synergized with MEK inhibition, whereas in lung and pancreatic cancer models the pan-RAF inhibitor RAF265 did not cause paradoxical MAPK activation and did not synergize with MEK inhibitors.

Methods: We set out to define molecular and functional effects of single and combined MEK (GSK1120212B, MEK inhibitor), mTOR (RAD001, mTOR inhibitor), BRAF (GSK2118436A, BRAF inhibitor) and RAF (Sorafenib and RAF265, pan-RAF inhibitors) inhibition, using WB analysis to dissect signaling and fixed dose-ratio experimental design to assess functional synergism by conservative isobologram analysis.

Conclusions: Overall, our data indicate that in appropriate cellular contexts both *lateral* and *vertical* MEK inhibition-based combination strategies exert highly synergistic antitumor effects across a spectrum of different cancer models; putative genetic determinants of functional synergism (e.g. *PTEN* for MEK/mTOR and *KRAS* for BRAF/MEK combinations, respectively) are currently being investigated.

The glycolytic phenotype of cancer cells modulates resistance to anti-angiogenic therapy in tumor xenografts

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Anti-angiogenic therapy is increasingly used in cancer patients but therapeutic responses are often short-term and predictive biomarkers for patients stratification are currently lacking. We previously showed that levels of "glucose addiction" modulate the pathologic response of tumor xenografts to VEGF neutralization. Here we investigated whether the glycolytic phenotype affects therapeutic responses to anti-angiogenic therapy. We found that highly glycolytic tumors become necrotic but rapidly resistant to VEGF neutralization. In contrast, poorly glycolytic tumors regressed following bevacizumab administration but, despite their small size, contained an hypoxic core and highly proliferative burden, as shown

by FAZA and FLT PET imaging and eventually developed acquired resistance. We also observed that protracted anti-VEGF therapy selects for highly glycolytic tumors and that silencing of AMPKa1 and a2 subunits in tumor cells increased glycolysis and accelerates development of secondary resistance. These results support the hypothesis that the glycolytic phenotype of tumor cells conveys resistance to anti-VEGF drugs.

HuR phosphorylation and doxorubicin: how a post-translational modification is involved in drug resistance

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BACKGROUND: HuR, an RNA binding protein involved in the post-transcriptional regulation of a wide spectrum of mRNAs, has been demonstrated to be a determinant of carcinogenesis and tumor aggressiveness in several cancer types. In this study, we investigated the role of HuR in the apoptosis and in the chemoresistance induced by the widely used anticancer drug doxorubicin in human breast cancer cells (MCF-7).

Material and method We challenged a small library of about 90 chemical compounds with an high content screening assay to quantitavely measure HuRtranslocation. **Results and discussion.** We showed that HuR acts in the early phase of cell response to doxorubicin, being induced to translocate into the cytoplasm upon phosphorylation. Reducing HuR levels diminished the apoptotic response to doxorubicin. We identified HA1004, AG494, U0126, AG490, Rottlerin and Erbstatin compounds that could inhibit HuR cytoplasmic accumulation and pointed to PKCδ, Rho kinase and ERK as potential HuR regulators. Among the hits rottlerin showed to be the most effective in blocking HuR nuclear export and in having correspondingly antagonistic effects with doxorubicin on cell toxicity. Co-immunoprecipitation of PKCδ and HuR upon doxorubicin confirmed the validity of HCS indications. In *in vitro* selected doxorubicin resistant MCF-7 cells (MCF-7/doxoR) overexpressing the multidrug resistance (MDR) related ABCG2 transporter, we observed a significant HuR downregulation that was paralleled by a corresponding downregulation of HuR targets as TOP2A and by loss of rottlerin toxicity. Restoration of HuR expression in these cells resensitized MCF-7/doxoR cells to doxorubicin, reactivating the apoptotic response. **Conclusions.** The present study shows that HuR is necessary to elicit the apoptotic cell response to doxorubicin, that restoration of HuR expression in resistant cells resensitizes them to the action of this drug. Moreover we suggest a novel mechanism of pharmacoresistance based on the interplay among the doxorubicin target TOP2A, its post-transcriptionally regulator HuR and the signaling control of PKCδ. Mol Cancer. 2012 Mar 21;11:13.

Combating Breast Cancer Brain Metastasis: The CTC Signature

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Brain metastatic breast cancer (BMBC) represents the most devastating and feared consequence of breast cancer since patients with BMBC have an exceptionally poor prognosis. Despite increasing incidence and being recognized as a problem of urgent clinical priority, mechanisms causing BMBC are largely unknown. Similarly, properties and biomarker identification of circulating tumor cells (CTCs), the "seeds" of metastasis, remain elusive. The current U.S. Federal Drug Administration - approved CellSearchTM technology for CTC capture does not detect the high proportion of CTCs that are not expressing the epithelial cell adhesion molecule (EpCAM) whose presence correlates with clinically diagnosed BMBC. Here we report novel strate-

gies investigating CTCs isolated from peripheral blood mononuclear cells (PBMCs) of patients with BMBC, including the development of CTC lines. We identified a unique BMBC CTC signature (HER2+/EGFR+/HPSE+/Notch1+/EpCAM-) by characterizing CTCs that could not be captured by CellSearchTM (EpCAM-negative CTCs). Second, we analyzed the invasive and metastatic competencies of isolated CTCs. Established CTC lines over-expressing the BMBC signature were highly invasive and capable to form brain metastasis in xenografts. Third, tumor cell morphologies of CTC - induced metastases closely resembled those of pathologically assessed tumors of patients whose blood was source of isolated CTCs. Lastly, the expression of proteins of the BMBC signature was detected in CTC - induced BMBC. Collectively, we provide first-time evidence of human CTCs isolation and long-term growth, and the establishment of CTC lines and their metastatic competency in the presence of a biomarker signature necessary to promote BMBC. These strategies and results can be of significance to develop novel therapeutics against breast cancer metastasis in general, BMBC in particular.

Oncogene-induced reactive oxygen species (ROS) fuel cell hyperproliferation, DNA damage response activation and cellular senescence

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Cellular transformation triggered by the activation of oncogenes is frequently associated with increased levels of reactive oxygen species (ROS) within the cells. Yet, the enzymatic origin and the contribution of ROS increase to the maintenance of the transformed phenotype are not yet fully understood. We discovered that NADPH oxidase 4 (Nox4) is necessary to allow hyperproliferation induced by H-Ras and that Nox4 inhibition reduces the production of ROS levels, cell-cycle progression and oncogene induced-senescence. Our data shows that ROS scavenging impairs the proliferation only of Ras-transformed cells, which are "addicted" to ROS for proliferation, while normal non-transformed cells were insensitive to ROS scavengers. Thus, we propose that oncogenes such as Ras exploit ROS as mitogenic second messengers and cause replicational stress and DNA damage response (DDR) activation. We also show for the first time that zebrafish is a valuable tool to study in a living organism the accumulation ROS induced by an oncogene and provide a simple assay to test *in vivo* the effects of anti-ROS drugs. Moreover, our results in human pancreatic cancer tumor samples provide a mechanistic explanation for the DNA damage generation and genome instability typically associated with solid tumors. Inhibiting the synthesis of mitogenic ROS may guide researchers in the design of therapeutic interventions based on ROS manipulation that may be more successful than so far achieved.

Everolimus reverts the drawback mTORC2 activation induced by sorafenib in preclinical models of human osteosarcoma potentiating the anti-tumoral, anti-angiogenic and anti-metastatic effects

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Background Osteosarcoma (OS) is the most common primary bone tumour in children and young adult. Despite improved prognosis metastatic or relapsing forms still have a fatal outcome. Extracellular Regulated Kinase (ERK)1/2 and mammalian Target of Rapamycin (mTOR) cooperate in conferring survival advantage to OS cells. ERK1/2 pathway communicates with mTOR through the oncosuppressor Liver Kinase B1 (LKB1) and the Adenosin monophosphate- activated kinase (AMPK). We previously demonstrated that the multikinase inhibitor sorafenib targeting ERK1/2 pathway showed antitumor activity in preclinical models of OS. Furthermore, in metastatic or relapsed OS patients we obtained some disease stabilization and tumor shrinkage. However, these responses were not long lasting and phenomenon of resistance occurred.
Aim In this work we aim exploring potential molecular mechanisms of escape from sorafenib treatment and counteracting this drawback effect. We here show the possibility to target both ERK1/2 and mTOR with the combined administration of sorafenib and the rapamycin analog everolimus in different preclinical models of OS.
Methods Immunoprecipitation, western blot analysis, and immunohistochemistry were conducted to explored biochemical effect of drug treatment in vitro and in vivo, respectively. Pharmacological combination effects were tested by CalcuSyn software and viability test after 72 hours treatment with scalar doses of sorafenib (10-0.62 mM), everolimus (100-6.2 nM) and their combination in 7 OS cell lines (MNNG-HOS, HOS, KHOS/NP, MG63, U-2 OS, SJSA-1, SAOS-2). Cell cycle, proliferation, and apoptosis were studied by flow cytometry in vitro, and with PCNA and TUNEL assay in vivo. Angiogenesis was evaluated with endothelial cell branching morphogenesis in matrigel and with chicken chorioallontoic membrane in vivo. Anti-tumor and anti-metastatic activity was tested in NOD/scid mice engrafted with MNNG-HOS subcutaneously and endovenously respectively treated with sorafenib (5 mg/kg/day), everolimus (1 mg/kg/day), and their combination for 28 days.
Results Two distinct protein complexes involve mTOR kinase: mTORC1 (controlling protein synthesis through S6 kinase) and mTORC2 (activated by phosphorylation in mTOR Ser2481 and inhibited by phosphorylation in Rictor Thr 1135). After prolonged sorafenib treatment (28 days) of MNNG-HOS xenografts, mTORC1 activity was significantly reduced (P-S6 Kinase expression, $p<0.05$) while mTORC2 activity was increased (P-mTOR Ser2481, $p<0.05$) if compared to vehicle- treated mice. Combining sorafenib with everolimus resulted in a complete abrogation of both mTORC1 and mTORC2 signalling in vitro and in vivo, thus preventing the drawback effect of single agent treatment. We demonstrated that the crosstalk between ERK1/2 and mTOR involving LKB1 was not interrupted. Nonetheless, combined treatment potentiated mTORC1inhibition through reactive oxygen species- mediated AMPK activation and induced mTORC2 complex disassembling. Functionally, sorafenib/everolimus combination resulted in (i) synergistic anti-proliferative and pro-apoptotic effects, (ii) impaired tumor growth, (iii) anti-angiogenic potentiation, (iv) reduced migratory and metastatic potential.
Conclusions Our findings provide new insights into the networking nature of ERK1/2-mTOR signal transduction circuits in OS, revealing in mTORC2 activation a possible mechanism of escape from sorafenib treatment. Everolimus potentiated preclinical activity of sorafenib sustaining the feasibility of testing this combination in clinical setting.

Targeting Insulin Receptor Substrates For Destruction as a Therapeutic Modality For Drug-Resistant Cancers

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Insulin receptor substrates IRS1 and IRS2 are scaffold proteins mediating mitogenic and anti-apoptotic signals mainly from IGF1R and IR, but also from other oncogenes like v-Src. The phosphorylation of IRS1/2 on tyrosine residues leads to the mobilization and activation of downstream signaling modules. The phosphorylation of IRS1/2 on serine residues leads to IRS1/2 degradation and termination of the signal. IRS1 and IRS2 are crucial for cell transformation by other oncoproteins, and the expression of IRS1 and IRS2 is often increased in human tumors. IRS1 is a major component in resistance to oncologic drugs. This was demonstrated for mTOR inhibitor drugs and EGFR inhibitor drugs. In both cases the drugs inhibited, as a side effect, the basal inhibitory phosphorylation of IRS1 on serine residues. This resulted in the stimulation of the IGF1R-IRS1 to PKB/Akt pathway, enhancing survival of the cancer cells and developing resistance to the drugs. We have recently shown that melanoma cells that became resistant to the lately approved drug PLX4032, an inhibitor of mutated $BRaf^{V600E}$, have significant higher levels of IRS1 and IRS2 compared to their parent PLX4032-sensitive melanoma cells. In addition we show that IRS1 and/or IRS2 levels increase following treatment of the PLX4032-sensitive melanoma cells with PLX4032. Correspondingly, the IGF1-induced PKB activation was higher in cells where IRS1/2 levels were enhanced, suggesting IRS1 and IRS2 as potential candidates for acquired drug resistance.
Here we report, for the first time, a novel proprietary family of low molecular weight inhibitors with a unique mechanism. These inhibitors induce IRS1/2 phosphorylation on serine residues, photolytic degradation of IRS1/2 and a long lasting anti-tumor effect. We demonstrate the efficacy of our selected lead, named NT157, in inhibiting tumor growth and metastasis of human melanoma in nude mice, and validate its unique mechanism of action in vivo by immunohistochemistry. Correspondingly we show that NT157 induces the depletion of IRS1 and IRS2 in PLX4032-resistant melanoma cells, and efficiently blocks the growth of these cells as tumors in mice. We believe that NT157 is a potential drug candidate for melanoma patients who have developed resistance to BRaf inhibitors. Furthermore, by down-regulating IRS1 and IRS2, NT157 may prevent the emergence of resistance to these drugs. NT157 is an effective inhibitor of other cancer cell types in vitro and in vivo, suggesting it may have broad applicability, probably because of the role IRS1 and IRS2 play in many tumors.

Can be Circulating Tumor Cells (CTC) and Disseminated Tumor Cells (DTC) syncronously detected in early prostate cancer?

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Background:
Although more than 90% of prostate cancer is considered localized at the time of diagnosis, there is evidence that prostate cancer cells disseminate early from the primary tumor. Indeed, tumor progression following radical prostatectomy occurs in 10% to 30% of patient. Unlike breast cancer, however, in which prognostic and predictive impact of tumor cells in peripheral blood (CTC) or bone marrow (DTC) was largely provided, their role in early prostate cancer is far from clear.
Study aims:
By an automated platform (CellSearch) which has permitted serial testing with good sensitivity and reproducibility, a strict correlation was established between CTC count and prognosis in metastatic breast, colon-rectal and prostate cancer. Conversely, whether CTC detection may be used as surrogate marker in early stage remain to be determined. Moreover, lacking a reproducible methodology to improve their detection rate, DTC have been difficult to apply in routine clinical practice.

Occurring prostate cancer metastases predominantly in the bone, we have refined a sensitive assay that enriches and identifies DTC from the bone marrow of men with prostate cancer. CTC and DTC count will be compared in early stage prostate cancer.

Methods and Materials/Patients:

The pros and cons of an manual vs. semi-automated quantitative DTC assay was firstly considered, with special attention to feasibility and reproducibility, false positive results, biological and phenotypical heterogeneity of DTC.

Adapting CellSearch procedure to bone marrow processing, semi-automated quantitative DTC assay was developed to count absolute number of DTC in 2 ml bone marrow.

The first clinical objective of the study is to correlate CTC and DTC count in early stage prostate cancer with major prognostic factors as determined at diagnosis before radical prostatectomy.

CTC and DTC changes will be synchronously determined under neoadjuvant setting to test whether their detection could be used for monitoring treatment efficacy in these patients.

Results/Conclusions:

The DTC assay was developed *in-vitro* using *MCF7* cell line spiked into bone marrow samples and then extended in *ex-vivo* samples from healthy donors and cancer patients. Data shows that DTC can be detected in the great majority of early prostate cancer, also in CTC-negative patients. Enrolling started at April-2011. Accrual is ongoing. Updated data including evaluations on CTC and DTC will be presented at the meeting.

The interaction between SPARC expressing tumor and myeloid cells determines breast cancer tumor aggressiveness and resistance to chemotherapy

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Epithelial-mesenchymal transition (EMT) has been associated with increased drug resistance. A number of distinct molecular processes, intrinsic to the tumor cell, are engaged in order to initiate an EMT and to enable its completion, including activation of transcription factors, expression of specific cell-surface proteins. In addition to cell-intrinsic molecular events, myeloid cells present in the tumor stroma can contribute to EMT. The matricellular protein SPARC (secreted protein acidic and rich in cysteine) is a master stromal regulator that is expressed during tissue repair and remodeling with key roles in orchestrating fibrotic responses, determining the composition of the tumor-associated stroma and somewhat unexpected in regulating the immune response. Extracellular matrix (ECM) gene expression profile of human breast carcinomas correlates SPARC expression with prognosis and response to therapy. The aim of this study was to create an experimental model suitable to test whether SPARC has a true role in resistance to therapy related to EMT and immune suppression. Primary mouse mammary carcinoma cell line obtained from *Sparc* $^{-/-}$ mice, SN25, has been transduced to over-express SPARC (SN25SP) and analyzed for EMT features *in vitro* and *in vivo*. Myeloid cells expansion was evaluated by IHC and flow cytometry. WT and *Sparc* $^{-/-}$ mice have been injected with SN25 or SN25/SP cells and treated once a week with 10 mg/Kg of DXR for two consecutive weeks. The efficacy of DXR was evaluated as percentage of tumor reduction at the end point (day 40) in comparison to untreated mice.

We found a direct correlation between SPARC expression resistance to DXR treatment and EMT feature: We have evidence that myeloid cell recruitment is functional for EMT and that SPARC determines the phenotype of recruited myeloid cells favoring tumor production of GM-CSF over G-CSF. The role of myeloid cells recruited in a milieu rich in GM-CSF in DXR resistance and EMT has been proven adding bisphosphonate to DXR during treatment. Biphosphonate has been shown by us and other to inhibit induction and function of myeloid suppressor cells. Indeed, bisphosphonate addition reverted EMT, myeloid cell phenotype and rendered SPARC-producing tumors sensitive to DXR.

Call for 2013 Pezcoller Foundation-AACR International Award for Cancer Research

The prestigious Pezcoller Foundation-AACR International Award for Cancer Research was established in 1997 to annually recognize a scientist:

- who has made a major scientific discovery in basic cancer research or who has made significant contributions to translational cancer research;
- who continues to be active in cancer research and has a record of recent, noteworthy publications;
- whose ongoing work holds promise for continued substantive contributions to progress in the field of cancer.

The Award is intended to honor an individual scientist. However, more than one scientist may be co-nominated and selected to share the Award when their investigations are closely related in subject matter and have resulted in work that is worthy of the Award. In the rare event that there are dual winners of the Award, the cash award will be shared equally between them, and the AACR Executive Committee will determine which of the two co-recipients will present the Pezcoller-AACR Award Lecture at the AACR Annual Meeting.
Candidates for the Award will be considered by a prestigious international Selection Committee of renowned cancer leaders appointed by the President of the AACR and the Council of the Pezcoller Foundation. The Committee will consider all nominations as they have been submitted; the Committee may not combine submitted nominations, add a new candidate to a submitted nomination, or otherwise make alterations to the submitted nominations. After careful deliberations by the Committee, its recommendations will be forwarded to the Executive Committee of the AACR and the Council of the Pezcoller Foundation for final consideration and determination.
Selection of the Award winner will be made on the basis of the candidate's scientific accomplishments. No regard will be given to race, gender, nationality, or religious or political view.
The Pezcoller Foundation was established in 1980 by Professor Alessio Pezcoller, a dedicated Italian surgeon who made important contributions to medicine during his career and who, through his foresight, vision and generous gift in support of the formation of the Foundation, stimulated others to make significant advances in cancer research. Previously the Pezcoller Foundation, gave a major biennial award for outstanding contributions to cancer and cancer-related biomedical science, in collaboration with the ESO-European School of Oncology.
The American Association for Cancer Research (AACR) was founded in 1907 by eleven physicians and scientists dedicated to the conquest of cancer and now has over 33,000 laboratory, translational, clinical and epidemiological scientists engaged in all areas of cancer research in the United States and in more than 97 other countries around the world.
The AACR is dedicated to its mission of preventing and curing cancer through the communication of important scientific results in a variety of forums including publications, meetings and training and educational programs. Because of the commitment of the Pezcoller Foundation and the AACR to scientific excellence in cancer research, these organizations are now collaborating annually on the presentation of the Award. This will strengthen international collaborations and will be a catalyst for advancements in cancer research internationally.

The winner of the Pezcoller Foundation-AACR International Award for Cancer Research will give an award lecture during the AACR Annual Meeting (April 2013), and the memorial Korsmeyer lecture at the VIMM in Padua and will receive the award in a ceremony at the Foundation's headquarters in Trento, Italy (May, 2013). The award consists of a prize of € 75.000 and a commemorative plaque.

Nomination Deadline: September 12, 2012
Questions about the nomination process:

Monique P. Eversley, M.S., Senior Coordinator, Scientific Achievement Awards - American Association for Cancer Research, 17th Floor, 615 Chestnut Street, Philadelphia, PA 19106-4404 - Tel. +1 (267) 646-0576;
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The Pezcoller Foundation Journal

Six-monthly review of the
Pezcoller Foundation
Via Dordi 8 - 38122 Trento - Italy
Tel. (39) 0461 980250
Fax (39) 0461 980350
e-mail: pezcoller@pezcoller.it
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Proprietario/editore:
Fondazione Prof. Alessio Pezcoller - Trento
n.36 - Registro delle Persone Giuridiche
presso il Commissario del Governo
della Provincia di Trento
Redazione: Via Dordi 8 - 38122 Trento
Direttore Responsabile: Gios Bernardi

"The Pezcoller Foundation Journal"
year 22, n. 38, Semestrale giugno 2012
Poste Italiane spa
Spedizione in abbonamento postale
D.L. 353/2003 (conv. in L. 27/02/2004 n. 46)
Art. 1, comma 2, CNS Trento
taxe percue / tassa riscossa