The Pezcoller Foundation

Journal

Summary

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- 27th Pezcoller Symposium:
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- Call for 2016 International Award
  For Cancer Research

News from the
Pezcoller Foundation World

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It's a great pleasure to report that the recipient of the 2015 Pezcoller Foundation-AACR International Award for Cancer Research is James P. Allison , Ph.D.,Chairman, Department of Immunology Executive Director, Immunotherapy Platform Deputy Director, David H Koch Center for Applied Research of Genitourinary Cancers, Vivian L. Smith Distinguished Chair in Immunology, The University of Texas, MD Anderson Cancer Center

The Selection Committee met in Philadelphia on November 22nd, 2014 and was chaired by Joan S. Brugge, PhD (2015) Professor and Chair Department of Cell Biology Harvard Medical School, Boston (MA). The other members of the Committee were: Alberto Bardelli, PhD, Director, Laboratory Of Molecular Genetics, Associate Professor, Department of Oncological Sciences, The Institute for Cancer Research and Treatment, IRCC University of Torino - Dominique L. de Valeriola, MD, General Medical Director - Inst. Jules Bordet, Brussels, Belgium - Richard M. Marais, PhD, Director Cancer Research UK, Manchester Institute, UK - Stephen B. Baylin, MD, Professor of Oncology & Medicine, Johns Hopkins University School of Medicine, Cancer Biology Division, Baltimore (MD) - Lisa Coussens Associate Director for Basic Research Knight Cancer Institute Oregon Health & Science University, Portland, OR -

Dr. James P. Allison was recommended as the recipient of the Award as a world-renowned cancer immunologist who has made seminal discoveries in basic immunology. These discoveries established a new paradigm in basic cancer immunologic research that has also led to the development of a new class of immunologic anticancer therapeutics. Dr. Allison's discovery and elucidation of the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) T cell signaling pathway and the development of the inhibitor of this pathway, ipilimumab (Yervoy) has been approved by the FDA for the treatment of malignant melanoma in March 2011. Dr. Allison began his career focusing on delineating the molecular events involved in T cell development, activation, and recognition of antigens. These more basic immunologic discoveries led to the identification of the first competing dual receptor T cell regulatory pathway that provides both activating and inhibiting signals. The CD28/CTLA-4 dual receptor pathway has become the prototype of a growing list of dual receptor systems that constitute immune checkpoints of T cell activation. Dr. Allison had cloned the murine homology of CD28 while CTLA-4 promotes inhibition of T cell activation, both by interacting with the B7.1/B7.2 receptors on antigen presenting cells. Dr. Allison continues to push the field forward by further elucidating the mechanisms that regulate the CD28/CTLA-4 signaling pathway, and by the identification of additional checkpoint signaling pathways that regulate T cells. He also identified a second molecule in the B7 family, B7x (B7-H4), a widely expressed B7 family member that has been shown to also inhibit T cell activation and to be associated on some cancer cell types in association with a poorer prognosis. He currently focuses on understanding additional family members including the PD-1/B7-H1 signaling pathway.
James Allison was introduced at the 2015 Annual Meeting in Philadelphia (PA) where he delivered to a large audience the Pezcoller Lecture: "Immune Checkpoint Blockade in Cancer Therapy: New Insights, Opportunities and Prospects for a Cure".

Afterwards the award was solemnly given in the prestigious hall of the Buonconsiglio Castle in Trento on May 15. In this occasion James P. Allison was introduced by Carlos Arteaga, 2014-2015 President of AACR.
Two days before the ceremony in Trento Dr. Allison gave the "Korsmeyer Lecture" in Padova at the VIMM Venetian Institute for Molecular Medicine to honor the memory of the late Stanley Korsmeyer winner of the 2004 Pezcoller-AACR Award.
This issue of our Journal is dedicated to the 27th Pezcoller Symposium entitled "Challenging Roadblocks to Cancer Cures" to be held in Trento from June 18 to June 20, 2015 co-chaired by David Livingston, Angelika Amon, Anne-Lise Børresen-Dale, Massimo Loda, Stefano Piccolo, William Sellers and Enrico Mihich. The focus of the symposium is the following: Cancer therapy is a topic of vigorous and increasingly promising research. However, only in rare instances is it curative, especially in advanced cancer. This symposium will focus on why greater success has not occurred and how a group of exceedingly perceptive leaders in the field are attempting to confront this problem. As a major part of the meeting, successful endeavors in major research areas will be discussed in detail. This meeting will attempt to illuminate major forces-both known and hypothesized- that block the discovery and development of curative cancer treatment. It will also articulate scientific advances and new areas of research aimed at overcoming these roadblocks. In addition, with significant audience participation, the leading edges of current therapeutics research work will be actively discussed and assessed.

Session I, Tumor Evolution
Session II, Tumor Microenvironment
Session III, Targeted Therapeutics
Session IV, Cancer Immunology
Session V, Clinical Research Roadblocks

The invited participants are:
Amon Angelika, Ph.D MIT Dept. of Biology, Cambridge MA
Baltimore David, Califonia Institute of Technology, Pasadena, CA
Barbacid Mariano, Centro Nacional de Investigaciones Oncologicas (CNIO)Madrid, Spain
Baselga Jose, Memorial Sloan Kettering Cancer Center, New York, NY
Biller Scott Ph.D., Agios Pharmaceuticals, Cambridge, MA
Caldas Carlos, UK Cambridge Institute University, Cancer Research, Cambridge UK
De Palma Michele, Swiss Institute for Experimental Cancer Research(ISREC) Swiss Federal Institute of Technology Lausanne CH
de Sauvage Frederic, Genentech Inc. San Francisco, CA
Disis Nora, University of Washington, Fred Hutchinson Cancer Research Center, Seattle WA
Elledge Stephen J., Harvard Medical School, Depart.of genetics, Boston, MA
June Carl H. M.D., University of Pennsylvania, School of Medicine, Philadelphia, PA
Loda Massimo, Dana Farber Cancer Institute, Boston, MA
Malumbres Marcos, Centro National de Investigaciones Oncolòigicas, Madrid, Spain
Piccolo Stefano, University of Padova School of Medicine, Department of Molecular Medicine, Padua, Italy

Polyak Kornelia M.D. Ph.D., Dana Farber Cancer Institute, Boston, MA
Rudensky Alexander, Memorial Sloan Kettering Cancer Center, New York, NY
Sellers William R. M.D.Novartis Institutes of Bio Medical Research, Cambridge, MA
Sharpe Arlene, Harvard Medical School, Dept. of microbiology and Immunology, Boston MA
Sotillo Rocio, EMBL Monterotondo, Italy
Swanton Charles, University College London Hospitals, London UK
Vankitaraman Ashok, University of Cambridge, MRC Cancer Unit, Cambridge UK
Weaver Beth A., University of Wisconsin, Madison WI
Walchock Jedd M.D.Ph.D., Memorial Sloan Kettering Cancer Center, New York, NY

The abstracts of the symposium are in the following pages.

Gios Bernardi M.D.
Editor and President Emeritus

Picture on front page: 2015 Pezcoller Foundation-AACR International Award for Cancer Research
From the left: Gios Bernardi, President Emeritus - Davide Bassi, President - Carlos Arteaga, AACR President - James P. Allison, winner - Anna De Poli, interpreter - Margaret Foti, AACR CEO.

27th Pezcoller Symposium

Challenging roadblocks to cancer cures

Trento, Italy, June 18-20, 2015

ABSTRACTS OF ORAL PRESENTATIONS

Paths to the Curative Treatment of Cancer

William R. Sellers
Novartis Institute for Biomedical Research

The last several decades have witnessed the emergence of multiple validated therapeutic modalities that have changed the course of cancer treatment and progression. In addition, to active chemotherapeutic agents, small molecule targeted therapeutics, antibody-based therapeutics, antibody-drug conjugates, immune modulators and cellular therapeutics have in one or more instances shown substantial single agent activity in distinct cancer states.

The genetic complexity of cancer both across tumors, within tumor types, and within single patients makes it clear that monotherapy is unlikely to bring substantial cures to the majority of patients and that combinations will be critical for curative therapy. In the pursuit of targeted therapy the emergence of genetic resistance has thwarted the activity of single agents, but has also illuminated a potential rationale route to the development of novel highly active combinations.

Based on the understanding of resistance in CML we have developed a novel allosteric site inhibitor (ABL001) of BCR-ABL that binds non-competitively with catalytic site inhibitors. ABL001 selectively inhibits the growth of CML and Ph+ ALL cells with potencies ranging from 1-10nM, while BCR-ABL-negative cell lines are unaffected at 1000-fold higher concentrations. ABL001 was also tested for its activity against clinically observed catalytic site resistance mutations and found to be active in the low nM range. In order to follow the outgrowth of clones harboring resistance to targeted therapeutics, a genetic bar-coding system was developed (ClonTracer) that enables the independent tracking of millions of individually "labeled" cells. Here, studies showed that distinct catalytic and allosteric inhibitors share overlapping resistant clones within their respective class, but have divergent clonal resistance when compared between the two classes. These data suggest that tumors might need to co-evolve independent mutations to defeat combinations between these two classes. In *in vivo* settings, single agent regimens led to tumor regressions however, all tumors quickly relapsed. In contrast, animals treated with the upfront combination of ABL001 and nilotinib achieved complete tumor regression with no evidence of disease relapse during the 70 days of treatment and for >100 days after treatment cessation.

These data have led to ongoing clinical trials testing the hypothesis that this rationally elucidated combination may lead to substantial curative activity and point to a potential pre-clinical route to evaluating the curative potential of candidate combination therapeutics including those using immunomodulation and CART therapies.

Maximum 2 pages, written by IB compiler

Cellular Consequences of Aneuploidy

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Aneuploidy is a hallmark of cancer. Understanding how aneuploidy impacts cell physiology is thus vital for understanding the principles underlying tumor formation. We developed yeast and mouse models to study the effects of aneuploidy on cell physiology. Our analyses revealed that the condition causes chromosome-specific phenotypes, and, remarkably, phenotypes shared by many different aneuploid yeast and mouse cells, which we collectively call the aneuploidy-associated stresses. Among, these stresses, proteotoxic stress caused by aneuploidy-induced proteomic changes appears especially prominent. We will discuss how aneuploidy affects protein quality control systems in both yeast and mammalian cells with a focus on autophagy. We will also discuss the paradox that despite the adverse effects of aneuploidy on cell physiology, tumor cells, who are characterized by high proliferative potential, are highly aneuploid. Our data indicate that whole chromosome aneuploidy causes further genome instability, providing a potential explanation for how the condition contributes to tumorigenesis. Finally, we will discuss strategies to exploit the stresses associated with aneuploidy to develop new cancer therapeutics.

Surviving and Dying during Cell Division

Marcos Malumbres
Spanish National Cancer Research Centre (CNIO) Madrid

Inhibition of mitotic progression has been proposed as an attractive therapeutic strategy to impair proliferation of tumor cells. Current cancer targets include several mitotic kinases and kinesins. Preclinical data also suggest that preventing mitotic exit may be highly efficient for killing mitotic cells. However, how cells survive during prolonged mitotic arrest is not well understood. Cell fate upon mitotic arrest is determined by the balance between mitotic slippage pathways and cell death. We therefore reasoned that interfering with the pathways that determine survival in mitosis could improve the efficiency of mitotic-targeted therapies. Although death in mitosis is thought to be at least partly mediated by apoptosis, the molecular pathways that control survival or death in mitosis are not well understood. By using a genetic model of defective mitotic exit in mammalian cells, we have shown that survival during prolonged mitotic arrest is modulated by apoptosis and autophagy-dependent cell death mechanisms. These pathways are modulated in response to the special energetic requirements of cells during mitotic arrest. Prolonged mitotic arrest results in loss of ATP and multiple cellular pathways sense and response to this defect. Alteration of these mechanisms results in enhanced cell death in mitosis and increases the anti-tumoral efficiency of microtubule poisons in breast cancer cells. Thus, survival of mitotic-arrested cells is limited by their metabolic requirements, a feature with critical implications in cancer therapies aimed to impair mitotic progression in tumor cells.

Consequences of Mad2 overexpression on early tumorigenesis

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Chromosomal instability (CIN), the inability to correctly segregate sister chromatids during mitosis, is a predominant feature of cancer and is strongly associated with patient survival and therapy outcome. Despite this correlation, the time, impact and importance of genomic instability during tumor initiation and progression is not well understood. Overexpression of the mitotic checkpoint protein Mad2, commonly found in human

tumors, leads to CIN and the development of aneuploid tumors in mouse models. However, recent observations from various laboratories suggest that aneuploidy can promote or suppress tumorigenesis depending on the genetic and cellular context. In fact, while overexpression of Mad2 cooperates with oncogenic Kras in lung tumorigenesis, its overexpression is detrimental in a Kras breast cancer model. Simultaneous induction of Mad2 and mutant Kras in mammary glands of adult female mice leads to checkpoint over-activation, and ultimately a delay in tumor onset. Time-lapse imaging of three-dimensional organotypic cultures derived from primary mammary cells revealed the consequent steps of mitotic arrest, apical extrusion and clearance accompanied by an increased occurrence of mitotic errors. Despite these initial cycles of enhanced cell turnover, elevated Mad2 levels are not selected against, but continue throughout tumorigenesis with no obvious impact on tumor phenotype. However, when faced with the strong selective pressure of oncogene withdrawal via de-induction of the genetic system, tumors previously affected by Mad2 have a higher probability of overcoming the initial regression phase and relapse. These results suggest that the impairment of cellular fitness is outweighed by the evolutionary advantage of karyotypic diversity allowing for the fast adaptability of the cancerous populations. This not only accounts for the correlation between CIN and therapy success, but also points out the importance of hidden CIN induced mutations as key players for disease progression.

Effects of chromosomal instability on tumor progression

Beth Weaver
University of Wisounsin, Madison, WI

Aneuploidy has been recognized as a hallmark of human tumors for over 100 years, leading to the hypothesis that it promotes tumor initiation and/or progression. We were surprised to find that aneuploidy due to reduction of the mitotic kinesin CENP-E can suppress tumors as well as promote them. Closer inspection revealed that it is the rate of chromosome missegregation/ chromosomal instability (CIN), rather than the presence or overall level of aneuploidy that determines the effect of mitotic defects on tumors. Low rates of CIN caused by reduction of CENP-E are weakly tumor promoting, while high rates of CIN resulted in cell death and tumor suppression. Notably, two insults that each cause low CIN could be combined to result in high CIN, cell death and tumor suppression. However, these experiments could not distinguish whether high CIN suppressed tumor initiation, tumor progression, or both. To test this question, we utilized animals expressing the Multiple Intestinal Neoplasia (Min) allele of the Adenomatous Polyposis Coli (Apc) tumor suppressor. $Apc^{Min/+}$ animals develop tens to hundreds of intestinal tumors and also develop mammary tumors when mutagenized with ENU. Effects on tumor initiation versus tumor progression can be distinguished in both contexts. $Apc^{Min/+}$ cells and animals exhibit a low rate of CIN that is increased by reduction of CENP-E. High CIN in CENP-$E^{+/-}$; $Apc^{Min/+}$ doubly heterozygous mice suppressed tumor growth and progression without inhibiting tumor initiation. This suggested that increasing the rate of CIN could be of potential use in treating existing tumors. Indeed, our data from a human clinical trial suggest that paclitaxel (Taxol™), the best selling chemotherapy drug in history, works via this method. Paclitaxel was shown to cause mitotic arrest prior to entering clinical trials three decades ago. It was widely accepted that paclitaxel caused death in patient tumor cells subsequent to mitotic arrest, as it does at commonly used concentrations in cell culture. However, we found that mitotic arrest is neither necessary nor sufficient for tumor response to paclitaxel. Indeed, paclitaxel accumulates in patient tumors at levels that are too low to cause mitotic arrest. Instead, cells in clinically relevant concentrations of paclitaxel proceed through mitosis on multipolar spindles, resulting in aneuploidy and CIN. Although paclitaxel is considered highly effective, only ~50% of patients benefit from its use. Currently, there is no way to predict which patients will respond. Together with our data from cell culture and animal models, our clinical data suggest that the 50% of patients that benefit from paclitaxel are the same 50% with tumors that have pre-existing rates of CIN. Hopefully, these insights can be leveraged to improve therapeutic outcomes by developing a biomarker to identify patients that will benefit from paclitaxel treatment.

How Aneuploidy Drives Cancer

Teresa Davoli, Andrew Wei Xu, Hajime Uno, Nicole L. Solimini, Kristen E. Mengwasser, Laura M. Sack, John C. Yoon, Peter J. Park & Stephen J. Elledge
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Aneuploidy has been recognized as a hallmark of cancer for over 100 years, yet no general theory to explain the recurring patterns of aneuploidy in cancer has emerged. We developed Tumor Suppressor and Oncogene (TUSON) Explorer, a computational method that analyzes the patterns of mutational signatures in tumors and predicts the likelihood that any individual gene functions as a tumor suppressor (TSG) or oncogene (OG). By analyzing >8200 tumor-normal pairs we provide statistical evidence suggesting many more genes possess cancer driver properties than anticipated, forming a continuum of oncogenic potential. Integrating our driver predictions with information on somatic copy number alterations, we find that the distribution and the potency of TSGs (STOP genes), OGs and essential genes (GO genes) on chromosomes can predict the complex patterns of aneuploidy and copy number variation characteristic of cancer genomes. We propose that the cancer genome is shaped through a process of cumulative haploinsufficiency and triplosensitivity. We are now assessing how aneuploidy drives cancer and the potency with which it does so relative to point mutant drivers. Ramifications of aneuploidy will be discussed.

Targeting intestinal stem cells in cancer

Frederic de Sauvage, Genentech Inc.
Genentech Inc. San Francisco, CA

The Notch, Wnt and Hedgehog signaling pathways play critical roles during embryonic development through modulation of proliferation, cell migration and differentiation. In adults, these pathways remain important in regulating stem cell function during normal tissue homeostasis. Inappropriate activation either through mutation or misexpression can result in tumorigenesis. The gastrointestinal tract is an example of tissue that remains dependent on Notch, Wnt and Hedgehog signaling for normal homeostasis. Intestinal epithelium is turned over every 5 days on average and is very dependent on stem cell activity. Multiple stem cell compartments, including crypt based columnar cells, the "+4" cells or cells in other more committed compartments, have been identified and contribute to intestinal homeostasis and/or regeneration. However, the respective interplay between these cells and their relative contribution to tumorigenesis is only beginning to be understood. As Notch, Wnt and Hedgehog pathways regulate stem cell function, normal intestinal homeostasis, and promote tumorigenesis, they are attractive targets for the development of anti-cancer stem cell therapeutics. The development of inhibitors targeting these pathways is therefore of the highest interest. We will discuss the role these developmental pathways in regulating intestinal stem cell populations in normal and tumor tissues.

YAP/TAZ cancer biology: hippo signaling and beyond

Stefano Piccolo
University of Padova, School of Medicine, Department of Molecular Medicine, Padua, Italy

We study how cells sense their environment and use this information to build and maintain tissues with specific form, size and function through the transcriptional coactivators YAP and TAZ. We are also interested in the cell and tissue-level mechanisms that lead to unrestrained YAP/TAZ activity and to tumor formation, progression and metastasis.
Our research focuses in three areas: (1) Mechanotransduction; (2) Molecular attributes of cancer stem cells; (3) Growth factor signaling. We are interested to elucidate how these contextual signals also regulate stem cell biology in adults, and act as elements of the tumor microenvironment essential to maintain cancer stem cells as well as the tumor's complex and "organ-like" cellular organization/hierarchy. Our approach to these goals entails both hypothesis-driven and data-driven investigations.

The role of the tumor microenvironment in breast tumor progression

Kornelia Polyak
Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA

The progression of DCIS (ductal carcinoma in situ) to invasive carcinoma is a poorly understood key step of breast tumorigenesis characterized by the loss of the myoepithelial cells and basement membrane. We previously described that the gene expression and DNA methylation profiles of normal and DCIS-associated myoepithelial cells are distinct potentially due to perturbed myoepithelial cell differentiation induced by signals from tumor epithelial and stromal cells such as leukocytes. Leukocytes may contribute to invasive progression by degrading the basement membrane and secreting chemokines/cytokines that promote cancer cell invasion and angiogenesis. Leukocytes may also suppress or promote DCIS progression via anti-tumor or pro-tumor immune responses. There is very limited knowledge about leukocyte populations in the normal breast, how these may change during physiologic events and tumor progression, and what role they may play in tumorigenesis. To investigate this, we have characterized the composition and molecular profiles of leukocytes infiltrating normal breast tissues of women with different risk of breast cancer and in situ and invasive breast carcinomas. Our results suggest key roles for microenvironmental alterations in DCIS, which is also likely shape the evolution of subsequent invasive tumors. Thus, understanding the selection pressures operating in the in situ to invasive transition would aid the design the more effective therapies for advanced stage disease.

microRNA control of tumor-associated macrophages

Mario Leonardo Squadrito, Caroline Baer & Michele De Palma
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microRNAs (miRNAs) are short, single-stranded RNAs that promote gene silencing at the post-transcriptional level. The enzyme DICER is a key component of the miRNA-processing machinery, which cleaves double-stranded precursor miRNAs to produce mature miRNAs. The miRNA-processing machinery is expressed in most eukaryotic cells, including macrophages (1). Extensive pre-clinical research has implicated tumor-associated macrophages (TAMs) in the facilitation of cancer progression; importantly, these immune cells also appear to influence the activity and efficacy of various anticancer therapies (2). Recent work has indicated that individual miRNAs expressed by TAMs, such as miR-511-3p, miR-142-3p and miR-155, have a role in fine-tuning pro- versus antitumoral programming of the tumor microenvironment (3-5). However, it is unclear if the endogenous miRNA network that is active in macrophages (1, 6) broadly operates to foster or attenuate TAM's protumoral functions. In order to explore the significance of endogenous miRNAs for TAM's development, differentiation and activation, we generated mice lacking *Dicer* specifically in macrophages. Efficient miRNA depletion in macrophages did not alter TAM's abundance in the tumors, but markedly reprogrammed their transcriptomes and effector functions from a pro- to an antitumoral type. This translated into profound Th1 skewing of the tumor microenvironment, which enhanced cytotoxic T-cell infiltration, abated tumor progression, and increased tumor responsiveness to immunotherapy. Collectively, these data identify a mechanism of TAM programming to an immunostimulatory phenotype that relies on defective miRNA processing and may be exploited to enhance the efficacy of cancer immunotherapies.

1. Squadrito, M.L *et al*. MicroRNA-mediated control of macrophages and its implications for cancer. *Trends Immunol* 34, 350-359 (2013).
2. De Palma, M. & Lewis, C.E. Macrophage regulation of tumor responses to anticancer therapies. *Cancer Cell* 23, 277-286 (2013).
3. Squadrito, M.L. *et al*. miR-511-3p modulates genetic programs of tumor-associated macrophages. *Cell Rep* 1, 141-154 (2012).
4. Sonda, N. *et al*. miR-142-3p prevents

macrophage differentiation during cancer-induced myelopoiesis. *Immunity* 38, 1236-1249 (2013)
5. Zonari, E. *et al*. A role for miR-155 in enabling tumor-infiltrating innate immune cells to mount effective antitumor responses in mice. *Blood* 122, 243-252 (2013).
6. Squadrito, M.L. *et al*. Endogenous RNAs modulate microRNA sorting to exosomes and transfer to acceptor cells. *Cell Rep* 8, 1432-1446 (2014).

Identification and functional validation of therapeutic targets in K-Ras driven lung and pancreatic tumors

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K-*RAS* oncogenes have been implicated in about one fifth of all human cancers including those with worse prognosis including lung adenocarcinomaand pancreatic ductal adenocarcinoma. We have developed genetically engineered mouse (GEM) strains that closely recapitulate the natural history of these human neoplasias. We have used these strains to validate targets of potential therapeutic value with the ultimate goal to translate these findings to the clinic. Briefly, we have crossed these GEM strains with mice that carried either germ line or conditional knock out mutations in loci encoding potential therapeutic targets. These targets were ablated by genetic means at the time of tumor initiation using the Cre-lox inducible recombinase system. Then, we followed the fate of the tumor in the absence of the target. This genetic-based strategy has significant advantages over classical pharmacological studies since it does not rely on the quality of the drug/inhibitor. For instance, the observed effects are always mechanism-based and not off-target effects. Moreover, if the target is eliminated systemically, our studies offer relevant information regarding potential toxic effects that may occur when the target would be pharmacologically inhibited in normal tissues. Using this experimental approach, we have interrogated each of the K-Ras downstream kinases including those of the Raf/Mek/Erk pathway as well as the cell cycle Cdks, the ultimate targets of K-Ras mitogenic signaling. Our results revealed some unexpected results that may have important implications for the development of future targeted therapies. For instance, we have established that whereas c-Raf is essential for the development of lung adenocarcinomas and pancreatic ductal adenocarcinomas, the closely related B-Raf and A-Raf kinases are completely dispensable, indicating that these enzymes do not have compensatory activities in tumor development, as previously thought (Blasco et al., Cancer Cell, 2011). Likewise, lung adenocarcinomas do not progress in the absence of Cdk4. However, ablation of the highly related cell cycle kinases Cdk2 and Cdk6 have no significant effect on tumor development (Puyol et al., Cancer Cell, 2010). These observations underscore the need to carry out target validation studies using mouse models in order to design selective therapies that will have a greater chance of success in the clinic. Based on early clinical results, we have also interrogated the anti tumor consequences of ablating the EGF receptor, a target for which there is already selective inhibitors. Unexpectedly, whereas ablation of EGF receptors has no therapeutic value in K-*Ras* driven lung adenocarcinomas and intestinal tumors (as previously observed in the clinic), it is essential for the initiation of pancreatic tumors (Navas et al., Cancer Cell, 2012). These findings indicate that K-*Ras* oncogenic signaling proceeds through different effector pathways in a tumor specific manner, an observation that should also help to design more selective therapies. More recently, we have generated new strains of mice in which expression of the resident K-Ras oncogene, as well as ablation of the p53 tumor suppressor, is mediated by the frt-FLp(o) recombinase system. Thus, allowing us to temporally separate tumor induction from target validation. Moreover, we have generated Cre-lox based, conditional knock-in alleles that direct the expression of kinase dead isoforms instead of the classical conditional knock outs that cause protein ablation. This new experimental system is allowing us to inactivate the previously validated targets c-Raf, Cdk4 and EGF receptor (as well as other kinases such as PI3Kinase and mTOR), in tumor-bearing mice to better evaluate their

therapeutic potential with the ultimate goal to serve as guide for the design of future clinical trails.

Extending the reach of target discovery for cancer and other diseases

Ashok Venkitaraman
Medical Research Council Cancer Unit
University of Cambridge, UK

Cellular pathways are controlled by interactions between biological macromolecules, which may be perturbed by disease-associated changes in the concentration, localization or structure of the reactants. As a result, many of the most attractive therapeutic targets for the modulation of disease-associated cellular pathways involve macromolecular interactions between proteins, rather than conventional drug targets like the active sites of enzymes. It is widely accepted that the ability to modulate protein-protein interactions using small molecules is fundamental both for the development of chemical tools to explore biological mechanisms ('chemical biology'), and to seed the future creation of new drugs with improved selectivity and therapeutic index. I will discuss the approaches we are taking to systematically scrutinize disease pathways for target identification, to develop drug-like chemical probes against protein-protein interactions, and to define the mechanisms through which these novel compounds exert their biological effects. The toolkit emerging from our work offers opportunities to extend the reach of target discovery for cancer and other diseases. Recent publications:

1. Venkitaraman, A.R. (2014) Cancer suppression by the chromosome custodians, BRCA1 and BRCA2. (2014) Science 343(6178):1470-5.
2. Liang H, Esposito A, De S, Ber S, Collin P, Surana U, Venkitaraman AR. (2014) Homeostatic control of polo-like kinase-1 engenders non-genetic heterogeneity in G2 checkpoint fidelity and timing. Nature Commun. 5:4048.
3. Ibbeson B.M., Laraia L., Alza E, O' Connor C.J., Tan Y., Davies H.M., McKenzie G., Venkitaraman A.R.*, Spring D.* (2014) Diversity-oriented synthesis as a tool for identifying new modulators of mitosis. Nature Commun. 5:3155
4. Jeyasekharan, A.D., Liu, Y., Hattori, H., Pisupati, V., Jonsdottir, A.B., Rajendra, E., Lee, M., Sundaramoorthy, E., Schlachter, S., Kaminski, C., Rosenfeld, Y., Sato, K., Savill, J., Ayoub, N.& Venkitaraman, A.R. (2013). A cancer-associated BRCA2 mutation reveals masked nuclear export signals controlling localization. Nature Str Mol Biol. 20, 1191-8.
5. Wickramasinghe, V., Savill, J. Chavali, S., Jonsdottir, A.B., Rajendra, E., Gruner, T., Laskey, R., Babu, M., & Venkitaraman, A.R. (2013). Human inositol polyphosphate kinase regulates transcript-selective nuclear mRNA export to preserve genome integrity. Molecular Cell. 51, 737-43.

Inhibitors of mutated isocitrate dehydrogenase as a potential new therapy for AML: from the bench to the clinic

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Somatic mutations in the metabolic enzymes isocitrate dehydrogenase 1 and 2 (IDH1/2) confer neomorphic activity in cancer cells, resulting in accumulation of the oncometabolite, R-2-hydroxyglutarate (2-HG). High levels of 2-HG result in epigenetic changes and impaired cellular differentiation. IDH mutations have been identified in a spectrum of solid and hematologic malignancies. For example, approximately 25% of patients with acute myelogenous leukemia have either IDH1 or IDH2 mutations. AG-120 and AG-221 are first-in-class, oral, potent, reversible, selective inhibitors of the IDH1 and IDH2 mutant enzymes, respectively. In a primary human IDH2 mutant positive acute myeloid leukemia (AML) xenograft model, AG-221 treatment reduced 2-HG levels and demonstrated a dose-dependent survival benefit. In addition, both molecules showed differentiation promoting effects in IDH mutant patient AML samples

ex vivo, with no affect on IDH wild type AML samples. Independent clinical trials are underway for both AG-221 and AG-120 in IDH2 and IDH1 mutant positive malignancies, respectively, in both the hematologic and solid tumor contexts. This presentation will focus on the discovery of these investigational medicines, as well as the early promising clinical results from the human AML trials.

Developing curative therapeutic combinations for cancer

William R. Sellers
MD Novartis Institutes for BioMedical Research, Cambridge, Ma

Recent advances in genetic-based and immunology-based therapeutic approaches to cancer have led to dramatic treatment responses in a variety of cancers many of which were largely refractory to standard of care chemotherapeutics. Nonetheless, these advances have led mostly to increases in disease-free survival rather than out right treatment-free cures. Cancer heterogeneity, manifest primarily as pre-existing and acquired sub-clonal genetic alterations, makes it likely that highly active combination therapy will be required to move to curative treatments. How to assemble, dose and sequence the right combinations remains a problem of high complexity due to the number of distinct cancers and the large number of combination possibilities even when one only considers therapeutics that are in clinical trials today.
We are using a variety of approaches to guide the development of rationale combinations. Two concepts are foremost in these approaches 1) identify combinations that lead to synergistic cell death through large-scale compound and pooled shRNA screens and 2) identifying combinations that have non-overlapping mechanisms of resistance as determined through the use of high-complexity bar-code screens (clonal tracing studies) and 3) the study of resistance in ongoing clinical trials. These approaches will be illustrated in the context of the ongoing development of novel small molecule programs targeting BCR-ABL, BRAF and EGFR along with immunology based therapeutics.

Multifaceted functions of the PD-1 pathway

Arlene H. Sharpe
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The immune response plays an important role in fighting cancer; however, the tumor environment is immunosuppressive and limits effective anti-tumor immunity. A new and promising strategy of tumor immunotherapy blocks pathways used by tumors to inhibit anti-tumor immunity. This inhibitory strategy is called checkpoint blockade. One key pathway that inhibits tumor-specific immunity is the PD-1 co-inhibitory pathway.
This pathway consists of the PD-1 receptor and its ligands PD-L1 (B7-H1) and PD-L2 (B7-DC). PD-1 is inducibly expressed on peripheral $CD4^+$ and $CD8^+$ T cells, NKT cells, NK cells, B cells, and monocytes, and some dendritic cell (DC) subsets upon activation. PD-1 is upregulated after T cell activation, but declines when Ag is cleared, and TCR is no longer engaged. If Ag is not cleared and T cells are repetitively stimulated (as in chronic infection or cancer), PD-1 expression remains elevated and T cells can enter a state of decreased effector function and proliferative capacity, termed “T cell exhaustion”. PD-1 is a marker for exhausted T cells, but PD-1 is not exclusively expressed by these cells. PD-1 is upregulated on activated T cells and constitutively expressed by natural and induced T_{regs}. PD-L1 is broadly expressed on hematopoietic (APC and T cells) and non-hematopoietic cells (including vascular endothelial and pancreatic islet cells), while PD-L2 is expressed mainly on macrophages and DCs. B7-1 is an additional binding partner for PD-L1 but does not bind PD-L2. RGMb is an additional ligand for PD-L2 but not PD-L1.
The PD-1 pathway plays critical roles in regulating the balance between T cell activation and tolerance. PD-1 engagement can inhibit T cell proliferation, cytokine production, cytolytic function, and survival. The PD-1 pathway also controls T cell tolerance at multiple checkpoints. PD-1:PD-L1 interactions inhibit 1) initial activation of self-reactive T cells, 2) effector T cell responses, and 3) target organ injury. PD-L1 on non-hematopoietic cells

can restrain self-reactive T cells in target organs, maintaining tolerance in tissues and protecting them from immunopathology. In addition, the PD-1 pathway is a key mediator of T cell dysfunction ("exhaustion") in cancer and chronic infections. This pathway is a promising therapeutic target in cancer. The remarkable effects of PD-1 pathway blockade in cancer demonstrate the key role of this pathway in inhibiting anti-tumor immunity. However, there are multiple co-inhibitory pathways that that limit T cell function, and these have become targets for cancer therapy. This talk will discuss the multifaceted immunoregulatory roles of PD-1 and its ligands in controlling T cell activation, tolerance and exhaustion. The role of the PD-1 pathway in cancer as well as therapeutic strategies that combine PD-1 blockade with other therapies also will be discussed.

Targeted therapy with engineered T cells

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The field of adoptive cell transfer (ACT) is currently comprised of CAR and TCR engineered T cells and has emerged from principles of basic immunology to paradigm-shifting clinical immunotherapy. ACT of T cells engineered to express artificial receptors that target cells of choice is an exciting new approach for cancer, and holds equal promise for chronic infection and autoimmunity. Using principles of synthetic biology, advances in immunology and genetic engineering have made it possible to generate human T-cells that display desired specificities and enhanced functionalities. Clinical trials in patients with advanced B cell leukemias and lymphomas treated with CD19-specific CAR T cells have induced durable remissions in adults and children. The prospects for the widespread availability of engineered T cells have changed dramatically given the recent entry of the pharmaceutical industry to this arena. Here, we discuss some of the challenges and opportunities that face the field of ACT.

Immune Modulation of Breast Cancer

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Breast cancer is immunogenic and there is significant evidence that as breast cancer develops, tumors elicit an adaptive immune response. T-cells infiltrate most breast cancers and in several studies, the presence of elevated intratumoral CD8+ T-cells or elevated CD8+ T-cells in conjunction with a limited T-regulatory cell infiltration is associated with a favorable prognosis. Moreover, patients with breast cancer have been shown to develop immunity against specific proteins expressed by their tumors. Recent studies demonstrate that the development of humoral immunity directed against tumor associated antigens can be detected prior to a breast cancer diagnosis indicating the ability of the immune system to act as a sensor for exposure to aberrantly expressed proteins.
Cellular immunity, directed against breast cancer antigens and elicited by exposure to malignancy, is present even while the disease progresses. The endogenous breast cancer specific cellular immune response is often of a Type II phenotype eliciting primarily antibody immunity rather than cytotoxic T-cells. Furthermore, type II cytokines secreted by T-cells actively dampen the development of a CD8 T-cell response. Type I cytokines, such as interferon gamma or TNF-alpha, are needed to activate antigen presenting cells allowing the presentation of tumor specific antigens via cross-priming, the primary mechanism by which cancer is recognized by T-cells. Immune cells which secrete Type I cytokines are rarely detected or found only in limited numbers in the tumors of most breast cancer patients. Several therapeutic modalities are under development and designed to increase or stimulate Type I T-cells capable of homing to breast tumors. Active immunization, targeting specific breast cancer antigens,

has been shown to directly modulate the breast cancer microenvironment by enhancing cross-priming resulting in the development of epitope spreading. The identification of developing immunity against multiple tumor antigens during the course of treatment has been associated with a survival benefit after vaccination targeting HER-2/neu (HER2). High levels of vaccine induced Type I HER2 specific T-cells have been shown to be inversely associated with serum TGF-beta levels in patients with advanced stage breast cancer. Significantly increasing the number of Type I T-cells targeting breast cancer, via strategies such as adoptive T-cell therapy, has been associated with a measurable clinical response in advanced stage breast cancer. Ex vivo expansion of HER2 specific Th1 cells, after vaccine priming, and infusion of those cells into patients resulted in a 40% clinical response rate in patients with Stage IV treatment-refractory breast cancer. After infusion of Type I HER2 positive T-cells, responding patients developed multiple clonal CD4+ and CD8+ T-cell populations in their peripheral blood, which may be an indication of epitope spreading. The level of HER2 specific T-cells achieved in vivo and the development of a diversity of clonal T-cells was significantly associated with clinical response.
Although breast cancer specific Type I T-cells may not be found in the malignancy at the time of diagnosis, such cells can be induced. The ability to circumvent the immune suppressive microenvironment opens the door for combination immune based therapies utilizing checkpoint blocking antibodies or specific chemotherapies shown to modulate immune suppression to further enhance the number and function of tumor specific Type I T-cells.

Combination Checkpoint Blockade and the Role of 'Passenger' Mutations in Clinical Response to Ipilimumab

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Given the activity noted with both CTLA-4 or PD-1 blockade, clinical trials are now investigating combination checkpoint blockade. The most mature data with a combination of ipilimumab + nivolumab in melanoma showed a response rate of 40% across dose cohorts with >50% in some cohorts in the context of manageable toxicity. Such responses are generally durable, even when treatment was stopped early for toxicity. Unlike in studies of PD-1 blockade monotherapy, there was no significant difference in clinical activity based on tumor expression of PD-L1. Phase 2 and 3 trials of this combination are ongoing in melanoma with phase 1 programs in numerous other tumor types.
Attention is being paid to the reasons underlying the efficacy of checkpoint blockade in certain malignancies. One hypothesis has been that cancers having a high mutational load may be more amenable to immune modulation by virtue of the larger number of potential neo-epitopes present, fostering baseline immune recognition that can then be potentiated by checkpoint blockade. We have found that melanoma patients having long term clinical activity with ipilimumab have a significantly greater median number of non-synonymous passenger mutations, compared with patients who do not respond or those who have only short-term regression. The use of whole exome sequencing has allowed us to explore the additional hypothesis that the higher likelihood of response observed may not simply be explained by a higher quantity of mutations but rather that the individual immunologic quality of mutations is also important. Using a novel bioinformatics platform, we have found sub-strings of class I epitopes (tetrapeptides) shared by clinical responders and not found in the non-responders. Intriguingly, the vast majority of these favorable substrings are also found in known epitopes derived from proteins found in bacteria and viruses.

Vectored Immunization by Targeting Dendritic Cells

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We showed some time ago that one of the most efficient methods of immunization to produce a CD8 cell response is direct immunization of dendritic cells in the skin using a lentiviral

vector programmed with the relevant antigen. We now know that the effectiveness of this procedure comes from a natural adjuvant effect of the vector due both to its fusion factor and to soluble components in the vector. To exploit this technology we have formed a company, Immune Design Corporation, which is concentrating on immunization with cancer antigens. It has initiated clinical trials in sarcoma patients. Both the methodology and the available results will be discussed.

Characterizing and modelling breast cancer subtypes

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We have recently re-defined breast cancer as a constellation of 10 genomic copy number driver-based subtypes (Curtis et al, Nature 2012; Dawson et al, EMBO J 2013; Dvinge et al, Nature 2013; Ali et al, Genome Biology 2014). These subtypes are remarkably stable along the pre-invasive-to-metastatic progression continuum, strongly suggesting the copy number drivers are prototypical 'stem' mutations in breast cancers. The frequency and patterns of SNVs across these 10 subtypes occur in characteristic patterns, and most of the clonal evolution in tumours occurs along the SNV landscape. We have now succeeded in generating patient-derived xenografts (PDTXs) representative of most of the 10 subtypes and have characterized the dynamics of genomic clones in these models (Eirew et al, Nature 2015). We are now using these models to perform pre-clinical drug screens and to study clonal dynamics. Perturbations of both the cell autonomous and non-cell autonomous compartments of these models provide a tractable platform for studying tumour progression, clonal evolution and dormancy.

Spatial and Temporal Dynamics of Cancer Evolution

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Increasing evidence supports complex sub clonal relationships in solid tumours, manifested as intratumour heterogeneity. Our group and others are finding evidence for spatial heterogeneity within individual tumours and the temporal dynamics of tumour evolution. Parallel evolution of sub clones, with distinct somatic events occurring in the same gene, signal transduction pathway or protein complex, suggests constraints to tumour evolution that might be therapeutically exploitable. Drivers of tumour heterogeneity appear to change during the disease course that contribute to the temporally distinct origins of cancer driver events. Genome doubling, occurring early or late in tumour evolution, exacerbates chromosomal instability contributing to intercellular heterogeneity and poor outcome. The finding of sub clonal driver events is likely to limit the efficacy of targeted mono therapies, suggesting the need for new approaches to drug development and clinical trial design. TRACERx, a longitudinal lung cancer evolution study and DARWIN clinical trials aimed at deciphering the relevance of sub clonal driver events to therapeutic outcome, will be discussed.

Tumor heterogeneity and acquired resistance to targeted therapies

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Tumor heterogeneity and acquired resistance to treatment via the emergence and growth of resistant clonal subpopulations is one of the major challenges facing the field of targeted therapeutics. The identification of these clones and their underlying mechanisms of resistance has proven extremely useful in some instances and, in turn, led to new therapies that either prevent their emergence or that can be utilized at the time of treatment failure. Therefore, a systematic approach to interrogate tumors at the time of progression may provide us with useful insights. However, in patients with advanced disease and multiple metastasic sites, a frequent situation in the clinic, it is challenging to analyze all tumor sites and obtain an integrated view on the mechanisms

of resistance. We are currently engaged in testing the antitumor activity of a novel, first in class, PI3K p110α inhibitor, BYL719, in patients with tumors harboring activating PI3K p110α mutations. *PIK3CA*, the gene encoding for the p110α subunit, is mutated in up to 40% of estrogen receptor (ER) and/or HER2 positive breast tumors. Hence, the rational to target PI3K is compelling. Selective PI3K p110α inhibitors have been shown to be preferentially active against cancer cell lines harboring PIK3CA mutations, a finding in contrast to pan-PI3K inhibitors. The observation of high selectivity in mutant models led us to initiate a first-in-human phase I clinical trial with BYL719, limited to patients with tumors harboring mutations in PIK3CA. In this clinical study we have observed clinical responses in breast, head and neck, and other tumors. As with other targeted therapies, we have eventually observed acquired resistance and tumor progression in all responding patients. In order to identify potential mechanisms of resistance we have obtained tumor tissue at the time of progression in patients participating in the clinical trial. We were able to sequence multiple metastasis sites, some of them still responding to therapy and others clearly progressing. Deep sequencing of the non-responding lesions revealed that selective treatment pressure resulted in a uniform loss of PTEN expression. Strikingly, the loss of PTEN expression, far from being mediated by a single genomic alteration, was the result of at least 6 distinct alterations involving three distinct mechanisms, occurring at different metastatic sites. This finding suggests the presence, under selective pressure, of genetic parallel tumor evolution of several clones towards a convergent phenotype of PTEN loss. This results in activation of PI3K signaling via an alternative PI3K isoform and, as a consequence, rescuing the tumor from the effects of PI3K p110α inhibition. Parallel evolution under selective pressure had been described in conditions where treatments are highly efficacious like HIV disease. We also observed disappearance of detectable PIK3CA mutations in post-treatment samples of two patients treated with BYL719, suggesting that a negative selection of clones bearing wild-type alleles of PIK3CA may also explain in other cases the emergence of resistance to BYL719.

Our work highlights the importance of using DNA sequencing as a tool to gain insights on mechanisms of resistance in patients treated with targeted therapies and how these tools may be used to design rational combinatorial therapy approaches that may pro-actively prevent the development of acquired resistance. Going forward, evolutionary analysis of tumors will elucidate predominant routes of drug resistance, identify alterations enriched during targeted therapy, and characterize the mutational background in which they may arise. Such knowledge should inform and increase our capability to predict combination therapies.

ABSTRACTS OF POSTERS

Patient-derived tumor xenografts as pharmacological model of human pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancer with an overall survival rate of 3-5% and a median survival of less than 6 months. Surgery remains the most effective treatment, with a limited efficacy of approved chemotherapy. Thus, there is a clear need for the development of new therapeutics but the research is braked by the limited value of the current preclinical PDAC models as predictors of patients response.
We are developing intrapancreas implanted orthotopic models of patient-derived xenografts (PDX-PDAC) that retain molecular and biological features of the patient tumors, and are amenable for testing novel therapeutics.
Ten surgically resected cancer specimens (histologically confirmed pancreatic adenocarcinoma) were implanted in immunocompromised NOD-SCID IL2Rgamma^{null} (NSG) mice. In 4 cases tumors were successfully engrafted and propagated by re-implanting tumor tissue fragments into the pancreas of severe combined immunedeficiency (SCID) mice.
Tumor growth after orthotopic implantation was monitored by abdominal palpation and by non invasive magnetic resonance imaging (7-TESLA small animal MRI scanner). T2-weighted high resolution sequences were analyzed using ImageJ software to calculate the tumor volume.
The histological analyses on explanted PDX-PDAC tumors confirmed the morphology and histology similarity to the original patient tumors, through multiple passages (up to five). The established xenografts retained a fair and constant amount of stroma through generations, despite exhibiting less desmoplastic reaction compared to the patient's tumor.
Research is ongoing to characterize mutational landscape of the PDX-PDAC models in comparison to the patient tumor.
Responsiveness to the existing approved chemotherapy against pancreatic cancer, gemcitabine, administered alone or combined with nab-paclitaxel, was evaluated.
Accordingly to patient's recommendations, drugs were given intravenously on days 1 and 8 of each 21-day cycle at the following human dose equivalent (HDE): nab-Paclitaxel 75 mg/m2 and Gemcitabine 450 mg/m2. While orthotopic growing PDX-PDAC were marginal responsive to single agent treatment, results show significant tumor response with the combination of the two drugs, reproducing the clinical setting.
In conclusion, PDX-PDAC growing orthotopically in the pancreas of mice provide a valuable tool for preclinical analysis of promising therapeutic regimens for pancreatic cancer, and might be used in drug discovery for hypothesis driven combination efficacy and biomarker studies.
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Anti-tumor immunization in mothers delays tumor development in cancer prone offspring

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Neonatal cancer is an issue of real urgency in oncology. Of the various therapeutic strategies used against this category of malignancy, immune-based therapies are the most promising. Significant effort has been spent on the recent successes of adoptive T-cell therapy, chimeric antigen receptors, and monoclonal antibodies. However, the prevention of paediatric cancer has not yet become a reality. Numerous examples have proven that maternal immunization represents the trump card against a number of life-threatening neonatal infectious diseases, as it can protect both mother and offspring via the passive transfer of maternal antibodies. In this work, we gauge whether maternal immunization can be used in neonatal cancer immune-prevention. In a previous study, we have shown that DNA vaccination-induced antibodies against rat Her2 (neu) protect heterozygous neu-transgenic female (BALB-neuT) mice from autochthonous mammary tumor development. Here, we have applied our immunization strategy to BALB/c mice that were mated with BALB-neuT males in an effort to study whether this process confers anti-tumor protection to their BALB-neuT offspring which have been genetically predisposed to develop mammary cancer. Significantly extended tumor-free survival has been observed in BALB-neuT offspring which were born and fed by mothers vaccinated against neu, as compared to controls. Maternally derived anti-neu IgG have been proven to successfully transfer from mothers to their offspring providing the observed protective effect. Vaccinated mother offspring also develop active immunity against neu as revealed by the presence of T-cell-mediated cytotoxicity against the neu immunodominant peptide. This active response is due to the transfer, in milk, of immune-complexes formed between the neu extracellular domain, which is shed from vaccine-transfected muscle cells, and the anti-neu IgG induced by the vaccine.
It is worth noting that preliminary results on a transgenic mouse model of neonatal neuroblastoma (NB), which overexpresses MYCN and ALK (anaplastic lymphoma kinase), have demonstrated that maternal vaccination using a plasmid which codes for human ALK may be effective in slowing the growth rate of NB abdominal masses and in increasing the overall survival of NB affected offspring, while this did not occur using the empty vector plasmid.
These findings demonstrate that maternal immunization possesses the potential to hamper mammary carcinogenesis in genetically pre-destined offspring and that it can be developed into treatment against lethal neonatal cancer for which powerful therapeutic options are currently unavailable.

Targeting Fibroblast Growth Factor Receptors in Small Cell Lung Cancer (SCLC)

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A large body of evidence supports the notion that fibroblast growth factor receptors (FGFRs) activation promotes growth and tumor cell survival. Hyperactivation of the FGFR pathway occurs almost in every type of cancer via overexpression of FGF ligands or via mutations, amplifications and fusions of the receptor's genes. In lung tumors, amplification of *FGFR1* is one of the most common genetic aberration and it is typically associated with addiction to MAPK signaling and responsiveness to FGFR inhibitors. In SCLC, a remarkably uniform *FGFR1* high-level copy number gain occurs in 5 -22% of all cases.
We developed a mouse model of FGFR1-expressing SCLC suitable for therapeutic interventions. The model is based on the delivery of a novel lentivirus encoding *FGFR1*, Cre recombinase, and secreted Gaussia luciferase to the lungs of mice carrying floxed alleles of P53 and RB. Combined loss of P53

and RB normally results in SCLC in mouse and occurs in 98% of human SCLCs.
In our model, the tumorigenic process initiated by the loss of P53 and RB is shaped by the concomitant hyperactivation of the FGFR1 pathway. Co-expressed secreted luciferase serves as an excellent surrogate marker of tumor development because of its short half-life (2hrs) and easy detection in blood.
A major problem with therapeutic interventions in mouse models, whether xenografts or genetically engineered, is measuring accurately how a given cancer therapy affects tumor growth and knowing the status/representation of the target in the course of therapy. This is not easily achievable with the current imaging and measuring techniques. For example, induction of necrosis upon therapy, followed by drug resistance would be very hard to quantify by caliper measurements or IVIS imaging in any model. In our system, by connecting the expression of a drug target with a secreted reporter produced exclusively by living cells, we can monitor closely viability changes in the tumor population of interest and therefore get valuable information about how tumors respond and resist to therapies.
We found that engineering lentiviruses with multiple genes of interests and secreted reporters is a sound strategy to induce and track the growth dynamics of novel, genetically-defined lung tumors in mice. The system is extremely flexible, because it allows testing the effect of multiple therapeutic targets, in our case *FGFR1* and its mutant forms, in relevant and already well-characterized genetically engineered mouse models of SCLC, (but also squamous cell carcinoma and non-small cell lung cancer models are suitable), requiring only cloning of new lentiviral constructs.

Getting around the roadblocks the widening path of angioprevention

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Introduction: A healthy lifestyle and diet have long been associated with smaller chances of developing non-familial malignancies. In recent years, research studies have turned this common sense-based concept into the proper strategy of chemoprevention, i.e. actively applying well-tolerated dietary supplements in order to reduce the risk of cancer formation or recurrence. Many natural compounds commonly found in foods and beverages (flavonoids such as carotenoids, green tea catechins...) as well as drugs used for other clinical practices (aspirin, metformin....) have been recognized as capable of preventing or retarding tumour development and progression. Several of these molecules attain chemopreventive effects by curbing angiogenesis, a crucial event in cancer progression. Olive mill wastewaters (OMWWs), a waste product from olive oil industry, are rich in polyphenols, particularly hydroxytyrosol (HT), known to possess anti-inflammatory and anti-oxydant properties. Xanthohumol (XN), the most abundant flavonoid in the hop plant (*Humulus lupulus L*), is also an antioxydant polyphenol and can be found in beer. Metformin, an anti-diabetic drug commonly used in type 2 diabetes patients, has been associated with decreased cancer risk in epidemiological studies.
Materials and methods: We tested the anti-angiogenic properties of OMWWs, XN (including synthetic derivatives) and Metformin on human umbilical endothelial cells (HUVECs). We evaluated cell viability curves via MTT, apoptosis by flow cytometry, migration/invasion in a Boyden chamber assay, and capillary-like network formation by the ability to form tubes and nodes on Matrigel. The effects of XN derivatives on cell proliferation were compared by calculating IC50 values at 96h. After treatment with Metformin or XN, we assessed the molecular mechanisms involved by analyzing AMPK activation by Western blotting. After treatment with OMWWs, we also evaluated ROS formation by flow cytometry.

Results: OMWWs inhibit ROS generation, but interfere with the proliferative, migratory and tube-formation abilities of HUVECs by inducing apoptosis. They have a stronger antiangiogenic effect than HT alone, possibly because of the synergistic action of different polyphenols present in the wastewaters. On the other hand, low doses of XN or Metformin affect HUVECs by activating AMPK, an ATP sensor and master regulator of cellular energy state, rather than inducing apoptiosis. Treated cells remain vital, but show reduced proliferation rate, migration rate and ability to form capillary-like networks. Although XN was previously found to be more effective on tumour cells than HUVECs, some of the XN derivatives we tested managed to achieve greater potency in endothelial cells as well.
Conclusions: Metformin, XN and OMWWs activity on HUVECs makes them good candidates for anti-angiogenic chemoprevention and confirm AMPK as an important molecular target, common to different antiangiogenic strategies. Our experience with modified XN further suggests that derivatization of these compounds can further tailor them to different prevention requirements - from weak, non-toxic molecules for general prevention, to stronger molecules for hampering tumour recurrence or hyperplasia progression to malignancy.

Role of SPARC and mast cells in non-Hodgkin B cell lymphomas

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B-cell lymphomas are a heterogeneous group of hematologic tumors that arise from the neoplastic transformation of different B-lymphocyte subsets. As for most neoplasms, the natural course of B-cell malignancies is characterized by tumor progression, featured by a flow of events leading to the enhancement of proliferative and invasive capabilities. Even if most of the processes involved in cancer progression are inherent to the neoplastic clone, this event seems to require the constant crosstalk with the surrounding microenvironment. Among matricellular proteins, the secreted protein acidic and rich in cysteine (SPARC) acts as a master regulator of extracellular matrix (ECM) deposition. We recently showed that SPARC deficiency, and consequent low ECM deposition, worsened the phenotype of the autoimmunity-prone *Fas*$^{lpr/lpr}$ mice towards uncontrolled lymphoproliferation, characterized by the expansion of CD5^{+} B-cells, mimicking human chronic lymphocytic leukemia (CLL). Indeed, CLL patients are characterized by low SPARC expression and low ECM deposition. On the other hand, some subtypes of B-cell non-Hodgkin lymphomas (B-NHLs), such as the diffuse large B-cell lymphoma (DLBCL), have been shown to express higher levels of SPARC and to deposit more abundant ECM. In DLBCL patients, high SPARC expression from histiocytic elements infiltrating the tumors has been associated to increased survival following standard treatments.
We performed a gene expression profile analysis of a cohort of B-NHL patients, and unraveled a significant association between high SPARC expression from the tumor clones and the activation of certain biological programs, including the inflammatory response, ECM remodeling and, very noticeably, mast cell function. Mast cells (MCs) are the key effectors in immunoglobulin E (IgE)–mediated allergic diseases, but also participate in a variety of IgE-independent biologic responses, and particularly, in tumorigenesis and cancer progression. Indeed, MC infiltration has been reported in several types of human solid and hematologic tumors and in animal cancer models, associated to either good or poor prognosis depending on the tumor type and stage. MCs may exert their regulatory function mobilizing and modulating the activity of different immune cells, as well as stromal cells. Very recently, MC infiltration in the bone marrow (BM) of splenic marginal zone lymphoma patients has been correlated with shorter time to progression; in this context, MCs established a crosstalk with CD40-expressing stromal cells that in turn promoted the proliferation of the lymphomatous clone through the production of B-cell trophic factors. Whether MCs have deleterious or protective effects in DLBCL is still debated, as MC infiltration in human tumors has been associated both with

favorable and unfavorable clinical outcomes. We used the murine A20 cell line that forms lymphomas *in vivo* in congenic BALB/c mice, expresses SPARC and mimics human DLBCL. To favor BM and spleen engraftment of A20 cells despite liver infiltration, mice where subjected to a sublethal irradiation prior to receiving A20 cells. Toluidine blue staining allowed identification of MCs infiltrating the spleen and BM of sublethally irradiated A20 cell-injected mice, which were very often degranulated and localized both peri- and intra-tumorally. Moreover, treatment of inoculated mice with cromolyn, a MC stabilizer, inhibited A20 cell infiltration of spleen and BM, corroborating the hypothesis of a possible role of MCs in favoring lymphoma establishment in those organs.

LincRNAs in mutant p53 Gain of Function phenotype

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Introduction: Somatic mutations of TP53 gene occur in more than 50% of all human cancers (1) and represent the most frequent genetic alterations in breast cancer (BC) (30% of all BC, 88% of basal-like BC) (2) and in high grade serous ovarian carcinoma (HGSOC) (~99% of cases) (3). TP53 somatic mutations are predominantly missense mutations that produce a transcriptionally deficient protein with loss of wild type (wt) p53 tumor suppressor activity; nevertheless, mutant p53 (mut_p53) is usually overexpressed in tumors with acquisition of pro-tumorigenic functions not displayed by the wild type (wt) form. Indeed, tumor cells that carry mut_p53 are more likely to invade, metastasize (4, 5) and be chemoresistant (6), with consequences for prognosis and response to therapy. However, the molecular mechanism that underlies mut_p53 gain of function is still an open chapter. Long intergenic non-coding RNAs (lincRNAs) participate in different molecular processes (e.g. epigenetic modification, protein folding and docking) and regulate several cellular functions (e.g. cell cycle, apoptosis, and migration). Interestingly, it has been shown that wt-p53 regulates the expression of several lincRNAs (e.g. lincRNA-p21 contributes to p53-dependent repressive transcriptional response) (7, 8). Yet, the role of lincRNAs in mut_p53 phenotype has not been explored so far.
Aim: To better understand the mechanism of mut_p53 oncogenic functions we propose to identify lincRNAs tied to mut_p53 in BC and HGSOC aggressive behavior.
Strategy: We either overexpressed mut_p53 in null cancer cells or silenced mut_p53 in cancer cells that carry mut_p53 and used these models for: 1) *in vitro* assays to evaluate cancer cells aggressiveness and invasiveness and 2) a qRT-PCR screening to evaluate the expression of selected lincRNAs, previously identified by an *in silico* approach.
Results: We used H1299 cells overexpressing mut_p53 (R273H) to perform a 3D-matrigel colony assay and observed that mut_p53 confers greater growth and invasive behavior to H1299.
We also used an HGSOC cell line (COV318) carrying mut_p53 (I195F) silenced (shRNA) or not for the endogenous protein to perform the mesothelial clearance assay (9) and observed that mut_p53 was associated with a higher clearance capability compared to cells silenced for mut_p53.
These results confirm that, in the cell models and assays used, mut_p53 is able to increase cell aggressiveness and invasiveness.
An *in silico* analysis (based on the mut_p53 gene expression signature found in ref. 5 intersected with lincRNAs genomic location) allowed us to identify 18 lincRNAs as putative targets of mut_p53. We tested, by qRT-PCR, the expression of the selected 18 lincRNAs in H1299 mut_p53 cells and in COV318 silenced for mut_p53 and found that in H1299 cells, overexpression of mut_p53 is associated with decreased levels of three of the 18 lincRNAs (AK092541, AK055332 and AI438961.1); instead, in COV318 mut_p53 correlates with higher levels of three different lincRNAs (MIAT, BC087857, RP11-517P14.2) that are reduced upon p53 silencing.
Conclusion and future steps: Expression of mut_p53, in the two *in vitro* models we have established, is associated with a greater aggressive and invasive behavior and with altered expression of six lincRNAs.

Now, we plan to investigate mut_p53 effect on cell aggressiveness and invasiveness in BC cell models and in other HGSOC cell lines. Finally, we plan to focus on the six lincRNAs differentially expressed by mut_p53, to evaluate their role (e.g. by silencing approaches) in mut_p53 dependent cancer cell aggressive behavior and study their mechanism of action.

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Mito-Luc Zebrafish model to visualise proliferation events in whole animals

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Recently we have generated a transgenic reporter mouse, called MITO-Luc, in which the luciferase reporter gene are placed under the control of cyclinB2 minimal promoter containing 3 CCAAT boxes conserved between mouse and human and tightly regulated by the transcription factor NF-Y. In these mice, bioluminescence imaging of NF-Y activity visualizes areas of physiological cell proliferation and regeneration during response to injury. Now we are generating a similar Zebrafish model which allows us to visualize through bio luminescence imaging (BLI) any proliferation events in the context of the entire alive animal during development and adult life. In this transgenic line, luciferase and GFP reporter genes are placed under control of the same proliferation dependent promoter that we have already employed in mice. Since 2 of these 3 CCAAT boxes are conserved in Zebrafish and the 3 NF-Y subunits are highly conserved between mouse, human and Zebrafish, mouse cyclinB2 minimal promoter should be sensitive in Zebrafish, too.
Moreover, our results in mice demonstrate that the expression of luciferase is fully detectable in the whole animal during development and in adult animals strongly suggesting that its expression could be detectable during Zebrafish life, too. In these Zebrafish model we have observed an ubiquitous GFP and bioluminescence signal in early living embryos while they become tissue specific at 33hpf embryos and in juveniles and adult zebrafish animals. Bioluminescence signal has been confirmed by luciferase assay in vitro. Moreover, we have confirmed the luciferase and GFP protein expression in proliferative tissues by immuno-fluorescence. To understand if the luciferase activity does occur in proliferating cells we have tested the effect of well-known antiproliferative drugs and treatments, and we have observed that the luciferase activity was inhibited by 5FU and X-Ray on zebrafish embryos. Finally we have analyzed the bioluminescence signal in alive adult Zebrafish after fin clip. We have observed an early systemic proliferation signal in the whole animal, and later a signal focused on the tail regenerating. The use of BLI on our zebrafish model as read out for proliferation events would speed up in the future the evaluation of anti- or pro-proliferative drug candidates.

Transposon based forward genetic screening in the study of colorectal cancer metastasis

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Colorectal cancer (CRC) is the second most lethal cancer, being metastasis the main cause of CRC death. According to a current hypothesis, metastasis requires plasticity of tumor cells that switch from an epithelial to a mesenchymal state (EMT)(1). Understanding the genetic elements that orchestrate EMT and metastasis is crucial to investigate novel treatments and to identify patients at higher risk to develop metastasis. Based on these premises, we aimed to identify novel genetic elements, mainly focusing on transcribed non-coding regions, that orchestrate tumor plasticity during CRC metastasis. To do so, we have combined a novel in vitro assay, that we named forced Single Cell Suspension Assay (fSCS), able to select cells with EMT/stem-cell traits and a transposon (TN) based mutagenic tool that only acts by insertional mutagenesis. We found that TN insertion generated in HCT116 cells, a colorectal cancer cell line with epithelial features, a cell clone, thereafter TN4-20, that is resistant to fSCS and has EMT traits (higher invasiveness, reduction of epithelial markers and increase of mesenchymal markers). By linker mediated PCR, we retrieved TN insertions from TN4-20 genomic DNA, and we focused on the TN insertion in the 3'UTR of BTBD7, a gene known to regulate salivary gland branching by EMT induction(2) and hepatocellular carcinoma invasiveness and metastasis(3). Interestingly, this TN insertion locates within the predicted target site of miR-23a/b, and miR-23b is a known anti-metastatic microRNA(4). We hypothesized that the TN insertion impairs miR-23b/BTBD7 interaction and that this interaction is important for TN4-20 fSCS resistance and EMT phenotype. Indeed TN4-20 cells show increased BTBD7 protein expression levels, and miR-23b over-expression in HCT116 induces a down-regulation of BTBD7 protein levels. In addition, we observed that miR-23b over-expression prevented fSCS survival in HCT116 but not in TN4-20 cells. Finally, by silencing BTBD7 expression, we observed a reduced fSCS survival, indicating BTBD7 importance in this phenotype. To further confirm our findings, we are currently using CRISPeR and Homologous Recombination techonology to obtain a phenocopy of fSCS resistance by targeted insertion of the transposon inside BTBD7 3'UTR. To understand the clinical significance of this interaction, we also will evaluate the expression of BTBD7 and miR-23b in CRC primary tumor specimens from patients with and without metastasis and in metastatic tissues.

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Combination of gefitinib and pemetrexed in preventing the acquisition of gefitinib-resistance in NSCLC cell lines with activating mutation of EGFR

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Introduction: EGFR tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, are clinically effective in treating non-small cell lung cancer (NSCLC) patients harboring EGFR activating mutations. Unfortunately, within 10 to 14 months from the beginning of therapy de novo resistance to TKIs is observed in the majority part of patients who initially have responded to therapy. Recent findings suggest that combined treatment with chemotherapy and EGFR-TKI offers advantages in term of inhibition of tumor progression when compared to chemotherapy alone or EGFR-TKI monotherapy. The NEJ005/TCOG0902 clinical trial presented at the ESMO meeting last year, indicate that the combination of gefitinib and chemotherapy in the EGFR-mutated setting has promising efficacy with predictable toxicities both with concurrent or sequential treatment, although concurrent regimen might provide better overall survival, and a randomized phase III study (NEJ009) comparing gefitinib alone versus gefitinib plus concurrent chemotherapy is ongoing. Considering these data, we hypothesize that EGFR-mutated patients could benefit of a combined treatment with gefitinib and pemetrexed in preventing or, at least, retarding the acquisition of EGFR-TKI resistance.
Methods: effects of the combined treatment with gefitinib and pemetrexed were evaluated on human HCC827 and PC9 gefitinib-sensitive EGFR exon 19 mutant NSCLC cell lines. G and P were administered following different schedules: gefitinib monotherapy (G); gefitinib intercalated with the combined therapy (G/G+P); gefitinib alternated with pemetrexed (G/P); gefitinib plus pemetrexed intercalated with gefitinib alone (G+P/G). Cell proliferation, cell death, colony formation and signaling transduction pathways have been evaluated.
Results: the concomitant treatment with gefitinib and pemetrexed produced an additive effect on the inhibition of cell proliferation and on the induction of apoptosis compared to gefitinib alone. Despite drug combination was not more effective in reducing EGFR activation and its downstream mediators such as p-ERK1/2 and p-AKT in respect to gefitinib alone, drug combination enhanced death signaling. The continuous treatment with gefitinib (G) generates gefitinib-resistant clones, both in HCC827 (epithelial mesenchymal transition) and PC9 cells (T790M). On the contrary the concomitant treatment with gefitinib and pemetrexed (G/G+P and G+P/G) intercalated every week with gefitinib alone prevented or retarded the acquisition of resistance towards gefitinib. The alternated schedule G/P, even though did not prevent drug resistance, resulted in a pronounced growth inhibition of gefitinib-resistant cells compared to gefitinib alone.
Conclusions: Our data suggest that concurrent combined treatment with pemetrexed and gefitinib, either as initial therapy or after a gefitinib treatment, might be of value in the treatment of selected NSCLC patients harboring EGFR activating mutation, preventing the acquisition of gefitinib-resistance. In vivo experiments on PC9 xenografts treated with gefitinib and pemetrexed, mimicking the in vitro schedules, are ongoing.
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GSK3B and GLI3 play a role in activation of hedgehog-gli pathway in human colon cancer

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We provide strong evidence of Hh-Gli signaling involvement in survival of colon cancer cells, with the main trigger of activation being deregulated GSK3β. Our clinical data reveals high expression levels of GSK3β and Gli3 in human colon cancer tissue samples, with higher GSK3β expression correlating with higher DUKES' stages. Further experiments on colon cancer cell lines show that Gli3 is the main Hh-Gli pathway effector in colon cancer cells. GSK3β, a Gli3 regulator, has a pro-tumorigenic role in colon cancer, it upregulates Hh-Gli signaling and positively affects colon cancer cell survival. We show that inhibition of GSK3β with lithium chloride enhances Gli3 processing into its repressor form, consequently downregulating Hh-Gli signaling, reducing cell proliferation and inducing cell death. Analysis of the molecular mechanisms revealed that lithium chloride enhances Gli3-SuFu-GSK3β complex formation leading to more efficient Gli3 cleavage and Hh-Gli signaling downregulation. This work proposes that activation of the Hh-Gli signaling pathway

in colon cancer cells occurs noncanonically via deregulated GSK3ß. Gli3 seems to be the main pathway effector, highlighting the activator potential of this transcription factor, which is mainly considered a negative regulator of the pathway.

The role of Fbxw7 expression in hepatocellular carcinoma and adjacent non-tumor liver tissue

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Background and aim: Fbxw7 is a tumor suppressor gene through ubiquitination and degradation of multiple oncoproteins. Loss of Fbxw7 expression is frequently observed in various human cancers. In the present study, we examined the role of Fbxw7 expression in both non tumor liver tissues and tumor tissues on clinicopathological significance.
Methods: Sixty six patients with hepatocellular carcinoma (HCC), who underwent hepatectomy, were divided into two groups: high and low gene expression group, based on the Fbxw7 expression level. We compared the clinicopathological factors between the high expression and low expression groups in both tumor and non tumor tissues.
Results: Fbxw7 mRNA expression level in the non tumor tissues was significantly higher than that in the tumor tissues. In the analysis of Fbxw7 expression in tumor and non tumor tissues, disease free survival rate in the Fbxw7 high expression group was significantly higher than that in the low expression group. In multivariable analysis, Fbxw7 low expression in both tumor and non tumor tissue was detected as the strongest independent risk factor for HCC recurrence.

Annexin A2 is a key determinant of GBM aggressiveness by controlling cancer cell dissemination and proliferation

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Increasing evidence underlines the importance of imaging techniques (MRI and PET) in order to improve the extent of resection of Glioblastoma (GBM) tumours, which is considered an important prognostic factor in these patients. However, despite the recent introduction of 5-ALA fluorescent imaging into the surgical management of GBM, progression free survival of patients has been increased of only 6 months. Thus, the identification and possible targeting of the molecular mechanisms involved in GBM cell heterogeneity and dissemination (responsible for resection escape) is becoming a particularly relevant issue. In this context, Annexin A2 (lipocortin II, ANXA2,) is a phospholipid-binding protein expressed in a variety of cell types, including cancer cells, which serves as a co-receptor for plasminogen and tissue plasminogen activator (t-PA).
For this reason, ANXA2 expression has been associated to a more invasive and migratory phenotype in many tumours, including leukemia, breast, gastric, pancreatic, lung and prostate cancer, hepatoma and also brain tumours.
In this study, we report that ANXA2 is over-expressed in gliomas in different cohorts of patients (from our centre and from public databases) and demonstrated that ANXA2 expression could be considered an independent prognostic factor in GBM. Moreover, we show that ANXA2 targeting, by using neutralizing antibodies or gene silencing techniques, is sufficient to dramatically inhibit primary GBM cell migration and extracellular matrix invasion together with a massive redistribution of intracellular actin filaments. In addition, ANXA2 down-regulation is associated to a partial induction of GBM cell differentiation as shown by a significant reduction of the stem cell markers CD133 and Nestin and the up-regulation of the astrocytic marker GFAP. Finally, we generated an ANXA2-dependent gene signature suggesting, beyond cell migration and invasion, its involvement

also in the regulation of the cell cycle and proliferation, thus making ANXA2 a reasonable target for GBM therapy.
In conclusion, our data support the idea that ANXA2 is a fundamental mediator of GBM cell aggressiveness by controlling cell dissemination and, in part, cell cycle dynamics.

T lymphocyte infiltration in neuroblastoma is a prognostic marker of clinical outcome

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We present here new evidence on tumor-infiltrating T lymphocytes as prognostic indicators in neuroblastoma, a solid tumor of childhood arising from neural crest cells involved in development of sympathetic nervous system. We investigated the relationship between the type, density and organization of tumor infiltrating T cells within a cohort of 84 neuroblastoma samples, based on the quantitative in situ immunohistochemical analysis of $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes, also differentiating for infiltration in tumor cell nests or surrounding fibrovascular septa. The dataset included patients from International Neuroblastoma Staging System (INSS) stages 1,2,3,4,4S (n = 34, 19, 5, 20, and 6, respectively) with up to 12 years of follow up after primary tumor resection. Available covariates included MHC class 1 profiling, MYCN amplification, and age at diagnosis. Brightfield microscopy images were first manually evaluated by experts to quantify T lymphocyte cell density; slides were additionally screened by automatic imaging analysis to extract other immunological spatial features, including proximity to proliferating cancer cells marked by Ki67. Neuroblastoma cells from tumors with favourable prognosis expressed high levels of MHC class I molecules. A significant association was detected between the density of infiltrating $CD3^+$ T cells and the levels of MHC class I molecules on tumor cells (linear regression P value: $3.5e^{-04}$), suggesting a possible interaction between infiltrating T cells and tumor cells. Moreover, T cell density was consistently higher in patients with good prognosis according to the Children's Oncology Group (COG) and the INSS stratification criteria. Survival analysis was performed by stratifying the patients according to the median cut-off value of T cell density. In general, we found that a higher density of T cell subtypes was significantly associated with a higher overall and relapse-free survival.
To determine the predictive value of T cell immunological features, we built a COG risk group predictor based on a logistic regression model using T cell species density as features. Feature selection based on cross-validation approach (CD3-nest, CD3-septa, CD8-nest, CD8-septa and CD4-septa). The model predicts the COG risk group with performance comparable to the predictors based only on MYCN amplification status (Area Under the Curve - AUC: 0.84 ± 0.07 vs 0.77 ± 0.08). As T cell density was not significantly associated to MYCN-amplification status and age at diagnosis (all P values > 0.09), the strongest markers of poor outcome in neuroblastoma, we also built an integrated predictor (AUC: 0.91 ± 0.07). These results show that 1) T cell density is a significant and independent predictor of clinical outcome of neuroblastoma, and 2) can usefully complement established prognostic markers. As the coordinated interaction of different T cell subsets is essential for promoting and maintaining an active and efficient adaptive immunity, we evaluated the degree of concurrent infiltration of $CD3^+$, $CD4^+$ and $CD8^+$ T cells, considering the *in-situ* structural organization and concurrent infiltration of T cell subsets in tumors with different outcome. Differential correlation network analysis revealed strong differences of co-infiltration patterns: low-risk neuroblastomas were characterized by a higher number of proliferating T cells and a more structured T cell organization, which was gradually lost in tumors with poor prognosis. Finally, an immunoscore based on $CD3^+$, $CD4^+$ and $CD8^+$ T cell densities was shown to associate with

favourable clinical outcome in MYCN-amplified tumors, improving stratification of patient prognosis (Cox regression P value: $1.54e^{-05}$). Our findings clearly establish a scenario where infiltrating T cells modulates progression of neuroblastoma, possibly offering new indicators of clinical interest.

Exploring m6A mRNA methylation for novel therapeutic chances in neuroblastoma

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Neuroblastoma is a tumor of the developing sympathoadrenal lineage of the neural crest that accounts for over 50% of all pediatric cancers and poses important challenges to establish more effective and less toxic therapies. In fact, despite industry is hardly engaged to develop new specific drugs and different clinical trials are underway, intensive doses of chemotherapy and radiotherapy are still the main option for neuroblastoma. The reason is predominantly due to the elusive nature of the tumor, the paucity of targetable driving mutations and a substantial lack of deep understanding of the molecular basis. Saying that, the embryonic derivation of neuroblastoma progenitor cells may account for a marked involvement of post-transcriptional control of gene expression in the phenotypic derangement of this tumor to maintain the undifferentiated state and promote rapid progression to oncogenesis. The importance of mRNA methylation has remained a mystery for decades, until rapid advances in the field of epitranscriptomics and RNA sequencing have raised the exciting hypothesis that reversible N6-methyl-adenosine (m6A) modification of mRNA may constitute an essential mechanism of post-transcriptional regulation. Currently, the function of mRNA methylation, the signaling pathways that control mRNA methylation, and methylated-mRNA binding proteins are still in the route of being uncovered, but m6A-mediated gene expression control has already proved to be an indispensable process guiding self-renewal and differentiation of embryonic stem cells.
We crossed the data of three major recent studies, which defined the mRNA-methylation landscape of the human transcriptome, with a dataset of more than 700 gene-expression profiles of neuroblastoma clinical samples, and we identified potential targets of methylase enzymes that could be associated with tumor progression and clinical outcome of neuroblastoma. We also found that increased levels of the METTL14 methyltransferase are associated with worse clinical features and poor prognoses, while levels of demethylases showed an opposite tendency. Further data will be required to substantiate the hypothesis that modulation of mRNA methylation might have direct impact on gene expression of methylase/demethylases target genes during oncogenic progression. The possibility of reversing m6A modifications through enzymatic inhibitors may then enable new insights for therapeutic intervention. We are on the way of investigating the cellular function of METTL14-dependent m6A in neuroblastoma cell models and during mouse embryogenesis.

Identification of a small molecule disrupting HuR:RNA Complex formation

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Post-transcriptional regulation is an essential determinant of gene expression programs in physiological and pathological conditions. HuR is a RNA-binding protein that coordinates the stabilization and translation of mRNAs critical in inflammation and tumor progression, including tumor necrosis factor-alpha (TNF). The low molecular weight compound D1 was identified through a validated high throughput screening on a set of anti-inflammatory agents for its ability to prevent HuR:RNA complex formation. We found that D1 interferes with the association step between HuR and the RNA with an equilibrium dissociation constant in

the nanomolar range in vitro (Ki=3.74±1.63 nM). While looking into the individual RRMs of the HuR, we found that main motifs responsible for binding to target RNA is RRM1 and RRM2(Kd= 2.5 nM) and our compound was able to halt the Protein RNA complex Binding (Ki=3.74±1.63 nM). The compound also lowered, TNF expression (HuR Target) in MDA-231 and SKBR3 breast cancer cell lines. Moreover HuD-RNA complex formation was affected by D1 at the reference doses, as expected considering the 78% structural similarity with HuR. Conversely, the binding of Lin28b, TTP, and TDP-43 to RNA did not appear substantially affected.

MicroRNA-146a exerts a dual role in melanoma controlling the Notch-Akt pathway and modulating metastasis-related molecules

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MicroRNAs (miRs) are small non-coding RNAs able to post-transcriptionally downregulate the expression of target mRNAs, acting as fundamental regulators of a variety of biological processes, including tumour establishment and progression. As malignant melanoma is the most aggressive form of skin cancer, it is crucial to disclose its underlying molecular mechanisms. In this work, we found that miR-146a, whose expression correlates with cell aggressiveness, exerts a major pleiotropic role, enhancing *in vivo* primary tumour growth while impairing lung metastatization. On the other hand, sponge-based miR-146a ablation completely reverts these phenotypes. miR-146a-dependent increased cell and tumour growth and adhesion is achieved through direct targeting of two negative regulators of Notch, Numb and Lfng, and subsequent activation of the Notch pathway, triggering increased HES-1, cyclin D1 and N-cadherin expression, as well as Fak and Src phosphorylation. Consistently, miR-146a strongly induces AKT phosphorylation; this effect could be ether direct or indirect, since we found that miR-146a-overexpressing cells show reduced Pten mRNA and protein levels, an effect that could depend on HES-1 transcriptional effect on the Pten promoter. miR-146a's opposite effect on cell metastatization is more unclear; so far we validated ROCK1 as a promising candidate target gene, and we found that various movement and aggressiveness-related molecules, like ADAM19, TRAF6, IRAK1, UHRF1 and SMAD3, are downmodulated upon miR-146a overexpression in melanoma cells. These evidences will be further investigated soon, with the purpose to solve the molecular mechanisms underlying the dual role of miR-146a in melanoma and potentially make it a good target for therapy.

DNA vaccination against human CSPG4 for the treatment of malignant melanoma: a comparative oncology trial in dogs

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Background: Melanoma is the sixth most common cancer worldwide, although it is highly curable with surgery in its early stage, approximately one/third of melanoma patients will experience disease recurrence and metastasis that may prove often fatal.

Given the inherent immunogenicity and the potential role played by immunological events in melanoma natural history, the development of DNA-based-vaccines for the treatment of this disease represents an appealing therapeutic option, alone, or in combination with checkpoint inhibitors such as anti-CTLA4 monoclonal antibodies (mAb). Despite the successful studies in murine-models, therapeutic efficacy of DNA vaccines has been so far disappointing. More appropriate pre-clinical models and vaccination targets are thus urgently needed.
Due to the many similarities with its human counterpart, canine (c) malignant melanoma (MM) represents a valuable clinical model to assess the efficacy of novel therapeutic strategies. As well as, chondroitin sulfate proteoglycan-4 (CSPG4) represents an attractive target for immunotherapy because of its high expression in human (h) melanoma and cMM tumors and its restricted distribution in normal tissues. These considerations and the translational power of veterinary clinical trials have prompted us to evaluate the safety, immunogenicity and therapeutic efficacy of a hCSPG4 DNA-based vaccine in client-owned dogs affected by CSPG4-positive, spontaneous MM.
Methods: Dogs with surgically-resected stage II-III CSPG4-positive, spontaneous oral MM received intramuscular hCSPG4 plasmid administration followed by electroporation (electrovaccination) monthly. The safety and the clinical efficacy of the DNA vaccine, as well as the specific humoral and cellular response induced by vaccination were evaluated.
Results: hCSPG4 electrovaccination caused no relevant side effects and resulted in significantly longer survival time in vaccinated dogs as compared to non-vaccinated controls. Indeed, all vaccinated dogs developed Abs against hCSPG4 and, more importantly, cCSPG4. Seven vaccinated dogs were also tested for cCSPG4-specific T cell response and only two gave positive results. Our results indicate that xenogeneic vaccination is a safe and effective way to overcome host unresponsiveness to the self-antigen, resulting in significantly increased overall and disease-free survival, thanks to the induction of specific CSPG4-antibodies. Moreover, in order to investigate in this model the power of combinatorial strategies between specific DNA-vaccines and immune checkpoint blockade, CTLA-4 expression on cMM PBMC and its functional recognition by mAb directed against hCTLA-4 (Ipilimumab) has been evaluated.
Conclusions: The validation of an effective cancer vaccine strategy in client-owned dogs with naturally occurring MM, recognized as a priceless model for predicting tumor behavior and response to immunotherapy in humans, will be of impact for the scientific community and lays the foundation to speed up the translation of this management modality to human melanoma patients, hopefully contributing to improve their survival.

Identification of a New Subclass of ALK Negative Anaplastic Large Cell Lymphoma Expressing Aberrant Levels of ERBB4 Transcripts

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Anaplastic Large Cell Lymphoma (ALCL) is a clinical and biological heterogeneous

disease including the ALK+ and ALK- systemic forms. While ALK+ ALCL are molecularly characterized and can be readily diagnosed, no specific markers and molecular events leading to ALK- ALCL transformation have been identified so far.
To discover biomarkers and/or genes potentially involved in ALK- ALCL pathogenesis, we applied the Cancer Outlier Profile Analysis (COPA) algorithm to a large gene expression profiling (GEP) data set including 249 cases of T-NHLs and normal T-cells. Among the top outliers, *ERBB4* and *COL29A1*genes were exclusively expressed in a subset of ALK- ALCL cases, and resulted highly correlated. Differential analysis comparing the expression profiles of ERBB4/COL29A1+ to ALK+ ALCL patients identified 48 genes up- and 37 down-regulated (qval < 0.0076). The most significantly over-represented functional category included transcriptional regulators. Gene-set-enrichment-analysis (GSEA) indicated that ERBB4/COL29A1+ samples shared activation of specific pathways such as inflammatory response, TNF-NF-κB, JAK-STAT, cytokine, and angiogenesis. RNA sequencing and 5'RNA Ligase Mediated Rapid Amplification of cDNA Ends (RLM-RACE) identified two ERBB4 truncated transcripts (referred as I20ΔERBB4 and I12ΔERBB4), displaying Transcription Starting Sites (TSS) in intron 12 and 20, respectively. On the contrary, full length COL29A1 mRNA was expressed in ERBB4/COL29A1+ samples. RT-qPCR analysis performed on 170 T-NHL samples (51 PCTL-NOS, 44 ALK+ ALCL, and 75 ALK- ALCL) highlighted specific expression of ERBB4 aberrant transcripts in 25% of ALK- ALCL (18 out of 75). ERBB4+ patients showed higher expression of I20ΔERBB4 as compared to I12ΔERBB4 transcript. ERBB4 expression at protein level was confirmed by immunohistochemistry and Western Blotting on selected cases. Inspection of intronic regions spanning two hypothetical TSS revealed the presence of Human Endogenous Retrovirus (HERV) Long Terminal Repeats (LTR) displaying promoter activity, as demonstrated by luciferase assays.
In conclusion, we defined a new subclass of ALK- ALCL characterized by ectopic expression of ERBB4 and COL29A1 genes. To the best of our knowledge, ERBB4 truncated transcripts carrying intronic 5'UTR have never been described before. Further studies are required to address whether the expression of ERBB4 aberrant transcripts may contribute to the ALCL transformation and lymphoma maintenance. This information might lead to more rational therapeutic approaches for ERBB4+ ALCL patients.

A HGF-induced multistep model of rabdomyosarcoma based on by passing stem cell quiescience, recapitulates the heterogeneity of the human erms subtype

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Embryonal Rhabdomyosarcoma (ERMS) is a rare pediatric cancer genetically heterogeneous, which still has a dismal prognosis when metastatic. Here we describe a fully penetrant multistep model of ERMS, based on inducible release of Hepatocyte Growth Factor (HGF) from mature skeletal fibers. In this system the satellite cells niche is enriched selectively for HGF, while additional cell-intrinsic

mutational events are left to chance. In an *Ink4a/Arf* null background stimulation of Met signaling caused widespread activation and proliferation of satellite cells that initially differentiated into new centro-nucleated myofibers intermingled with old ones, mimicking regeneration without a wound. At later stages, in a framework of progression, satellite cells first lost the ability to differentiate and finally formed full blown multifocal ERMS. We provide genetic evidence for satellite cells being the cell of origin of the tumors by showing a drastic reduction of tumor incidence in the *Pax7* null genetic background. Murine ERMS showed intertumoral and intratumoral heterogeneity, mimicking human ERMS. Some of the tumor-derived cell lines carried *c-met* or *Alk* amplification and were sensitive to Met and ALK inhibitors, other responded selectively to inhibitors of the PI3K pathway. Bioinformatic analysis of two large datasets of human RMS links the Met signature to the ERMS subtype, suggesting that HGF-induced activation of satellite cells may also trigger initiation of human ERMS. Overall our work emphasizes the emerging concept that quiescence is a powerful barrier to tumor development and highlights the role of the HGF/Met axis and of the muscle stem cell in ERMS initiation. Finally, our model represents a robust pre-clinical platform to deconstruct the complexity of ERMS, a pre-requisite for a precision-guided therapeutic approach.

Down-regulation of YAP expression reduces clonogenicity of pancreatic ductal adenocarcinoma.

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Hippo signaling pathway has emerged as a key regulator of organ size, cell proliferation and apoptosis. The yes-associated protein, YAP, is a transcriptional co-activator of the mammalian Hippo pathway acting *via* binding to the TEAD transcription factor. The Hippo pathway was found to mediate contact inhibition of growth through the regulation of E-cadherin. However, the role of YAP regulating E-cadherin mediated growth inhibition in pancreatic cancer is not well understood. In this study, we demonstrated the YAP functionality by knockdown of YAP. Loss of YAP significantly suppressed CTGF and CYR61 mRNA expression, which are main target genes of Hippo pathway. On the contrary, overexpression of YAP reversed the expression of those Hippo target genes. Interestingly, the anchorage-independent growth in soft agar was diminished in YAP silented cells through the activation of E-cadherin, suggesting a role of YAP associated with E-cadherin mediated growth inhibition. The clonogenic activity of PDAC was strongly correlated with the expression of endogenous E-cadherin level which explains the phenotypic effects we observed. Moreover, we also identified a compound that can specifically modulate YAP functionality: Protein kinase C inhibitor, Bisindolylmaleimide (GF 109203X). Mechanistically, GF 109203X activated YAP dependent TEA-reporter and B-catenin driven TCF/LEF reporter activity. However, it strongly suppressed CTGF mRNA expression and inhibited anchorage-independent cell growth in soft agar. Therefore, these findings indicate the potential role of YAP through E-cadherin-mediated anchorage-independent growth in PDAC.

HIPK2 deficency promotes cytokinesis failure and tumorigenicity: focusing on pancreatic adenocarcinoma

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HIPK2 is a multitalented kinase with oncosoppressive fuction. In addition to its role as apoptotic activator, we have demonstrated that HIPK2 is involved in abscission, the last step of cytokinesis and this function is relevant for cell ploidy preservation. Indeed, cytokinesis failure due to HIPK2 deficiency causes tetraploidization and chromosomal instability in murine embryo fibroblasts (MEF) leading to high tumorigenicity when MEF are transformed with E1A and ha-Ras. Tetraploidization and chromosomal instability are common features in several human tumors and pancreatic cancers are characterized by cytokinesis failure event in early steps of carcinogenesis. Performing a HIPK2 immunoistochemical analysis on tissue microarray of human pancreas normal and tumor samples, we have observed a strong inverse correlation between HIPK2 protein levels on one side and grade of malignancies and DNA cell content in pancreatic adenocarcinoma (PDA) progression on the oppose side. To further investigate the HIPK2 role in PDA, we will evaluate the impact of the homozygous and heterozygous deletion of hipk2 in a well-characterized mouse model of PDA (i.e., LSL-KRasG12D; pdx1-CRE) mice. Notably, we will use MITO-luc animals engineered to express the luciferase reporter gene in cells undergoing active proliferation by means of NF-Y, a transcription factor master regulator of proliferation. Thus, we will have the opportunity to follow PDA evolution in the entire living animal in a time frame process. Moreover, to better understand the molecular implication of HIPK2 in pancreatic tumorigenesis, we will set up a pancreatic organoid model. This will provide an in vitro tool to experimentally address whether aneuploidy and chromosomal instability are critical in PDA development.

Cell fate choices: when p53-directed translational control leads to apoptosis

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The p53 tumor suppressor gene still captures the scientific interest due to its involvement in a plethora of processes, including the ability to induce cell death. Considering p53 as a therapeutic target, studies have led to small molecules p53 activators, with the MDM2 inhibitor Nutlin-3A being a prototypical one. Nevertheless, clinical applications remain challenging mostly because Nutlin-3A treatment can result in phenotypes ranging from overt apoptosis to selective cell cycle arrest, which is a much less desirable outcome.
What is dictating differences in cellular responses to Nutlin-3A and how will it be possible to shift the balance towards the commitment to apoptosis? To address this issue, we employed three cell lines (HCT116, SJSA1 and MCF7) which are p53 wild type and exemplify the different responses to Nutlin-3A: cell cycle arrest, apoptosis, or both, respectively. Next to classical transcriptomic analysis, we examined the translatome by means of polysomal profiling which allows us to separate actively translated mRNAs, bound by polysomes, from those that are not actively translated.
Our RNA-seq data strongly support the hypothesis that p53-dependent transcriptional control by itself cannot fully explain the different cellular responses, in that expression of both cell cycle and apoptotic p53 target genes is equally induced after Nutlin-3A treatment in the three cell lines. On the contrary, we demonstrate translational selectivity to shape p53-dependent cell outcomes. Indeed, only in Nutlin-3A treated SJSA1 cells, several translationally enhanced mRNAs are enriched for apoptotic functions.
Examining what determines their translation, we found that these mRNAs carry a specific motif in their 3'UTR, which is sufficient to stimulate translation in a reporter gene assay upon Nutlin-3A treatment in SJSA1, but not HCT116 or MCF7 cells.
Ongoing experiments suggests that cell specific trans-factor(s) binding the identified motif can actively influence the translation of apoptotic mRNAs, thus contributing to establish a full commitment to programmed cell death upon Nutlin-3A treatment.

Identification of a YAP/TAZ/TEAD transcriptional program driving oncogenic growth

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YAP and TAZ are nuclear effectors of the Hippo pathway and key players in organ growth and tumorigenesis. Yet, their working as transcriptional regulators remains underinvestigated. By ChIP-seq analyses in breast cancer cells (MDA-MB-231 cells), we discovered that YAP/TAZ - together with their DNA-binding partner TEAD - mainly drive transcription by binding to enhancers (defined as H3K4me3+ chromatin regions). Considering that enhancers contact target promoters via chromatin looping, we used a new approach to identify the target genes controlled by YAP/TAZ/TEAD: we exploited a high-resolution map of chromatin interactions in human cells to identify the promoters that physically interact with YAP/TAZ/TEAD binding sites located on enhancers. By merging ChIP-seq and gene expression data, we identified >350 genes directly activated by YAP/TAZ/TEAD in breast cancer cells. This list comprises a complex repertoire of genes devoted to the control of cell proliferation, a critical process in most YAP/TAZ-dependent biological responses. The YAP/TAZ/TEAD cell proliferation program that we identified involves proteins playing pivotal roles in several specific steps of the cell cycle, such as the assembly of the licensing complex, DNA replication and repair, chromosome segregation and cytokinesis. The program also includes transcription factors potentially able to amplify the effects of YAP/TAZ. Overall, this work massively extends the previous knowledge on the transcriptional regulation of proliferation by YAP/TAZ in cancer cells.

Call for 2016 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 a group of 11 physicians and scientists interested in research, "to further the investigation and spread the knowledge of cancer." Today, the AACR accelerates progress toward the prevention and cure of cancer by promoting research, education, communication, and collaboration

The mission of the American Association for Cancer Research is to prevent and cure cancer through research, education, communication, and collaboration. Through its programs and services, the AACR fosters research in cancer and related biomedical science; accelerates the dissemination of new research findings among scientists and others dedicated to the conquest of cancer; promotes science education and training; and advances the understanding of cancer etiology, prevention, diagnosis, and treatment throughout the world.

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 16-20, 2016) in New Orleans, Louisiana 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 13, 2016). The award consists of a prize of € 75.000 and a commemorative plaque.

Nomination Deadline: August 12, 2015
Questions about the nomination process:
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