The Pezcoller Foundation

Journal

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- Call for nomination Scholar-In-Training Awards

News from the
Pezcoller Foundation World

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Picture on front page:
2019 Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research winner:
Prof. Alberto Mantovani

June 2019
Editorial

First of all we are reporting with a great pleasure that the recipient of 2019 Pezcoller Foundation – AACR International Award for Extraordinary Achievement in Cancer research is Prof. Alberto Mantovani, scientific Director at the Istituto Clinico Humanitas and President of the Fondazione Humanitas for Research in Milan.
Mantovani was chosen among 21 nominees, by the Selection Committee chaired by Michael B. Kastan MD, PhD, who met in Philadelphia on November 30 2018. The other members of the Committee were: Mariano Barbacid, PhD, FAACR; Nina Bhardwaj, MD, PhD; Carlos M. Caldas MD; Elisabetta Dejana PhD; Joe W. Gray PhD FAACR; Lorenzo Moretta MD; David A. Tuveson MD, PhD; Jennifer Wargo MD.
The Award to Mantovani was formally announced at the AACR annual Meeting in Atlanta, on Sunday, March 31, 2019, where he delivered his award lecture. The award prize was then officially presented to Mantovani by the President of the Pezcoller Foundation Prof. Enzo Galligioni, MD on May 11 in the Teatro Sociale of Trento, during the Award Ceremony at the presence of the of Elizabeth M. Jaffee, MD Past President of AACR, Margaret Foti PhD, MD, CEO of AACR, Gios Bernardi MD, President Emeritus of the Pezcoller Foundation and large part of the Trento Community.
Before the Award Ceremony Mantovani gave a Lectio Magistralis at the at the Department of Molecular Medicine of the University of Padova and a second lecture at the CIBIO (Centre for Integrative Biology) of the University of Trento.
In the Award Ceremony, President Galligioni highlighted the main points of Mantovani's researches and discoveries.
"Dr. Mantovani is an eminent physician-scientist, he said, a leader in the field of tumor immunology for decades. Presently Scientific Director at the Istituto Clinico Humanitas and Full Professor of General Pathology at the Humanitas University, Mantovani was for many years Head of the Department of Immunology and Cell Biology, Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy. Furthermore he holds the Chair of Inflammation and Therapeutic Innovation, at the Queen Mary University, London, UK. By identifying macrophages in tumors as corrupted policemen promoting cancer progression, and by discovering relevant genes and functions, Alberto Mantovani highlighted the role of inflammation and immunity in the tumor microenvironment, a paradigm shift fundamental for the development of tumor immunology and immunotherapy. In addition, his researches included Chemokines (CCL2), IL-1/Toll-like receptors (TLR) and Humoral innate immunity (PTX3)."
"Alberto Mantovani is the most cited Italian scientist working in Italy (Scopus, Web of Science, Google scholar) and a bibliometric analysis indicates that he is one of the 10 most quoted immunologists in the world.

He currently serves as president of International Union of Immunological Societies (IUIS) and is past president of the Italian Society of Immu-

nology and the International Cytokine Society. He serves as Editor-in-Chief, Seminars in Immunology and senior editor of Cancer Immunology Research

Mantovani is actively involved in the fostering of science and scientific policies in Italy and cofounded the association " Gruppo2003" of Italian highly cited scientists (http://www.gruppo2003.it) and the website http://www.scienzainrete.it. "

During the ceremony as President Emeritus I made some remarks where I tried to point out the human and social personality of the winner, referring to Mantovani's publications aimed to young scholars:
…"Alberto Mantovani want to convey the typical sense of adventure of science. He recommends a passion for the work, the modesty of learning from everyone, especially from collaborators and technicians…..
Mantovani, although deeply engaged on scientific researches, never forget his being a physician and that the ultimate concern, not only for a physician but also for any scientific researcher, must be the patient, from the bench to the hospital bed…"

In addition to the Award, I would like to mention that on the next June 17-18, we will hold our 31st Pezcoller Symposium. In this year's Symposium, the focus will be on the newest insights into the processes that give rise to cancer tissue and its persistence. Cancers are, in essence, products of organ and tissue development gone wrong. In fact, many critical cellular and organ-based operations from genome integrity, the behavior of one's own microbiome, oxygen utilization and cell metabolism, orderly tissue and organ anatomy, disciplined cell behavior, and the immune response may become disordered in the interest of tumor development. Also included will be recent findings that have the potential to influence clinical cancer therapeutics in novel ways. In the next pages you will find the program and the faculty.
Finally I'm pleased to particularly remember, among all other activities of the Pezcoller Foundation, the Agreement between the Pezcoller Foundation and the Museum of Science of Trento (MUSE), aimed to mutually collaborate to the diffusion of the scientific awareness among general population.

Gios Bernardi
Editor

31th Pezcoller Symposium

CANCER AS A CORRUPTED TISSUE

Trento, Italy • June 17 - 18, 2019

PROGRAM

MONDAY JUNE 17, 2019

8.00 Registration
8.30 Enzo Galligioni Welcome
8.40 David Livingston Focus & Goals

The Enrico Mihich Lecture
Chair: David Livingston
8.50 Sean Morrison
The metabolic regulation of cancer progression
9.35 Discussion

Session 1 - The Microbiome and Cancer
Chair: Maria Rescigno
09.50 Romina Goldszmid
Microbiota as a key modulator of the tumor microenvironment
10.15 Discussion
10.30 Jennifer Wargo
The role of the gut and tumor microbiome in response to cancer therapy
10.55 Discussion
11.10 Coffee Break

Session 2 - Cancer Metastasis
Chair: Stefano Piccolo
11.30 Christoph Klein
From early dissemination to manifest metastasis:Theoretical and practical challenges of an unsolved problem.
11.55 Discussion
12.10 Mikala Egeblad
Neutrophil extracellular traps generated during inflammation drive cancer cell proliferation and a pro-metastatic microenvironment
12.35 Discussion
12.50 Lunch

Session 3 - Genome Order and Disorder
Chair: Fabrizio D'Adda di Fagagna
14.00 Roger Greenberg
The necessity of noncanonical DNA damage reponses for human cancer
14.25 Discussion
14.40 Daniel Durocher
Charting the genetic architecture of the DNA damage response
15.05 Discussion
15.20 Simon Boulton
Maintaining telomeres in ALT cancers
15.45 Discussion
16.00 Fabrizio D'Adda di Fagagna
The role of non coding RNA in genome intgrity
16.25 Discussion
16.40 Poster View
17.30 Adjourn

19.30 Symposium Dinner

TUESDAY JUNE 18, 2019

Session 4 - Tissue and Organ Formation
Chair: Cathrin Brisken
08.30 Cedric Blanpain
Cancer cell of origin, tumor heterogeneity and EMT transitional state
08.55 Discussion
09.10 Stefano Piccolo
Tumors as wounds that never heals: a YAP/TAZ perspective
09.35 Discussion
09.50 Nikolaus Rajewsky
Principles of Gene Regulation by Single-Cell RNA Sequencing
10.15 *Discussion*
10.30 Coffee Break

Session 5 - Cancer and Metabolism
Chair: Massimo Loda
10.50 William Sellers
The Next Generation Characterization of the Cancer Cell Line Encyclopedia - implications for targeting metabolic alterations in cancer
11.15 Discussion
11.30 Matt Vander Heiden
Metabolic limitations of cancer progression
11.55 Discussion
12.10 Lunch

Session 6 - Cancer and Immunology
Chair: Alberto Bardelli
13.20 Andrea Ablasser
Expanding roles of cGAS in immunity and inflammation
13.45 Discussion
14.00 Catherine Wu
Addressing cancer heterogeneity: personalized cancer vaccines
14.25 Discussion
14.40 Poster Discussion and Poster Presentation (led by Massimo Loda)
15.40 David Livingston
Concluding Remarks

INVITED PARTECIPANTS

FACULTY

- **Ablasser Andrea**
  Swiss Federal Institute of Technology, Lausanne, CH
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  Université Libre de Bruxelles, BE
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- **Wu Catherine**
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31th Pezcoller Symposium

Cancer as a corrupted tissue

Trento, Italy, June 17-18, 2019

ABSTRACTS OF ORAL PRESENTATIONS

The metabolic regulation of melanoma metastasis

Sean J. Morrison

Investigator, Howard Hughes Medical Institute
Professor, Children's Research Institute
University of Texas Southwestern Medical Center

To study the mechanisms that regulate distant metastasis by melanoma cells, we developed a xenograft assay in which small numbers of cells from primary human melanomas engraft in NOD/SCID IL2Rγ^{null} (NSG) mice and spontaneously metastasize (Nature 456:593). We have now banked and characterized the metastatic potential of melanomas from more than 150 patients in this assay and observed a correlation between spontaneous metastasis in NSG mice and metastasis in patients: melanomas that are destined to form distant metastases in patients spontaneously form distant metastases in the mice ('efficient' metastasizers), while melanomas that do not form distant metastases in patients metastasize more slowly in mice ('inefficient' metastasizers) (Science Translational Medicine 4:159ra149). This demonstrates there are intrinsic differences among melanomas from different patients in their potential to metastasize.

Using this assay, we discovered that metastasizing melanoma cells experience high levels of oxidative stress and that the rare melanoma cells that successfully metastasize undergo reversible metabolic changes that increase their capacity to survive oxidative stress, including increased dependence on the folate pathway (Nature 527:186). Treatment of xenografted mice with anti-oxidants increases the frequency of circulating melanoma cells in the blood as well as metastatic disease burden, demonstrating that oxidative stress is one factor that limits distant metastasis. These data suggest that rather than treating cancer with anti-oxidants, which have promoted cancer initiation and progression in large clinical trials, that we should devise pro-oxidant therapies that inhibit cancer progression by exacerbating oxidative stress. A major focus of my lab is understanding the mechanisms cancer cells use to cope with oxidative stress, with a view to developing therapies that inhibit these mechanisms.

A recent discovery is that metabolic differences among melanomas, and even among melanoma cells from the same tumor, confer differences in metastatic potential by modulating the ability to cope with oxidative stress. We observe differences in Monocarboxylate Transporter 1 (MCT1) expression among melanomas that correlate with differences in metastatic potential and differences in survival among patients. The main physiological function of MCT1 is to transport lactate in and out of cells. We found that MCT1 function promotes melanoma metastasis by reducing oxidative stress. Efficiently and inefficiently metastasizing patient-derived xenografts similarly metabolized isotopically-labeled glucose and glutamine; however, efficient metastasizers took up more lactate. Efficient metastasizers expressed higher levels of MCT1 and MCT1 inhibition reduced lactate uptake in vivo. MCT1 inhibition had little effect on the growth of primary subcutaneous tumors but substantially depleted circulating melanoma cells and reduced metastatic disease burden. MCT1 inhibition suppressed the oxidative pentose phosphate pathway and increased reactive oxygen species (ROS) levels. Anti-oxidant treatment rescued the effect of MCT1 inhibition on metastasis. MCT1^{high} and MCT1$^{-/low}$ cells from the same melanomas had similar capacities to form subcutaneous tumors, but MCT1^{high} cells formed more metastases after intravenous injection. Metabolic differences among cancer cells htus confer differences in metastatic potential as metastasizing cells depend upon MCT1 to manage oxidative stress. MCT1 inhibition is thus a pro-oxidant therapy

that might impair disease progression in patients with high risk stage II and III melanomas.

Microbiota as a key modulator of the tumor microenvironment

Romina S. Goldszmid

Inflammatory Cell Dynamics Section, Cancer and Inflammation Program, CCR, NCI, Bethesda, MD.

Cancer has historically been viewed as a disease determined by genetic and environmental factors, however, it is now clear that inflammation affects all stages of the disease: initiation, progression and metastasis formation. The inflamed tumor microenvironment is in part sustained by infiltrating myeloid cells such as macrophages, monocytes, dendritic cells, and neutrophils. In cancer, as in infection, these cells can induce adaptive immune responses, but in cancer they mainly promote the tumor's immune evasion, progression, and metastasis. Moreover, the role of distinct myeloid cell populations in response to cancer therapy remains unclear. We and others have previously uncovered a role for commensal microbes in controlling the response to cancer immuno- and chemotherapy. In this presentation, we will discuss the role of the microbiota in regulating the composition and function of the myeloid cell compartment in the tumor microenvironment and the role of these cells in the response to cancer therapy. Targeting myeloid cells in the tumor microenvironment represents a powerful approach to manipulate the outcome of cancer therapy; therefore, a clear understanding of their regulation and functional organization may lead to rational novel cancer immunotherapeutic approaches.

The Role of the Gut and Tumor Microbiome in Response to Cancer Therapy

Wargo Jennifer

MD Anderson Cancer Center, Houston, TX
(missing)

From early dissemination to manifest metastasis:Theoretical and practical challenges of an unsolved problem.

Christoph A. Klein

Experimental Medicine and Therapy Research, University of Regensburg and Fraunhofer Institute of Toxicology and Experimental Medicine, Regensburg, Germany

Mutation, selection and adaptation are - by convention - thought to occur primarily within, and to a lesser degree, outside the primary tumour. However, we previously noted in breast cancer and melanoma that metastatic dissemination occurs often early and that advanced tumour stages seed relatively fewer cells. This indicates that metastatic founder cells may lodge and evolve considerable periods of time outside the primary tumour. However, it generates a plethora of questions: How do cancer cells survive at distant sites? From which disseminated cancer cells descent metastatic colonies? Can genomic evolution occur during dormancy in quiescent cells? Does the environment trigger progression of early-disseminated cancer cells or do late-arriving cancer cells take over incipient metastatic colonies? We try to address these questions by analysing single disseminated cancer cells isolated at various time points of disease. Our data suggest that available models and endpoint analyses – such as the comparison of primary tumours and metastases - are insufficient to reflect disease dynamics in patients. Therefore, we have to carefully consider the clinical and evolutionary stage of an individual disease when we try to address the underlying mechanisms.

Neutrophil extracellular traps produced during inflammation awakens dormant cancer cells

Mikela Egeblad

Cold Spring Harbor Laboratory
Cold Spring Harbor, NY

Every year, ~40,000 women in the US who had been successfully treated for primary breast cancer nonetheless have metastatic recurrence. Metastasis requires four key steps: 1) tumor cells leave the tumor; 2) tumor cells enter a new tissue; 3) disseminated tumor cells (DTCs) re-initiate proliferation; and 4) an inflammatory microenvironment is established to support the growing metastasis. Steps 1-2 are rarely amenable to intervention, as they usually occur before the primary tumor is detected and treated. However, we may be able to target steps 3-4 to ultimately reduce the occurrence of metastasis and its associated mortality.
Our research on neutrophil extracellular traps (NETs) has provided novel insights into how inflammation can cause a) DTCs to re-initiate

proliferation, and b) result in a metastasis-supporting inflammatory microenvironment. NETs are released by neutrophils to the extracellular space in response to infections and inflammation, and they consist of meshes of genomic DNA with ~40 associated proteins, including proteases and high mobility group box 1 (HMGB1). We recently reported that lung inflammation, induced by either tobacco smoke exposure or bacterial lipopolysaccharide (LPS), drove quiescent DTCs to re-initiate proliferation. This resulted in metastases that killed mice with experimental lung inflammation in <4 weeks, while control mice survived >8 months without metastasis. Using intravital imaging, we found that DTCs that exit quiescence after lung inflammation are surrounded by NETs. We further discovered that proteases on NETs cleave the basement membrane protein laminin, generating a b1-integrin-activating epitope that caused quiescent DTCs to re-initiate proliferation. Thus, NET-associated proteases drive step 3 of the metastatic process. Our new data now show NETs also drive a complex, feed-forward-loop between NETs, macrophages, and fibroblasts leading to the creation of a highly inflammatory microenvironment. We propose that this NET-induced microenvironment is critical for sustaining the proliferation and survival of the metastasizing cancer cells. By dissecting the mechanisms by which NETs promote re-initiation of proliferation of quiescent DTCs and generation of a sustained inflammatory microenvironment, we have identified several points of potential intervention to prevent dormant cell awakening and prolong the survival of cancer patients.

A novel chromatin directed vulnerability in BRCA mutated cancers

Priyanka Verma, Junwei Shi and Roger A Greenberg

Department of Cancer Biology, Basser Center for BRCA, Perelman School of Medicine, University of Pennsylvania, 421 Curie Boulevard, Philadelphia, Pennsylvania 19104, USA.

The DNA damage response encompasses acute and delayed signaling events that culminate in repair of genomic lesions and the production of inflammatory cytokines that attract immune responses. This multifaceted DNA damage response is critical to cancer etiology and response to therapy, particularly in light of the realization that DNA damaging therapies can synergize with immune checkpoint blockade to eradicate tumors. A central aspect of the DNA damage response is the induction of myriad homology directed DNA repair mechanisms that use templated DNA synthesis to execute either high fidelity restoration of lesions to their ground state or inherently error prone mechanisms that result in loss of genome integrity. This prominence of repair mechanism utilization is illustrated in the setting of homologous recombination deficiency due to mutation within a network of genes centered around the breast and ovarian cancer suppressor proteins. This BRCA network is required for high fidelity DNA repair by homologous recombination. While deficiency in this canonical homology directed DNA repair pathway is confers cancer susceptibility, it also creates vulnerability to agents that target orthogonal repair mechanisms. Poly(ADP) Ribose Polymerase (PARP) inhibitors are approved to treat homologous recombination deficiency breast and ovarian cancer, with demonstrated improvements in progression free survival in tumors with BRCA mutations. The efficacy of PARP inhibitors depends on expression of the PARP1 enzyme, which becomes trapped on chromatin and necessitates intact BRCA dependent HR for repair in S-phase. In the absence of BRCA1, toxic use of nohomologous endjoining repair mechanisms creates dicentric chromosomes that result in a loss of viability upon passage through mitosis. The intersection of these repair pathways in PARPi response leads to resistance mechanisms that entail either loss of PARP1 expression or deficiency in nohomologous endjoining repair. These are thought to account, at least in part, for failure of ~50% of BRCA mutated cancers to respond to PARPi. Ideally, additional targets could be identified that circumvent PARPi resistance regardless of mechanism and restore efficacy in BRCA mutated cancers.
This presentation will describe our unpublished results that implicate the chromatin remodeling protein CHD1L as a novel therapeutic target in BRCA mutated cancers. Functional domain directed CRISPR-Cas9 screens were used to identify CHD1L as a vulnerability in BRCA1 and BRCA2 mutated cells. Our findings demonstrate that CHD1L loss is synthetic lethal in combination with mutation to either BRCA1 or BRCA2, while conferring extreme PARPi hypersensitivity regardless of resistance mechanism. Models to conceptualize these findings will be presented as well as their implications for harnessing DNA damage responses to enhance therapy in homologous recombination deficient cancers.

Charting the Genetic Architecture of the DNA Damage Response

Durocher Daniel

Lunenfeld-Tannenbaum Research Institute
Mount Sinai Hospital, Toronto, Canada

The orchestration of DNA repair is of fundamental importance to the maintenance of genomic integrity and tumor suppression. DNA damage must be detected in the context of the varied chromatin landscape, its presence must be communicated throughout the cell to alter many ongoing processes, and the machinery that will mend the lesion must be recruited to the damage site. In my presentation, I will discuss our recent efforts in mapping genome maintenance pathways using genome-scale CRISPR/Cas9 screens in human cells. I will highlight how these screens can be used to identify new genome stability factors, characterize drug responses and provide new insights into the genetic architecture of the genome stability network by identifying potentially actionable synthetic lethal genetic interactions. I will argue that somatic genetic screens in human cells are powerful tools to study the DNA damage response and its integration within other cellular pathways.

Maintaining telomeres in ALT cancers

Simon J. Boulton

Tha Francis Crick Institute, London, UK

In non-malignant somatic cells, telomeres undergo progressive shortening after DNA replication, which eventually results in replicative senescence and checkpoint-driven cell death. In contrast, tumour cells achieve replicative immortality by activating one of two distinct telomere maintenance mechanisms; cancers either re-express telomerase or induce Alternative Lengthening of Telomeres (ALT), which involves recombination between telomeres. ALT is utilised in 10-15% of all tumours and is associated with poor prognosis due to their complex karyotype and lack of targeted therapies. Currently, the mechanisms underpinning ALT induction and maintenance remain poorly understood. Here, we show that ALT recombination requires coordinate regulation of the SMX resolvasome and BTR complex to ensure the appropriate balance of resolution and dissolution activities at recombining telomeres. Critical to this control is SLX4IP, which accumulates specifically at ALT telomeres and interacts with SLX4, XPF and BLM. Loss of SLX4IP results in a hyper-ALT phenotype that is incompatible with cell viability following concomitant loss of SLX4. Inactivation of BLM is sufficient to rescue toxic telomere aggregation and synthetic lethality in this context, suggesting that SLX4IP favours SMX-dependent resolution by antagonising promiscuous BLM activity during ALT recombination. The clinical importance of SLX4IP in the ALT process is highlighted by its inactivation in a subset of ALT positive osteosarcomas. Collectively, our findings uncover an SLX4IP-dependent regulatory mechanism critical for telomere maintenance in ALT cancer cells.

The role of non coding RNA in genome integrity

Fabrizio d'Adda di Fagagna1,2

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The DNA damage response (DDR) is a signaling pathway 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).
More recently, we reported that DNA double-strand breaks (DSBs) trigger the synthesis of damage-induced long non-coding RNA (dilncRNA) that can be processed into shorter DNA damage response RNAs (DDRNAs) (Francia et al Nature 2012). Such transcripts are essential for full DDR activation and their inhibition by antisense oligonucleotides (ASO) allows site-specific inhibition of DNA damage signalling and repair (Michelini et al Nature Cell Biology 2017; D'Alessandro et al Nature Communications).
We will discuss our progress in understanding the mechanisms of dilncRNA synthesis by the different factors associated with the RNA pol II holoenzyme in cells and in a novel reconstituted in vitro system, challenging the distinction between a DNA lesion and a transcriptional promoter.In addition, we will show how sequence-specific DDR inhibition can be achieved in vivo by targeting dilncRNA and DDRNA and we will discuss its potential therapeutic uses.

Cancer cell of origin, tumor heterogeneity and EMT transitional state

Cédric Blanpain

WELBIO, Laboratory of Stem Cells and Cancer, Université Libre de Bruxelles (ULB), 1070 Bruxelles, Belgium

Different theories have been proposed to explain tumour heterogeneity including the cancer cell of origin. Here, we developed new genetically engineered mouse models allowing lineage tracing together with oncogenic activation in different cell lineages of the skin epidermis and the mammary gland and assessed whether

the cancer cell of origin controls tumour heterogeneity. I will present evidence that the cancer cell of origin controls tumour heterogeneity and the underlying molecular mechanisms by which the cell of origin control tumor differentiation, stemness, EMT, resistance to therapy and metastasis. These results have important implications for our understanding of the mechanisms controlling tumor heterogeneity and the development of new strategies to block tumor initiation, progression, metastasis and resistance to therapy. This work is supported by the ERC, WELBIO, FNRS, TELEVIE, and the Fondation Baillet-Latour.

Tumors As Wounds That Never Heal: A YAP/TAZ Perspective

Stefano Piccolo

Dip. Medicina Molecolare Università di Padova

Enhanced YAP/TAZ activity is emerging as common trait of multiple solid tumors in humans. Strikingly, in mouse models, adult organs lacking YAP/TAZ are unable to develop tumors without overt side effects for normal tissue homeostasis, making YAP/TAZ prime candidates for cancer therapy. That said, YAP/TAZ have physiological functions during tissue repair, being essential for organ regeneration after injury. YAP/TAZ are typically inactive in normal tissues but potently induced by mechanical and physical cues that the cell receives from its microenvironment, such as extracellular matrix stiffness and topology, and 3D architectural features of the tissue. I will focus on new discoveries on the mechanisms by which YAP/TAZ are controlled by mechanotransduction, and on YAP/TAZ as reprogramming factors in epithelial cells. Indeed, in normal tissues YAP/TAZ activation turns more differentiated normal cells into cells endowed with stem-like properties; in tumors, YAP/TAZ convert non-stem tumor cells into cancer-stem cells, increasing tumor aggressiveness and fueling metastasis. I will also expand on these mechanisms, providing new hints for therapeutic intervention.

Principles of Gene Regulation by Single-Cell RNA Sequencing

Nikolaus Rajewsky

The Berlin Institute for Medical System Biology, Berlin, Germany
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The Next Generation Characterization of the Cancer Cell Line Encyclopedia implications for targeting metabolic alterations in cancer.

William Sellers
Broad Institute of MIT, Cambridge, MA

The Cancer Cell Line Encyclopedia, a large-scale collection of well annotated cell lines, has provided a rigorous backbone upon which to study genetic variants, candidate targets, small molecule and biologic therapeutics and to identify new marker driven cancer dependencies. With the goal of more fully understanding molecular features that contribute to cancer phenotypes including drug response we have expanded the cell line characterizations to include genetic, RNA splicing, DNA methylation, histone H3 modification, miRNA expression, metabolomic and reverse phase protein-array data for 1,072 cell lines from various lineages and ethnicities. Integrating these data with functional characterization including drug sensitivity, shRNA knockdown and CRISPR/Cas9 knockout data reveals potential new cancer drug targets and associated biomarkers. In addition, the unbiased association analysis linking cancer metabolome to genetic alterations, epigenetic reprogramming, and gene dependency provides a detailed description of metabolic consequences of the cancer dysregulated genome.
Importantly, in the metabolic space we observed distinct patterns of cell autonomous synthesis and secretion of kynurenine, an immune-suppressive metabolite. Furthermore, we found significant variation in the ability to suppress kynurenine with IDO1 selective inhibitors. Finally, by manipulating amino acid availability in large-scale screens of >500 barcoded cell lines, we demonstrated that aberrant ASNS hypermethylation sensitizes subsets of gastric and hepatic cancers to asparaginase therapy. Together, this dataset and an accompanying public data portal provide a resource to accelerate cancer research.

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Metabolic limitations of cancer progression

Matthew G. Vander Heiden

Koch Institute for Cancer Research, Massachusetts Institute of Technology, Cambridge MA USA

Complex regulatory mechanisms enable cell metabolism to match physiological state. The major pathways cells use to turn nutrients into energy and to synthesize macromolecules have been elucidated; however, there remain many unanswered questions regarding how metabolism supports cancer cell proliferation and thus how best to target metabolism for cancer treatment. Of note, many existing cancer therapies target metabolic processes that are thought to be essential in all cells. This raises a key question of how drugs which target these processes show differential effectiveness to treat cancer as well as a therapeutic window between cells. We have characterized the factors that drive sensitivity to disruption of specific metabolic processes, and find that both cancer cell extrinsic and intrinsic factors dictate metabolic vulnerabilities. Further, this leads to different cells being more reliant on limiting nutrients for different metabolic processes, creating a framework to understand how cancers become differentially sensitive to agents which target otherwise essential pathways.

Addressing cancer heterogeneity: personalized cancer vaccines

Catherine J. Wu, M.D.
Professor of Medicine
Chief, Division of Stem Cell Transplantation and Cellular Therapies
Department of Medical Oncology

Dana-Farber Cancer Institute and Harvard Medical School, Boston MA USA

The recent successes and challenges of cancer immunotherapy have motivated intense investigation of the molecular and cellular determinants of therapeutic response. The generation of broad computational and analytic tools to directly probe human samples has led to the emergence of systematic approaches to meet this challenge. At the heart of productive anti-tumor immune responses is the interaction of the T cell and the antigen presenting cell, with recognition of antigen by the T cell receptor (TCR); these interactions are further impacted by heterogeneous immune cell populations within the tumor microenvironment. While the search for immunogenic tumor antigens has been the subject of decades-long studies, multiple lines of evidence have convincingly demonstrated tumor neoantigens as an important class of immunogenic tumor antigens. Neoantigens arise from amino acid changes encoded by somatic mutations in the tumor cell and have the potential to bind to and be presented by personal HLA molecules. Using next-generation sequencing approaches, we can now systematically identify mutations leading to amino acid changes that can be potentially recognized immunologically through the implementation of neoantigen discovery pipelines. In recent studies, we have demonstrated that neoantigen load is associated with clinical outcome to immune-based therapies, and neoantigens can be safely and feasibly targeted to generate customized cancer vaccines. We have been undertaking pilot clinical trials to develop personal cancer vaccines in melanoma and glioblastoma that utilize synthetic long peptides as delivery approach for this therapy. Recent results and new directions will be discussed.

ABSTRACTS OF POSTERS

In-vivo reprogramming of postmitotic neurons induces medulloblastoma

Giuseppe Aiello CIBIO

PhD Student: Giuseppe Aiello Armenise-Harvard Laboratory of Brain Disorders and Cancer Centre for Integrative Biology - CIBIO (University of Trento)

It is widely accepted that the "cell of origin" of tumors has to possess a proliferative capacity. Particularly for brain cancer, the transition of neural progenitor to differentiated postmitotic neurons is considered irreversible in physiological and pathological conditions. Therefore, postmitotic neurons have not been considered as suitable cell of origin of brain cancer. Here, we show that neurons reprograming may occur upon Shh activation and it leads to Medulloblastoma (MB) formation in vivo. The Shh MB is a cerebellar tumor, found in infants and adults that is thought to originate from cerebellar granule neuron progenitors. More recently, it was discovered that the two different forms of SHH MB are distinguished by different transcriptome/methylome levels suggesting that the adult SHH MB may originate from a different cell of origin. Relying on these data, we use a conditional Cre-Lox recombination system that recapitulate the human adult medulloblastoma pathogenesis in mice and demonstrate that the post-migratory mature granule neurons can be reprogrammed in-vivo. This process leads to Shh medulloblastoma and the tumor formation is restricted to the cerebellum. Thus suggesting that cerebellar granule neurons have defined characteristics that allow the reprogramming process and cancer formation, upon specific mutational hits. Our novel model of cancer development could explain the human SHH medulloblastoma onset in adult individuals where granule neuron progenitors are no more present. We strongly believe that our model represents an important starting point to study other tissues where postmitotic cells might originate cancer and therefore this will open a new field in cancer and stem cell biology.

Pharmacological activation of TRPM8 channel overcomes innate resistance to standard-of-care therapies in prostate cancer

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Discovery of novel '*druggable*' targets is a primary goal in cancer translational research. Transient Receptor Potential subfamily M member 8 (TRPM8) is a cation channel almost exclusively expressed by the luminal compartment of the prostate epithelium in the human body. Primarily associated with calcium homeostasis, TRPM8 levels rise in primary and metastatic prostate cancer (PCa), which makes it a possible oncogenic factor and a suitable target of potential clinical interest.
Herein, we show that increased TRPM8 levels in

prostate cells favor calcium uptake and activation of pro-survival calcium/calmodulin-dependent kinase II (CaMKII). Nevertheless, by combining a multidisciplinary approach to an *in vitro* genetic platform modelling prostate tumorigenesis, we demonstrate that potent TRPM8 agonists synergize with X-ray treatments to induce massive apoptotic response in radioresistant pre-malignant and malignant preclinical prototypes of primary prostate lesions. As well, TRPM8 activation enhances docetaxel and enzalutamide efficacy in eradicating hormone naïve metastatic PCa cells. Overall, our findings identify TRPM8 as a valuable target for the treatment of PCa and provide a solid rationale to pursue the clinical testing of TRPM8 agonists in combination with standard-of-care therapies in PCa patients.

Establishment and Analysis of Patient-Specific Group3 Medulloblastoma Mouse Models

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Brain cancer is now the deadliest form of childhood cancer in the United States. In particular, Group3 Medulloblastoma (MB) is the pediatric brain tumor with highest morbidity and mortality. Patients with Group3 MB currently have the worst outcome and nearly 50% are metastatic at the time of diagnosis. However, the cellular and molecular mechanisms underlying Group3 MB are still unknown. What is still lacking in the field is the possibility to obtain tumors by direct genetic modification of mice and to be able to recapitulate the growth and metastasis formation of Group3 MB.
Exploiting in-vivo transfection of mouse cerebellar cells with CRISPR-Cas9 and PiggyBac transposase systems, we tested different combination of putative oncosuppressors and putative oncogenes, derived from human Medulloblastoma NGS data, for their ability to induce Group3 MB in mice. Surprisingly, concomitant overexpression of c-Myc and other transcription factors in mouse cerebellum is able to induce MB in few months. The newly generated mouse model is able to fully recapitulate human Group 3 MB.
Using this proposed patient-specific model, we were able to unravel the molecular aspects of Group 3 medulloblastoma tumorigenesis and identify molecules inhibiting tumor growth in a targeted manner.

Cell-autonomous and cell non-autonomous downregulation of the tumor suppressor DAB2IP by microRNA-149-3p promotes cancer progression by microenvironment remodeling.

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The dynamic crosstalk established between tumor cells and surrounding stroma cells is crucial in carcinogenesis (Quail and Joyce, 2013). In this context, signaling modulators and adaptors that dictate intrinsic cell responses to microenvironmental cues play a fundamental role, often underestimated. The tumor suppressor DAB2IP belongs to this category: it is a cytoplasmic Ras-GAP and adaptor protein that negatively modulates multiple oncogenic pathways, including canonical WNT signaling, VEGF signaling via PI3K/Akt, TNF signaling via NF-κB, and Androgen Receptor (AR) activity (Bellazzo et al., 2017). Not surprisingly, its expression is frequently reduced by gene methylation in several tumors, including breast and prostate cancer. In addition, various post-transcriptional mechanisms of DAB2IP inactivation have been reported; in particular, due to the long 3'UTR sequence of its main transcript, DAB2IP is a strong candidate for microRNAs (miRNAs)-mediated regulation (Bellazzo et al., 2017). Performing an high-throughput screening of a large collection of human miRNAs, we have identified miR-149-3p as a negative modulator of DAB2IP (Bellazzo et al., 2018). By efficiently downregulating DAB2IP, miR-149-3p enhances NF-κB signaling activation in prostate cancer cells, promoting cell motility, and improving the secretion of pro-inflammatory and pro-angiogenic factors. Importantly, we found that the inhibition of endogenous miR-149-3p restored DAB2IP tumor suppressive functions, and efficiently reduced tumor growth and dissemination of prostate cancer cells in vivo (Bellazzo et al., 2018). DAB2IP loss can also affect the behavior of endothelial cells surrounding the tumor: in fact, conditional DAB2IP knockout in vascular endothelial cells was shown to potently support formation of a pre-metastatic niche, facilitating tumor growth and dissemination in mouse models of melanoma and breast cancer (Ji et al., 2015). We discovered that cancer cells can reduce DAB2IP levels in neighboring endothelial cells and fibroblasts by exosome-mediated secretion of miR-149-3p, stimulating their proliferation and motility, and potentially remodeling the tumor microenvironment. Notably, experiments with miR-149-3p in-

hibitors clearly indicate that additional signals released by cancer cells also contribute to DAB2IP downregulation in nearby endothelial cells and fibroblasts, and these need to be uncovered.
These results have various implications; they contribute to clarify the complex mechanisms that govern the dynamic crosstalk between tumor cells and surrounding stroma, and they may suggest promising therapeutic strategies in cancer. Indeed, approaches aimed to upregulate DAB2IP levels would offer the unique therapeutic opportunity to act on a single protein that can negatively modulate multiple oncogenic signals, both in cancer cells and in stromal cells. In this perspective, factors involved in cell non-autonomous downregulation of DAB2IP might be optimal targets to develop pharmacologic strategies to limit cancer progression by potentiating DAB2IP functions in multiple cellular components of the tumor tissue.

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A novel platform to study and target undruggable Ewing onco-chimeras.

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Cancer in children is relatively rare, yet it is the leading cause of death by disease in developed countries. Development of efficient therapeutic strategies in the past years has drastically reduced the lethality of several common types of pediatric and juvenile forms of cancer such as leukemia and lymphoma, but such a tremendous success does not include sarcoma's treatment, whose mortality rate remains the same as twenty years ago. Ewing sarcoma is an aggressive type of bone tumor representing 1% of all childhood cancers, and its initial oncogenic event is represented by a balanced chromosomal translocation originating a chimeric oncoprotein, which in 90% of the patients derives from the fusion between EWS and FLI1 genes. Ewing sarcoma's treatment is currently based on the use of generic chemo-agents such as doxorubicin, vincristine, cyclophosphamide, and dactinomycin, and novel targeted treatments are urgently required. The identification of novel drugs through preclinical studies is however heavily influenced by the model enrolled in the studies. Faithfull preclinical models able to recapitulate the biological characteristics of the human disease should be employed to improve the efficiency of the preclinical tests and to decrease the percentage of subsequently failing clinical trials. Currently, however, no faithful preclinical model of Ewing Sarcoma is available. Therefore, first objective of this study will be the development of reliable models able to faithfully recapitulate Ewing sarcomagenesis by inducing the expression of Ewing oncoproteins in the correct cellular microenvironment, which is considered to be the mesenchymal stem cell. As exclusive hallmark of tumor cells and driving force of the disease, oncochimeras are ideal targets in medical oncology. Among them, however, transcription factor oncoproteins such as Ewing sarcoma oncoproteins are classified as "undruggable" from a conventional pharmacological point of view, lacking in their structure convenient targeting pockets, and even though much is known regarding the oncogenic functions of different chimeras, the success rate at which this advanced knowledge has been translated into effective therapies is pitifully low. Second objective of this study will therefore be the enrollment of the generated models in preclinical screenings to identify those molecular mechanisms whose pharmacological tuning will tear down Ewing sarcoma lethality by modulating oncochimera's stability.
By succeeding, our project will definitely break down the ancient dogma postulating the undruggability of oncogenic chimeras belonging to the class of transcription factors, unveil new therapeutic strategies towards the eradication of Ewing sarcoma, and, finally, provide a pivotal platform easily exportable to all those tumors driven by "undruggable" oncogenic chimeras.

Contribution of centriole appendages to PIDDosome activation

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Failure in the physical separation of two cells at the end of cell division (i.e. cyctokinesis) is one of the most common malfunctions of the cell division cycle, predisposing cells to neoplastic transformation. Cytokinesis failure is invariably followed by a reduction in the propensity of cells to commit to additional cell cycles. Fava et al. demonstrated that this p53-dependent cell cycle arrest depends on the activation of the PIDDosome, a multiprotein complex comprising PIDD1, RAIDD and Caspase-2 (Fava et al, 2017). Moreover, centrosome abundance appears crucial for determining the cellular behavior in response to cytokinesis failure and PIDD1 physically associates with the centrosome.
A genetic screen for centrosomal proteins revealed the PIDD1 position within the epistatic map of centriole appendage proteins. Moreover, delocalization of PIDD1 from the centrosome results into compromised PIDDosome activation and p53-dependent cell cycle arrest. Taken together, our data shed light on the role of the PIDDosome as a centrosome counting entity.

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Targeting tumor-associated macrophages in osteosarcoma: depletion versus re-direction

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The pro-tumorigenic role of tumor-associate macrophages (TAM) has been widely demonstrated in several tumor types. On the contrary, very few data exist on the activity of TAM and of other immune cells in osteosarcoma (OS), the most common primary bone tumors in young adolescents and children.
We have recently demonstrated in immuno-competent OS mouse models that trabectedin, a marine-derived chemotherapic agent, exerts a potent anti-tumor activity that is further enhanced by the combination with anti-PD-1 antibody. Besides directly affecting neoplastic cells, trabectedin modifies the tumor immune landscape by recruiting T lymphocytes at the tumor site. However, contrary to what expected from the literature, the treatment did not affect TAM.
To better investigate the role of TAM in OS, we performed a "proof-of-concept" co-injection experiment with OS cells mixed with macrophages differentiated *in vitro* toward classical M1 or M2 phenotype, or left undifferentiated (M0). While the presence of M1 macrophages inhibited OS, neither M0 nor M2 macrophages affected significantly tumor growth. This result indicated that the presence of TAM per se does not influence OS growth and suggested that their re-direction toward a M1 phenotype could exert therapeutic activity. To clarify this issue, mice bearing OS tumors on both flanks were treated locally, only in one lesion, with either liposome-encapsulated clodronate to deplete TAM, or with SD101, a synthetic oligonucleotide with immunostimulatory CpG motifs. Despite clodronate reduced TAM infiltration, tumor growth inhibition was limited, on both flanks; on the other hand, SD101 efficiently halted the growth of both treated and untreated lesions. TAM number was not affected by SD101 treatment, but they showed a significant reduction in the expression of the M2 marker CD206. Additionally, tumor infiltration by CD8 T cells was enhanced in both treated and untreated tumors by SD101, but unaffected by clodronate.
Overall these preliminary results support the hypothesis that re-directing the phenotype of TAM in OS could be therapeutically more efficient than their direct elimination.

The glutamine addiction of multiple myeloma cells shapes the metabolic microenvironment of the bone marrow niche impairing osteoblastic differentiation

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Metabolic alterations of cancer cells are final-

ized to satisfy the increased nutrient needs due to uncontrolled proliferation but also impact on normal cells of tumor microenvironment. Glutamine addiction of multiple myeloma (MM) cells (Bolzoni, Chiu et al., Blood 2016; 128:667-679) leads to decreased glutamine levels in the bone marrow (BM) plasma of patients. Osteolytic bone lesions are a hallmark of active MM, and several mechanisms have been implied in their pathogenesis. However, the role of MM-related alterations of glutamine metabolism in bone lesions has not been investigated yet. Bone lesions in MM are characterized by loss and impaired differentiation of osteoblasts. Interestingly, Glutamine Synthetase (GS), the only enzyme able to catalyze intracellular glutamine synthesis, is down-regulated during osteoblast differentiation. Moreover, it has been recently demonstrated that the inhibition of glutamine metabolism decreases bone mass in mice (Yu et al., Cell Metabolism 2019; 29, 966–978). Thus, we hypothesized that the low-glutamine microenvironment imposed by MM plasma cells negatively affects osteoblastogenesis.

Human MM cell lines (HMCLs), immortalized BM mesenchymal stem cells (MSC) and osteoblastic cell lines (HOBIT and HOB-01) were grown in DMEM supplemented with 2 mM Gln and 10% fetal bovine serum. Changes in extracellular Gln were measured with a commercial kit or with mass spectrometry, and amino acid uptake was assessed as previously described (Bianchi et al. Neuroscience. 2008; 151:1042-1052). The expression of osteoblastic markers (ALP, COL1A1 and RUNX2), ALP activity and positivity to ALP staining were then assessed during osteoblast differentiation.

As expected from data obtained ex vivo, HMCLs consumed large amounts of glutamine (750 ± 50 nmol/10^6 cells/day) and exhibited fast initial uptake of the amino acid. When co-cultured with MSC or osteoblasts, HMCLs accelerated the depletion of extracellular glutamine (+25%/day, compared to monocultures of MSC/osteoblasts), promoted GS expression in MSC and hindered viability of osteoblasts but not of MSC. Consistently, osteoblasts were more sensitive to Gln depletion (EC_{50} of 240 ±15 μM for HOBIT cells and 300± 5 μM for HOB-01cells) than MSC (EC_{50} 80 ± 20 μM). The expression and the activity of the concentrative glutamine transporter SNAT2 (SLC38A2) were induced during osteoblastogenesis, while the expression of other transporters, such as ASCT2 and SNAT1, was unchanged. SNAT2 induction was also associated with the increased expression of Glutaminase 1 (KGA, long-transcript form), suggesting higher glutamine demand and consumption in differentiating osteoblast precursors. In agreement with the experimental hypothesis, MSC osteoblastic differentiation was substantially impaired in the absence of glutamine; moreover, the decrease of extracellular glutamine concentration from 0.6 mM (the average physiological BM plasma concentration) to 0.4 mM (the average concentration in the BM plasma of MM patients) was sufficient to cause a significant reduction of osteoblastic markers as well as of ALP activity and staining.

These preliminary results suggest that glutamine addiction of MM contributes to osteoblast impairment in the bone lesions of the MM BM niche.

JMJD6-mediated Regulation of Estrogen Receptor in Breast Cancer and its role in Tamoxifen response

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Introduction

Breast cancer (BCa) is one of the most common cancer types in women worldwide, and estrogen receptor (ER) is expressed in the vast majority of these tumors. ER-positive BCa relies on estrogen levels for the development and progression of the disease, as activation of ER signaling regulates the expression of critical genes involved in proliferation and migration of tumor cells. Thus, anti-hormonal treatment, through the inhibition of ER action, is the mainstay therapeutic option for high-risk ER-positive breast cancer patients. It is well known that modulation of chromatin conformation via epigenetic modifications regulates transcription of many genes, including hormone receptors (HRs). The bifunctional arginine demethylase and lysyl-hydroxylase, JMJD6, is an epigenetic modifier often associated with tumor progression in many tumors, including BCa. A crucial role of JMJD6 is to demethylate arginine residues 3 and 2 of histone 4 (H4R3) and 3 (H3R2), respectively, thus affecting structural conformation of the chromatin and transcription and function of HRs.

Hypothesis and aims

We hypothesized that modulation of the methylation status of these histones by JMJD6 is critical for the regulation of ER expression and signaling in BCa, thus possibly affecting proliferation and metastatic potential of tumor cells. On the other hand, we expect that increased ER expression would improve efficacy of hormone therapy in BCa patients. This project aims to explore the molecular and functional role of JMJD6 in regulating ER signaling and response to Tamoxifen in BCa cell lines and in suitable mouse models. Ultimately, we aim to validate our findings in hormone treated and not treated BCa patients cohorts.

Results
Our preliminary data reveled that CRISPR-Cas9 JMJD6 knock-out (KO) ER-positive MCF7 showed a strong increased methylation of H4R3, as well as increased ER expression, and expression of ER-responsive genes, RARA and GREB1. In agreement with this, proliferation of JMJD6 KO cells was strongly increased compared to wild-type (WT) control. As expected, stimulation with estradiol (E2) further enhanced proliferation of both JMJD6 KO and WT MCF7 cells, however, Tamoxifen treatment equally suppressed proliferation of both cell types. Furthermore, JMJD6 KO MCF7 cells showed increased migration when stimulated with E2 due to loss of E-cadherin expression, nevertheless, Tamoxifen treatment successfully suppressed cell migration. Importantly, increased ER expression and improved response to Tamoxifen was also observe in ER-low BT474 BCa cell line that poorly respond to Tamoxifen treatment in WT condition, suggesting that inhibition of JMJD6 levels can improve response to hormone therapy by modulating ER levels. Supporting this hypothesis, we found that reduced levels of JMJD6 were significantly correlated with longer overall survival of BCa patients treated with hormone therapy in the METABRIC database compared to hormone treated patients expressing high levels of JMJD6, suggesting that JMJD6 could be a predictive marker for hormone therapy response in these patients.
Conclusion
Our data suggest that inhibition of JMJD6 in BCa patients could potentially improve response to hormone therapy by increasing ER expression, thus providing a rational for the development of JMJD6 inhibitors.

Ultrasensitive detection of cancer biomarkers from blood circulating NBI-isolated vesicles.

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Extracellular vesicles (EVs) are secreted membranous particles intensively studied for their potential cargo of diagnostic markers. An efficient and high-throughput study of EVs in the routine clinical practice is needed to understand their clinical utility. We designed the nickel-based isolation (NBI) procedure to rapidly isolate EVs to preserve their integrity and original dispersity in solution. Then, we combined it with ultrasensitive homogeneous assays, such as amplified luminescent proximity homogeneous assay (alpha) or droplet digital PCR (ddPCR), to detect known cancer biomarkers from retrospectively analysed oncological patients.
By applying alpha-NBI, we detected picomolar concentrations of prostate-specific membrane antigen (PSMA) in fractions of EVs isolated from the plasma of prostate cancer patients, discriminating them from control subjects. Directly from oil-encapsulated EVs for digital PCR, we identified somatic BRAF and KRAS mutations from the plasma of metastatic colorectal cancer (CRC) patients, matching 100% of concordance with tissue diagnostics and higher sensitivity and specificity compared with immune-enrichment of tumor-derived EVs. We propose NBI-combined approaches as a further tool to develop liquid biopsy studies and gain advantages from the possibility of probing tumor heterogeneity from circulating EVs.

New platform for the direct profiling of micrornas in biofluids

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Circulating microRNAs have been identified as potential biomarkers for early detection, prognosis and prediction of several diseases. Their use in clinical diagnostics has been limited by the lack of suitable detection techniques. Most of the current technologies suffer from requiring complex protocols, not yet able to deliver robust and cost-effective assays in the field of clinical diagnostics. In this work, we report the development of a breakthrough platform for profiling circulating microRNAs. The platform comprises a novel silicon photomultiplier-based reader in conjunction with a chemical-based method for nucleic acid detection. Accurate microRNAs profiling without extraction, pre-amplification or pre-labelling of target is now achievable. We designed and synthesized a set of reagents that combined the chemical-based method with a chemiluminescent reaction. The signals generated were read using a novel, compact silicon photomultiplier-based reader. The platform sensitivity was determined by measuring known concentrations of hsa-miR-21-5p spike-ins. The limit of detection was calculated as 4.7 pmol/L. The platform was also successfully used to directly detect hsa-miR-21-5p in eight non-small cell lung cancer plasma samples. Levels of plasma hsa-miR-21-5p expression were also measured via TaqMan RT-qPCR.

The successful integration of a unique chemical-based method for nucleic acid detection with a novel silicon photomultiplier-based reader created an innovative product (ODG platform) with diagnostic utility, for the direct qualitative and quantitative analysis of microRNA biomarkers in biological fluids.

The SWI/SNF complex is a mechanoregulated inhibitor of YAP and TAZ

Inactivation of ARID1A and other components of the nuclear SWI/SNF protein complex occurs at very high frequencies in a variety of human malignancies, suggesting a widespread role of the SWI/SNF complex in tumour suppression[1]. We show that ARID1A-containing SWI/SNF complex (ARID1A-SWI/SNF) operates as an inhibitor of the pro-oncogenic transcriptional coactivators YAP and TAZ (also known as WWTR1)[2]. YAP and TAZ are necessary to mediate the effects of the inactivation of the SWI/SNF complex, such as cell proliferation, acquisition of stem cell-like traits and liver tumorigenesis[2].

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Pro-tumoral role of Complement activation in murine tumor models

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Cancer related inflammation (CRI) plays a fundamental role in fueling tumor appearance and development. Although the important contribution of complement activation to inflammation, its role in CRI still remains understudied. Recently our group demonstrated the pro-malignant role of complement activation in models of mesenchymal carcinogenesis, induced by 3-methycolanthrene (3-MCA), and epithelial inflammation-driven skin carcinogenesis, induced by dimethylbenz-α-anthracene/terephthalic acid treatments (DMBA/TPA). Our results showed that mice deficient for the central complement component C3 were protected from tumor development. Further experiments revealed that C3-cleavage products were deposited on vessels and tumor cells of tumor tissues, while they were absent in normal subcutaneous tissues. The C3 deposition on tumor cells was also observed *in vitro*, both on 3-MCA-derived sarcoma and on different murine cancer cell lines. Further, *in vivo* experiments suggested that complement activation was mainly due to the activation of the classical pathway. Then, we investigated C3-downstream mechanism(s) of protection in three different murine tumor models. We observed that C3aR- but not C5aR1- and C5L2-deficient mice were protected from tumor growth in a transplantable model of sarcoma (MN-MCA1), as well as in the 3-MCA-induced carcinogenesis model, suggesting that the C3a/C3aR axis was most likely responsible for the phenotype showed by C3-deficient mice in sarcoma models. However, in the DMBA/TPA model we observed that the C5a/C5aR1 dependent signaling was mainly responsible for the phenotype showed by C3-deficient mice, since C5aR1$^{-/-}$ mice were protected from tumor development, and that the underlying mechanism involved the hematopoietic compartment Our results indicate that complement activation occurs in tumor and contributes to tumor development, although the mechanism/s implicated could be different in the mouse models.

Targeted NGS in pharmacogenes to identify novel rare variants related to fluoropyrimidines toxicity

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Fluoropyrimidines (FL) are frequently used in several solid tumours treatment. Unfortunately, about 26% of patient treated with FL will develop unpredictable severe to life-threatening toxicity (grade ≥3). Common single nucleotide polymorphisms (SNP) provide partial explanation to the overall adverse reactions (Toffoli et al, 2015). However, most variants affecting proteins phenotype in humans, including those involved in pharmacokinetics, were reported to be novel and rare (Lek et al, 2016; Kozyra et al, 2017; Ingelman-Sundberg et al, 2018).

We aimed to screen 54 pharmacogenes involved in the FL absorption, distribution, metabolism, and excretion (ADME) and folate pathway to identify novel markers of severe FL-related toxicity in a selected group of patients with severe FL-related toxicity phenotype.

One hundred twenty patients treated with a FL-based regimen were retrospectively selected based on grade ≥3 toxicity occurrence and absence of acknowledged DPYD risk variants (DPYD*2A, DPYD*13, DPYD-c2846, and DPYD-HapB3). PharmGkb resources and literature data were used for genes selection. A custom NimbleGen SeqCap EZ Choice (Roche, Inc.) which covers the CDS and UTRs of target genes was designed including the flanked splice junctions regions. An additional region of 3kbp was included to address the promoter genetic variants for 3 genes (DPYD, MTHFR and TYMS). The libraries were sequenced with MiSeq instrument (Illumina, Inc.). The resulting fastQ files were analyzed using different tools in in-house pipeline.

By a preliminary analysis, eight singleton variants on DPYD were identified in eight different patients. Seven of them were missense and one was located in the 3'UTR. In addition, three previously reported very rare missense variants were detected. Variants were analytically validated by Sanger sequencing and were evaluated with several amino acid change and splicing defect prediction tools. It is interesting to note that the identified missense variants have high damage score and nine of these may modify the exonic splicing sequences (ESE/ESI). Furthermore, eighty-five new variants were detected in the FL-ADME genes, including a deletion of 43bp in the 3'UTR of the TYMS and its overlapped regulator ENOSF1. rTS (ENOSF1) RNA may downregulate TS (TYMS) RNA expression via 3'UTR interaction (Dolnick, R. et al., 2005).

In conclusion we preliminarily demonstrated that rare and novel genetic variants in DPYD can be detected in patients with an extreme toxicity phenotype and can potentially account for a defective FL detoxification. We will try to demonstrate the functional role of these variants through specific functional assays.

Metabolic re-programming by malat1 depletion in prostate cancer cell lines and organotypic slice cultures

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BACKGROUND: Prostate cancer (PCa) is the most common malignancy in men worldwide with an increased global burden. The continuous improvement of next-generation sequencing methods shed light on the direct involvement of multiple non-coding RNA (ncRNA) transcripts in cell biology and homeostasis, cancer growth and progression as well as in other pathophysiological contexts. Contextually, cancer metabolism recently emerged at the upfront edge of anticancer research with the promise to develop novel therapeutic approaches. Interestingly, very recent evidences linked long (>200nt) ncRNAs (lncRNAs) to metabolism opening up to novel therapeutic strategies based on lncRNAs. Therefore, the study of their effects on metabolism might unravel overlooked pathways to target in an anticancer perspective.

HYPOTHESIS & AIM: Although MALAT1 has been one of the earliest identified lncRNAs and its role in cancer as promoter of tumour progression and metastasis is well defined, little or no information is available about its direct involvement in mitochondrial metabolism regulation. In this light, our working hypothesis is that MALAT1 might play a pivotal role in PCa as a metabolically active signal integrator. For this reason, the main goal of our study is to investigate whether MALAT1 might represent a metabolically active target for PCa inhibition in Organotypic Slice Cultures (OSCs), an ex vivo human model suitable for gene expression, gene targeting, and drug testing analyses.

EXPERIMENTAL DESIGN: Our experimental strategy is made of a multi-pronged approach aimed at defining a novel role of MALAT1 in PCa metabolism. Specifically, we characterized MALAT1-dependent transcriptome in human PCa cells with aggressive/metastatic phenotype and in OSCs and identified putative MALAT1-dependent metabolic enzymes and metabolites that were further investigated by NMR-based metabolomics analysis before and after ex-vivo gene targeting.

RESULTS: Transcriptomics and metabolomics performed in PCa cell lines (PC-3, DU145, and C27IM) pointed out malate dehydrogenase (ME3), pyruvate dehydrogenase kinases (PDK1 and PDK3) as well as choline kinase A (CHKA) as important metabolic enzymes under MALAT1 transcriptional control. Both transcriptomics and metabolic profiles were validated by RT-PCR, western blot and specific enzymatic analysis (lactate production, NAD+/NADH assays, and assessment of mitochondrial OXPHOS complexes). These findings were confirmed in a large number of human OSCs (n=47) obtained upon surgery from a selected cohort of patients with diverse stage of the disease. Currently, we are exploring whether these enzymes belonging to the tricarboxylic acid (TCA) cycle and phosphatidylcholine pathway are involved in MALAT1 regulatory function and whether a direct modulation of TCA cycle enzymes or phosphatidylcholine metabolites may reproduce the MALAT1-associated metabolic environment in PCa.
In conclusion, our findings suggest an unprecedented role of MALAT1 as regulator of cell metabolism and potentially active target for PCa inhibition at single patient level.

Mechanisms of telomere maintenance in zebrafish brain tumors: correlation with pediatric glioblastoma

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Alternative lengthening of telomeres (ALT) occurs in pediatric brain tumours and may develop as a result of chromosomal instability promoted by altered histone H3 modifications in subtelomeric regions and/or in association with ATRX mutations/downregulation. However, development of ALT may require additional genetic and epigenetic changes, at present largely unknown.
Here we have investigated whether ALT develops as a result of the coordination between DNA damage accumulation and chromatin status at telomeres in brain cancers. We used a model of juvenile zebrafish brain tumour based on the conditional expression of human RAS oncogene in brain progenitor cells (*Mayrhofer et al. 2017*). Zebrafish brain tumours have long and irregular telomeres, undergo sister chromatid exchange and are positive for C-Circles, suggesting that ALT mechanisms are predominant. Following ALT phenotype, DNA damage at telomeres was significantly higher than in control cells. We found that during the progression of tumours from single cancer initiating clone to a full tumour, downregulation of tert through hypomethylation of its promoter precedes the development of ALT and is linked to an increase of Terra expression. To study the contributions of telomerase in the development of ALT, we established the same model in a background of co-overexpressed *tert* and *terc*. The tumour generated were similar in location but less aggressive respect tumours without *tert/terc* overexpression. The analysis of telomere and ALT biomarkers showed an ALT rescue in the function of tert and terc over-expression. We also found a reduction of telomeric DNA damage, downregulation of genes of the pre-replication complex and the status of heterochromatin of telomeres was re-established in telomerase positive brain tumours. We suggest that the activity of telomerase can reduce the replication stress at telomeres by regulation of telomeric heterochromatin. Finally, we characterized telomere maintenance mechanisms in a cohort of 20 human brain tumours, where we measured telomeric DNA damage in association with telomeric chromatin. Besides reporting the first in vivo genetic model of ALT+ brain tumour, this study identifies telomeric maintenance mechanisms as significant drivers of the coordination between DNA replication and chromatin status at telomeres in brain cancers.

Adenocarcinoma-neuroendocrine transition of castration resistant prostate cancer depends on SPARC down-regulation in stromal accessory cells

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Adenocarcinoma-neuroendocrine transition of castration resistant prostate cancer can occur in a relevant subset of patients as a mecha-

nism to resistance to androgen deprivation and androgen-receptor targeted drugs. Tumor cell plasticity toward neuroendocrine differentiation (NED) can be sustained by signals provided by the tumor microenvironment. We previously identified the matricellular protein SPARC as a crucial modulator of cancer progression and phenotype, exerting different functions depending whether expressed by tumor or stroma cells. Therefore, we aimed at elucidating the different contribution of tumor- or stroma-derived SPARC in prostate cancer, utilizing the TRAMP mouse model. Crossing TRAMP mice with Sparc-/- mice, we observed appearance of areas of NED, similarly to what occurs in TRAMP mice relapsing after surgical castration. Areas of NED were positive for both adenocarcinoma (CK8) and neuroendocrine (SYP) markers, suggesting trans-differentiation from adenocarcinoma. In TRAMP prostates, we observed SPARC positivity in scattered tumor cells and in infiltrating fibroblasts. Moreover, adenocarcinoma cells injected in Sparc-/- mice acquired SYP expression. This suggested a role for stromal-derived SPARC in limiting NED of prostate cancer cells. Accordingly, prostate cancer cell lines co-cultured in presence of Sparc-deficient fibroblasts acquired neuroendocrine features. This likely occurs through the effect of IL-6, which is released by SPARC deficient, but not sufficient, fibroblasts. Our data indicate that stromal SPARC down-regulation controls prostate cancer transition from adenocarcinoma to neuroendocrine phenotype. A deeper understanding of the molecular mechanisms governing NED according to extracellular matrix composition will provide important insights for the development of new therapeutic strategies in prostate cancer.

Pro-tumorigenic role of ETS-related gene (ERG) in pre-cancerous prostate lesions

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Prostate Cancer (PCa) is the second most common cancer in men. While several genomic, genetic and molecular alterations characterizing human PCa have been functionally associated with tumor onset, progression and resistance to therapy, the role of many other molecular events remains still unclear. By combining prostate organoids technology with genetic engineering and CLICK-chemistry coupled Mass Spectrometry approaches, we identified a panel of secreted proteins regulated by ETS-related gene (ERG). Based on the nature of the identified factors, ERG activity in pre-cancerous human prostate lesions may prime prostate cells and the surrounding stroma to create a tolerant and supportive environment during the initial steps of tumorigenesis.

Cross-talk with lung epithelial cells regulates Sfrp2 expression enabling disseminated breast cancer cell latency

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The process of metastasis is highly complex. In the case of breast cancer, there are frequently long timespans between cells leaving the primary tumour and the growth of overt metastases. During this period, cancer cells persist in an indolent or latent state before transitioning back to an aggressive growth. Possible reasons for disease indolence include interplay with myeloid and fibroblastic cells in the tumour microenvironment and ongoing immune surveillance. However, the signals causing actively growing cells to enter into an indolent state, and enabling them to survive for extended periods of time, are not well understood. In this project, we propose that the behavior of indolent breast cancer cells in the lung is determined by their interactions with alveolar epithelial cells. In vivo data, as well as a newly developed lung organotypic system, revealed that lung epithelial cells promote the formation of fibronectin (FN) fibrils by indolent cells that

drive integrin-dependent pro-survival signals. Combined in vivo RNA sequencing and drop-out screening identified Secreted frizzled-related protein 2 (Sfrp2) as a key mediator of this interaction. Sfrp2 is induced by lung epithelial cells and promotes FN fibril formation, integrin activation and survival, while blockade of Sfrp2 expression reduces the burden of indolent disease. Treatment of mice with an integrin-specific inhibitor, reduces the survival of disseminated indolent breast cancer cells in vivo, suggesting FDA-approved integrin-inhibitors as a valuable adjuvant therapies to eradicated these cells and prevent metastasis.

F-actin dynamics regulates mammalian organ growth and cell fate maintenance

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in vitro, cell behavior can be potently regulated by the mechanical properties of cells and of their microenvironment. Cells sense these features through integrin receptors and focal adhesions and counteract external forces by developing internal pulling forces via their actomyosin cytoskeleton, in turns regulating intracellular pathways, including the transcriptional coactivators YAP/TAZ. Whether mechanical cues are relevant for in vivo regulation of adult organ homeostasis, and whether this occurs through YAP/TAZ, remains largely unaddressed. We developed Capzb conditional knockout mice and obtained primary fibroblasts to characterize the role of CAPZ *in vitro*. *In vivo*, functional analyses were carried out by inducing Capzb inactivation in adult hepatocytes. We found that the F-actin capping protein CAPZ restrains actomyosin contractility: Capzb inactivation alters stress fiber and focal adhesion dynamics leading to enhanced myosin activity, increased traction forces, and increased liver stiffness. *in vitro*, this rescues YAP from inhibition by a small cellular geometry; in vivo, it induces YAP activation in parallel to the Hippo pathway, causing extensive hepatocyte proliferation and leading to striking organ overgrowth. Moreover, Capzb is required for the maintenance of the differentiated hepatocyte state, for metabolic zonation, and for gluconeogenesis. In keeping with changes in tissue mechanics, inhibition of the contractility regulator ROCK, or deletion of the Yap1 mechanotransducer, reverse the phenotypes emerging in Capzb-null livers. These results indicate a previously unsuspected role for CAPZ in tuning the mechanical properties of cells and tissues, providing a missing genetic evidence for mechanical properties as potent and specific regulator of liver organ growth, cell-fate and tissue metabolism in vivo. More generally, it indicates for the first time that mechanotransduction has a physiological role in maintaining liver homeostasis in mammals. Our genetic system will thus open the possibility for testing, in the future, the effective and functional role of mechanostransduction in multiple other tissues and in altered context, such as cancer development.

Histone chaperone ANP32E as a proto-oncogenic factor in MYC-driven tumorigenesis

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Breast cancer consists of highly heterogeneous tumors, whose driver oncogenes result difficult to be uniquely defined. We have recently reported the central role of MYC in initiating and sustaining a step-wise epigenetic reprogramming process in mammary luminal epithelial cells (IMEC) toward a stem cell-like condition, which favors cell transformation and tumor initiation. Among the chromatin players that may synergize with MYC, we identified the H2A.Z-specific chaperone ANP32E, whose expression is induced in MYC-transformed IMEC (*t*-IMEC). Interestingly, analysis of the TCGA dataset showed that ANP32E alteration is specifically enriched among basal-like breast cancers characterized by MYC deregulation and this combination correlates with a worst prognosis. These observations suggested a possible cooperation between MYC and ANP32E in tumor formation and maintenance. Of note, ANP32E overexpression in *t*-IMEC was associated with increased tumorigenic potential both *in vitro* and *in vivo*. Considering that MYC overexpression is cause of replication stress (RS) and that ANP32E-mediated eviction of H2A.Z is required for DNA-damage repair (DDR), we investigated whether their simultaneous deregulation could further impact on RS and result in DNA damage accumulation. Of note, t-IMEC overexpressing ANP32E (t-IMEC-ANP32E) are characterized by increment of transcriptional R-Loops and high-

er number of γH2A.X-marked DNA-damage foci, respect to t-IMEC. A critical component of the DDR is represented by ATR, a factor activated by regions of single-stranded DNA, which can occur as a result of oncogene-induced RS. Interestingly, both *in vitro* and in vivo treatment with an ATR inhibitor showed enhanced sensitivity in association with ANP32E overexpression. This study supports the notion that ANP32E alteration in cells undergoing MYC-related RS can promote the development and maintenance of a tumorigenic phenotype by interfering with the DNA repair machinery. The correlation between ANP32E deregulated expression and increased cell sensitivity to ATR inhibition could establish a therapeutic rational for targeted treatment of basal-like breast cancers characterized by combined MYC and ANP32E alteration.

Decoding the role of glutaminolysis in developmental and tumor angiogenesis

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Metabolism is emerging as an important regulator in co-determining endothelial cell (EC) behavior and function. Little is known about a possible role of glutamine metabolism during new vessel sprouting in normal and pathological conditions. The key rate-limiting step of glutaminolysis is the conversion of glutamine to glutamate by the mitochondrial glutaminases GLS1 and GLS2. Given the very low expression level of GLS2 in ECs, in the present study we investigate the role of GLS1 at cellular level and how it affects angiogenesis in vivo. At first we characterized the effect of GLS1 on EC behavior. The absence of glutaminase activity by GLS1 specific blocker CB-839 or expression by shRNA alters cell cycle progression and ultimately impairs proliferation, with no cytotoxicity. In addition, we found that the absence of GLS1 induces a stress response via ATF4, resembling the aminoacid starvation response, and affects the total level of VEGF R2 receptor and its activation after stimulation with VEGF A. To determine whether GLS1 plays a role during angiogenesis in vivo, we generated endothelial conditional GLS1 knockout mice by crossing floxed Gls1 mice (Gls1flox/flox) with VE-cadherin CreRT2 transgenic mice. In xenograft tumors we found that knock-out of GLS1 in vivo significantly inhibited the tumor growth and vascular vessels formation. These findings underscore the importance of glutamine metabolism in ECs and during angiogenesis *in vivo*.

Bone marrow hematopoietic adaptation to distant breast cancer begins early in transformation in association with deregulated circulating microRNAs

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During tumorigenesis, newly transformed cells initiate an active cross-talk with bystander cells, mostly of bone marrow (BM) origin, to establish a pro-tumorigenic microenvironment. We hypothesized that signs of this cross-talk can be identified in the BM at the very early phases of cancer development, being finalized to the instruction of a tumor-promoting hematopoiesis. In the MMTV-NeuT model of spontaneous mammary carcinoma we showed that gene expression profiling of the bone marrow along disease progression indicates modifications in the hematopoietic compartment already at early disease stages that become more relevant with tumor progression. The transcriptional profile of the adapted hematopoiesis revealed the induction of programs related with innate immunity and responses to danger signals, alongside the down-modulation of adaptive immune response. These transcriptional reprogramming is paralleled by an expansion of the myeloid populations at the expense of erythroid and B lymphoid fractions. The finding of such modifications in the BM microarchitecture even at earlier stages of cancer development (high-grade dysplasia/in situ cancer stage) provided the first evidence of the BM acting as a very early sensor of peripheral transformation. Moreover, we profiled plasmatic microRNAs at late and early stages of tumor progression and found, already at early time points, differentially expressed microRNAs, which could potentially be used as early biomarkers of cancerogenesis. In conclusion, our data lay a first demonstration that BM hematopoietic adaptation to cancer is not confined to a general immunosuppressive state associated with advanced cancers, rather it represents an early

process co-evolving with malignant clone expansion.

Anti-CSPG4 DNA vaccination reveals potential therapeutic effects for the treatment of CSPG4$^+$ tumors: a comparative oncology study

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Among the most interesting targets for immunotherapeutic approaches, the Chondroitin Sulfate Proteoglycan (CSPG)4 stands out, with low expression in healthy tissues, high expression in several solid tumors and a key role in cancer progression. Because of the translational power of dogs as pre-clinical models for human malignancies and the CSPG4 over-expression by both human and canine malignant melanoma (MM), we demonstrated the safety and the clinical effectiveness of a xenogeneic human (Hu)-CSPG4 DNA vaccine in client-owned canine patients with stage II-III surgically resected CSPG4$^+$ MM. However, Hu-CSPG4 vaccine was barely effective in activating human T cells from healthy donors *in vitro*. Based on these results, we aimed to increase the translational power of our approach and to extend it for the treatment of other CSPG4+ tumors besides MM.
As a step forward in this direction, we generated a hybrid plasmid, derived in part from the Hu- and in part from the dog (Do)-CSPG4 sequences (HuDo-CSPG4). We tested the safety, immunogenicity and anti-tumor potential of HuDo-CSPG4 DNA vaccine in mice, in dogs with stage II-IV surgically resected CSPG4$^+$ MM and in a human setting *in vitro*.
Chimeric HuDo-CSPG4 vaccination resulted strongly immunogenic in mice. In canine patients, the procedure was safe and induced antibodies against both Hu- and Do-CSPG4, with a higher affinity and anti-tumor potential as compared to Hu-CSPG4. Clinically, HuDo-CSPG4 was effective in increasing the overall survival of vaccinated canine MM patients as compared to controls. Data obtained *in vitro* with T cells from human healthy donors suggested HuDo-CSPG4 is more immunogenic than Hu-CSPG4.
Moreover, we started to investigate CSPG4 role in both canine and human osteosarcoma (OSA) to eventually propose CSPG4 DNA vaccination as an innovative comparative therapy for OSA treatment, too. We found a strong correlation between CSPG4 over-expression and a worse prognosis in both human and canine OSA patients. The potentiality of CSPG4 immune-targeting for OSA treatment was demonstrated by the ability of anti-CSPG4 monoclonal antibodies (mAbs) to significantly inhibit both canine and human CSPG4$^+$ OSA *in vitro* proliferation, migration and osteospheres generation. In addition, anti- CSPG4 mAbs potentiated the anti-proliferative effect of doxorubicin. Interestingly, sera derived from canine MM patients enrolled in our previous veterinary trials, were also able to *in vitro* inhibit OSA cells tumorigenic potential in both adherent and non-adherent conditions.
Overall, these results provide the rationale to propose HuDo-CSPG4 vaccination for the treatment of canine CSPG4$^+$ tumors, to be successfully translated in a human setting.

A novel integrative strategy to prevent colorectal cancer within the diet-host-microbiota triangle: from organoids to human in vivo reality

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Colorectal cancer (CRC) is one of the most common cancers in the western world. Several hundreds of thousands people are diagnosed annually with CRC and over half of patients die or have comorbidities. Research has suggested that dietary patterns, dysbiosis and microbial metabolites may play a pivotal role in, leading to increasing interest among scientists. However, despite the fact that microbial metabolites play a crucial role in many biological cases, adequate tools for deciphering the relationship between diet-microbiome-host are not yet available. TRIANGLE aims to provide new insight into the mechanisms by which microbial metabolites may prevent CRC. The first objective is targeted at designing in vitro models mimicking human organogenesis and tumorigenesis to evaluate the role of microbial metabolites. To accomplish this, human colon organoids/tumoroids will be established. The second objective is to identify microbial metabolites that can act as cancer-preventive agents. These

metabolites will be produced with a gastrointestinal model inoculated with faeces from both healthy and CRC patients and determined by mass spectrometry. Released microbial metabolites from a food model will then be tested in the colon organoids/tumoroids, and metabolite signatures of organoids will be studied. Metabolomics analysis and 3D cell assays of colon organoids/tumoroids will provide valuable new insights into the mechanisms by which nutrient-gene interaction influences colon stem cell niche and CRC, and will open up new possibilities for CRC understanding and prevention. In summary, the results from TRIANGLE through the integration of microbial metabolites (diet), gut microbiota and colon organoids (host), will be highly multidisciplinary, and will undoubtedly set the stage towards the identification of mechanisms contributing to CRC understanding and will ultimately pave the way to phytochemical use and prevention.

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A screen for drugs that affect brain tumor development and microglia recruitment in zebrafish

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One of the hallmarks of brain cancer is the formation of an immune-microenvironment infiltrated with tumor associated macrophages (TAMs), which contribute to establish a status of chronic inflammation. Thus, the production of high amount of immune inhibitory cytokines or inflammatory mediators leads to an increase in tumor cells proliferation and invasion.
The most frequent and aggressive form of brain tumor, glioblastoma multiforme (GBM), is resistant to standard of care therapies because of its heterogeneity, infiltration properties and immune suppressive microenvironment, therefore innovative therapies are being investigated, among which immunotherapies, whose aim is to alleviate GBM-associated immune suppression and to boost anti-tumor immune responses.
In this project we use a zebrafish model of brain tumor to investigate the immune microenvironment of healthy and tumoral brains and the effect of compound treatments both on brain tumor development and immune cells, mainly microglia. Leukocytes, including macrophages and microglia are normally present in the brain at homeostatic conditions at larval and adult stages. Both in the larval and adult tumor model an increase in the number of immune cells in the region expressing the HRAS oncogene, together with an increased percentage of amoeboid-like active microglia was observed.
Given that an altered epigenetic landscape is very common in brain tumors, we have screened zebrafish larvae with a library of compounds targeting epigenetic factors and, as a result, 3 hits were found to have an effect in slowing down the development of brain tumors, but only one, an HDAC inhibitor, led to changes in microglia morphology.
In order to characterize gene expression changes in tumor cells after treatment with the HDACi, we have optimized the TRAP (Translating Ribosomes Affinity Purification) technique to specifically pull down translating ribosomes in the cytoplasm of oncogene-expressing brain cells. The translatome of HDACi treated tumor cells was compared with the transcriptome from FACS sorted zebrafish brain tumor cells; from the analysis of the differentially translated or transcribed genes, the translatome emerged as more precisely reflecting the biological phenotype that had been observed after HDACi treatment. Pathway analysis of the differentially translated genes revealed that the drug treatment caused the downregulation of many factors involved in purine metabolism, which could explain the decrease in brain tumor growth in the zebrafish model treated with HDACi.
Taken together, these results reinforce the knowledge that the zebrafish provides a useful model to investigate the effects of HDACi in brain tumors and associated microglia.

miRNAs as potential predictive biomarkers of metastases in thin and thick primary cutaneous melanomas

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Background: The early detection of primary cutaneous melanomas at high-risk of metastatic dissemination is essential to improve clinical management and outcomes of melanoma patients. The high potential of miRNAs as diagnostic, prognostic and therapeutic biomarkers in melanoma is well established. The aim of this study was to characterize miRNAs expression profile in relation to metastatic process of cutaneous melanoma and correlate miRNAs expression with clinical and pathological factors.
Materials and Method: Expression levels of six miRNAs, known to be involved in metastatic process (miR-145-5p, miR-150-5p, miR-182-5p, miR-203-3p, miR-205-5p and miR-211-5p), were analyzed by quantitative Real-Time PCR in a series of 32 metastatic and non-metastatic primary cutaneous melanomas, including thin and thick melanomas. Eight samples of metastases were also examined. Associations between miRNA expression levels and clinical-pathologic characteristics of primary tumors were also evaluated. All statistical analyses were performed with the R software (*www.r-project.org.*).
Results: A lower miR-205-5p expression was observed in metastases when compared with primary metastatic melanomas (p=0.04). Furthermore, a progressive downregulation of miR-205-5p expression was observed from loco-regional metastasis to distant metastasis.
Significantly lower miR-145-5p and miR-203-3p expression levels were found in cases with Breslow thickness >1mm (p = 0.002 and p = 0.005, respectively), high Clark level (p = 0.007 and p = 0.005, respectively), ulceration (p = 0.00001 and p = 0.0002, respectively) and mitotic rate ≥1/mm^2 (p = 0.02 and p = 0.001 respectively).
Conclusion: Our findings add insights into the characterization of miRNAs expression profile of thin and thick melanomas, pointing to miR-205-5p as potential marker of distant metastases and to miR-145-5p and miR-203-3p as markers of aggressiveness in primary tumors.

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Polyunsaturated fatty acids reduces in vitro tumor growth of colorectal cancer patient-derived organoids

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Epidemiological data support that obesity, due to a high-fat intake typical of Western diet, increases the risk of colon cancer (CRC). Recently it has been demonstrated that, high fat diet (HFD) and exposure to saturated fatty acids - such as palmitoleic acid (PA) - augments the numbers of intestinal progenitors and their tumorigenicity. Instead, polyunsaturated fatty acids such as docosahexaenoic acid (DHA) are shown to be protective against CRC in epidemiological studies; however experimental evidence supporting this anticancer effect, as well as the mechanism, is still lacking. We investigated the potential anti-CRC activity of polyunsaturated fatty acids in our collections of patient-derived CRC organoids.
DHA decreased CRC growth in vitro, while PA did not show any effect respect to control. Tumor growth inhibition was mainly caused by cell proliferation rate reduction (determined by ki-67 staining). Notably, this effect was much more prominent in KRAS-mutated compared to KRAS-wild type tumors. We further analyzed the effect of DHA or PA on organoids tumor initiating cells by assessing stem cell markers (by qPCR) and clonogenic capacity. DHA treatment diminished both Lgr5 and SOX2 RNA levels in KRAS-mutated organoids while PA treatment did not modify expression levels significantly. To assess the colony-forming efficiency we dissociated CRC-organoids to single cells and quantified the number of growing organoids. DHA treatment decreased colony formation respect to control while PA did not. Even DHA-pretreated organoids showed decreased colony efficiency compared to those that were not pre-treated, supporting a role for DHA in modulating the stem cell number in organoids. Our results indicate that polyunsaturated fatty acids affect organoid-initiating capacity and tumor growth supporting a potential role for prevention and treatment of CRC.

Pezcoller Foundation–AACR International Award for Extraordinary Achievement in Cancer Research

2019 Program guidelines and nomination instructions

NOMINATION DEADLINE
August 1, 2019

NOMINATION PROCESS
Nominations may be submitted by any invdividual, whether an AACR member or nonmember, who is currently or has previously been affiliated with any institution involved in cancer research, cancer medicine, or cancer-related biomedical science. Self-nominations are prohibited.
Nominators must maintain strict confidentiality of their nominations and all nominations must be submitted online to myaacr.aacr.org. Paper nominations will not be accepted.
Eligible nominations must include the following nomination materials:

- A letter of recommendation written in English (Max: 1,000 words) that comprehensively describes the nominee's major scientific achievement(s) in basic cancer research and/or their significant contributions to translational cancer research. This letter must also outline the nominee's current research activity and indicate how this research holds promise for continued substantive contributions to the cancer field.
- A brief scientific citation (Max: 50 words) highlighting the major scientific contribution(s) justifying the award candidate's nomination.

AWARD ELIGIBILITY AND CRITERIA
The prestigious Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research was established in 1997 to 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.
Eligible candidates must continue to be active in cancer research, have a record of recent, noteworthy publications, and be conducting ongoing work that holds promise for continued substantive contributions to progress in the field of cancer.
The Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research is intended to honor an individual scientist. However, more than one scientist may be co-nominated and selected to share the award in the event that their investigations are intimately related in subject matter and have resulted in work that is worthy of the award and a joint nomination.
Cancer researchers affiliated with institutions in academia, industry, or government involved in cancer research, medicine, or cancer-related biomedical science anywhere in the world are eligible. Institutions and/or organizations are not eligible to receive the award.

AWARD SELECTION PROCESS
All eligible nominees will be considered by a prestigious Pezcoller Foundation-AACR International

Award for Extraordinary Achievement in Cancer Research Selection Committee consisting of an international cohort of renowned cancer leaders appointed by the AACR President in consultation with the Pezcoller Foundation Council.
The Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research Selection Committee will consider all nominations as they have been submitted and are restricted from combining submitted nominations, adding new nominees, or otherwise making alterations to any submitted nomination.
Once chosen, the primary and alternate award recipient selections made by the Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research Selection Committee shall be sent to the AACR Executive Committee and the Pezcoller Foundation Council for final consideration and ratification.
Selection of the award winner will be made on the basis of the candidate's scientific accomplishments. No regard shall be given to race, gender, nationality, religion or political preference.
Selected award recipients will receive an unrestricted grant of €75,000, a commemorative award, and be invited to present a scientific lecture in conjunction with the AACR Annual Meeting immediately following their selection.

THE AWARD RECIPIENT

The winner of the Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research will present an Award lecture at the AACR Annual Meeting 2020 in San Diego, CA (April 24-29, 2020). The winner will also present the Twenty-third Annual Pezcoller Foundation-AACR International Award for Cancer Research Lecture, just prior to the official Award ceremony to be held in Trento, Italy in May 2020. Should the recipient be unable to participate in either event, the award must be forfeited and will instead be presented to the selected Award alternate.
In the rare event that there are dual winners of the Award, the monetary award will be shared equally between both recipients while the AACR Executive Committee will determine which of the two co-recipients will present the Pezcoller-AACR Award Lecture at the AACR Annual Meeting.

2020 Scholar-In-Training Awards

The **AACR** has been proud to offer Scholar-in-Training Awards to enable the participation of meritorious early-career scientists at the Annual Meeting 2019. Since its inception in 1986, the AACR Annual Meeting Scholar-in-Training Award program has provided more than 4,580 grants to young investigators and has received support from more than 55 cancer research foundations, corporations, individuals and other organizations dedicated to the fight against cancer. Scholar-in-Training Awards are highly competitive and recognize outstanding young investigators presenting meritorious proffered papers at the AACR Annual Meeting .

2020 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.
Selections are made by the criteria from Pezcoller (i.e. European scientists with at least one awardee representing Italy) and based on the meritorious score of the submitted abstract and application.
Any questions can be directed to sita@aacr.org

Picture:
2019 Scholar-In-Training Awardees with President Galligioni in Atlanta, March 31, 2019

The Pezcoller Foundation

Journal

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