The Pezcoller Foundation

Journal

Summary

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Editorial

It's a great pleasure to report that the recipient of the 2017 Pezcoller Foundation-AACR International Award for Cancer Research is David Livingston M.D., Emil Frei Professor of Medicine, Harvard Medical School, Boston, MA and Professor of Genetics, Deputy Director Dana-Farber Cancer Institute, current Chairman of the Pezcoller Symposia.

The Selection Committee met in Philadelphia on December 5th 2016 and was chaired by Jedd D. Wolchok, MD, PhD of the Memorial Sloan Kettering Cancer Institute of New York. The other members of the Committee were: Donatella del Bufalo, PhD, Senior Researcher Regina Elena National Cancer Institute Rome, Italy; Manel Esteller, MD, PhD, Laboratory Director, Cancer Epigenetics Group Institute d'Investigació Biomèdica de Bellvitge (IDIBELL), Barcelona, Spain; Judy Garber, MD, MPH, FAACR, Director Center for Cancer Genetics and Prevention Dana-Farber Cancer Institute Boston, MA; Mien-Chie Hung, PhD, VP for Basic Research, Professor and Chair Department of Molecular & Cell Oncology, UT MD Anderson Cancer Center Houston, TX; Karen Knudsen, PhD Director Thomas Jefferson University Sidney, Kimmel Cancer Center, Philadelphia, PA; Daniel S. Peeper, PhD, Professor of Functional Oncogenomics Netherlands Cancer Institute, Amsterdam, Netherlands; Stefan M. Pfister, MD Professor Division of Pediatric Neurooncology, German Cancer Research Center, Heidelberg, Germany.

Dr. Livingston's work has been pivotal to our understanding of the retinoblastoma pathway of cell cycle control as well as the transcriptional co-activation function of the key regulatory proteins, p300 and CBP. Furthermore, he is credited with the landmark discovery of the central functions of the tumor suppressors, BRCA1 and BRCA2, which has revolutionized the fields of breast and ovarian cancer research. Dr. Livingston is lauded by his colleagues as the founder of a wide range of scientific fields, far beyond the realm of cancer research.
Dr. Livingston's tremendous reputation as a respected and admired scientist, colleague, and mentor is evident through his continued impact on the scientific endeavor.
Dr. Livingston has presented the Pezcoller Lecture, "Brca1, Other Fanconi-Gene, And Swi/Snf Function Co-Sustain-Mammary Epithelial Differentiation Through Dna Damage Repair" at the Washington Convention Center during the AACR Annual Meeting.
Dr. Livingston has presented the Pezcoller Foundation-AACR International Award for Cancer Research Lecture at the University of Padova, hosted by the Department of Molecular Medicine directed by Prof. Stefano Piccolo.
He also gave the Pezcoller Foundation-AACR International Award for Cancer Research Lecture at the University of Trento, at the Centre for Integrative Biology directed by Prof. Alessandro Quattrone.
Afterwards the award was solemnly given in the prestigious hall of the Buonconsiglio Castle in Trento on May 5. In this occasion David Livingston was introduced by the President of the Pezcoller Foundation Dr. Enzo Galligioni. The ceremony was also attended by dr. Nancy Davidson, past President of the AACR.

Gios Bernardi M.D.
Editor and President Emeritus

Picture on front page:
2017 Pezcoller Foundation-AACR International Award for Cancer Research in the Main Hall of the Buonconsiglio Castle, Trento
From the left: Enzo Galligioni , President - David Livingston, winner - Nancy Davidson, AACR

In memory of Enrico Mihich by Gios Bernardi

Enrico Mihich MD, true friend and fundamental support for Pezcoller Foundation, died December 29, 2016 at the age of 88, in Boston MA.
Mihich was born in Fiume (Rijeka) in 1928. He graduated from the University of Milan in 1951 and then moved to the Sloan Kettering Cancer Center, in New York City, in 1952.
He joined Roswell Park Cancer Institute in 1957 and from 1969 to 2006 he was Chair of the Molecular Pharmacology and Cancer Therapy Program at the Buffalo University.
Starting from 1984, he served six years as a member of the NIC, National Institute of Cancer.
Dr. Mihich retired from Roswell Park in 2007, moved to Brookline, MA, and joined the Dana Farber Cancer Institute as a Presidential Scholar, serving as a special assistant to the president for sponsored research.
He published more than 400 scientific papers and worked in the editorial boards of prestigious peer-reviewed journals.
He was a true friend and partner of the Pezcoller Foundation for almost thirty years. Together with the Foundation's presidency, he was the architect of the fundamental agreement between Pezcoller and AACR to establish the Pezcoller Foundation-AACR International Award for Cancer Research in 1997, a partnership that continues today. He was the inventor and initiator of the Pezcoller Symposia, and served as chairman of the Pezcoller Symposium Committee until 2008 and since then together with David Livingston. Every year he took part also in the Symposia Standing Committees.
Despite the more than evident difficulties, he continued to participate in all our symposia, while this year, on the occasion of the 29th Symposium, he will not be with us and we will surely miss him.
The Board of Pezcoller Foundation, with its President Galligioni and myself, would like to extend our heartfelt condolences to his wife Marisa, his daughter Sylvia and his granddaughters.

Picture: dr. Enrico Mihich, 2010

29th Pezcoller Symposium

Building New Bridges Between Basic and Cancer Science

Trento, Italy, June 22-23, 2017

FOCUS AND GOAL

This issue of our Journal is dedicated to the 29th Pezcoller Symposium entitled "Building New Bridges Between Basic and Cancer Science" to be held in Trento from June 22 to June 23, co-chaired by David Livingston, Alberto Bardelli, Massimo Loda and Stefano Piccolo.

This Symposium will focus on a growing set of observations in the cancer field and their implications for the future. The past decade has witnessed the development and application of groundbreaking technologies that have improved cancer diagnosis, prognosis making, and therapy. As a result, the outlook for cancer patients has brightened. Chief among these new approaches are a strong and productive thrust towards the discovery and development of powerful targeted agents for cancer therapy; the discovery and development of novel, immunologically-directed therapies that have led to extraordinarily positive therapeutic effects in certain cancer patients; and the growing value of deciphering the sequences of cancer genomes and its contribution to the design of more precise and, hence, effective therapy for individual patients. These major advances, notwithstanding, cure still remains elusive, especially for advanced and particularly aggressive cancers. One major reason is that the basic scientific forces that drive cancer development, establish its aggressive and sometimes lethal nature, and wall it off from immunological killing remain incompletely or poorly understood. Thus, without the growth of new, basic cancer science knowledge, frequent cures of most advanced or aggressive cancers will be difficult to achieve. Hence, this Symposium aims to define the limits of our understanding of the basic biology of cancer cells and of the cells of the tumor microenvironment in which cancers arise and proliferate. It will also propose and describe new opportunities for moving beyond these limits. In essence, this meeting has been built upon the premise that ongoing progress in these endeavors will be needed to achieve ever better cancer therapy and prevention.

29th Pezcoller Symposium

BUILDING NEW BRIDGES BETWEEN BASIC AND CANCER SCIENCE

Trento, Italy • June 22 - 23, 2017

PROGRAM

THURSDAY JUNE 22, 2017

8.00 Registration

8.35 Enzo Galligioni Welcome

8.45 David Livingston Focus & Goals

08.55 **The Enrico Mihich Lecture**
Robert Weinberg
Mechanisms of Malignant Progression

Session I, part 1 The Roots of Cancer
Chair/Moderator: Pier Giuseppe Pelicci

09.40 Gerard Evan
Targeting the Engines of Cancer, not the Drivers
10.05 Discussion
10.20 Alan D'Andrea
PARP Inhibitor Resistance and Acquired Vulnerability in Ovarian Cancer
10.45 Discussion
11.00 Fabrizio D'Adda di Fagagna
DNA Damage Response in Cancer and the Role of non coding RNA
11.25 Discussion
11.40 *Coffee Break*
11:50 Pier Giuseppe Pelicci
Biological Heterogeneity of Tumors
12.15 Discussion
12.30 Richard Treisman
Probing the Transcriptional Consequences of Ras and Rho Activation
12.55 Discussion
13.10 *Lunch*

Session I, part 2 The Roots of Cancer (continues)
Chair/Moderator: Stefano Piccolo

14.10 Richard Gilbertson
Mapping Cancer Origins: a Window into Cancer Prevention
14.35 Discussion
14.50 Galit Lahav
Therapy guided by Protein Dynamics in Single Cells
15.15 Discussion
15.30 Karen Vousden
Metabolic Pathways and Cancer Development
15.55 Discussion
16.10 Andrea Califano
Elucidating and Targeting Mechanisms of Cancer Cell State Maintenance
16.35 Discussion
16.50 Poster Session
17.30 Adjourn

20.00 Symposium Dinner

FRIDAY, JUNE 23, 2017

Session I, part 3 The Roots of Cancer (continues)
Chair/Moderator: Stefano Piccolo

08.00 Gioacchino Natoli
Transcriptional Control of Differentiation in Cancer Cells
08.25 Discussion
08.40 Valerie Weaver
Tissue Tension Reprograms the Tumor
09.05 Discussion

Session II, Frontiers of Immunological and Therapeutics Science
Chair/Moderator: Alberto Bardelli

09.20 Robert Schreiber
Personalizing Cancer Immunotherapy
09.45 Discussion
10.00 Laurie Glimcher
Stressed out: A Novel Approach to Cancer Immunotherapy
10.25 Discussion
10.40 *Coffee Break*
10.50 Giulio Draetta
Targeting Critical Dependencies in Pancreatic Cancer
11.15 Discussion
11.30 Giovanni Parmigiani
Novel Approaches to Clinical Trial Design in Cancer
11.55 Discussion

Session III, Next Generation Questions
Chair/Moderator: Massimo Loda

12.10 Joe Gray
Exploring the Spatial Systems Biology of Cancer
12.35 Discussion
12.50 Maria Rescigno
Microbiota, Immune System and Cancer Development
13.15 Discussion
13.30 Lunch
14.30 Garry Nolan
A Defined "Structure" for the Immune System That Reflects Immune Surveillance & Mechanistic Processes
14.55 Discussion
15.10 Ruedi Abershold
The Modular Proteome and its Clinical Significance
15.50 Nico Thomä
BRCC36 Moonlighting between BRCA1 and Metabolism
16.15 Discussion
16.30 Poster Discussion
17.30 David Livingston
Concluding Remarks
17.40 Adjourn

INVITED PARTECIPANTS

- **Aebersold Ruedi**
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- **Weinberg Robert**
  Whitehead Institute/MIT, Cambridge, MA

29th Pezcoller Symposium

Building New Bridges Between Basic and Cancer Science

Trento, Italy, June 22-23, 2017

ABSTRACTS OF ORAL PRESENTATIONS

Malignant Progression, the Epithelial-Mesenchymal Transition and Cancer Stem Cells

Robert A. Weinberg

Whitehead Institute for Biomedical Research, MIT Department of Biology, Cambridge MA

Many of the phenotypes of carcinoma cells are organized by non-genetic (i.e., epigenetic) programs that operate independently of the mutant alleles that cancer cells acquire during the course of tumor progression. In the case of carcinoma cells, aggressive phenotype are often and perhaps invariably orchestrated by a cell-biological program termed the epithelial-mesenchymal transition (EMT), which imparts phenotypes such as invasiveness, motility, and an ability to disseminate to distant sites. In the case of mammary carcinoma cells, the EMT, at least in a mouse model of the disease, also imparts tumor-initiating traits, which are critical when a disseminated carcinoma cells undertakes to form metastatic colonies. These phenomena raise the question of the relationship between normal stem cells in the mammary gland and their neoplastic derivatives. As we have found, an EMT-inducing transcription factor — Slug — is critical to the physiology of normal mammary stem cells, while the derived tumors form tumor-initiating cells (i.e., also known as cancer stem cells, CSCs) that are driven by the paralog of Slug termed Snail. These cell-biological programs are critical to the clinical behavior of tumors, since the more mesenchymal carcinoma cells created by the EMT program, including the CSCs, show an elevated resistance to a variety of chemotherapeutic regimens. This provokes the question of how to attack these therapy-resistant cells. Agents that specifically target the CSCs can be identified. However, an alternative and potentially more effective therapeutic strategy is to induce CSCs to differentiate into more epithelial non-CSCs. Indeed, we have found that agents that induce an elevation in the intracellular concentrations of cyclic AMP will facilitate the exit of cells —both normal and neoplastic—from the more mesenchymal, CSC/SC state into the more epithelial non-SC state, which may render them more responsive to a variety of existing therapeutics. Observations like these illustrate the importance of non-genetic programs in governing the responsiveness of cancer cells to various types of therapy.

Targeting the Engines of Cancer, not the Drivers

Gerard I. Evan, Trevor Littlewood, Nicole Sodir, Roderik Kortlever and Luca Pellegrinet

Department of Biochemistry and Cambridge Cancer Centre, University of Cambridge, UK

Cancers are genetically complex, rogue somatic clones driven by mutations that disrupt the intra-- and extracellular networks that restrain untoward growth, proliferation, survival and invasion. Because of their pivotal importance in tissue ontogeny and homeostasis, such networks have evolved to be highly robust and functionally redundant, and are possessed of remarkable plasticity and capacity to self--organize and self--correct. While these properties confer great stability on tissue architecture and design, they also underlie the capacity of cells and tissues to adapt and evolve in response to pharmacological perturbation, so contributing to the high rate of relapse in patients treated

with targeted anti--cancer drugs. To circumvent this, our approach is to target critical non--redundant signaling nodes (engines) that act as the hubs for the diverse inputs of upstream oncogenic drivers, in effect serving as GO/NO--GO switches that decide whether oncogenic signals are transduced. Examples of such common engines are Ras (integrates outputs of RTKs), Myc (transcriptionally integrates outputs of intracellular kinases), E2F (integrates outputs of cell cycle CDKs) and p53 (monitors network integrity, flux and dysfunction). Each of these engines is pharmacologically intractable at present. Nonetheless, it is possible to use novel switchable mouse genetics to explore the therapeutic efficacy and side effects of reversibly inhibiting such engines. Using in vivo models of lung and pancreatic adenocarcinoma, we show how targeting Myc is highly effective at driving regression of established cancers, in part by triggering death of tumor cells and rapid collapse of the tumour stroma, while inflicting minimal collateral damage on normal tissues. Unexpectedly, our data also uncover a hitherto unexpected role for Myc in regulating higher order, tissue--specific regenerative programs, potently modulating innate and adaptive tumour immunity and paving the way for combining Myc--targeting therapies with those that disrupt immune blockade.

PARP Inhibitor Resistance and Acquired Vulnerability In Ovarian Cancer

Alan D. D'Andrea

Center for DNA Damage and Repair, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

Large-scale genomic studies have demonstrated that approximately 50% of high-grade serous ovarian cancers (HGSOCs) harbor genetic and epigenetic alterations in homologous recombination repair (HRR) pathway genes. The most commonly altered HRR genes are BRCA1 and BRCA2, followed by other Fanconi Anemia Genes including FANCN/PALB2, FANCO/RAD51, FANCJ/BRIP, and FANCA. Loss of HRR causes genomic instability, hyperdependence on alternative DNA repair mechanisms, and enhanced sensitivity to platinum analogues, topoisomerase inhibitors, and PARP-inhibitors (PARPi). The synthetic lethal interaction with PARPi is being exploited therapeutically in diverse clinical contexts and most notably in ovarian cancer where the PARPi olaparib is FDA approved for use in patients with germline BRCA1/2 mutations. PARP inhibitor resistance has already emerged as a vexing clinical problem for the treatment of BRCA1/2 deficient tumors. The most prevalent mechanism of PARPi resistance is secondary events that cancel the original HRR alteration and restore HRR proficiency. However, PARPi resistance may still develop without restoration of HRR proficiency via disruption of multiple proteins, such as PTIP or CHD4, that leads to replication fork (RF) stabilization. Importantly, this latter mechanism-namely, the restoration of RF stability- appears to be a highly prevalent mechanism of PARP inhibitor resistance in vitro and in vivo, particularly in tumor cells with an underlying BRCA2 deficiency. Due to their underlying deficiency in BRCA2 and inability to generate RAD51 nucleofilaments, these tumor cells are unable to restore HRR mechanisms. Instead, these cells acquire PARP inhibitor resistance by limiting the nucleolytic degradation of their stalled replication forks. We have recently made the surprising observation that BRCA2-deficient tumors can become resistant to PARPi by downregulating the expression of the polycomb repressive complex PRC2, a methyltransferase complex containing EZH2, SUZ12, EED, and RbAp48. Importantly, downregulation of PRC2 results in the reduced recruitment of the nuclease MUS81 to the RF, thereby providing a novel mechanism of RF protection and PARPi resistance. A molecular understanding of PARP inhibitor resistance mechanisms may allow the generation of a new class of drugs, or a re-purposing of existing drugs, which may reverse this resistance and extend the use of PARP inhibitors to more tumor types.

DNA Damage Response in Cancer and the Role of non coding RNA

Fabrizio d'Adda di Fagagna

IFOM Foundation, Milan, Italy; Istituto di Genetica Molecolare, National Research Council, Pavia, Italy

The DNA damage response (DDR) is a signaling pathway that arrests the proliferation of cells undergoing genotoxic events to coordinate DNA repair efforts. We previously demonstrated that DDR is physiologically activated in cancer initiation (Di Micco et al Nature 2006) and ageing (d'Adda di Fagagna et al. Nature 2003, Fumagalli et al. Nature Cell Biology 2012). Recently, we reported that a novel class of small non-coding RNA (termed DDRNA) is necessary to activate the DDR upon various sources of DNA damage (Francia et al., Nature 2012; d'Adda di Fagagna, Trends in Cell Biology 2014).

We will show that DNA double-strand breaks trigger the local transcription of the damaged locus by RNA polymerase II and that full DDR activation depends on a network of site-specific RNA:RNA interactions.

This discovery allowed us to demonstrate that sequence-specific oligonucleotides designed

against non coding-transcripts generated at a damaged genomic locus inhibit DDR activation both in cultured cells and in vivo in relevant animal models.

Regulation of self-renewal in Cancer Stem Cells

Verga Falzacappa M.V., Insinga A., Tanaskovic O. and Pelicci P.G.,

Istituto Europeo di Oncologia (IEO), Milan, Italy Department of Oncology and Haemato-oncology, University of Milan, Italy

Recent findings support the concept that cells with the properties of stem cells (SC) are integral to the development and perpetuation of several forms of human cancer, and that eradication of cancer stem cells (CSC) may be essential to achieve cancer cure. However, direct proof of these concepts is still lacking, mainly due the scarcity of appropriate model systems. We have recently defined a number of CSC-specific biological properties and underlying molecular mechanisms, using mouse models of i) leukaemia, obtained by transgenic expression of the PML-RAR, mutant NPM or AML1-ETO leukemia-associated oncogenes; and ii) mammary tumor, obtained by transgenic expression of the ErbB2 oncogene. We found that self-renewing divisions of CSCs are more frequent than normal counterparts, unlimited and symmetric, thus contributing to increasing numbers of SCs in tumoral tissues. SCs with targeted mutation of the tumor suppressor p53 possess the same self-renewal properties of cancer SCs, and their number increases progressively in the p53-null pre-malignant mammary gland. We showed that p53 signaling is attenuated in ErbB2-driven tumors, and that pharmacological re-activation of p53 induced restoration of asymmetric divisions in cancer SCs and tumor growth reduction, without affecting rates of apoptosis or proliferation on additional cancer cells. These data demonstrate that p53 regulates polarity of cell division in mammary SCs and suggest that loss-of-p53 in epithelial cancers favors symmetric divisions of CSCs, contributing to tumor growth. As a further mechanisms of extended self-renewal in cancer stem cells, we have demonstrated that up-regulation of the cell-cycle inhibitor p21 is indispensable for maintaining self-renewal of leukaemia SCs (LSCs). Expression of leukaemia-associated oncogenes in normal hematopoietic SCs (HSCs) induces DNA damage and activates a p21-dependent cellular response that, in turn, imposes cell-cycle restriction and triggers repair of the damaged DNA. This effect of p21 prevents the physiological exhaustion of HSC self-renewal, which occurs in time owing to accumulation of DNA damage, and confers an advantage to HSCs when they hyper-proliferate, as it occurs during stress or after full transformation (for example, in the LSCs), thus explaining the role of p21 in the maintenance of the self-renewal potential of LSCs. Finally, I will discuss unpublished data showing the contribution of immune-surveillance to the elimination of DNA-damaged SCs, and the underlying role of p21.

Probing the Transcriptional Consequences of Ras and Rho Activation

Richard Treisman

Francis Crick Institute, Midland Road, London UK

Signalling by small GTPases of the Ras and Rho families controls proliferation, adhesion and invasion of both normal and transformed cells. We study how signalling from these regulators interfaces with transcriptional programmes, focussing on the transcriptional regulator SRF. SRF works in partnership with two families of signal-regulated cofactors, which compete for a common site on its DNA-binding domain: the TCFs are regulated by Ras-MAP Kinase signalling, while the MRTFs respond to Rho GTPase signalling. The MRTFs contain novel G-actin binding element, the RPEL motif, and represent a new paradigm for signal transduction, in which G-actin itself acts as a signalling molecule.
Genomic studies indicate that MRTF-SRF signalling controls the transcription of scores of genes involved in invasion, metastasis and mechanosensing. Recent studies on MRTF-SRF signalling in cancer-associated fibroblasts, and the control of other RPEL proteins by G-actin will be discussed.
The SRF network, and particularly TCF-SRF signalling, directs most of the initial transcription response to MAP kinase activation. The TCFs also act as general antagonists of MRTF-dependent SRF target gene expression, and competition between TCFs and MRTFs for SRF determines the balance between antagonistic proliferative and contractile programmes of gene expression. Recent work shows that this system can be used to establish a functional hierarchy between transcription factor activity, chromatin modifications and transcription.

Mapping Cancer Origins - a Window Into Cancer Prevention

Prof. Richard J. Gilbertson

Li Ka Shing Chair of Oncology, Head of Dept. of Oncology, Director, Cambridge Cancer Centre, CRUK Cambridge Institute, Li Ka Shing Centre, Cambridge, UK

Cancers are distributed unevenly across the body, but the importance of cell intrinsic factors such as stem cell function in determining organ cancer risk is unknown. Over the last 15 years we have developed the technique of cross-species genomics to map cells of origin of brain tumours in the developing nervous system. These studies have revealed that brain tumours arise from matched combinations of susceptible cell types and oncogenic mutations. More recently we have built on these data to use Cre-recombination of conditional lineage tracing, oncogene, and tumour suppressor alleles to define populations of stem and non-stem cells in multiple mouse organs and test their life-long susceptibility to tumorigenesis. We show that tumour incidence is determined by the life-long generative capacity of mutated cells. This relationship held true in the presence of multiple genotypes and regardless of developmental stage, strongly supporting the notion that stem cells dictate organ cancer risk. Using the liver as a model system, we further show that damage-induced activation of stem cell function markedly increases cancer risk. Therefore, we propose that a combination of stem cell mutagenesis and extrinsic factors that enhance the proliferation of these cell populations, creates a "perfect storm" that ultimately determines organ cancer risk and provides insights into approaches of cancer prevention.

Therapy Guided by p53 Dynamics in Single Cells

Galit Lahav

Professor of Systems Biology, Harvard Medical School, Boston MA

Many signaling molecules exhibit complex dynamical behaviors, yet little is known about how these dynamics affect cellular responses. In this talk I will focus on the dynamics of p53, a critical tumor-suppressive protein that controls genomic integrity and cell survival. I will present recent studies from the lab demonstrating how studying the dynamics of p53 in response to radiation and chemotherapy in individual cells can help with optimizing the schedule of combination therapy and with increasing the efficacy of anticancer treatments.

Metabolic Vulnerabilities in Detached Cancer Cells

Christiaan F Labuschagne and Karen H Vousden

Francis Crick Institute, London, UK

Tissue culture models of cancer cell have proven invaluable in allowing the dissection of molecular pathways underlying many aspects of cancer cell behaviour. However, it is clear that they do not accurately represent the growth conditions encountered by cancer cells in vivo, and that these differences can have a profound impact on the cell's behaviour. While systemic influences of the whole organism and the complex interplay between cancer and stromal cells can be difficult to model in vitro, changes in the adherence of cells and nutrient availability can be more easily modulated. Epithelial cells grown in monolayer cultures benefit from matrix attachment signals that promote survival and nutrient uptake, and loss of attachment can promote a stress response leading to cell death. Oncogenic alterations can protect cells during detachment and may help cancer cells survive as they invade out of their normal environment. One of the key factors in allowing cells to survive detachment is the ability to limit ROS, which is increased under such conditions. We have explored whether detachment from matrix provokes other metabolic changes that might provide additional targetable vulnerabilities in cancer cells. Our results show that in multiple cancer cell lines, loss of attachment leads to an accumulation of 2HG and a drop in ATP levels, likely reflecting an inhibition of ATP synthase. Consistently, the detached cells show a block in fatty acid oxidation and are dependent on glucose to support viability.

Elucidating and Targeting Mechanisms of Cancer Cell State Maintenance

Andrea Califano

Professor of Systems Biology , Columbia University, New York, NY

Use of targeted inhibitors in precision cancer medicine is largely predicated on the identification of actionable oncogene mutations. Yet, only ~25% of human malignancies present with actionable alterations, and only 5% to 10% of all patients benefits from targeted therapy in terms of progression free survival. Most critically, even among patients who initially respond, a majority will eventually relapse with drug-resistant disease. Thus, there is urgent need for complementary precision cancer medicine approaches that focus on protein targets representing individual and synergistic tumor vulnerabilities, independent of their mutational status. To address this challenge, we have developed network-based methods for the systematic identification and validation of tumor checkpoint modules, comprising Master Regulator proteins, whose concerted aberrant activity is both necessary and sufficient for implementation and maintenance

of specific tumor cell states. We have identified and validated tumor checkpoints for multiple tumor types, from glioblastoma and lymphoma to breast and prostate adenocarcinoma and shown that they implement complex regulatory bottlenecks, whose genetic or pharmacologic inhibition abrogates tumor viability in vitro and in vivo. Finally, we have developed methodologies that leverage large-scale drug-perturbation assays to systematically identify drugs and drug combinations whose mechanism of action is specifically effective in abrogating tumor checkpoint activity on an individual patient basis. To systematically evaluate this approach, we have opened a novel N-of-1 study, which has already enrolled >80 patients, across 14 tumor subtypes, who have failed multiple lines of therapy. Therapeutic value of computationally predicted therapies is first evaluated in patient-derived xenografts (PDX) and/or organotypic cultures and ultimately used to guide patient therapy. So far, of 30 drugs prioritized by this approach for six malignancies – from metastatic pancreatic tumor to KRAS/SDHB-mutant GIST – more than 1/2 produced objective response in PDX models and in three out of four treated patients.

Molecular Control of Cellular Heterogeneity in Human Pancreatic Cancer

Gioacchino Natoli, MD.

European Institute of Oncology (IEO) and Humanitas University (Hunimed), Milan - Italy

Pancreatic ductal adenocarcinoma (PDAC) is nearly always an incurable disease, with a median survival time after diagnosis of four months. The causes of this extremely aggressive behavior are both the advanced stage of the disease at diagnosis and the peculiar biological properties of this tumor type. While a certain degree of heterogeneity is common to virtually all solid cancers and can impact therapeutic responses, a remarkable feature of human PDACs is the co-occurrence within the same tumor of completely different and morphologically identifiable components: well-differentiated (low-grade) epithelial structures and nests of poorly differentiated (high-grade) quasi-mesenchimal tumor cells, whose coexistence reflects distinct underlying gene regulatory networks and transcriptional outputs. We have recently undertaken a detailed molecular analysis of the gene regulatory networks that control maintenance of differentiation in human PDACs and we have identified and validated a small number of critical regulators. We will discuss the relevance of these findings and the future developments in this area.

Tumor Neoantigens as Targets for Cancer Specific Immunotherapy

Robert D. Schreiber, Ph.D.

Washington University School of Medicine St. Louis, MO

Our previous work led to the elaboration of strong experimental data demonstrating the existence of a cancer immunoediting process that functions in both mouse models of cancer and human cancer patients. In the course of these studies we used a combination of exome sequencing and epitope prediction algorithms to show that tumor specific mutant proteins expressed in highly immunogenic d42m1 tumor cells derived from methylcholanthrene (MCA) treated immunodeficient mice represent immunodominant, tumor specific mutant antigens (TSMA) for CD8+ T cells and that immunoselection is a major mechanism underlying the immunoediting process. More recently, we used a similar approach to identify tumor neoantigens in progressively growing T3 MCA sarcomas that render these mouse tumors susceptible to T cell-dependent checkpoint blockade immunotherapy. This work lead to the identification of two immunodominant neoantigens among the 700 point mutations in T3 tumor cells encoding mutant Laminin α subunit 4 (mLama4) and a mutant glucosyltransferase (mAlg8). Therapeutic vaccination of mice bearing established T3 tumors with synthetic long peptides (SLP) vaccines consisting of mLama4 plus mAlg8 peptides plus poly IC resulted in tumor rejection. The therapeutic protection afforded by the SLP vaccine was equal to that induced by checkpoint blockade therapy. Since we did not see evidence of T cell responses to other T3 TSMA, we explored whether the immunodominant antigens masked responses to weaker TSMA. Using CRISPR/Cas9, we reverted mLama4 and mAlg8 back to their nonimmunogenic wild type counterparts in T3 tumor cells. The tumor cells lacking mutant forms of either Lama4 or Alg8 remained susceptible to checkpoint blockade therapy with evidence of TIL skewing towards the remaining TSMA. We then generated T3 tumor cells lacking both mLama4 and mAlg8. The “fixed” T3 tumor cells also remained susceptible to anti-PD-1/anti-CTLA-4-mediated rejection, with evidence of enhanced T cell responses to at least two additional TSMAs—a point mutant form of Gapvd1 and a second novel mutant protein caused by an indel. We are currently testing whether SLP vaccines comprised of these two subdominant TSMA can effectively treat mice bearing either parental or “fixed” T3 tumors. We have also explored the minimal TSMA requirement for immune mediated tumor rejection. Using oncogene driven sarcomas that were completely devoid of mutant neoantigens, we found that protective im-

mune responses to tumors requires the presence of both MHC-I and MHC-II restricted epitopes. The implications of these findings to development of effective neoantigen vaccines will be discussed.

Stressed Out: A Novel Approach to Cancer Immunotherapy

Laurie H. Glimcher - CEO and President

Dana Farber Cancer Institute, Boston, MA

Cancer cells induce a set of adaptive response pathways to survive in the face of stressors due to inadequate vascularization1. One such adaptive pathway is the unfolded protein (UPR) or endoplasmic reticulum (ER) stress response mediated in part by the ER-localized transmembrane sensor IRE1 and its substrate XBP1. We have shown that the transcription factor XBP1 promotes intrinsic tumor growth directly in the setting of triple negative breast cancer, and now have established that this signaling pathway also regulates the host anti-tumor immune response. Dendritic cells (DCs) are required to initiate and sustain T cell-dependent anti-cancer immunity. However, tumors often evade immune control by crippling normal DC function. Constitutive activation of XBP1 in tumor-associated DCs (tDCs) drives ovarian cancer (OvCa) progression by blunting anti-tumor immunity. XBP1 activation, fueled by lipid peroxidation byproducts, induced a triglyceride biosynthetic program in tDCs leading to abnormal lipid accumulation and subsequent inhibition of tDC capacity to support anti-tumor T cells. Accordingly, DC-specific XBP1 deletion or selective nanoparticle-mediated XBP1 silencing in tDCs restored their immunostimulatory activity in situ and extended survival by evoking protective Type 1 anti-tumor responses. Targeting the ER stress response should concomitantly inhibit tumor growth and enhance anti-cancer immunity, thus offering a unique approach to cancer immunotherapy.

Targeting Critical Dependencies in Pancreatic Cancer

Giulio F. Draetta

The University of Texas MS Anderson Cancer Center, Houston, TX

A major focus of our laboratory is the study of the genetic and functional heterogeneity of pancreatic adenocarcinoma. During the past few years, we have appreciated that distinct populations of cells in each tumor, whether of human or mouse origin, are selectively dependent of specific biochemical cascades for survival. Driving impactful clinical results will require an improved understanding of gene functions playing rate limiting functions in each tumor subpopulation at time of treatment. Through functional genomics screens across multiple primary human PDAC samples, we are starting to appreciate the existence of vulnerabilities that are shared across tumor types compared to others representing more unique events. Targeting strategies will have to balance the opportunity to broadly affect the largest representation of tumor cells in a patient with the challenge of overcoming toxicity for normal cells, with which certain functions may be shared.

A discovery our group recently made was that populations resistant to KRAS* inactivation can emerge upon suppression of signaling. These cells remain alive after treatment, show activation of mitochondrial respiration and autophagy, and are vulnerable to treatment with OXPHOS inhibitors. Since these initial findings, we have been able to show that treatment with gemcitabine/NAB-paclitaxel or other combined chemo radiation regimens, also results in induction of mitochondrial dependency. The discovery of IACS-10759, a novel OXPHOS inhibitor of high potency, selectivity and optimized pharmaceutical properties, has given us the opportunity to verify this hypothesis in the clinic with a planned initiation of solid tumor clinical trials in 2017.

Through a combination of mouse genetics and human tumor analysis, we have also recently discovered that a subset of pancreatic cancer tumors is enriched for cells showing a mesenchymal-like phenotype that have lost their dependency on KRAS* for survival, have suppressed the expression of the SNF5/SMARCB1 chromatin-remodeling complex subunit, show c-Myc activation, and an enhanced dependency on proteostasis. These tumors are indeed highly sensitive to proteasome, HSP90 and JNK inhibitors and we are currently planning to exploit combinations of these agents in the clinic.

Novel Approaches to Clinical Trial Design in Cancer

Giovanni Parmigiani

Dana-Farber-Cancer Institute, Boston MA

Do we need novel approaches to clinical trial design in cancer? How far can technological innovation in trial design can potentially take us towards addressing the vexing lack of speed in drug development? To stimulate discussion on these issues, in this presentation I will give a brief review of recent progress on trial design, focusing particularly on Bayesian methodologies for adaptive trials. I will focus on increasing flexibility in trial desing by adding and dropping arms dynamically, learning across cancer sites,

prioritizing biomarker subgroups, and testing drug combinations ---as examples. I will present quantifications of the potential benefits of these innovation, with the goal of supporting a discussion about their wider adoption.

A Defined "Structure" for the Immune System That Reflects Immune Surveillance & Mechanistic Processes

Garry P. Nolan, Ph.D.

Rachford and Carlota A Harris Professor, Department of Microbiology & Immunology, Baxter Laboratory for Stem Cell Biology, Stanford University School of Medicine, Stanford, CA

High parameter single cell analysis has driven deep understanding of immune processes. Using a next-generation single-cell "mass cytometry" platform we quantify surface and cytokine or drug responsive indices of kinase target with 45 or more parameter analysis (e.g. 45 antibodies, viability, nucleic acid content, and relative cell size). Similarly, we have developed two advanced technologies that enable deep phenotyping of solid tissue in both fresh frozen and FFPE formats (50 – 100 markers). We have recently extended this parameterization to mRNA with the capability to measure down to 5 molecules per cell in combination with any other set of previously created markers.
I will present evidence of deep internal order in immune functionality demonstrating that differentiation and immune activities have evolved with a definable "shape". This shape is altered during immune surveillance and "imprinted" during, and after, pathogen attack, traumatic injury, or auto-immune disease. Hierarchies of functionally defined trans-cellular modules are observed that can be used for mechanistic and clinical insights. I will focus upon pathogen attack, traumatic surgical intervention in human, and auto-immune processes for the presentation.

The Modular Proteome and its Clinical Significance

Ruedi Aebersold

Department of Biology, Institute of Molecular Systems Biology, ETH Zurich and Faculty of Science, University of Zurich, Switzerland

The question how genetic variability is translated into phenotypes is fundamental in biology and medicine. Powerful genomic technologies now determine genomic variability at unprecedented scale, speed, accuracy and (low) cost. This technology has been particularly effectively used in the field of oncology in attempts to relate genome variability to disease phenotypes.
Generally, (clinical) phenotypes are the consequence of perturbed biochemical processes which are largely catalysed by proteins that are, in turn, organized in functional modules such as complexes and pathways. The main objective of our work is therefore to quantitatively describe the prototype, defined as the acute state of the proteome (composition and organization of a tissue or cell.
We approach this objective by developing and using a range of mass spectrometric and computational methods for the reproducible quantification of the proteome and for the systematic analysis of the connectivity of proteins in modules.
In this presentation we will discuss the current state of this technology. We will additionally discuss selected applications of the technology in which we use genetic reference strain compendia of structured genotypic variability and clinical cohorts to test the notion that the thus determined proteotype complements genomic information to determine (clinical) phenotypes.

Microbiota, Immune System and Cancer Development

Elena Zagato, Chiara Pozzi and Maria Rescigno

European Institute of Oncology and University of Milan, Italy

The microbiota is emerging as an important environmental factor influencing several functions of our body. Many disorders have been associated to a disequilibrium of the microbiota that is called dysbiosis. Colorectal cancer (CRC) is the third leading cause of cancer-related deaths in industrialized countries. It is a multifactorial disorder influenced by genetic, environmental and lifestyle factors, including excessive alcohol use, smoking, dietary habits and reduced physical activity. Dysbiosis has also been associated with the pathology, but whether this is a cause or an effect of the disease is less clear. Reports in animal models have shown that several bacterial strains may promote tumor development via increasing the stability of oncogenes, releasing genotoxic toxins, driving cell proliferation and accumulation of suppressive immune cells within the tumor or via an exacerbation of the inflammatory response. However, contrasting results have been obtained on the role of the microbiota using germ-free mice. In some models the microbiota seems to be protumorigenic,

while in others antitumorigenic. This suggests also the existence of tumor-protecting bacterial strains, whose identity remains to be identified. We have studied the role of the microbiota in the ApcMin/+ mouse model of spontaneous intestinal tumorigenesis and demonstrated that dysbiosis and changes in mucus composition are concomitant to tumorigenesis. We identified a taxon most strongly underrepresented during tumorigenesis. Reconstitution of ApcMin/+ mice with a C57BL/6 mice isolate of this taxon protected mice from tumor development indicating the existence of tumor-protecting bacteria. These bacteria have a direct effect on tumor cell proliferation.

Multiscale Molecular Pathology – at the Interface Between Biology and Computer Science

Young Hwan Chang, Michel Nederlof, Tyler Risom, Spencer Watson, Danielle Jorgens, Mark Dane, Claudia Lopez, Guillaume Thibault, Damir Sudar, Rosalie Sears, James Korkola, Laura Heiser, Gordon Mills, and Joe Gray.

OHSU Center for Spatial Systems Biomedicine, Oregon Health & Science University, Portland, Oregon, USA

Cancers arise and advance as a result of genomic and epigenomic changes that alter the hierarchical, molecular organization of cells and tissues in ways that affect tumor cell function and response to treatment. These changes, which affect structures ranging in size from a few Angstroms, can now be measured using a suite of new imaging devices including electron microscopy, conventional and super resolution fluorescence microscopy, confocal microscopy and lightsheet microscopy after preparation using new multicolor tumor staining procedures. Interpretation of the images and omic information requires sophisticated new algorithms including machine learning and sparse clustering. This talk will focus on insights gained from integrative analyses of tumor omic properties and multiscale, multicolor images of human breast tumor cells and the proximal and distal microenvironments in which they live. These include (a) spatial analysis of the consequence of proximal and distal interactions between cells and molecular structures, (b) elucidation of how tumor cells escape therapeutic control through epigenomic evolution, and (c) discovery of nanoscale structures that influence information exchange between metastatic tumor cells and with the microenvironments in which they reside.

BRCC36 Moonlighting between BRCA1 and Metabolism

Nicolas Thomä

MetabolismFriederich Miescher Institute for Biomedical Research, Basel, Switzerland

The K63-linkage specific deubiquitinase complexes BRCA1A and BRISC share a common core of subunits including the enzymatically active subunit BRCC36, yet they starkly differ in their biological function: BRCA1A is essential for genomic stability, while BRISC is involved in immune regulation. We found that the substantial difference in activity, regulation and assembly originates form the scaffold proteins ABRAXAS and ABRO1, which modulate the activity of BRCC36 and recruit additional subunits to functionalize the common core. A C-terminal domain of ABRAXAS recruits RAP80 to BRCA1A and integrates it as an obligate subunit, while the phosphorylated C-terminus binds BRCA1, turning BRCA1A into a multifunctional DNA repair machine. We discovered that BRISC, but not BRCA1A, is inhibited by SHMT2, a moonlighting metabolic enzyme, which binds to scaffold domain ABRO1 and sterically prevents substrate binding.

ABSTRACTS OF POSTERS

Investigating the telomeric long noncoding RNA TERRA in cancer cells at single cell resolution

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The immortal potential of cancer cells depends on their capability to maintain their telomeres by activating telomere lengthening mechanisms. 90% of human cancers express the enzyme telomerase which adds telomeric repeats to the 3' end of chromosomes, while 10% of tumors activate homologous recombination based mechanisms known as alternative lengthening mechanisms of telomeres (ALT). The inhibition of telomerase or the activation of ALT mechanisms induces apoptosis in telomerase-positive and ALT cancer cells, respectively. For these reasons, telomeres and telomerase represent important drug targets for controlling tumorigenesis and proliferation of cancer cells.

The long noncoding RNA TERRA (telomeric repeat-containing RNA) is a key component of telomere biology. TERRA is transcribed from the subtelomeric regions of chromosomes and its transcription proceeds towards the chromosome ends, terminating within the telomeric repeat tract. TERRA expression is induced in human and mouse tumors. Nevertheless, the mechanisms regulating TERRA expression in cancer cells remain to be elucidated.

TERRA transcripts have been proposed to act as scaffold molecules promoting the recruitment of enzymatic activities, including chromatin remodelling factors and the enzyme telomerase, at telomeres. Furthermore several studies have indicated TERRA as regulator of telomere maintenance, in both telomerase-positive and ALT cancer cells. However, the mechanisms of action of TERRA remains to be defined. Interestingly, it has been observed that an impaired localization of TERRA at chromosome ends associates with dysfunctional telomeres and genome instability. Nevertheless, the dynamics of TERRA transcripts is still poorly known.

In order to gain insight into the dynamics of TERRA, we were wondering if TERRA transcripts could be detected *in vivo*, in human cancer cells. To answer this question we employed the MS2-GFP system, which relies on the high affinity binding between the phage MS2 coat protein and the MS2 RNA stem-loop sequence. We used the CRISPR/Cas9 genome editing tool to generate clones containing MS2 stem-loop sequences integrated at a single telomere. As expected, upon expression of an MS2-GFP fusion protein, we observed discrete MS2-GFP foci only in cells containing MS2 sequences at a single telomere. Our results indicate that TERRA is expressed in a small population of cells and show predominant nuclear localization. To investigate the localization of TERRA/MS2GFP foci at telomeres, we expressed a cherry-fused TRF2 protein, a key component of telomere biology, in TERRA-MS2 clones. Interestingly, preliminary two-colors confocal microscopy experiments in live-cell indicate that TERRA-MS2 transcripts only transiently colocalize with telomeres. Furthermore, our findings indicate that TERRA molecules expressed from a single chromosome end co-localize with multiple telomeres. By using MS2 sequence-specific chemically modified antisense oligonucleotides we are able to down-regulate MS2-tagged TERRA transcripts expressed from a single telomere. At this point, one of our future goal is to combine live-cell imaging and biochemical approaches in order to characterize the dynamics of TERRA molecules. In particular our aim is to explore the dynamics and functions of TERRA in cancer cells, at single cell resolution.

Tumor infiltrating (TINKs) and tumor associated (TANKs) Natural Killer cells: new paradigm for colorectal cancer progression and angiogenesis.

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Introduction. NK cells are effectors lymphocytes of innate immunity, primarily involved in immunosurveillance against tumors through their cy-

totoxic activity. We previously reported that NKs from Non-Small Cell Lung Cancer (NSCLC) can acquire the decidual-like CD56brightCD16-VEGFhighPlGFhighIL-8+IFNγlow phenotype and that TGFß1 is a relevant orchestrator in the NK angiogenic switch. We investigated whether tumor associated (TANKs; peripheral blood NK cells) and tumor infiltrating (TINKs) NK, isolated from patients with colorectal cancer (CRC), are subjected to the inflammatory angiogenic-switch. Material and methods. NK subset distribution and cytokine profiling were performed by flow cytometry, on peripheral blood and tissue samples from CRC patients. Supernatants from FACS-sorted NKs were used either for secretomic profiling, using antibody membrane array or in functional in vitro angiogenesis assay. Biochemical approaches were used to determine molecular pathways modulated in different CRC TINK/TANK phenotype and function. Results and discussion. We found that TINK/TANKs from CRC patients show impaired degranulation activities and decreased NKG2D expression. CRC TINK/TANKs express the decidual NK markers CD9 and CD49a and induced endothelial cell proliferation, migration, adhesion and formation of capillary-like structures on Human Umbilical Vein Endothelial Cells (HUVEC) in vitro. Secretome and flow cytometry analysis on CRC peripheral blood NK cells showed up-regulation of several pro-angiogenic factors, such VEGF, Angiogenin, Angiopoietin-1, Timp1-2, MMP-9. Molecularly, we observed p-STAT3 and p-STAT5 up-regulation in CRC TANKs and their chemical inhibition resulted in both inhibited pro-angiogenic factor production and formation of capillary-like structures in vitro. Conclusions. Our data demonstrate that TINK/TANKS from CRC patients are switched toward a pro-angiogenic/pro-tumor phenotype and function. Therefore, we propose TINK/TANKs as a new hallmark ad relevant target in CRC inflammation.

Exploring prostate tissue homeostasis and tumorigenesis with 3D organoids

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Prostate cancer (PCa) is one of the most frequent forms of cancer in men, characterized by high clinical and genetic heterogeneity. Many uncertainties remain about the choice of treatment and, crucially, the metastatic disease is still incurable. Despite intense efforts, PCa research has been hampered by a limited understanding of prostate homeostasis and by long-standing problems in establishing accurate experimental models of healthy and transformed epithelium. Currently, 2D cell cultures and animal models poorly recapitulate key PCa features, including progressive epithelial transformation, heterogeneity and (poly)clonal origin.

In the last decade, 3D organoid cultures have been developed for many tissues, enabling a rapid expansion of more reliable and physiological *in vitro* models. Prostate organoids, however, still present some limitations and do not faithfully recapitulate their tissue of origin. Indeed, they are predominantly made up of cycling progenitor cells, whereas the adult prostate epithelium is largely quiescent and enriched with luminal cells with intense secretory activity. Strikingly, the efficiency of PCa organoid derivation (<20%) is reported to be the lowest across human carcinomas.

For these reasons, we have begun to explore how soluble factors supplied to the medium affect signalling pathways that mainly contribute to the survival, proliferation and differentiation of prostate organoids. In particular, the role of Wnt and TGF signalling pathways is under investigation. Moreover, we present our progresses in the genetic engineering of organoids in order to model two genetic alterations, PTEN loss and TMPRSS2:ERG fusion, found at high frequency in PCa patients. *In vitro* phenotyping – including extracellular vesicle profiling – and *in vivo* modelling - via orthotopic transplantation - will be employed to model and understand the variety of mechanisms underlying the progressive transformation of prostate epithelium into aggressive cancer types.

Proline Dehydrogenase Expression And Regulation In Non Small Cell Lung Carcinoma

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Introduction

Non-Small Cell Lung Cancer (NSCLC) is one of the most frequent and deadly cancers and comprises two main histotypes, adenocarcinoma (ADC) and

squamocellular carcinoma (SCC). Identification of markers to improve diagnosis, prognosis and guide therapeutic options for NSCLC is needed. Proline dehydrogenase (PRODH) is a mitochondrial flavoenzyme that catalyzes the key step in proline degradation and is involved in the regulation of cell survival, autophagy and apoptosis. PRODH has a role as a tumor suppressor in renal and colorectal cancer and as a metastasis suppressor in breast cancer.

Materials and methods

We characterized expression of PRODH in a panel of ADCs and SCCs by immunohistochemistry and qPCR and tested if there was correlation between expression of PRODH and clinical features of the tumors or expression of other markers. We also tested the role of TTF-1 as a possible transactivator of the PRODH gene in two NSCLC cell lines, by transfection experiments and expression analyses. Moreover, four putative TTF-1 response elements, bioinformatically predicted in the PRODH promoter, were cloned and tested in luciferase reporter assays.

Results and discussion

We found PRODH immunostaining in the majority (70%) of lung ADCs. Patients with PRODH positive tumors showed a better survival than patients with negative tumors. Protein staining in tumors was paralleled by high transcript levels. TTF-1, a homeodomain-containing transcription factor essential for thyroid and lung development and physiology, was found to have a similar expression pattern in normal lung tissues and in NSCLCs. Based on their similar expression, their involvement in the same tumors or genetic pathologies, and the presence of putative TTF-1 response elements in PRODH promoter, we hypothesized that PRODH and TTF-1 may act in the same pathway and that TTF-1 could directly transactivate the PRODH gene. We show that this is indeed the case and that transactivation in A549 and NCI-H1299 ADC cell lines occurs by binding of TTF-1 to a response element in the PRODH promoter.

Conclusion

Our data support a possible application of PRODH immunostaining as a prognostic marker and opens up new research perspectives aimed to investigate PRODH transactivation by TTF-1 and the role of PRODH in the biology of NSCLC.

Identification and characterization of new anti-angiogenic compounds from natural sources

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The inhibition of angiogenesis has attracted broad attention in the field of pharmacological research, not only for cancer, but for other angiogenesis dependent diseases including ophthalmic, cutaneous and inflammatory diseases, as well as a number of rare diseases. Our research group has characterized multiple new natural bioactive compounds with multitargeted antiangiogenic effects by employing a well-established set of in vitro, in vivo and ex vivo preclinical models of angiogenesis. Most of them have been isolated from plants and terrestrial microorganisms, mainly due to their higher availability and because their therapeutic effects had been previously known in folk traditional medicines. In vitro primary screening includes cell differentiation and toxicity and proliferation assays. Secondary screening involves several experiments to evaluate effects on adhesion, migration, invasion, apoptosis or cell cycle analysis, among others. Additionally, we perform a further molecular characterization analyzing possible signaling pathways that are affected to elucidate their mechanism of action. The characterization is completed with the ex vivo aortic ring assay, and in vivo assays, as CAM and zebrafish assays, to ensure the anti-angiogenic ability. As a fruit of the mentioned screening, a number of compounds with remarkable anti-angiogenic activity have been identified and characterized.

Our experimental work is supported by grants BIO2014-56092-R (MINECO and FEDER) and P12-CTS-1507 (Andalusian Government and FEDER) and funds from group BIO-267 (Andalusian Government). The “CIBER de Enfermedades Raras” is an initiative from the ISCIII (Spain)]. This communicaction has the support of a travel grant “Universidad de Málaga. Campus de Excelencia Internacional Andalucía Tech”.

ETV7-mediated DNAJC15 repression leads to Doxorubicin resistance in breast cancer cells

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Breast cancer treatment often includes Doxorubicin as adjuvant as well as neoadjuvant chemotherapy. Despite its cytotoxicity cells can develop drug resistance to Doxorubicin. Here we show that Doxorubicin and other chemotherapeutic drugs induce expression of ETV7, a transcriptional repressor member of the large ETS family of transcription factors. The ETV7 expression led to down-regulation of DNAJC15, a co-chaperone protein whose low expression was previously associated with drug resistance in breast and ovarian cancer. There was a corresponding reduction in Doxorubicin sensitivity of MCF7 and MDA-MB-231 breast cancer cells. We identified the binding site for ETV7 within the promoter of DNAJC15 and we also found that DNA methylation may be a factor in ETV-mediated transcriptional repression at the DNAJC15 promoter. These findings of an inverse correlation between ETV7 and DNAJC15 expression in breast cancer cells in terms of Doxorubicin resistance, correlated well with treatment responses of breast cancer patients with recurrent disease, based on our analyses of reported genome-wide expression arrays. In addition, we demonstrated that ETV7-mediated Doxorubicin resistance involves increased Doxorubicin efflux via nuclear pumps, which could be rescued in part by DNAJC15 up-regulation. A better understanding of the opposing impacts of Doxorubicin could improve the design of combinatorial adjuvant regimens with the aim of avoiding resistance and relapse.

Palbociclib, a CDK4/6 inhibitor, as a new strategy of treatment in Triple-negative breast cancer (TNBC) and in Malignant pleural mesothelioma (MPM)

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Background: The inhibition of cell-cycle, a process undergoing a progressive de-regulation due to molecular alterations during carcinogenesis, is becoming a new therapeutic frontier for the treatment of several tumors. Palbociclib (PD-0332991) is a reversible CDK4/6 inhibitor and acts by inhibiting the transition from G1 to S phase of the cell cycle. Cell sensitivity to palbociclib can be predicted by the presence of high levels of p-Rb and cyclin-D1 and by a contemporary reduced expression of the cell cycle inhibitor p16INK4 (Finn et al., 2009). Recently, the FDA granted accelerated approval to palbociclib, to be used in combination with letrozole for postmenopausal women with ER+/HER2-metastatic breast cancer (Ozaki et al., 2015). Early evidence reported that both in TNBC as well as in MPM patients, the cdkn2a/arf gene that encoded for p16ink4, is deleted in a high percentage of patients. Considering that to date there are no targeted therapies for these patients and chemotherapy remains the main treatment option, the identification of novel therapeutic strategies are urgently needed and palbociclib may represent a new target option.
Results: In a panel of TNBC and MPM cell lines, we firstly demonstrated that palbociclib inhibits cell viability only in cell lines with elevated levels of p-Rb and cyclin-D1 and low levels of p16ink4. As palbociclib is a cytostatic drug, we demonstrated that the treatment of sensitive TNBC and/or MPM cell lines with the drug for 24 hours, induces cell cycle arrest in G0/G1 phase and consequently a down-regulation of p-Rb, Rb and p-CDK6, c-myc protein levels and an up-regulation of cyclin-D1 were observed, both in dose- and time-dependent manner. Considering that the treatment with palbociclib in TNBC as well as MPM sensitive cell lines induced an up-regulation of p-AKT (ser478), we studied different schedules of treatments of palbociclib with PI3K/AKT inhibitors (BYL719 or BEZ235) showing both additive and synergistic effects. In addition, the cytostatic effect of palbociclib was associated with the induction of cellular senescence both in TNBC and MPM cells; the association with PI3K inhibitors strongly increased the percentage of senescent cells through a p53 and p21 up-regulation. Finally, in TNBC models, we evaluated the effects of palbociclib on cell energy metabolism both in normoxic and hypoxic conditions. Palbociclib was able to down-regulate hypoxia-mediated induction of HIF-1α and GLUT-1 expression as well as glucose uptake and glucose consumption, and the association with PI3K/AKT inhibitors enhanced these effects.
Conclusions: Our results provide a rationale to use the CDK4/6 inhibitor palbociclib as an effective treatment in TNBC and MPM patients in association with PI3K/AKT inhibitors.

A one-step method to isolate intact and disperse extracellular vesicles with biological function

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Extracellular vesicles (EVs) are membranous entities basically distinguished in exosomes and microvesicles according to their size, although different biogenetic mechanisms have been also suggested. The range of diameters is typically 80-200 nm for exosomes and 400-600 nm for microvesicles. It is widely recognized that EVs are secreted in the body fluids by various cell types including tumour cells. They encapsulate nucleic acids, proteins, lipids, or metabolites that are produced from the cell of origin and mediate functional transfer of biological material to recipient cells. When EVs are shed from their parental tumour cells, the metabolism of recipient cells can be influenced implicating a number of aspects of tumour progression and metastasis, most of them attributed to transferred RNA. We developed a scalable and easy-to use method, NBI, that shows great selective profile for EVs and overcomes limits of the gold standard differential ultracentrifugation. NBI preserves integrity of EVs, improves their stability in solution and, coupled with tunable resistive pulse sensing (TRPS), offers accuracy for correlation analyses and relative normalization of EVs from liquid biopsy specimens. Interestingly, our preliminary in vitro data show that EVs produced from aggressive breast cancer cells (MDA-MB-231) confer unexpected migratory properties either to non-invasive MCF-7 or non-tumorigenic MCF10A cells: treatment with RNAses abrogate this effect on recipient cells, presenting a novel approach to study functional RNA transfer by tumour-released EVs.

mTORC1 Drives TCA Cycle Alterations and Accumulation of the Oncometabolite Fumarate in Renal Cell Carcinoma

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Renal Cell Carcinomas (RCCs) are common cancers diagnosed in more than 350,000 people worldwide each year. Several pathways are found de-regulated including the mTORC1 cascade and profound metabolic alterations. Investigation over the years and identification/testing of novel potential therapies has been limited by the lack of faithful animal models. Here we intercrossed mice harboring a Tsc1 floxable allele with a Kidney-specific Cre mouse line (Ksp-Cre). The animals are born with normal kidneys and slowly develop renal cysts which gradually undergo tranformation with formation of papillae, cystadenomas and papillary type II carcinomas within the first three months of life. The phenotype is fully penetrant and highly reproducible. Upregulation of mTORC1 is observed in all stages, as expected. Global metabolomic profiling of these kidneys at P20, P50 and P80 revealed the presence of alterations in metabolic pathways previously ascribed to mTORC1 de-regulation such as glycolysis, the pentose phosphate pathway, pyrimidine and fatty acids biosynthesis and glutamine anaplerosis. Notably, we also observed defective TCA cycle regulation paralleled by a marked accumulation of the oncometabolite fumarate. Cell lines lacking or downregulated for Tsc1 confirmed that mTORC1 directly regulates the expression levels of fumarate hydratase and accumulation of fumarate. Interestingly, renal biopses of clear cell RCC with mTORC1 pathway upregulation display reduce expression of Fh enzyme. Thus, our mouse model recapitulates key features of renal carcinogenesis and unveils accumulation of the oncometabolite fumarate in response to mTORC1 upregulation as an essential player in disease progression.

MEN1309/OBT076: a novel Antibody-Drug Conjugate targeting the Ly75/CD205 antigen

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MEN1309/OBT076 is a novel fully humanized antibody drug conjugate (ADC) which binds to Ly75/CD205 with high affinity. The antibody is conjugated through a cleavable linker to a maytansinoid, DM4, a potent tubulin inhibitor.
The MEN1309/OBT076 target, Ly75/CD205, is a C-type lectin receptor presenting a short cytoplasmic tail that contains motifs for amino acid-based endocytosis, making this receptor an ideal target antigen for an ADC-based antitumoral therapy.
In the present study, we investigated the expression level and the functional role of the novel target antigen Ly75/CD205 in a tumoral context and the in vitro and the in vivo antitumor activity of MEN1309/OBT076 was characterized.
Ly75/CD205 mRNA expression was determined in human cancer cell lines derived from different histotypes. In these samples the expression levels of intergenically spliced isoforms between Ly75/CD205 and DCL-1 were also analyzed showing that chimeric mRNAs were expressed on average 30 fold less than Ly75 mRNA. In order to characterize the functional role of Ly75/CD205 in tumor cells, its expression was downregulated by siRNA in bladder, pancreatic and triple negative breast cancer cell lines showing that its inhibition leads to a decrease in the proliferation rate .
MEN1309/OBT076 cytotoxic activity demonstrated a potent effect against cells having strong as well as low or moderate antigen expression.
The in vitro results were also confirmed in vivo, where MEN1309/OBT076 showed an impressive antitumor activity, resulting in complete and long lasting responses in most of the xenograft models evaluated (NHL, TNBC, bladder and pancreatic cancers). In particular, TNBC patient-derived xenograft model, MEN1309/OBT076 (5 mg/kg q21dx3) showed an excellent antitumor activity inducing a complete tumor regression in four out of six mice.
Finally, in a pancreatic adenocarcinoma xenograft model HPAFII, the pharmacokinetics profile in serum of MEN1309/OBT076 at 5 mg/kg was characterized and it was qualitatively correlated, using immunofluorescence, with the occurrence of phosphorylation of Serine 10 of H3 Histone in cancer cells, as a pharmacodynamic (PD) marker of DM4 activity on microtubules.
Overall, our data suggest that MEN1309/OBT076 is a selective and potent novel ADC and it deserves to enter in Phase I study for a variety of Ly75/CD205 positive tumor histotypes.

Mechanisms of telomere maintenance in brain tumors

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Glioblastoma is the most common high grade tumor of the central nervous system with a median survival of 12-15 months post-diagnosis. Approximately 30% of these tumors employ a strategy called Alternative Lengthening of Telomeres (ALT) to attain cellular immortality.
ALT is a non-canonical mechanism developed by cancer cells with no-functional telomerase, which involves telomere extension using telomeric DNA as template.
Several therapies targeting telomerase pathways are being tested in the clinics, but no ALT-targeted therapies are known, because the mechanisms of ALT are largely unknown.
We have developed a zebrafish model of brain tumor based on the conditional expression of human-relevant oncogenes in brain progenitor cells in Tert-proficient and Tert-deficient backgrounds, that may be ideal for drug screening and drug discovery targeting telomere maintenance mechanisms (TMM).
We investigated which TMM mechanisms are employed during tumor development and progression and whether they are dependent on contributions of the catalytic (Tert) subunits of telomerase or on ALT processes.
We found that brain tumors develop in our model in the absence of functional telomerase, albeit smaller and less invasive, but with the same frequency of tumors with functional telomerase.
Using terminal restriction fragment (TRF) assay we studied telomere length distribution and correlated it with Q-FISH analysis to verify the presence of telomeres with irregular lengths as a function of ALT; zebrafish GBM cells show high telomere content and strong and irregular telomere FISH signals, typical of telomeres with variable lengths found in ALT+ cells, suggesting

that ALT mechanisms may be important in these tumors even in the presence of functional telomerase.
We confirmed that, in the zebrafish brain tumor model, telomere maintenance is due to ALT, as quantitative c-circle assay showed higher levels than in controls. We are now analysing the expression of the long non coding RNA, TERRA, which levels correlate with ALT, as it may provide an additional marker for ALT+ tumors.

p53-induced apoptosis is specified by a translation program regulated by PCBP2 and DHX30

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Activation of p53 by the small molecule Nutlin-3a can result in a combination of cell cycle arrest and apoptosis. The relative proportion of these events is difficult to predict, leaving uncertainty as to Nutlin-3a therapeutic benefits. Here, we report a new translational control mechanism shaping p53-dependent apoptosis. We studied translational control by means of polysomal profiling in two distinct cell lines SJSA1 and HCT116, of which only the first undergoes robust cell death. While both cell cycle and apoptotic p53 target genes are coordinated in their transcription and translation in both cell lines, there is little overlap among mRNAs that are modulated only at the level of relative polysome association. In particular, mRNAs that were enhanced in translation only in Nutlin-treated SJSA1 cells are enriched for apoptotic functions. A high proportion of those mRNAs carry a specific motif in their 3'UTR (consensus: 5'-CCCCA/CT/GGGCCCT, defined as CG-motif). The CG-motif was sufficient to stimulate mRNA translation in response to Nutlin treatment in SJSA1, but not in HCT116 nor in a SJSA1 p53-KO clone. Using protein pull-down followed by quantitative mass spectrometry, we identified the RNA helicase DHX30 as specific interactor of the CG-motif in HCT116 cells, while PCBP2 was found as CG-motif binding protein in both cell lines. The binding of DHX30 to the CG-motif is dependent on the binding of PCBP2, and depletion of either DHX30 or PCBP2 in HCT116 cells enhanced: i) the polysome association of endogenous mRNAs containing the motif; and iii) the induction of apoptosis not only in response to Nutlin, but also to 5-fluorouracil and doxorubicin. Our work reveals that translation control contributes to p53-dependent apoptosis, with DHX30 acting as a negative modulator of mRNA translation potential together with PCBP2 through a newly identified 3'-UTR cis-element.

Modulation of hepatic cancer stem cells markers following the induction of extrinsic apoptosis pathway by CD95

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Background: Hepatocelullar carcinoma (HCC) remains one of lethal malignancies that have poor prognosis and high recurrence rate. Although HCC is a heterogeneous disease, dysregulation of molecular profiling related to apoptosis also contributes to the disease progression. This study reports on relevant function of CD95 death receptor that can induce apoptosis via modulation of apoptosis genes and cancer stem cell markers in HCC.
Method: For the in vivo study, genes CD90, CD95, and CD95L from 47 samples (14 HCC, 9 peri-HCC, 13 cirrhosis, and 11 normal) from patient undergoing liver resection without any prior treatments was analysed. For the in vitro study, HCC the human cell lines HepG2, JHH6, and HUH7 were used representing high to low basal CD95 expression. Apoptosis-induction was performed by using anti-CD95 (DX2) at a concentration of 250 ng/ml and 500 ng/ml for 24 hours. Flow cytometry and quantitative real time PCR were performed to analyse the data.
Results: The expression of CD95 and CD95 genes were highly variable in human tissues. A significant increase for CD95L was noticed in HCC as compared to normal tissues, as observed for CD90. After in vitro treatment of anti-CD95, extrinsic apoptosis genes TNF-α and TRAIL2R were upregulated in all cell lines, as well as pro-apoptotic gene Puma; anti-apoptotic gene Bcl2b was down-regulated. Cancer stem cell marker CD24 was highly upregulated in HepG2 and to a lower extent in JHH6, while CD13 was slightly increased in all cell lines. There was no significant changes for CD44 and CD90.
Conclusion: We observed a modulation of ap-

optotic genes Puma, TNF-α, and TRAIL2R and cancer stem cell markers CD24 and CD13 after induction by CD95 antibody in acute phase treatment.
Keyword: HCC, CD95, apoptotic genes, cancer stem cell markers.

Inactivation of DNA repair triggers dynamic neoantigen evolution and impairs cancer growth

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Molecular alterations in genes involved in DNA mismatch repair (MMR) promote cancer initiation and foster tumor progression. MMR deficient cancers frequently show favorable prognosis and indolent progression. The functional basis of the clinical outcome of patients with MMR deficient tumors is not clear. To address this, we genetically inactivated MutL homolog 1 (MLH1) in colorectal, breast and pancreatic mouse cancer cells. The growth of MMR deficient cells was comparable to their proficient counterparts in vitro and upon transplantation in immune-compromised mice. In contrast MMR deficient cancer cells grew poorly when transplanted in syngeneic mice. Exome sequencing of MMR proficient cells revealed mutational loads and neo-antigen profiles that were stable over time. MMR inactivation led to dynamic mutational profiles, resulting in persistent renewal of neoantigens. Using a pharmacological screen we find that exposure of mouse cancer cells to the clinical approved agent temozolomide (TMZ) leads to inactivation of DNA repair, dynamic neoantigen profile and effective immune surveillance. MMR proficient and deficient human colorectal cancer cells show stable and dynamic neoantigen profiles respectively. Development of resistance to TMZ in human colorectal cancer cells leads to MMR inactivation and continuous renewal of neoantigens. These results provide the rationale for developing innovative anticancer therapies that target DNA repair proteins.

Short and long transcripts variant of KRAS at 3'UTR region as genetic biomarkers in glioblastoma

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3'UTR shortening has been established as a key mechanism of oncogene activation and has demonstrated promising potential as a prognostic marker (Mayr et al 2009; Lembo et al 2012; Singh et al 2009; Lapuk et al 2010). Generally, rapidly proliferating cells preferentially express mRNAs with shortened 3′UTRs (Elkon et al 2012; Sandberg et al 2008). As a general mechanism, the shortening of 3'UTRs enables key genes to escape microRNA repression, thus leading to higher expression and promoting proliferation. Moreover, shorter 3′ UTRs produce higher levels of protein. The KRAS gene is an important regulator of cellular proliferation and its 3'UTR region has recently attracted attention as genetic biomarkers predictive for prognosis, diagnosis and treatment of many cancers. mRNA transcripts of the KRAS gene differing in the lengths of their 3' untranslated regions (UTRs) are reported but their function is still largely unknown. In contrast to the well-studied SNP and MRE (Chung et al. 2014; Smits et al. 2011; Luong HT et al. 2012; Kumar et al. 2011) , the properties and dynamics of the emerging shortening 3'UTR KRAS remain elusive. Moreover, recent reports indicate that brain tissue possesses the longest 3′UTRs (Miura P 2013; Ulitsky I 2012). Mutations in KRAS are rare in human gliomas and particularly rare in WHO grade III and IV gliomas in adult patients (as reviewed by Cox-Fesik 2014; Hellinghoff 2012). Interestingly, expression of activated HRAS in breast cancer cells results in significant changes in the 3'UTR expression pattern toward shortening (64%) as well as lengthening (36%) (Lianoglou et al 2013). Brain tumors are KRAS-driven cancer and can originate from specific brain regions and from different cell types (Sanai et al. 2005). Although in mouse models of Kras-induced glioma, expression of oncogenic Ras is often insufficient for malignant gliomagenesis (Holland et al 2000). More recently, mouse model by Holmen have shown the essential role for KRAS signalling in glioblastoma maintenance and progression (Holmen et al 2005; Song et al 2013) and in zebrafish oncogenic KRAS promotes malignant brain tumors (Ju et al 2015). We investigated whether 3′UTR shortening can produce biologically relevant stratification of glioma cancers. Here, we report transcriptional analysis with specific prim-

ers for long and short transcripts of the KRAS gene at 3'UTR region. We measured differential expression of three transcript variants at 3'UTR respectively in size of 5.2 kb, 1.2 kb and 2.3 kb across a panel of primary and established cultures of human glioblastoma. Glioblastoma multiforme samples exhibited significantly higher KRAS mRNA levels compared to established cultures. Moreover, expression of long transcript variant correlates with reduced aggressiveness in primitive tumours and mitogenic stimulation induced preferentially this variant in almost all samples. Further analysis will determine its potential functional role as genetic biomarker in predicting glioblastoma outcome.

A comprehensive exploration of mutually exclusive genomic aberrations in tumor samples to identify synthetic lethal pairs

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Human tumors are characterized by the accumulation of genomic aberrations of which some are crucial for tumor initiation and progression while other are not. The growing accumulation of large-scale patient-derived data sets allows for the setup of data-driven approaches to exploit these mutations for cancer treatment. Mutual exclusivity of a specific pair of aberrant genes might suggest that their concomitant aberration leads to cell death (synthetic lethality, SL). Therefore, the identification of synthetic lethal gene aberrations within pathways that are typically altered in cancer provides a potential therapeutic window for tumor cells specific targeting, for identification of novel drug targets, for rational design of drug combinations, and for strategies to avoid drug resistance.
To identify potential synthetic lethal gene combinations, we developed an in silico methodology (SPICE: Synthetic Lethal Phenotype Identification through Cancer Evolution Analysis) by mining genomic and transcriptomic data belonging to 29 datasets of primary untreated tumor samples from The Cancer Genome Atlas (TCGA). Each dataset included from 36 to 1080 samples (median 370) and was comprehensively explored for mutual exclusivity relation between all possible pair of genes affected by homozygous deletions, amplification and single nucleotide variants (SNVs). To manage this huge amount of data, we implemented FaME, Fast Mutually Exclusive algorithm, exploiting state of the art parallel techniques. Each dataset is represented as a matrix and each cell reports the genomic status of a gene in a sample. Fast matrix multiplication based on openBLAS libraries, logarithm-based implementation of Fisher's exact test, and massive parallelization of code allows testing hundreds of millions of gene pairs in few minutes. While the FaME approach is agnostic (it ignores gene functions), literature data suggest that SL genes tend to belong to the same pathway or have similar biological function; we, therefore, applied our implemented version to available pathway collections, including BioCyc, KEGG, REACTOME, Pathways Interaction Database, Wiki Pathways, as well as gene ontology sets describing biological processes, cellular compartments, and molecular functions.
We identified 1,160 pairs of mutually exclusive aberrations in 18 tumor types. To validate our approach, we first verified if our method was able to nominate known SL pairs; we identified SNVs and combination of homo-deletion and SNVs of the EGFR and K-RAS genes as an SL pair in lung adenocarcinoma (LUAD) (p= 0.005 and p= 0.0029, respectively). Such pair has been previously identified by Unni (Unni et al. eLife 2015;4:e06907. DOI: 10.7554/eLife.06907). A second known call was the absence of concomitant SNVs and/or amplification of the isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) genes in low grade glioma (LGG) (p=8.14*E-9 and p= 7.55*E-9, respectively). Such pair has been previously identified in glioblastoma multiforme (GBM), acute myeloid leukemia (AML), and thyroid carcinomas by extensive experimental work (Hui Yang et al, Clin Cancer Res. 2012). A last example is the synthetic lethality between TP53 homozygous deletion and MDM2 amplification as previously reported in GBM (Nature. 2008 October 23; 455(7216): 1061–1068. TCGA consortium). Interestingly, our analysis indicates that this mutual exclusivity may also be significant for soft sarcomas (p=0.002). We are now in the process of experimentally validating previously unidentified pairs of SL nominated by SPICE.

Generation and characterization of zebrafish models of uveal melanoma

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Uveal melanoma (UM) is the most common primary cancer of the eye and its prognosis is strongly influenced by the occurrence of me-

tastasis, which are both rapidly developing and mostly fatal. The most frequent driver mutations occur in a small number of genes, including GNAQ, GNA11, BAP1, SF3B1 and EIF1AX, and have been identified through NGS. Due to a lack of suitable animal models, the mechanism through which mutations in these genes cause or cooperate in UM initiation and progression is still largely unknown. We focused our attention on the cloning of some relevant mutant construct ($GNAQ^{Q209P}$, $GNAQ^{R183Q}$, $GNA11^{Q209L}$, $SF3B1^{R625H}$) for the generation of transgenic strains expressing the human mutant protein in uveal melanocytes, using the kita promoter. We started to generate zebrafish models of UM using a binary Gal4/UAS system, to express the human mutant genes in uveal melanocytes and in their progenitors, to recapitulate the human disease. The models will be useful for: a) studying the effects of these mutations on the functions of the encoded proteins in the context of UM; b) investigating the signalling pathways and cellular responses affected by specific mutations; c) using the same approach to test the role of additional mutations/genetic alterations that will be discovered in UM patients; d) using the data obtained to predict suitable therapeutic strategies. In parallel, we will perform a chemical screen using a transgenic model previously generated in our lab, where oncogenic RAS is expressed under the kita promoter. Larvae of this transgenic line develop uveal melanocytic aggregates by 3 dpf that can be easily scored by testing fluorescent melanocytes proliferation and migration in the eye. The positive hits found in the chemical screen will be useful to identify pathways involved in UM initiation and will be also tested to treat experimental metastasis in adult zebrafish models.

ZAR1 is a novel epigenetically inactivated tumour suppressor in lung cancer

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Background: Lung cancer is the leading cause of cancer related deaths with 1.8 million new cases each year and poor 5-year prognosis. Promoter hypermethylation of tumour suppressors leads to their inactivation and thereby can promote cancer development and progression.
Results: In this study, we analysed ZAR1 (zygote arrest 1), which has been said to be a maternal-effect gene and its expression mostly limited to certain reproductive tissues. Our study shows that ZAR1 is expressed in normal lung but inactivated by promoter methylation in lung cancer. ZAR1 is hypermethylated in primary lung cancer samples (22 % SCLC and 62 % NSCLC) but unmethylated in normal control lung tissue. In lung cancer cell lines ZAR1 was methylated in 75 % of SCLC and 83 % of NSCLC (significant vs. normal tissue $p<0.05$). In matching tumours and control tissues we observed, that NSCLC primary tumour samples exhibited a strong tumour specific promoter methylation of ZAR1 in comparison to the normal control lung tissue. Demethylation treatment of various lung cancer cell lines reversed ZAR1 promoter hypermethylation and subsequently re-established ZAR1 expression. In addition, we could show the growth inhibitory potential of ZAR1 in lung cancer cell lines and cancer cell lines. Exogenous expression of ZAR1 inhibited colony formation and blocked cell cycle progression.
Conclusion: Our study shows for the first time the lung tumour specific epigenetic inactivation of ZAR1 due to DNA methylation of its CpG island promoter. Furthermore ZAR1 was characterised by the ability to block tumour growth through the inhibition of cell cycle progression in cancer cell lines. We propose that ZAR1 could serve as an epigenetically inactivated biomarker in lung cancer.
Keywords: lung cancer, ZAR1 (zygote arrest 1), tumour suppressor, DNA methylation, epigenetics.

The Hnrnp Raly Binds Rna and Transcriptionally Active Chromatin and Regulates RNA Transcription

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RALY is a member of the heterogeneous nuclear ribonucleoprotein family (hnRNP), a large family of RNA-binding proteins involved in many aspects of RNA metabolism. The role of RALY in RNA maturation is still not characterized, but RALY was observed to interact with the spliceosome and the Exon Junction Complex. Furthermore, RALY was found upregulated in different types of cancer.
In the first part of the study, we identify RALY interacting RNAs through RIP-seq analysis and assessed the role of RALY in gene expression. We demonstrate that RALY binds specific coding and non-coding RNAs and associates with translating mRNAs of mammalian cells. Among the identified transcripts, we focused on ANXA1 and H1FX mRNAs, respectively encoding for the markers of tumorigenesis Annexin A1 and histone H1X. We observed that the downregulation of RALY induced changes in the levels of H1FX and ANXA1 mRNAs and proteins in an opposite manner. We provide also evidence for a direct binding of RALY to the U-rich elements present within the 3´UTR of both transcripts. Finally, we observe a significant arrest in cell proliferation in RALY downregulated cells.
In the second part of the work, we show that RALY can interact with transcriptionally active chromatin in a transcription-dependent manner and that its downregulation causes a global decrease of RNA Polymerase II (RNAPII)-mediated transcription, without affecting RNAPII elongation rate. Through microarray analysis on RALY-downregulated HeLa cells, we detect an altered expression of numerous genes involved in transcription promotion and cell cycle regulation, including the E2F transcription factors family. Due to its relevant role as transcription factor, we focused on the proliferation-promoting factor E2F1. We demonstrated that the stability of E2F1 mRNA is reduced in cells lacking RALY expression, with a consequent reduction of E2F1 protein levels.
Taken together, our results characterize the RNA-binding and transcriptional control activity of RALY, successively highlight the importance of RALY expression for transcription and cell proliferation.

Targeting 'trunk' pathway mutations overrides secondary resistance to targeted therapies in colorectal cancers

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Human tumours, including colorectal cancers (CRC), develop through a branched rather than a linear pattern of evolution. Molecular alterations that occur early are present in every subclone (trunk mutations); on the contrary, different geographically separated regions of the tumour (subclones) carry heterogeneous mutations (branched mutations). Targeted therapies exert a strong selective pressure allowing pre-existing low frequency mutated subclones to expand and heterogeneously repopulate the neoplastic lesion, leading to treatment failure. We used CRC as a model system to test the hypothesis that genetic or pharmacological modulation of trunk oncogenic events may overcome the heterogeneous mechanisms of secondary resistance to targeted therapies. To test our assumption, we first generated CRC cells resistant to commonly used targeted agents including EGFR and BRAF inhibitors. We also exploited patient-derived CRC cell models obtained from individuals that responded and then relapsed to EGFR or BRAF blockade. Alterations in the Wnt pathway occur very early during CRC progression, are shared by all tumour cells when the disease spreads to distant organs, representing therefore the truncal CRC oncogenic event. We found that functional restoration of wildtype (WT) WNT signaling in CRC cells carrying defective APC alleles, inhibits proliferation and leads to rapid cell death regardless of the heterogeneity of resistance mechanisms. Analogously, restoration of WT APC impairs formation and growth of CRC patient-derived organoids, leading to caspase activation. On the other hand, we show that in CRC cells in which APC is functional but the APC-WNT pathway is constitutively active due to RSPO3 translocations or ZNRF3 mutations, pharmacological inhibition of WNT signaling is likewise effective at intercepting heterogeneous resistance mechanisms both in vitro and in vivo. Down-regulation of ß-catenin activity, through porcupine inhibitor LGK974, is sufficient and necessary to counteract/bypass constant hyperactivation of prosurvival MAPK pathway effectors, responsible of the inefficacy of targeted agents. Importantly, combination of targeted therapies and WNT pathway inhibitor LGK974 delay the development and outgrowth of heterogeneous resistant clones.In summary we show that in colorectal cancers, dependency from trunk oncogenic mutations is maintained in branched sub-clones that develop divergent mechanisms of resistance, therefore representing a promising clinical opportunity upon secondary resistance to targeted agents.

CRISPR-Cas9 editing to study the effect of germline variants on the genesis of early somatic events in Prostate cancer

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Prostate cancer (PCa), the second most common cancer among men, is a highly heritable molecularly and clinically heterogeneous disease. In this work we focused on the study of germline variants as putative trigger for early somatic events in PCa. Our previous in silico analysis identified a non-coding polymorphic regulatory element at the 7p14.3 locus associated with DNA repair and hormone regulated transcript levels and with an early recurrent prostate cancer specific somatic mutation in the Speckle-Type POZ protein (SPOP) gene (OR=5.54 for A allele, P=1.22e-08). ChIP-seq and luciferase assay analyses demonstrated that androgen receptor (AR) and CCAAT/Enhancer Binding Protein (C/EBP) beta (CEBPB) bind to the locus and that their transcriptional activity is allele-specific. To further investigate the enhancer activity of the 7p14.3 locus we deleted 731bp of this region in PC-3 cells with CRISPR-Cas9. RNA-seq was performed upon AR overexpression and/or CEBPB silencing. A strongly deregulation of transcriptome, confirmed by qPCR, was observed in edited versus non-edited cells (control cells) when compared to the same analysis in edited versus edited and control versus control (P=1e-04 and P=3e-05 Mann-Whitney test, respectively). Differential transcript expression analysis of edited versus non-edited cells showed significant concordance with genes predicted to physically interact with the 7p14.3 locus by previously generated Hi-C chromosome conformation capture data from benign prostate cells (Romanel, Garritano, et al Nat Comm, in press). To elucidate the specific role of the SNP in the regulatory region, we are now editing PC-3 cells (G/G) in order to generate isogenic cell lines with AG and AA genotype. To achieve the allele specific editing we are following two different strategies via CRISPR-Cas9. The first strategy includes transient transfection with pSpCas9, sg-RNA and chemical modified ssDNA-PS as a donor template for homologous recombination (HR). In the second strategy we will use a previously edited clone (deletion of 731bp of the region of interest), a specific sg-RNA encompassing the deletion, and a dsDNA in order to perform the precise insertion of the deleted region via non homologous end joining (NHEJ) repair. We will next assess if DNA damage response is differentially perturbed in the presence of the alternative genotypes. This work is a proof of concept of germline predisposition to molecularly distinct cancer subclasses and has the potential to nominate new mechanisms of cancer development.

SiC/SiOx core/shell nanowires functionalized with porphyrin derivatives: new multifunctional photosensitizer for photodynamic therapy

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The photodynamic therapy (PDT) is a non surgical therapeutical approach, commonly used in dermatological oncology, capable to kill the tumoral cells inducing a localized tissue necrosis through a chemical process light-mediated. The photodynamic reaction between a photosensitizer molecule (porphyrin, chlorine, bacteriochlorin) and the light in the visible range is oxygen dependent, giving rise to reactive oxygen species, i.e. singlet oxygen, which induces cell death by apoptosis and microvascular damage [1]. In the last years, the interest toward this medical approach to treat deep tumors is increasing, reflecting the necessity to develop more effective approaches in order to implement or even replace the common chemotherapy and radiotherapy techniques in the treatment of disabling diseases [2].
In the present work, we show a highly promising multi-functional and multi-excitable nanohybrid material, i.e. SiC/SiOx core-shell nanowires (NWs) functionalized with porphyrin derivatives [3], for a X-ray and IR induced PDT, in order to reach both deep and shallow tumors. NWs have been functionalized via wet-chemistry with a tetraphenylamino porphyrin (TAPP), whose amino (NH2) ending groups can be exploited to covalently bond the folic acid, in order to specifically target cancer cells that overexpress the folate receptor. Herein, the synthesis and char-

acterization of the optical and electronic properties of NWs + TAPP will be presented, together with preliminary cytotoxicity biological tests and the evaluation of the induction of oxidative stress, i.e. singlet oxygen generation. Our findings provide new insights in developing effective strategies to solution-based functionalization of SiC/SiOx core/shell nanowires for application in biomedical field.

Integrative Transcriptome-Wide Analysis of RNA Binding and Splicing Reveals Complex Loss and Gain of Function Alterations by SRSF2 P95 Mutations in Myeloid Malignancies

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Recurrent mutations in the core splicing factor Serine Arginine Rich Splicing Factor 2 (SRSF2) are associated with poor clinical outcomes in myelodysplastic syndromes (MDS) and secondary acute myeloid leukemia (sAML). SRSF2 mutations almost exclusively change proline 95 (P95), within the C-terminal region of the RNA binding domain, into histidine, leucine, or arginine. Despite their high frequency, the mechanisms by which SRSF2 mutations promote MDS and AML are not yet known. Here we use RNA crosslinking and immunoprecipitation (HITS-CLIP) coupled with RNA deep sequencing (RNA-seq) to determine at a transcriptome wide level how SRSF2 mutations affect RNA binding and splicing in vivo in a syngeneic hematopoietic cell context. SRSF2 P95 mutations skew RNA consensus recognition and binding in vivo confirming our previous in vitro findings that mutant SRSF2 exhibits higher affinity for the CCNG than the GGNG consensus motif. Surprisingly, the majority of differential binding events do not translate into differential splicing and only a subset of differential binding events dominated by altered recognition of the CCNG- versus the GGNG- consensus motifs result in alternative splicing of exons, while others result in intron retention and alternative splice site choices. Importantly, differentially bound and alternatively spliced $SRSF2^{P95H}$ targets are enriched in RNA processing and splicing genes, including several members of the hnRNP and SR families of proteins, creating a "hyper-splicing" phenotype wherein mutation of a single splicing factor leads to widespread modifications in multiple RNA processing and splicing proteins. The complexity of splicing dysregulation in SRSF2 mutant cells has important implications for targeting splicing for therapeutic purposes.

2018 Pezcoller Foundation–AACR International Award For Cancer Research

AWARD SUMMARY

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

- 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; and
- 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.
The award consists of an unrestricted honorarium of €75,000, a commemorative plaque, and full support to the winner and a guest to attend the AACR Annual Meeting. The winner of the 21st Annual Pezcoller Foundation-AACR International Award for Cancer Research will give an award lecture at the AACR Annual Meeting 2018 in Chicago, IL, USA (April 14-18, 2018).

ELIGIBILITY

- Eligible candidates are cancer researchers affiliated with institutions in academia, industry, or government that are involved in cancer research, cancer medicine, or cancer-related biomedical science anywhere in the world.
- Institutions or organizations are not eligible for the award.
- Receipt of other major awards does not preclude a candidate from eligibility for the award.
- No regard shall be given to race, gender, nationality, geographic location, or religious or political views.

NOMINATION PROCESS

Nominations may be made by any scientist, whether an AACR member or nonmember, who is now or has been affiliated with any institution involved in cancer research, cancer medicine, or cancer-related biomedical science. Candidates may not nominate themselves.
Nominators are asked to maintain the confidentiality of the nomination process and to refrain from informing the candidate about the nomination. There is no restriction on the number of candidates that may be nominated by any institution or individual.
There is no restriction on the number of candidates that may be nominated by any individual scientist. There is no restriction on the number of nominators that may write nomination letters or that may sign a single nomination letter on behalf of a candidate.
Nominations must be emailed to awards@aacr.org no later than 4 p.m. U.S. ET on Wednesday, August 9, 2017. Paper nominations cannot be accepted. Full nomination instructions and program guidelines are available through the link below.
www.aacr.org/scientificawards

NOMINATION MATERIALS

1) Nomination Letter. The letter must:

- be addressed to the Selection Committee; be written in English; and not exceed 1,000 words;
- specify the AACR Award for which the candidate is being nominated;
- contain a concise description of the candidate's major scientific discovery in basic cancer research or significant contributions to translational cancer research, and the impact of these

accomplishments on the field, with publications supporting these accomplishments directly referenced within the letter;
- contain a concise description of the candidate's ongoing work which holds promise for continued substantive contributions to progress in the field of cancer; and
- be signed with a handwritten signature by the nominator.

If more than one candidate is nominated to share the Award, the nomination letter must clearly outline how the work of the individuals is closely related in subject matter and warrants a joint nomination.

2) Candidate's CV.
   The candidate's curriculum vitae in English, including a complete list of the candidate's publications.
3) Summary Statement.
   A statement of no more than 50 words summarizing the candidate's research accomplishments for which he or she is being nominated.

SELECTION PROCESS

Candidates for the award will be considered by a prestigious international Selection Committee of renowned cancer leaders appointed by the president of the AACR in consultation with 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.

RESPONSIBILITIES OF THE AWARD RECIPIENT

The recipient will also present the Pezcoller Foundation-AACR International Award for Cancer Research Lecture, both at the University of Padua and at the University of Trento in Italy, just prior to the official Award ceremony in Trento, in early May 2018. Remarks to be made during the ceremony must be delivered to the Pezcoller Foundation at least four weeks prior to allow sufficient time for translation into Italian. Should the recipient be unable to participate in either event, the award must be forfeited and may be presented instead to the alternate.
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.

CHANGES TO THE NOMINATION

Withdrawal of nomination: Please advise the AACR promptly, in writing, should you decide to withdraw your nomination for any reason. Your letter (or e-mail) should include the nominator's name and institution, the title of the Award and name of the candidate, and the reason for withdrawal.

INQUIRIES

Questions regarding eligibility of a candidate may be directed to the AACR Scientific Achievement Awards office at *awards@aacr.org* or by calling (215) 446-7128, prior to submitting an application. Inquiries about the "Program Guidelines and Nomination Instructions" may be directed to Ms. Linda Stokes.

2018 Pezcoller Foundation–EACR Cancer Researcher Award

Celebrating academic excellence and achievements in the field of cancer research

ABOUT THE AWARD AND AWARD LECTURE

The Pezcoller Foundation – EACR Cancer Researcher Award celebrates academic excellence and achievements in the field of cancer research. The award is presented biennially to a researcher of excellence with no more than 15 years post-doctoral experience (or equivalent degree), with at least five years spent in Europe.
The winner gives the prestigious Pezcoller Foundation – EACR Cancer Researcher Award Lecture at the Biennial EACR Congress. The next award will be presented at the EACR25 Congress to be held from 30 June to 03 July 2018 in Amsterdam, Netherlands.
The award winner will receive a €10.000 honorarium, a free registration for the EACR Congress, plus accommodation and travel costs as an invited speaker.

CALL FOR NOMINATIONS

(see also the website: www.pezcoller.it/en/awards/pezcoller-eacr-award.html - www.pezcoller.it/en/awards/pezcoller-eacr-award.html)
We invite you to nominate a cancer researcher who has demonstrated academic excellence and achievements in the field of cancer research. Self-nominations cannot be accepted.
The nominee should:

- Have no more than 15 years post-doctoral experience (or equivalent degree)
- Presently be employed in a European institution
- Have a record of employment in Europe of at least five years

DOCUMENTS TO SEND:

Completed Nomination Form

- A one page letter of support giving the reasons for the nomination
- A concise curriculum vitae from the nominee
- Details of the nominee's relevant publications

HOW TO SUBMIT YOUR NOMINATION:

Please email your completed nomination form and accompanying documents to Laura Strachan at the EACR Secretariat (mail to: l.strachan@nottingham.ac.uk) by 03 January 2018.

Deadline for receipt of nominations: 03 January 2018

NOMINATION FORM MUST INCLUDE

Name of Proposer, Work address, Phone, Email

Name of Award Nominee, Work address, Phone, Email, Date Doctorate Awarded

Letter of Support giving the reasons for the Nomination, using the following headings

1. What have been the most noteworthy achievements of the Nominee in the field of cancer research?
2. How has the Nominee's research contributed to the ultimate goal of preventing loss of life to cancer?
3. What research is the Nominee currently undertaking and what is its significance?

Maximum of 1 page in total.

2018 Scholar-In-Training Awards

The AACR is proud to offer Scholar-in-Training Awards to enable the participation of meritorious early-career scientists at the Annual Meeting 2017. Since its inception in 1986, the AACR Annual Meeting Scholar-in-Training Award program has provided more than 4,400 grants to young investigators and has received support from more than 50 cancer research foundations, corporations, individuals and other organizations dedicated to the fight against cancer. This year, fourteen organizations or individuals generously provided the funding to support this program. To commemorate the AACR's 110th Anniversary, this funding recognizes 110 Scholar-in-Training Awardees.

2018 AACR-PEZCOLLER FOUNDATION SCHOLAR-IN-TRAINING AWARDS
The Pezcoller Foundation supports these awards to enhance participation in the programs and activities of the AACR by early-career investigators residing in Europe and to provide these outstanding Scholar-in-Training Awardees with an opportunity to share their research findings with the international cancer research community at the AACR Annual Meeting.

Picture:
2017 Scholar-In-Training Awardees with President Galligioni in Washington DC, April 1, 2017

Topics of all Pezcoller Foundation Symposia

Trento 19 – 21 June 1989
DRUG RESISTANCE: MECHANISMS AND REVERSAL

Rovereto: 11 – 13 June 1990
THE THERAPEUTIC IMPLICATIONS OF THE MOLECULAR BIOLOGY OF BREAST CANCER

Trento: 5 – 7 June 1991
TUMOR SUPPRESSOR GENES

Rovereto: 24 – 26 June 1992
ADHESION MOLECULES: CELLULAR RECOGNITION MECHANISMS

Trento: 9 – 11 June 1993
APOPTOSI

Rovereto: 29 June – 1 July 1994
NORMAL AND MALIGNANT HEMATOPOIESIS: NEW ADVANCES

Trento: 14 – 16 June 1995
CANCER GENES. FUNCTIONAL ASPECTS

Trento: 17 – 19 June 1996
GENOMIC INSTABILITY AND IMMORTALITY IN CANCER

Rovereto: 4 – 7 June 1997
THE BIOLOGY OF TUMORS

Trento: 29 June – 1 July 1998
THE GENETICS OF CANCER SUSCEPTIBILITY

Rovereto: 5 – 7 June 1999
MOLECULAR HORIZONS IN CANCER THERAPEUTICS

Trento: 1 – 3 June 2000
SIGNALING CROSS-TALKS IN CANCER CELLS

Rovereto: 31 May – 2 June 2001
FOCUSING ANALYTICAL TOOLS ON COMPLEXITY IN CANCER

Trento: 30 maggio – 1 June 2002
THE NOVEL DICHOTOMY OF IMMUNE INTERACTIONS WITH TUMORS

Rovereto: 12 – 14 June 2003
MOLECULAR IN VIVO VISUALISATION OF CANCER CELLS

Trento: 10 – 12 June 2004
STEM CELLS AND EPIGENESIS IN CANCER

Trento: 16 – 18 June 2005
MOLECULAR UNDERSTANDING OF SOLID TUMORS

Trento: 27 – 29 June 2006
TUMOR MICROENVIRONMENT: HETEROTYPIC INTERACTIONS

Trento: 14 – 16 June 2007
HYPOTHESIS DRIVEN CLINICAL INVESTIGATION IN CANCER

Trento: 11 – 13 June 2008
MOLECULAR BIOLOGY OF CANCER: 20 YEARS OF PROGRESS PUNCTUATED BY THE PEZCOLLER SYMPOSIA

Trento: 11 – 13 June 2009
UNCONVENTIONAL THERAPEUTIC TARGETS IN CANCER

Trento: 10 – 12 June 2010
RNA BIOLOGY AND CANCER

Trento: 16 – 18 June 2011
ENGINEERING AND NANOTECHNOLOGY IN CANCER

Trento: 14 – 16 June 2012
MOLECULAR BASIS FOR RESISTANCE TO TARGETED AGENTS

Trento: 20 – 22 June 2013
METABOLISM AND TUMORIGENESIS

Trento: 19 – 21 June 2014
CANCERS DRIVEN BY HORMONES

Trento: 18 – 20 June 2015
CHALLENGING ROADBLOCKS TO CANCER CURES

Trento: 20 – 21 June 2016
INITIAL STEPS ON THE ROUTE TO TOMORIGENESIS

Trento: 22 – 23 June 2017
BUILDING NEW BRIDGES BETWEEN BASIC AND CANCER SCIENCE

The Pezcoller Foundation

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