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Editorial

This 34th Pezcoller Symposium, is the first under the scientific direction of Dr. William G. Kaelin and the renewed Scientific Standing Committee. We are deeply indebted to all of them for having accepted this challenging task, to ensure to the symposia, topics at the cutting edge of cancer science and the best experts in the field.
Both this year's topic and faculty indeed, fit perfectly with the tradition of excellence, begun in 1989 by Enrico Mihich and continued for many years by David Livingston.
A great deal of interest in fact has been raised in this symposium, aimed at **"identifying and clarifying the advances that have changed, or will change the way we study cancer and the ways we treat patients"**, as stated by Dr. Kaelin in focus and goals.
We have more than 260 registered participants this year, with the largest number of submitted posters and 57 of them have been accepted for presentation, by the selection committee coordinated by Dr. Massimo Loda.
In addition to the traditional formula of 25 minutes for each presentation, followed by a robust discussion, we have added this year a new session: the Career Development Panel Discussion. This will be chaired by Dr. Kaelin itself, to emphasize the special focus of the Pezcoller Symposia on young researchers.
Once again we have the pleasure of hosting the editors of 3 leading scientific journals and providing all interested researchers with the Symposium's highlights. Thanks to the collaboration with the CIBIO Department of the University of Trento and the European School of Oncology (ESO), they will be presented in an ESO international event on September 7th 2023.
Among other major activities of the Foundation over the course of this year, I would like to mention the Pezcoller – AACR International Award for Extraordinary Achievement in Cancer Research, given to Tak W. Mak from Toronto, a true giant in cancer research. Moreover, in collaboration with EACR, we awarded young European researchers with the Pezcoller – EACR Women in Cancer Research Award, won by Johanna Joyce, the Translational Cancer Researcher Award, won by Nicola Aceto and the new EACR – Mark Foundation – Pezcoller Foundation Rising Star Award, won by Isidro Cortes-Ciriano.
The Pezcoller Foundation also continues to support young researchers in Italy, through the7 two-year fellowships, awarded in collaboration with SIC, and the travel scholarships, in collaboration with AACR.
We are especially proud of all these collaborations and activities, aimed at Excellence in Cancer Research and support to young researchers, that are the fundamental pillars of our activities.
In the current issue of the Pezcoller Journal, we have included some information on the call for nominations for the Awards, the fellowships and the scholarships that may potentially interest some participants and their institutes.
Finally, I can't thank enough all those who made a key contribution to the organization and management of this Symposium: the chairman William Kaelin, the members of the Standing Committee and of the Poster selection Committee, the staff of the Pezcoller Foundation, the Orikata agency and Jam Session technical services and, last but not least, the Humanities Department of the University of Trento, for hosting the Symposium in this so prestigious and comfortable venue.

Enzo Galligioni
President

Picture on front page:
Dr William G. Kaelin, Chairman
of Pezcoller Symposia since 2022

34th Pezcoller Symposium

June 19–20, 2023
University of Trento, Auditorium of Humanities Department
Via Tommaso Gar, 14, Trento

New technologies for studying and treating cancer

Moderators:

Kaelin William G. (2019 Nobel Laureate)
Ambrogio Chiara
Bardelli Alberto
Bonini Chiara
Del Sal Giannino
Demichelis Francesca
Draetta Giulio
Loda Massimo
Piccolo Stefano
Schulman Brenda A.

Day 1 – Monday, June 19, 2023
Central European Summer Time (CEST)

8.00 ***Registration***

8.30 ***Welcome*** Enzo Galligioni

8.40 ***Focus and Goals*** William G. Kaelin

8.50 ***Moderator:*** Giulio Draetta
The David Livingston Lecture
"Micromapping: A new approach to mapping of intra- and extracellular microenvironments."
David W. MacMillan, Ph.D.
(2021 Nobel Laureate)
(Princeton University)

9:30 Discussion

9.45 ***"Inspiration from Nature on Drugging "Undruggable" Targets"***
Gregory Verdine, Ph.D.
(Harvard University)

10.10 Discussion

10.25 Coffee break & poster exhibition

10.40 ***Moderator:*** Francesca Demichelis
"Integrating Genomics and Computation for Cancer Target and Drug Discovery"
Xiaole Shirley Liu, Ph.D
(GV20 Therapeutics)

11.05 Discussion

11.20 ***"Machine learning for identifying translatable biomarkers and targets in oncology"***
Daphne Koller, Ph.D. (*Insitro, Inc.)*

11.45 Discussion

12.00 ***"The power of ONE: Immunology in the age of single cell genomics"***
Ido Amit, Ph.D.
(Weizmann Institute of Science)

12.25 Discussion

12.40 Lunch & poster exhibition

14.20 ***Moderator:*** Chiara Ambrogio
"Ubiquitin ligases and signaling"
Brenda A. Schulman, Ph.D.
(Max Planck Institute of Biochemistry)

14.45 Discussion

15.00 ***"Single cell spatial proteomics: Technology and applications to oncology"***
Matthias Mann, Ph.D.
(Max Planck Institute of Biochemistry)

15.25 Discussion

15.40 ***Moderator:*** Giannino Del Sal
"Illuminating dark proteome-mediated transcriptional control in cancer by live-cell single-molecule imaging"
Shasha Chong, Ph.D.
(California Institute of Technology)

16.05 Discussion

16.20 ***"Fighting cancer via identification and characterization of molecular glue degraders"***
Georg Winter, Ph.D.
(CeMM- Research Center for Molecular Medicine of the Austrian Academy of Sciences)

16.45 Discussion

17.00 ***Moderator:*** William G. Kaelin
Career Development Panel Discussion

17.45 ***END OF DAY 1***

20.00 Symposium dinner, at Grand Hotel Trento

Program

Day 2 – Tuesday, June 20, 2023
Central European Summer Time (CEST)

8.30 ***Moderator:*** Alberto Bardelli
The Enrico Mihich Lecture
"Updates with CAR T cells"
Carl H. June, M.D.
(Parker Institute for Cancer Immunotherapy at the University of Pennsylvania)

9.10 Discussion

9.25 ***"NK cell engineering for cancer immunotherapy"***
Katy Rezvani, M.D., Ph.D.
(MD Anderson Cancer Center)

9.50 Discussion

10.05 ***"Targeting KRAS mutants using covalent chemistry and the immune system"***
Kevan Shokat, Ph.D.
(University of California, San Francisco)

10.30 Discussion

10.45 Coffee break & poster exhibition

11.00 ***Moderator:*** Chiara Bonini
"Engineering next-generation tumoroids for precision medicine"
Matthias Lütolf, Ph.D.
(EPFL)

11.25 Discussion

11.40 ***"Spatially resolved single-cell genomics & cell atlas of the brain"***
Xiaowei Zhuang, Ph.D.
(Harvard University)

12.05 Discussion

12.20 Lunch & poster exhibition

13.20 ***Moderator:*** Massimo Loda
Maria Begnudelli Awards: oral talks of the 3 best poster presenters

14.20 ***Moderator:*** Stefano Piccolo
"Resolving variant effects at scale with Saturation Genome Editing"
Greg Findlay, Ph.D., M.D.
(The Francis Crick Institute)

14.45 Discussion

15.00 ***"Where Does He Get Those Wonderful Toys? A Tour of the Functional Genomics Toolbox"***
John Doench, Ph.D.
(Broad Institute of MIT and Harvard)

15.25 Discussion

15.40 ***"Early detection of cancer using cell-free DNA fragmentomes"***
Victor E. Velculescu, M.D., Ph.D.
(Johns Hopkins University School of Medicine)

16.05 Discussion

16.20 ***Moderator:*** Brenda A. Schulman
"New Approaches for Finding Tiny Tumors: Towards Early Detection, Intervention, and Treatment of Ovarian Cancer"
Angela M. Belcher, Ph.D.
(Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology)

16:45 Discussion

17:00 ***"Using chemistry to find new cancer targets"***
Deepak Nijhawan, M.D., Ph.D.
(UT Southwestern Medical Center, Harold C. Simmons Comprehensive Cancer Center)

17:25 Discussion

17.40 Closing remarks

Faculty

- **Amit Ido**
  Weizmann Institute of Science, Rehovot, ISR
- **Belcher Angela M.**
  Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA
- **Chong Shasha**
  California Institute of Technology, Pasadena, CA
- **Doench John**
  Broad Institute of MIT and Harvard, Cambridge, MA
- **Findlay Greg**
  The Francis Crick Institute, London, UK
- **June Carl H.**
  Parker Institute for Cancer Immunotherapy, University of Pennsylvania, Philadelphia, PA
- **Koller Daphne**
  Insitro, Inc., San Francisco, CA
- **Liu Xiaole Shirley**
  GV20 Therapeutics, Cambridge, MA
- **Lütolf Matthias**
  EPFL, Lausanne, CH
- **MacMillan David W.**
  Princeton University, California Institute of Technology, Princeton, NJ
- **Mann Matthias**
  Max Planck Institute of Biochemistry, Munich, DE
- **Nijhawan Deepak**
  UT Southwestern Medical Center, Harold C. Simmons Comprehensive Cancer Center, Dallas, TX
- **Rezvani Katy**
  MD Anderson Cancer Center, Houston, TX
- **Schulman Brenda A.**
  Max Planck Institute of Biochemistry, Martinsried, DE
- **Shokat Kevan**
  University of California, San Francisco, CA
- **Velculescu Victor E.**
  Johns Hopkins University School of Medicine, Baltimore, MD
- **Verdine Gregory L.**
  Harvard University, Cambridge, MA
- **Winter Georg**
  CeMM, Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, AT
- **Zhuang Xiaowei**
  Howard Hughes Medical Institute, Harvard University, Cambridge, MA

Discussants

- **Kaelin William G.**
  Dana-Farber Cancer Institute, Boston, MA
- **Ambrogio Chiara**
  University of Torino, ITA
- **Bardelli Alberto**
  IFOM, Milano, ITA
- **Bonini Chiara**
  Università Vita-Salute San Raffaele, Milano, ITA
- **Del Sal Giannino**
  ICGEB, University of Trieste, ITA
- **Demichelis Francesca**
  CIBIO Department, University of Trento, ITA
- **Draetta Giulio**
  MD Anderson Cancer Center, Houston, TX
- **Loda Massimo**
  Weill Cornell Medicine, New York, NY
- **Piccolo Stefano**
  University of Padova, ITA
- **Schulman Brenda A.**
  Max Planck Institute of Biochemistry, Martinsried, DE

34th Pezcoller Symposium

New Technologies for Studying and Treating Cancer

Trento, Italy, June 19-20, 2023

ABSTRACTS OF ORAL PRESENTATIONS

Micromapping: A New Approach to Mapping of Intra- and Extracellular Microenvironments

David W. C. MacMillan

Merck Center for Catalysis, Princeton University, Princeton, NJ 08544, USA

This lecture will describe µMap, a new photoredox-based approach to microenvironment mapping that provides a powerful means to probe biological pathways at the subcellular level. µMap uses a light-driven energy transfer mechanism to activate warheads proximal to localized iridium catalysis, which, in turn, label neighboring biomolecules in a complex intra- or extracellular environment. We will discuss the development of this new technology and its application in a number of biological contexts, including intrasynaptic labeling, target identification, mapping of the interactome of checkpoint inhibitors and CAR-T cells, and mapping of chromatin state changes.

Inspiration from Nature on Drugging "Undruggable" Targets

Gregory L. Verdine

Harvard University and Harvard Medical School, LifeMine Therapeutics, FogPharma

There currently exists a substantial Actionability Gap separating the ability of biological science to identify human disease drivers versus the ability of interventional science to invent therapeutics that modulate those drivers. The Actionability Gap pervades all therapeutic areas, but is particularly striking in cancer, because so much is known about the molecular details of disease etiology, most of the truly impactful targets are intracellular where the druggability challenge is particularly high, and the unmet medical need warrants extreme urgency. How best to tackle this problem? For the past several decades, the speaker and his research teams have turned to nature as inspiration for fundamentally new approaches toward tackling intractable drug discovery challenges. These approaches and their tangible outcomes will be reviewed.

Integrated Genomics and Computation for Cancer Target and Drug Discovery

X. Shirley Liu, Ph.D.

GV20 Therapeutics, Cambridge, MA, USA

Despite the exciting clinical benefits of immune checkpoint inhibitors, only a minority of cancer patients respond to treatment. Addressing resistance to immune checkpoint inhibitors is an urgent unmet need and requires new approaches for target identification and drug discovery.
GV20 Therapeutics adopts an interdisciplinary approach integrating functional genomics, big data AI, and cancer immunology for cancer target identification and drug discovery. Our platform computationally extracts antibodies from large cohorts of patient tumor RNA-seq profiles, and uses AI to pair targets and corresponding antibodies in silico, de novo with speed and scale. We then leverage in-house and public functional genomics and proteomics data to de-risk the AI-identified targets from patient tumors and provide insights on target function, before we conduct systematic in vitro and in vivo validation experiments. We used this approach to discover our lead program, GV20-0251, which is a first-in-class monoclonal antibody against a novel immune checkpoint IGSF8. In multiple syngeneic tumor models, anti-IGSF8 antibody has single-agent efficacy and is synergistic with anti-PD1 in controlling tumor growth, and the safety of GV20-0251 is currently being tested in the clinic. Our efforts represent the beginning of rationally combining genomics and AI to unlock the hidden information from patient tumors to develop cancer immunotherapeutics.

Machine learning for identifying translatable biomarkers and targets in oncology

Daphne Koller

Insitro, Inc.

Modern medicine has been transformative to the care of patients with certain types of cancers, turning a death sentence into a chronic disease or even (occasionally) a complete cure. However, progress recently has slowed considerably, with many of the new interventions providing limited benefit or highly heterogeneous responses. A key factor in this trend is that we simply don›t understand the underlying biology of disease, and are therefore unable to predict which interventions might meaningfully modulate clinical outcomes and in which patients. At insitro, we are pursuing an oncology discovery strategy that relies on primary human data at its core, bypassing reductionist cell systems or non-translatable animal models. These high content, multi-modal data are interpreted via cutting edge machine learning models to create a meaningful representation of human pathophysiological states, which enables us to identify novel targets and biomarkers for coherent patient segments, and use those to accelerate the development of effective therapeutic interventions.

The power of ONE: Immunology in the age of single cell genomics

Ido Amit

Weizmann Institute of Science

The immune system is a complex, dynamic and plastic network composed of various interacting cell types that are constantly sensing and responding to environmental cues. From very early on, the immunology field has invested great efforts to characterize the various immune cell types and elucidate their functions. However, accumulating evidence indicates that current technologies and classification schemes are limited in their ability to account for the functional heterogeneity of immune processes. Single cell genomics hold the potential to revolutionize the way we characterize complex immune cell assemblies and study their spatial organization, dynamics, clonal distribution, pathways, and crosstalk. This emerging field can greatly affect basic and translational research of the immune system. I will discuss how emerging single cell genomic studies are changing our perspective in cancer immunology. Finally, I will consider recent and forthcoming technological and analytical advances in single cell genomics and their huge potential impact on the future of immunology research and immunotherapy.

Ubiquitin ligases and signaling

Brenda A. Schulman[1], [2]

[1] Max Planck Institute of Biochemistry, Martinsried, Germany
[2] St. Jude Children›s Research Hospital, Memphis, TN, USA

The specificity of ubiquitylation depends on a vast collection of E3 ligase enzymes that modify particular protein substrates at the right time and place in a cell. With approximately 300 different family members in humans, and even more in plants and other organisms, the largest cohort of E3 ligases are the modular, multisubunit Cullin-RING ligases (CRLs). CRLs comprise nearly half of all E3 ligases; they mediate a large fraction of all protein degradation, and regulate virtually every facet of cell biology, including the cell cycle, DNA repair, stress responses, signaling, immunity, circadian rhythms, and a plethora of other pathways. Many bacterial and viral pathogens subvert and/or hijack CRL pathways to promote infection and evade host defense systems. Moreover, defects in specific CRL pathways subunits underlie numerous cancers. Meanwhile, CRLs are amongst the hottest platforms for therapeutic targeted protein degradation. To understand this immense regulation, a major focus of our lab is to identify pathways and determine molecular mechanisms underlying CRL activities.
In will present our latest data investigating the underlying molecular mechanisms, and profiling, CRL responses to biological stimuli including metabolic signals, cytokines, and drugs mediating targeted protein degradation. From a conceptual perspective, our data reveal a systemwide mechanism for multiprotein complex formation: a limiting component is recycled from idling complexes to fuel mix-

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ing-and-matching of parts and transient stabilization of the subset of complexes needed at a given time. This averts supply chain problems, obviates a need for producing new parts, prevents buildup of superfluous and potentially toxic molecular machines, and allows rapidly establishing degradation pathways needed for cellular regulation.

Single cell spatial proteomics: Technology and applications to oncology

Prof. Dr. Matthias Mann

Max Planck Institute of Biochemistry, Martinsried, Germany

Breakthroughs in mass spectrometry-based proteomics, computational biology, and deep learning have recently come together to enable new biomedical insights. In this talk, I will summarize these advances and how they enable a broad range of systems-wide interrogations of the proteome. One of these emerging directions is single cell proteomics. When coupling new mass spectrometric instrumentation with low flow chromatography, thousands of proteins can be quantified specifically and accurately, allowing us to describe cellular heterogeneity. We leverage this sensitivity in our new workflow termed Deep Visual Proteomics (DVP), combining high-content microscopy, AI-driven image recognition, and ultrahigh sensitivity MS in order to connect visual, spatial and molecular proteomics data at single cell type level. We are applying DVP to precision medicine in a variety of diseases, using archived formalin-fixed paraffin embedded tissue. These include single cell-type analysis of borderline ovarian cancers where we complement our DVP proteomics approach with transcriptomics data to uncover distinct proteomic and transcriptomic signature that reflect the malignant progression and disease outcome. In the field of dermatology, we are applying DVP to analyze different cell-types present in the skin of patients suffering from severe cutaneous drug reactions and melanoma, in order to identify disease drivers that may ultimately lead to new therapeutic options. We believe that the spatial resolution of our DVP approach has great potential for the characterization of cell types in cancer helping to pave the way towards precision oncology.

Illuminating dark proteome-mediated transcriptional control in cancer by live-cell single-molecule imaging

Shasha Chong

Caltech, California Institute of Technology

Mammalian transcription factors (TFs) ubiquitously contain intrinsically disordered low-complexity domains (LCDs), but how these LCDs perform transactivation functions remains unclear. Using quantitative single-cell and single-molecule imaging, we found that TF LCDs undergo dynamic, multivalent, and selective protein-protein interactions, which drive the formation of local high-concentration TF “hubs” at synthetic and endogenous genomic loci. The formation of LCD interaction hubs plays an essential role in transactivation. We next tuned the level and localization of LCD-LCD interactions of the oncogenic TF EWS::FLI1 in Ewing sarcoma cells and found that efficient activation of endogenous human genes by EWS::FLI1 requires narrow optimum of LCD-LCD interactions at the genes. Increasing LCD-LCD interactions toward putative liquid-liquid phase separation (LLPS) represses transcription of these endogenous genes. Likewise, ectopically creating LCD-LCD interactions to sequester EWS::FLI1 into a well-documented LLPS compartment, the nucleolus, represses EWS::FLI1-driven transcription and inhibits malignant transformation of Ewing sarcoma. We further found that a TF diffuses significantly more slowly in the nucleolus than in the nucleoplasm, demonstrating an important impact of LLPS on TF dynamic behaviors and single-molecule diffusion measurement as an effective approach to diagnose LLPS in living cells. Our findings reveal fundamental mechanisms underpinning transcriptional control and suggest potential therapeutic strategies for targeting oncogenic TFs.

Fighting Cancer via Identification and Characterization of Molecular Glue Degraders

Georg E. Winter

CeMM Research Center for Molecular Medicine of the Austrian Academy of Science, Vienna, Austria

Targeted protein degradation is a pharmacological paradigm that is based on small molecules that induce molecular proximity between disease-causing proteins and effectors of the cellular degradation machinery. Here, I will discuss our efforts in employing multi-omics approaches in order to identify and characterize small-molecule degraders that target previously undruggable proteins for degradation by the proteasome or for clearance via macroautophagy. Additionally, I will discuss how we are using deep mutational scanning to experimentally infer functional hotspots in E3 ubiquitin ligases and review the associated implications for the emergence of resistance mechanisms for small-molecule degraders.

Updates with CAR T cells

Dr. Carl H. June

Parker Institute for Cancer Immunotherapy at the University of Pennsylvania

Advances in the understanding of basic immunology have ushered in two major approaches for cancer therapy over the past 10 years. The first is checkpoint therapy to augment the function of the natural immune system. The second uses the emerging discipline of synthetic biology and the tools of molecular biology and genome engineering to create new forms of engineered cells with enhanced functionalities. The emergence of synthetic biology approaches for cellular engineering provides a broadly expanded set of tools for programming immune cells for enhanced function. Barriers to therapy of solid tumors will be discussed.

Reference: Good CR, Aznar MA, Kuramitsu S, et al. An NK-like CAR T cell transition in CAR T cell dysfunction. Cell. 2021;184(25):6081-100.e26.

NK cell engineering for cancer immunotherapy

Dr Katy Rezvani

MD Anderson Cancer Center

Dr. Rezvani will discuss a new frontier in NK cell therapeutics: engineering NK cells with chimeric antigen receptors. She will discuss the opportunities and challenges of NK cell CAR engineering, and present pre-clinical and early phase clinical data on cord blood-derived NK cells expressing CD19 CAR and IL-15 to enhance their in vivo persistence in patients with relapsed or refractory blood cancers. In addition, she will discuss novel strategies for the gene editing of CAR NK cells to enhance their function by targeting immune checkpoints. Finally, she will discuss the approach of precomplexing NK cells with an anti-CD16 bispecific antibody targeting CD30 to redirect their specificity, and updates on a clinical trial using this approach in patients with CD30-expressing lymphoma.

Targeting KRAS mutants using covalent chemistry and the immune system

Kevan Shokat

University of California, San Francisco

Somatic mutations in the small GTPase K-Ras are the most common activating lesions found in human cancer, and are generally associated with poor response to standard therapies. Efforts to directly target this oncogene have faced difficulties due to its picomolar affinity for GTP/GDP and the absence of known allosteric regulatory sites. I will discuss the development of small molecules that irreversibly bind to a common oncogenic mutant, K-Ras G12C. These compounds rely on the mutant cysteine for binding and therefore do not affect the wild type protein (WT). New covalent molecules targeting K-Ras G12S and G12R are currently under development and will be discussed. I will also discuss ways to leverage the immune system to overcome drug resistance.

Engineering next-generation tumoroids for precision medicine

Matthias Lütolf

EPFL

Organoids form through poorly understood morphogenetic processes in which initially homogeneous ensembles of stem cells spontaneously self-organize in suspension or within permissive three-dimensional extracellular matrices. Yet, the absence of virtually any predefined patterning influences such as morphogen gradients or mechanical cues results in an extensive heterogeneity. Moreover, the current mismatch in shape, size and lifespan between native organs and their in vitro counterparts hinders their even wider applicability. In this talk I will discuss some of our ongoing efforts in developing next-generation organoids that are assembled by guiding cell-intrinsic self-patterning through engineered stem cell microenvironments. In particular, I will highlight how we are using these technologies to build next-generation patient-based tumor models for precision medicine.

Spatially resolved single-cell genomics & cell atlas of the brain

Xiaowei Zhuang

Howard Hughes Medical Institute, Harvard University

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Inside living organisms, thousands of different genes function collectively to give rise to cellular behavior and tissue function. Understanding the behaviors and functions of cells and tissues thus require imaging at the genome scale, which will advance our understanding in many areas of biology, ranging from the regulation of gene expression in cells to the development of cell fate and the organization of cell types in complex tissues. We developed a single-cell transcriptome and genome imaging method, multiplexed error-robust fluorescence in situ hybridization (MERFISH), which allows RNA, DNA, and epigenetic marks to be imaged at the genome scale. This approach enabled spatially resolved transcriptomic profiling, epigenomic profiling, and 3D-genome organization mapping in single cells. The ability to perform single-cell gene expression profiling in intact tissues further enabled the identification, spatial mapping, and functional annotation of distinct cell types in intact tissues. In this talk, I will describe the MERFISH technology and its applications, with a focus on mapping the molecular, spatial, and functional organizations of cell types in the mouse and human brain.

Resolving variant effects at scale with Saturation Genome Editing

Greg Findlay

The Francis Crick Institute, London

Our incomplete understanding of how rare variants contribute to disease phenotypes substantially limits the clinical utility of genomic data. To address this, we developed Saturation Genome Editing (SGE), a CRISPR-based method to assay all possible single nucleotide variants across targeted genomic regions. We've used SGE to functionally characterise over 10,000 variants across the tumour suppressor genes BRCA1 and VHL. The resulting variant effect maps reveal loss-of-function variants acting via diverse mechanisms and predict human disease risk with extremely high accuracy. Ongoing work centres on scaling SGE and related technologies to more cell types, phenotypic assays, and genes, towards the ultimate goal of being able to predict the consequences of any variant encountered clinically.

Where Does He Get Those Wonderful Toys? A Tour of the Functional Genomics Toolbox

John Doench

Broad Istitute of MIT and Harvard

We will cover two technologies that allow exploration of the genome at broad scale and fine resolution. For the former, Cas12a, unlike Cas9, allows for multiplexing guide RNAs from a single transcript, simplifying combinatorial perturbations, and we will share work mapping synthetic lethal interactions with knockout screens, as well as progress in developing Cas12a for CRISPR activation screens. Additionally, base editor technology enables nucleotide-level manipulation, and we will present screens using this approach to map cancer-relevant genes and pathways.

Early detection of cancer using cell-free DNA fragmentomes

Victor E. Velculescu

MD, PhD. Johns Hopkins University School of Medicine

Cell-free DNA in the blood provides a non-invasive diagnostic avenue for patients with cancer. However, characteristics of the origins and molecular features of cell-free DNA are poorly understood. We have developed an approach to evaluate fragmentation patterns of cell-free DNA (cfDNA fragmentomes) across the genome using low coverage whole genome sequencing. We have found that cfDNA fragmentomes reflect an integrated view of chromatin, genome, and transcriptome states of normal and cancer cells of an individual. Machine learning models of multifeature fragmentome data show high performance for early detection of many common cancers, including lung, liver, ovarian, pancreatic and others, in individuals who are at average or high-risk of developing these diseases. These findings provide a biological basis for changes in cfDNA fragmentation in patients with cancer and provide an accessible approach for noninvasive cancer detection.

Developing Tools to Detect Millimeter and Sub-millimeter Ovarian and Fallopian Tumors. Towards Early Detection and Interception of Ovarian Cancer

Angela M Belcher

Massachusetts Institute of Technology
Koch Institute for Integrative Cancer Research

Recent studies have suggested that ovarian cancer has a probable origin at the fallopian tube (FT) epithelium, through the emergence of early pre-cancerous lesions called serous tubal intra-epithelial carcinoma (STICs). We currently lack a high-throughput and reproducible way to identify and study STICs and other precancerous lesions in the FTs. We have developed a suite of imaging instruments to image sub-millimeter sized tumors in whole animals and human tissues. For imaging, we have focused on a particular form of near-infrared (NIR) optical imaging, in the second near-infrared optical window (NIR-II: 1000 - 1700 nm), which offers significant advantages for high-resolution imaging over other imaging techniques. NIR operates within the biological "window" increasing the ability to see through and within biological tissues use of non-radioactive molecular probes; has low tissue autofluorescence background; reduced scattering; large Stokes' shift allowing for greater contrast; and insensitivity to photobleaching; and relatively low cost for wide-scale deployment. In order to leverage these advantages of our NIR-II nanoprobes, we have also designed a hyperspectral NIR-II optical imaging system, which can perform spectral analysis without requiring a priori knowledge of the photo-physical origins of the signal, resulting in improved signal-to-noise. Previously we have demonstrated the ability to identify clusters as small as a few cells, ~ 100 µm in size, well beyond the resolution of macroscopic (whole body) imaging systems such as CT or MRI. By suitably adapting our hyperspectral imaging system for whole-organ imaging of excised FT specimens ex vivo, we aim to detect the presence of STIC clusters located anywhere within the entire tube, instead of relying on chance detection from sectioning only ~ 0.5% of the organ. In addition, this will allow us to identify, characterize and collect live precancerous cells to better understand the biology, including the variability in their malignant potential and genetic makeup.

Using chemistry to find new cancer targets

Deepak Nijhawan

UT Southwestern Medical Center, Harold C. Simmons Comprehensive Cancer Center

Orphan cytotoxins are small molecules for which the mechanism of action (MoA) is either unknown or ambiguous. Unveiling the mechanism of these compounds may lead to useful tools for biological investigation and in some cases, new therapeutic leads. In select cases, the DNA mismatch repair-deficient colorectal cancer cell line, HCT116, has been used as a tool in forward genetic screens to identify compound-resistant mutations, which have ultimately led to target identification. To expand the utility of this approach, we engineered cancer cell lines with inducible mismatch repair deficits, thus providing temporal control over mutagenesis. By screening for compound resistance phenotypes in cells with low or high rates of mutagenesis, we increased both the specificity and sensitivity of identifying resistance mutations. We have used these cells as a platform to identify compound resistant mutations for novel orphan cytotoxins emerging from high throughput phenotypic screens of large chemical libraries. These results have led to new chemical strategies to target proteins important for cancer cell growth.

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Abstracts Of Posters

An arginine to citrulline conversion controls the epigenome and the proteome of cancer cells leading to the acquisition of resistance

F. Agostini, A. Ferino, Y. Cortolezzis, L. Xodo, E. Di Giorgio

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KRAS mutations are frequently observed in pancreatic ductal adenocarcinoma (PDAC), in colorectal adenocarcinoma (CRC) and in non-small cell lung cancer (NSCLC). These tumors were reported to be KRAS-addicted, but differently from what thought at the beginning a relevant part of KRAS dependency is metabolic. We have recently demonstrated that the blockage of KRAS signaling achieved by knocking-out the down-stream target NRF2 or by forcing the degradation of KRAS with LC-2 causes a metabolic reprogramming of pancreatic cancer cells, which switch off glycolysis and become auxotrophic to arginine. A similar metabolic resetting was observed in patient's derived organoids treated with neoadjuvant FOLFIRINOX therapy suggesting that targeting the dependency from exogenous arginine could represent an exploitable strategy to overcome cancer resistance to therapy. Interestingly, in all these models in which a boost in arginine metabolism was observed the expression of Peptidyl Arginine Deiminases (PADIs) was switched-on. PADIs comprise five members (PADI1,2,3,4,6) which catalyze the deimination of peptidyl-arginine into citrulline. Histone citrullination promotes gene expression by creating a relaxed chromatin, while citrullination of enzymes can affect their activity because it modifies their secondary and tertiary structure. We found that hypoxia triggers the expression of PADI1 and PADI3, both in normal and in PDAC, CRC and NSCLC cancer cells and this up-regulation is linked to a perturbation of pyruvate kinase activity. Inhibition of PADI enzymes with BB-Cl amidine perturbed the viability of cancer cells. CRC and PDAC patients with higher mRNA expression levels of PADI1 and PADI3 were characterized by a worse prognosis.
With this project we aim to: a) define the citrullinome by identifying the citrullinated proteins in hypoxia and normoxia; b) identify the signature of genes under the control of PADI1 and PADI3 by evaluating the transcriptome in BB-Cl amidine treated cells and knocked-down for PADI1 and PADI3; c) identify the direct transcriptional targets of PADI1 and PADI3 by obtaining the H3R-8cit profile in PADI1 and PADI3 knocked-down cells in respect to control cells; d) identify the epigenetic and post-transcriptional mechanisms that control the expression of PADI1 and PADI3; e) evaluate the contribution of PADI1 and PADI3 enzymes in controlling the metabolic resetting of cancer cells that have acquired resistance to KRAS inhibition, NRF2 blockage and FOLFIRINOX therapy and that are all characterized by a re-expression of PADI1 and PADI3 enzymes.
Even though we are only at the beginning of this project, a better comprehension of the role of PADIs is of particular interest because it will allow to understand more deeply the molecular mechanisms that sustain the metabolism of cancer cells. This could also allow the identification of new and more specific targets for therapy.

EMID2 selected through in vivo secretome screening for its tumor microenvironment-modulating and anti-invasive properties

Alfi Edoardo

In the past, strategies to combat cancer have primarily focused on impeding the proliferation of cancer cells. However, contemporary approaches have shifted their attention to the surrounding environment in which the tumor is situated. Notably, the extracellular matrix of cancer cells differs in composition and structure from that of normal cells. To identify proteins capable of restraining tumor growth and expansion by modulating the tumor microenvironment, we conducted an in vivo screening of an Adeno-Associated Vector secretome library in a mouse model of cancer. Among the proteins tested, EMID2 demonstrated the strongest ability to inhibit cancer cell invasiveness. The integration of EMID2 in the extracellular matrix altered its mechanical properties and structure, which resulted in reduced cancer cell migration and angiogenesis. These changes were primarily attributed to the protein›s capacity to impede the maturation of TGFß and the activation of cancer-associated fibroblasts.

Gold Nanoparticles and endothelial progenitor cells: a win-win alliance for targeting tumours

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INTRODUCTION: Plasmonic photothermal therapy utilizes biologically inert Near Infrared (NIR) gold nanoparticles (AuNPs) that convert light into heat capable of eliminating cancerous tissue. This approach has lower morbidity than surgical resection and can potentially synergize with other treatment modalities including chemotherapy and immunotherapy. In this work, we propose tumor tropic cellular vectors, called Endothelial Colony Forming Cells (ECFCs), enriched with gold chitosan-coated nanorods (AuNRs). ECFCs display a great capability to intake AuNRs without losing viability and exerting an in *vitro* and *in vivo* antitumor activity per se.

METHODS Conventional optical and Transmission electron microscopes (TEM), Photoacoustic imaging (PA) were used to evaluate AUNRs intracellular uptake in Melanoma cells (M6) and ECFCs. Melanoma spheroids were employed to investigate the behavior of AuNRs-ECFC in 3D-culture. The tumor tropic properties of AuNRs-ECFC were confirmed in vivo, using a human melanoma xenograft rat model.

RESULTS: The PA signal provided from ECFC loaded with AuNRs exhibited a stronger enhancement compared to AuNRs-M6. As expected, ECFCs loaded with AuNRs, thanks to their ability to enter the spheroid, exert their antitumor activity by reducing the volume of the sphere, compared to control spheroids plated with unloaded ECFCs. Besides, the PA signal provided from AuNR-ECFCs inside spheroids exhibited a strong enhancement compared to M6-AuNRs ones. Histological analyses of explanted tumor mass demonstrate that gold is still retained after 1 week from injection and organs including liver, spleen, kidney, and lung did not show any morphological alteration compared to control rats treated with unloaded ECFCs.

CONCLUSIONS: We demonstrated in vitro that AuNRs-loaded ECFCs are able to generate higher photoacoustic signals than AuNRs loaded in M6 cells. 3D cultures confirm the cytostatic effect of AuNRs-ECFC on tumor. In vivo, we show, via immunohistochemical analysis, a great tumor-homing efficiency of AuNRs–ECFCs after a bolus intravenous administration and their permanence inside the tumor masses 1 week after administration. These important AuNRs properties will be exploit in the so called "Trojan Horse Strategy" to perform NIR-infrared photothermal ablation and re-sensitize melanoma cells to radiotherapy.

Targeting carbonic anhydrase ix to overcome gastric cancer drug resistance and stimulate immunogenic cell death

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Background. Gastric cancer (GC) represents the fifth most frequently diagnosed malignancy and the fourth leading cause of cancer-related death worldwide. When upfront surgery is not pursuable, multimodal perioperative chemotherapy (pCT) is used to improve patients› overall survival. However, GC progressively gains chemoresistance, limiting therapies, thus the identification of suitable targets to overcome drug resistance is of fundamental interest. Amongst the potential biomarkers, the carbonic anhydrase IX (CAIX) has gained the most attention.
Methods. GC patients who underwent pCT FLOT (i.e., Leucovorin, 5-Fluouracil, Docetaxel, and Oxaliplatin) followed by gastrectomy at the Careggi hospital in Florence were classified as responder and non-responder, depending on the tumor regression grade, and formalin-fixed paraffin-embedded GC sections were analyzed by immunohistochemistry to detect the CAIX levels. GC cells were sorted at FACS for the CAIX expression or forced to overexpress CAIX by using the pRP-EGFP/Neo-CMV>hCA9 plasmid. FLOT-resistant GC cells were generated according to the "high-level laboratory models". The ureido-benzene-sulphonamide SLC-0111 was used to inhibit CAIX activity in GC cells. Colony formation, MTT, and caspase 3/7 flow cytometry assay were performed following GC cell treatment with single or combined SLC-0111/FLOT. GC spheroids were treated as well and evaluated with the ReViSP software. The expression or release by GC cells of damage-associated molecular patterns (DAMPs) as Annexin A1 (ANXA1), Calreticulin (CALR), High Mobility Group Box 1 (HMGB1), and C-X-C motif chemokine ligand 10 (CXCL10) was evaluated in flow cytometry and ELISA following the single and combined SLC-0111 treatment.
Results. CAIX expression in GC patients was significantly higher in the non-responder group than in the responder one. In the experimental setting, the CAIX-high-expressing AGS GC cells were more resistant to the pCT FLOT than the CAIX-low-expressing counterpart. In line with this observation, the forced overexpression of CAIX in AGS cells significantly impaired therapy response compared to control cells. Moreover, FLOT-resistant AGS and ACC-201 GC cells overexpressed CAIX compared to control cells. SLC-0111 significantly improved the therapy response in terms of cancer cell death of both wild-type and resistant GC cells. Moreover, the SLC-0111 treatment was able to induce in GC cells a death with immunogenic potential, as suggested by the increased level of the DAMPs CALR, ANXA1, HMGB1, and CXCL10 following the SLC-0111 treatment.
Conclusion. Overall, these data suggest a correlation between CAIX and GC drug resistance highlighting the efficacy of SLC-0111 in boosting the therapy response of GC cells, in re-sensitizing resistant GC cells to pCT, and potentially in warming up the tumor by inducing immunogenic death of GC cells.

Characterization of glioblastoma heterogeneity by spatial transcriptomics

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IDH1 wild-type glioblastoma (GBM) is the most frequent and aggressive brain tumor in adults, whose peculiar hallmark is a marked subclonal heterogeneity resulting in strong adaptation ability. The source of dynamic reorganization within the spatial context of GBM still needs to be characterized.
To this aim, we describe GBMs by integrating topological data derived by spatially resolved transcriptomics, patient-derived glioblastoma organoids (GBOs), and glioma stem cells (GSCs). We sampled four areas per tumor: the tumoral core, the inner edge of the tumor, the external edge of the tumor, and a distal area from the tumor. We analyzed each area through spatial transcriptomics using the 10X genomics Visium technology, which allows the analysis of samples' transcriptomes within their morphological context at a high cell resolution. Spatial transcriptomics relies upon using a slide filled with barcoded spots that specifically capture transcriptomic information within the spatial organization of tissue.
After integration of st-RNAseq data from the areas through canonical correlation analysis, the tumoral core area and the inner edge of the tumor area cluster together in the low dimensional embedding, as do the external edge and the distal area, but separately from the previous areas.
The paired distribution is consistent among the analyzed patients. We observed a differential content of cancer cells and a peculiar profile of chromosomal aberrations within the different areas.
GBOs are a new and powerful *in vitro* model; we derived them from the four areas. We appreciated different efficiencies of the derivation, and different growth rates between the four areas consistent with the preliminary st-RNAseq results: the tumoral core and the inner edge of the tumor areas showed both greater GBO derivation efficiency and growth rates with respect to the other two areas.
The derivation of GSCs from the different areas has been successful for only two of the four areas, the tumoral core area and the inner edge of the tumor, with different proliferation capacities for each patient.
We finally performed a preliminary characterisation of both GBOs and GSCs through immunofluorescence analysis of widespread GBM markers, comparing the GBOs with the original tissue sections and highlighting a different expression of the markers between the areas.

YAP mediates the sensitivity to FK866 in Triple Negative Breast Cancer

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INTRODUCTION: Disruption of the balance between cell proliferation and cell death disturb cell and tissues homeostasis and often drive

pathological conditions. YAP/TAZ are the downstream effectors of the Hippo pathway, and function as transcriptional co-activators of context-specific pro or anti-surval target genes. YAP and TAZ coordinate several NAD+-dependent processes like glycolysis, fatty acid oxidation and glutaminolysis in nutrient-deprivation conditions. However, correlations between NAD(H) levels and YAP/TAZ metabolic function are still poorly understood.

MATERIAL AND METHODS: YAP stable silenced cells were obtained by infecting the triple negative breast cancer (TNBC) cell line MDAMB231 (MDA) with lentiviral particules containing pLKO-shYAP. Metabolic and non-metabolic viability assays were used to assess the sensitivity to to the NAD+-depleting agent FK866 in attachement conditions, while the soft agar assay was performed to evaluate the ability of cells to grow in ancorage-independent conditions. Mitochondrial respiration was assesed through the Seahorse MitoStress test. Real-Time PCR and Western Blot were performed to determine the expression levels of genes/proteins involved in NAD+ and mitochondrial metabolism as well as in YAP transcriptional programs.

RESULTS AND DISCUSSION: We showed that FK866 treatment decrease YAP phosphorylation status at serine 127, thus increasing its nuclear translocation, in a cancer cell model of TNBC which is sensitive to FK866. The stable silencing of YAP in MDA rescued FK866 toxicity both in 2D and in 3D culturing, promoting a dose dependent FK866-resistant like phenotype. At the metabolic level, the silencing of YAP on MDA p. induces the FK866-dependent expression of the mitochondrial biogenesis gene PGC1-α, and an increase of the mitochondrial respiratory capacity and mass.

CONCLUSION: We show a relation between sensitivity to FK866 and YAP activation, whose dampening can sustain the acquirement of resistance to this small molecule in TNBC, likely through the modulation of mitochondrial metabolic traits.

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Gene co-expression networks' hubs as therapeutic targets for basal-like breast cancer

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Breast cancer (BC) is a highly heterogeneous disease for which no general treatment is currently available, in particular, the aggressive basal-like BC (BLBC) subtype still represents an unmet medical need. Intrinsic features determining aggressiveness of tumor cells, such as survival, proliferation and invasion are the result of the orchestrated activity of many components interacting with each other, determining specific gene regulations. Gene co-expression networks are considered useful tools to define prognostic gene signatures, and identify centrally connected genes as potential therapeutic targets.

To reconstruct BC transcriptional co-expression networks, we applied Weighted Gene Coexpression Network Analysis to the METABRIC dataset, a large primary BC data collection comprising 1981 samples belonging to all molecular subtypes. This led to the identification of 21 modules, representing groups of genes topologically close in the whole gene expression network, significantly correlated with survival and tumor grade. We next searched for modules more tightly interconnected in the BLBC subtype and identified a module, named E2F targets. The expression levels of this specific module strongly correlate with clinical features and poor prognosis, supporting its relevant biological functions. We next posited network centrality (kWithin) as a reliable parameter to identify module's regulators, selecting as potential activators of the module the most central Transcriptional Factors (TF). Analysis of available datasets describing gene expression perturbations upon TFs modulation revealed coherent regulation of the module's genes, with no effect on other modules, confirming that genes with high kWithin are upstream regulators of the module. Five of the most central and not yet studied TF hubs of E2F target module, namely PTTG1, TEAD4, E2F3, TFDP1 and CEBPG, were chosen as potential regulators of the entire gene set and inactivated in multiple BLBC cell lines, by means of both RNA silencing and CRISPR-mediated KO. *In vitro* validations showed that targeting the chosen TFs reduced aggressiveness features in BLBC cells.
In conclusion, our work allowed to identify gene expression modules highly relevant in BC as well as their central TF regulators. Upon experimental validation, this approach could be successfully extended to other BC subtypes, and to all tumors for which gene expression data are increasingly available, allowing for the identification of druggable targets.

Circular RNAs can regulate cancer-related protein expression through direct interaction with mRNAs.

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Circular RNAs (circRNAs) are a subfamily of ncRNA characterized by the absence of 3' nor 5' ends as they are covalently linked in a post-transcriptional process known as a back-splicing reaction. During the last years, interest in circRNAs increased as they are deregulated in a myriad of pathological processes such as cancer. Many studies suggest that circRNAs act as miRNA 'sponges', although due to stoichiometric issues, this mechanism cannot be generalized and might apply only to very specific cases.
Our project tries to understand the molecular mechanisms in which these molecules drive the regulation of specific genes via circRNA:mRNA interaction and the impact of this interplay on oncogenic development. Our approach to finding direct mRNA interactors of circRNAs consists of treating cells with trimethylpsoralen, which crosslinks base-paired RNA regions in vivo, pulling-down circRNAs followed by RNA sequencing to capture mRNA interactors. Firstly, we discovered that circZNF609, through a direct interaction which overlaps its back-splicing junction, can increase CKAP5 mRNA stability and translation in several cancer cell lines by favouring the recruitment of the transcript of the RNA-binding protein ELAVL1. CKAP5 is a key factor in microtubule dynamics stability, therefore, through this mechanism, circZNF609 can regulate microtubule function in cancer cells and sustain cell-cycle progression. CircZNF609 knockdown or circZNF609/CKAP5 interaction blocking can increase different cancer cells' sensitivity to microtubule-targeting chemotherapeutic drugs. More recently, we discovered that also other circRNAs can regulate protein translation of interacting mRNAs coding for factors with a crucial role in cancer. These observations point out that circRNAs directly regulate gene expression through circRNA/mRNA interactions and explore the possibility of modulating those interactions to improve cancer therapies.

Glutamine regulates BCR/Abl expression in hypoxic chronic myeloid leukemia cells via fatty acids metabolism

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INTRODUCTION
Under very low oxygen tension, Chronic Myeloid Leukemia (CML) cells undergo the suppression of the BCR/Abl oncoprotein, whereas a BCR/Abl-independent subset of cells, commonly referred to as leukemia stem cells, is maintained. Such cell population retains the capacity, when transferred to normoxic conditions, to generate a BCR/Abl-expressing progeny which is, in vivo, responsible for the relapse of the disease, demonstrating to be also resistant to the tyrosine kinase inhibitors (TKi) by lacking their molecular target. Moreover, under oxygen restriction, glutamine plays a major role, stabilizing c-Myc expression and inducing cancer cells to diverge towards a more pronounced fatty acids (FA) metabolism.

MATERIAL AND METHOD
K562 and KCL22 cell lines were subjected to glucose and/or glutamine deprivation in hypoxic conditions (96hrs at 0.1% O2). Cells metabolic profile was generated through the Seahorse XFe96 Analyzer while L-Glutamine-13C5 was exploited via LC/MS to determine its contribution in FA de novo synthesis. BODIPY 493/503 was used to measure the intracellular neutral lipid

droplets in confocal microscopy and flow cytometry whose presence and morphology were also determined via transmission electron microscopy. BCR/Abl was evaluated via Western Blotting whilst CD36 was determined through flow cytometry.

RESULTS AND DISCUSSION
We observed that glutamine is capable to boost glycolysis, leading to a faster BCR/Abl downregulation in hypoxic conditions, and decrease the basal and maximal cell respiration capacity. We also identified that under oxygen and glucose shortage, CML cells were characterized by numerous lipid droplets. Such an augmented neutral lipid content was due to a glutamine-dependent CD36 upregulation, which is capable to uptake FA from the extracellular milieu. In these conditions, CML cells rapidly lose BCR/Abl expression, a phenomenon which was validated by the treatment with exogenous BSA-Palmitate, capable to reduce BCR/Abl expression, while the use of the sulfosuccinimidyl oleate, a specific CD36 inhibitor, sustained the oncoprotein maintenance instead.

CONCLUSION
Our results suggest that FA may play a fundamental role in hypoxic-induced BCR/Abl suppression and that such FA degradation might be needed for the oncoprotein re-expression once normoxic conditions are restored. This phenomenon might be therefore exploited to sustain BCR/Abl expression in hypoxic cells to be more susceptible to TKi.

Long-range oncogene regulation by 3D chromatin conformation in acute T cell leukemia

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In the last decade, the three-dimensional (3D) architecture of chromatin has emerged as a fundamental mechanism to control gene expression, and therefore virtually any cell function. Alterations of chromatin structure may happen at different levels, such as A/B compartments, topology associated domains (TADs) and chromatin loops, where the folding of chromatin allows for long-range interactions between enhancers and promoters of oncogenes, thus favoring cell growth. Independently from their genetic driver lesions, about 2/3 of acute lymphoblastic leukemia cases show alterations of epigenetic elements, most of them controlling growth and differentiation. Our group focused on characterizing the enhancer/promoter activity and interactivity at the genome-wide level in a set of patients affected by canonical or high-risk acute T cell leukemia (T-ALL) and demonstrated that chromatin conformation can correctly stratify disease subgroups and provide important information on their oncogenic program. For instance, we identified long-range enhancer elements driving the expression of surface markers of diagnostic value (at the CD1 locus) or oncogenes that associate with disease aggressiveness (at the MYCN locus) in specific T-ALL subtypes.
In our study, we show that 3D enhancer-promoter interactivity analysis reveals key oncogenic networks. By ranking interactions in terms of number of connections and their strength we were able to identify hubs that regulate the expression of subtype-specific genes, directly connected with cell proliferation and differentiation stages. The results of this study allowed us to identify chromatin loops that correlate with differential expression of new potential disease markers and pharmacological targets at the genome-wide level. Taken together, these results produced a better understanding of T-ALL disease mechanisms and their heterogeneity, thus predicting potential therapeutic vulnerabilities and hopefully the development of more targeted regimens.

Single-cell meta-analysis of glioblastoma reveals a fast-cycling cell population expressing markers of alternative lengthening of telomeres.

Vittorio Bontempi, Emma Busarello, Irene Pecchini, Toma Tebaldi, Marina Mione

Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor with a life expectancy of 12 months and a 5-year survival of 6.8%. It is characterized by a high level of both inter and intratumoral heterogeneity, resulting in treatment failure. Recent studies on the mechanisms of telomere maintenance (TMM) in cancer have added an additional layer of complexity, reporting two main mechanisms depending on the activation of telomerase reverse transcriptase (TERT) or Alternative lengthening of Telomeres (ALT) in tumor cells. Around 10-15% of GBMs use the ALT mechanism, which relies on homologous recombination-based telomere elongation. This mechanism is characterized by heterogenous telomere length, reduced TERT activity and expression, increased c-circles, increased DNA damage, high telomeric recombination and increased levels of the telomeric transcript TERRA. Two previously established isogenic models of GBM in zebrafish in our lab, which differ only for the TMM they use (ALT- and ALT+), allowed us to look at transcriptome-wide differences, revealing an overexpression of genes involved in the pre-replicative complex and cell cycle in the ALT+ brain tumors. Among these, the pre-replicative complex genes ORC4, ORC6, MCM2, RPA3 and CDC45 showed a strong correlation with the ALT phenotype.
Given the complexity just described, our aim is to dissect transcriptome diversity at the level of single-cell RNA-seq, to possibly identify specific subpopulations of ALT+ cells and find novel markers and therapeutic targets for ALT+ glioblastoma, improving patient stratification. We analyzed 4 different datasets of single cell RNA-seq of GBMs, comprising a total of 230k cells and 59 patients. Importantly, 14% of glioma patients showed a distinct subset of glioma cells with an increased expression of the pre-replicative complex genes, compared to the other glioma cells in the same tumor. Putative ALT+ cells accounted for 15% of the tumor cells. Analysis of cell cycle markers revealed that all cells in this subpopulation are in the S or G2 phases, and Gene Ontology analysis confirmed an enrichment of cell cycle processes among highly expressed genes. Lastly, at least one patient in every dataset contained the cluster of putative ALT+ cells, except for the one composed only of GBMs with mutant IDH, which is a widely used marker of good prognosis. These findings suggest that a specific subset of fast cycling glioma cells in IDH wild type tumors might drive the ALT phenotype.
Further studies will compare single-cell RNA-seq of the two GBM models in zebrafish to validate the association between ALT and specific transcripts and correlate them with the levels of TMM heterogeneity in GBMs.

Switching off CK2-mediated activation of survivin offers new therapeutic opportunities in neuroblastoma

Cazzanelli Giulia

CK2 is a Ser/Thr kinase involved in multiple cellular processes, including apoptosis, cell cycle progression and DNA damage control, found overexpressed in a variety of cancers, especially hematological malignancies. Recently it has been shown that CK2 has a very relevant role in medulloblastoma (MB) cancer progression. The inhibition or downregulation of CK2 has been proven efficacious in cancerous cells, but currently only one CK2 inhibitor, silmitasertib (CX-4945), is in clinical trials for the treatment of various malignancies.
We discovered three novel CK2 inhibitors that, although less potent in kinase assay with respect to CX-4945, showed superior efficacy in cancerous cells. The most active inhibitor (CK2-TN03) was tested on cell lines derived from tumors of neuroepithelial tissues, i.e. MB, neuroblastoma (NB) and glioblastoma (GB). CK2-TN03 was significantly more active than CX-4945 in MB and NB cell lines and both inhibitors induced cell death in NB cells more dramatically than in MB cells. CK2-TN03 and CX-4945 only marginally impacted GB cells viability, indicating a cancer-specific effect. The analysis of the viability following CK2-TN03 treatment of various NB cell lines harboring different genetic backgrounds and the stall in mitosis before going to apoptosis of CK2-TN03 treated cells identified the anti-apoptotic protein survivin as the most probable CK2-downstream target of our inhibitor. Importantly, survivin is amplified in all high-risk neuroblastomas and its function is affected by direct inhibition of its phosphorylation by CK2. We demonstrated that following CK2-TN03 treatment, survivin RNA and protein levels are reduced through CK2 regulation of the MDM2/p53 balance via AKT1 and BRD4/MYCN. We also showed that CK2-TN03 caused an increased in caspases 3/7 activity in actively replicating NB cells, but did not affect the viability of non-cycling cells. Finally, CK2-TN03 significantly reduced tumor growth in mice xenografts without any apparent toxicity.
As the anti-NB effect of CK2 inhibition has never been reported before, we believe we could provide an additional therapeutic indication for CK2 inhibitors. CK2-TN03 could be an alternative, possibly better, option for the treatment of this pediatric tumor.

Chemical inhibition of the m6A methyltransferase METTL3 sensitizes triple-negative breast cancer to DNA damaging therapy

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Among the different types of breast cancer, triple-negative breast cancer (TNBC) is the one with the most unfavourable prognosis and high risk of recurrence. Conventional chemotherapy and DNA damaging agents are the main treatment for this cancer. METTL3 methyltransferase is responsible for the deposition of N6-methyladenosine (m6A) modification, which is the most abundant internal mRNA modification, regulating different aspects of RNA metabolism, including splicing, export, decay, and translation. METTL3 was found up-regulated in different types of cancer, including breast cancer, and it has been reported to play a crucial role in cancer progression and drug resistance. TNBC is characterized by high METTL3 methyltransferase activity, and this correlates with invasiveness and metastasis. Notably, the inhibition of METTL3 by small molecule has shown promise as a potential therapeutic approach in acute myeloid leukemia. However, the specific cancer types in which inhibitors of METTL3 may be most effective have yet to be fully determined. Here, we show that STM2457, a specific METTL3 inhibitor, strongly affect TNBC cells proliferation and migration. Moreover, STM2457 strongly synergizes with DNA damaging agents utilized in TNBC therapy, such as platinum-salts and the PARP1/2 inhibitor Olaparib. By using two TNBC patient-derived organoids with wild-type BRCA1 and BRCA2 genes, we show that the catalytic inhibition of METTL3 significantly synergizes with DNA-damaging chemotherapy and, more importantly, sensitizes TNBC organoids to PARPs inhibition. In conclusion, our data indicate that the use of small molecule inhibitors of METTL3 may provide great benefit in conventional treatment for TNBC and pave the way for novel targeted therapies.

Plasma-derived EV RNA cargo deconvolution is informative for assessing metastatic prostate cancer response to treatment.

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Prostate cancer patients almost inevitably develop resistance to hormonal therapy, entering a state referred to as castration-resistant prostate cancer (CRPC). Liquid biopsies allow for the interrogation of all components shed by tumor cells into biofluids, including extracellular vesicles (EV) and cell-free DNA (cfDNA). In the framework of the multi-institutional PRIME (PRostate cancer plasma Integrative Multi-modal Evaluation) program, we profiled the EV-associated transcriptome from the plasma of 58 CRPC patients upon Enzalutamide treatment at multiple time points (n=3; baseline, 12 weeks, and progression) and of 16 healthy donors (HD). Plasma samples were collected across multiple Italian institutions with harmonized procedures and processed using ONCE (ONe Aliquot for Circulating Elements), an in-house developed approach for the concomitant isolation of EVs and cfDNA from a single plasma aliquot.
RNA was extracted from plasma EV with the IZON qEV RNA Extraction kit and treated with RNase-Free DNAse I. Libraries were prepared using the Takara Bio SMARTer smRNA-Seq kit for total RNA and sequenced on NovaSeq 6000 with a SR150 protocol.
We leveraged transcript and read-specific integrity metrics with EV biophysical features and transcriptional deconvolution approaches to dissect the contribution of different tissues to the circulating EV-associated RNA signal in the plasma. We observed that transcript reads' length and coverage uniformity depend on the putative cell of origin and the RNA biotype. Specifically, YRNAs, mitochondrial transcripts, and blood-related transcripts show uniform coverage and longer reads compared to other RNA biotypes and to RNAs from other tissues of origin. Stratifying the CRPC patients by the time of response to Enzalutamide, we detected significantly larger EV diameters in the short responders (p-value<0.01) and identified 732 genes differentially represented in plasma EV between short- and long-treatment responders (FDR<0.05; n=677 and n=55 upregulated in short and in long responders, respectively). Most of the highly represented genes in short responders (n=434) are concordantly more expressed in CRPC tissues than the whole blood, in line with the presence of prostate cancer cell-specific transcriptome within the plasma-derived EV-RNA signal. Furthermore, the integration of EV transcriptomics with cfDNA genomics (through targeted sequencing) of oncogenes confirmed the identification of circulating tumor-derived EV signal in the plasma.
We ultimately aim to develop an analytical platform that allows the identification of circulating putative markers for treatment stratification of patients by integrating EV transcripts quantification and read-specific features, EV biophysical characteristics, and cfDNA genomics.

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Identifying and characterizing novel HSP90 inhibitors with senolytic activity in a hormone-induced breast cancer-senescence model.

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Cell senescence is characterized by halted cell proliferation and the acquisition of a pro-inflammatory profile termed Senescence Associated Secretory Phenotype. A wide array of intrinsic and extrinsic stimuli can trigger cell senescence. Cancer cells can enter into senescent state following treatments that inflict DNA damage. Intriguingly, these cells can evade the senescent state, gaining enhanced proliferative capabilities and resistance to further treatment, thereby prompting tumor recurrence. Senolytics offer the potential to eradicate selectively senescent cells, thus preventing tumor recurrence and even catalyzing tumor regression. Several senolytics with varying targets have been recognized.
Nevertheless, these drugs, particularly those aimed at HSP90 (such as 17-DMAG), pose safety and specificity challenges. Our research, therefore, strives to identify and characterize innovative HSP90 inhibitors with senolytic features for application in hormone-driven cancers. We utilized in silico strategy to pinpoint several new, optimized HSP90-inhibiting compounds with minimal cytotoxicity, exploiting a combined structure- and ligand-based virtual screening approach. Among them, two HSP90α inhibitors, K4 and K5 (IC50 155 nM and 372 nM, respectively), exhibited senolytic activity in vitro without any observable cytotoxicity in non-senescent cells (patent pending). Furthermore, proliferation and mortality assays performed on various tumor cell lines derived from hormone-driven cancers revealed that these drugs elicit a cytostatic effect rather than a cytotoxic one.
Consequently, due to its pronounced responsiveness, we selected the MCF7 cell line as our in vitro model. Senescence was induced via treatment with 4-Hydroxytamoxifen (Tam, 10uM 96h). Growth curve and beta-galactosidase (ß-gal) assays confirmed that Tam effectively blocked MCF7 cell proliferation and triggered senescence up to 35-40% of cells compared to control. To assess the potential senolytic activity of K4 and K5, we treated senescence-induced cells with both drugs (10 uM, 96h). ß-gal staining was notably reduced, with only 10% of senescent cells remaining with K4 or K5 compared to DMSO.
Interestingly, compared to control, the mortality rate rose significantly with K5 (up to 2.5 fold) but not with K4. Western blot analysis confirmed that p21 and γH2AX were stabilized by Tam, thereby corroborating the senescence status and induction of DNA damage. Moreover, Cyclin D1 was reduced by Tam, indicating cell cycle arrest, but its expression was revived after treatment with K5 or DMSO. Our findings suggest that the two newly identified HSP90 inhibitors, particularly K5, display senolytic properties and may be leveraged with tamoxifen to inhibit proliferation and stimulate cell death in breast cancer cells.

Profiling of metastases in resectable lung cancer reveals a key role for miRNA-PD-L1 axis in the resistance to neoadjuvant chemotherapy

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Neoadjuvant chemotherapy (NACT) followed by surgery is the treatment of choice for resectable locally advanced non-small-cell lung cancer (NSCLC) (Stage IIIA). However, the majority of NACT-treated patients show resistant lymph nodal metastases (LNmets) and poor prognosis. Therefore, there is an urgent need to understand molecular mechanisms underlying NACT-resistance in LNmets and to identify predictive biomarkers. To this purpose, we performed coupled miRNome and transcriptional profiling of LNmets and found that a microRNA signature (aka LN-signature) accurately predicts NACT response in treatment naïve LNmets (AUC=0.81-0.82). Mechanistically, we discovered using in vitro and in vivo NSCLC experimental models that miRNA/PD-L1 regulatory axis drives NACT resistance, hallmarks metastases with active IFN-γ response pathway (an inducer of PD-L1 expression), and impacts T cells viability and relative abundances in tumor-microenviroment (TME). We further confirmed our findings in independent cohorts of NSCLC patients as well as using external NSCLC transcriptome datasets. Our data provides reliable miRNA-based biomarkers that predict heterogeneity of response to NACT treatment in LNmets and novel mechanistic insights that connect NACT response and anti-tumor immunity.

CDKN1B/p27kip1 expression is critical for growth, progression and treatment response of luminal breast cancer

Dall'Acqua Alessandra

Breast cancer (BC) is the most frequent malignancy among women worldwide. About 70% of BC belong to the luminal subtype (LBC, estrogen receptor, ER^+, progesterone receptor, $PR^{+/-}$, HER2 not amplified) and patients rely on hormonal therapy and CDK4/6 inhibitors (CDK4/6i), as most common therapeutic approaches.
The cell cycle inhibitor $p27^{Kip1}$ (CDKN1B, hereafter p27) has been involved in LBC growth, progression and treatment response, but data regarding its role are still controversial.
To study p27 role in LBC, we exploited the MIND (Mouse INtraDuctal) injection technique, uniquely maintaining in mice the features of human LBC. We intraductally injected p27WT and p27 knock-out (KO) LBC cells, in mammary glands of 6-8 weeks old NSG female mice.
Nine months after injection, mice were sacrificed, mammary glands collected, distant organs evaluated for the presence of metastasis. First, absence of p27 induced a substantial anticipated onset (20 *vs* 25 weeks). Further, the ducts injected with p27KO LBC cells were engorged with tumor cells and tumors were bigger in size than those from p27WT cells. Strikingly, distant dissemination to the lung tissue was observed only when p27KO LBC cells were injected.
Pathological analyses of the tumors indicated that loss of p27 induced a strong downmodulation of PR, both at transcriptional and protein level, coupled with an upregulation of ER expression. Clinical data indicate that $PR^{low/-}$ LBC are more aggressive and respond less to hormonal therapy. We thus tested whether the variation in the hormonal repertoire impacted on p27KO LBC response to therapy and assessed that loss of p27 conferred resistance to tamoxifen and CDK4/6i, either alone or in combination.
At mechanistic level, we observed that ER stability was altered in p27KO cells. Both CDK4 and CDK6 were able to bind ER, especially under hormonal stimulation, and this binding was greatly increased in p27KO cells. Treatment with CDK4/6i in the presence of hormones impacted on ER ubiquitination and stability, rescuing ER S118 phosphorylation and suggesting a possible reprogramming of ER/PR transcriptional program.
Altogether, here we discover a new p27/ER/PR axis and crosstalk that establish a prognostic and predictive value for p27 in the response of LBC to therapies. Future experiments, integrating these findings with ChIPseq molecular profiling data will also provide novel insights to improve the clinical management of LBC patients.

Fusion transcriptome profiling defines the monoclonal nature of multifocal epithelioid hemangioma of bone

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AIMS: Vascular tumors of bone are rare tumors characterized by unusual morphology, variable classifications, and unpredictable behavior. Among these, epithelioid hemangioma (EH) of bone remains a highly controversial entity due to frequent multifocal presentation. Indeed, the WHO classifies EHs of soft tissues as benign tumors, whereas bone EHs are considered intermediate-locally aggressive tumors.
To gain insights into the clinical behavior and biology of bone EH we retrospectively analyzed 42 patients treated in a single institution from 1978 to 2021.

METHODS AND RESULTS: Multifocal presentation was detected in 17 of 42 patients (40%) primarily as synchronous lesions. Patients were treated with curettage (57%), resection (29%) or biopsy followed by radiotherapy or embolization (14%). Follow up (minimum 24 months) was available for 38 patients, with only 5 local recurrences (13%) and no death of disease.
To clarify whether the synchronous bone lesions in multifocal EH represent multicentric disease or metastases, 4 cases were profiled by RNA-sequencing. Separate lesions from the same patient showed a very similar transcriptional profile and expressed the same fusion transcript (involving FOS or FOSB) with identical gene breakpoints.

CONCLUSIONS: These results indicate that in EH of bone, multifocal lesions are clonally related and therefore represent metastasis of a same neoplastic clone rather than simultaneous independent tumors. This finding is an apparent contradiction with the benign clinical course of the disease and suggests that tumor dissemination in bone EH likely reflects a phenomenon of passive spreading, with tumor cells colonizing distal sites while maintaining their benign biological nature.

Lipid metabolism rewiring by long noncoding RNA malat1-targeting in prostate cancer: an integrated omics and mathematical modeling approach.

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This study stems from our experimental evidence revealing a key role of MALAT1 in Prostate cancer (PCa) metabolism. MALAT1 targeting restored PCa glucose metabolism with consequences on pyruvate transformation and cell proliferation, bringing it a step closer to that of normal prostate epithelial cells.
Our aim is deciphering how MALAT1 affects the regulation of crucial lipid metabolic enzymes, focusing on Choline Kinase A (CHKA) and Ceramide Kinase (CERK), two key enzymes involved in lipogenesis and bioactive lipid synthesis, and the Androgen Receptor (AR) signaling, in terms of transcriptional regulation.
In MALAT1-depleted cells, transcriptomics and metabolomics showed a decrease in CHKA expression associated with reduced phosphocholine-containing metabolites. Phospholipid biosynthesis and sphingolipid metabolism were the most enriched pathways associated with MALAT1 targeting, according to the decrease of CHKA and CERK mRNAs (30-50%).
Besides, CHKA and CERK genes are regulated positively and negatively by dihydrotestosterone (DHT)/AR, respectively. Combining MALAT1 targeting with DHT, hormone responsiveness was retained in both androgen-sensitive (LNCaP) and castration-resistant PCa (22RV1) cells. Of interest, MALAT1 targeting repressed PSA in both cell lines, while catalytic subunit of human telomerase was induced in LNCaP and inhibited in 22RV1 cells, respectively. Conversely, combination of MALAT1 targeting with AR antagonists did not change the effect of MALAT1 silencing on CHKA and CERK mRNAs, while it enhanced PSA repression. Mechanistically, nuclear translocation by immunofluorescence and recruitment of AR onto CHKA, CERK and PSA regulatory regions by chromatin ImmunoPrecipitation was observed upon MALAT1 targeting in LNCaP cells, mimicking that induced by DHT. In 22RV1 cells, upon MALAT1 targeting alone, recruitment of AR variant 7 (ARV7) rather than AR was found. Addition of

DHT, instead, caused recruitment of both AR and ARV7 along target genes regulatory regions, suggestive of a MALAT1-/DHT-dependent chromatin remodeling. Next, RNA-IP revealed that AR associates with MALAT1 in basal conditions and this interaction is abrogated upon DHT. Moreover, we developed a mathematical model based on Ordinary Differential Equation able to describe and predict the modulation of the leading players in metabolic reprogramming upon MALAT1 depletion. We validated this model's first version on pyruvate and glucose metabolism data, aiming to extend it to lipid metabolism.
In conclusion, these findings support an unprecedented role of MALAT1 in choline metabolism and ceramide phosphorylation. MALAT1 targeting unveils a novel unliganded AR-dependent transcriptional repression of CHKA, CERK and PSA and pave the basis of a RNA-based therapeutic option, specifically suitable for castration-resistant PCa.

TrkB is a potential therapeutic target for invasive cutaneous squamous cell carcinoma.

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Cutaneous squamous cell carcinoma (SCC) is one of the most common age-associated malignancy. Although most of SCCs are treated by surgery, a subset of them displays a higher likelihood of recurrence and metastasis causing death. Nowadays, no satisfactory diagnostic biomarkers for high risk SCCs has been proposed.
We previously demonstrated that the age-related expression switch of the neurotrophin BDNF and its receptor TrkB in fibroblasts and keratinocytes could make aged skin prone to tumorigenesis. Although TrkB is frequently overexpressed in various tumors and its upregulation promotes tumor progression, still little is known about SCC. Based on our results, the aim of the study was to identify new SCC biomarkers and therapeutic approaches.
The correlation between the expression levels of TrkB and/or specific downstream proteins (E-cadherin, Yap1, Notch1), and histological characteristics of SCCs was investigated. Immunohistochemical analysis of tumor specimens showed that the expression levels of TrkB and nuclear Yap1 were significantly higher in invasive SCC than in situ lesions. On the contrary, E-cadherin and Notch1 expression was significantly lower in invasive SCC. Notably, the ROC analysis indicated that the combination of these four proteins is an effective diagnostic signature able to discriminate both the SCC types and high risk invasive SCC.
To investigate if TrkB may be a therapeutic target, two SCC cell cultures were treated with a TrkB-specific inhibitor (ANA-12). ANA-12 treatment inhibited the STAT3 pathway, reduced the expression of the EMT-transcription factor Slug, induced E-cadherin expression and, in turn, recovered cell-cell adhesion properties. In parallel, Yap1 translocated into cytoplasm and Notch1 to membrane similarly to normal keratinocytes. Furthermore, ANA-12 treatment induced p21 translocation in the nucleus where the protein plays an anti-proliferative role. Indeed, ANA-12 treated SCC cells displayed reduced proliferation and migration. p21 can also suppress IL-6 transcription. Notably, ANA-12 reduced IL-6 expression and secretion from SCC cells. ANA-12 administration significantly inhibited SCC invasion in 3D SCC in vitro models.
Therefore, our data indicate that TrkB signaling pathway regulates tumor progression, the specific signature "TrkB, E-cadherin, Yap1, Notch1" may be a diagnostic biomarker for invasive SCC risk, and TrkB may be an attractive target for new SCC therapies.

Multi-omics profiling of epithelioid sarcomas

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Epithelioid sarcoma (ES) is a very rare mesenchymal neoplasm characterized by the loss of expression of the chromatin remodeling factor SMARCB1/INI1. According to the WHO classification, ES is distinguished into two distinct subtypes: the classic type (C-ES) and the proximal type (P-ES), with P-ES generally pursuing a more aggressive clinical course. Little is known about ES biology and the molecular determinants that account for the distinctive pathological and clinical features of ES variants.
To shed light on these issues, we performed a multi-omics profiling (RNA-, miRNA-, target DNA-sequencing and DNA methylation array) of 24 primary, untreated ES. Unsupervised clustering of the transcriptome identified two separate groups corresponding to the two histopathological ES subtypes, indicating that the morphological classification pinpoints two molecularly distinct entities. P-ES showed over-expression of genes involved in cell cycle and chromatin remodeling, denoting that, in addition to SMARCB1/INI1 deficiency, this variant exhibits a wider deregulation of chromatin metabolism. Conversely, C-ES over-expressed genes associated with cell adhesion, migration, and vessel development.
Targeted DNA-sequencing failed to highlight recurrent gene alterations, besides SMARCB1 loss. Thus, to gain insight into these transcriptomic differences, we evaluated the involvement of epigenetic mechanisms. Unsupervised clustering of the miRNome showed a trend towards separation of P-ES and C-ES, similar to what observed in RNA-sequencing. The integration of miRNA- and RNA-sequencing data revealed that miRNAs play an important role in shaping the distinctive transcriptomes of the two subtypes. In particular, our results point to a miR-137:EZH2:miR-24:MYC/E2F/HELLS circuit as possibly implicated in the promotion of cell proliferation and chromatin remodeling in P-ES. Interestingly, HELLS has been also reported to assist DNMTs in both maintenance and de novo DNA methylation. Accordingly, P-ES and C-ES showed different DNA methylation profiles, with P-ES being characterized by a higher level of genomic DNA methylation, mainly affecting genes involved in cell migration and vessel development.
Overall, our study demonstrates that P-ES and C-ES are biologically different entities, characterized by distinctive miRNA:gene circuitries and DNA methylation profiles that cooperate in shaping the clinicopathological features of this disease.

Efficacy of small molecule HuR inhibitors in breast cancer models

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The Human antigen R (HuR) protein is an RNA-binding protein, ubiquitously expressed in human tissues, that orchestrates the maturation and processing of target RNAs both in the nucleus and in the cytoplasm. HuR binds AU-rich elements of its target transcripts modulating their half-lives and translatability. HuR is also crucial in promoting a proper differentiation of several cellular lineages. Due to its pleiotropic role and the incredibly high number of target genes (approximately 7% of the human protein-coding gene transcripts), it is not surprising that an altered expression or localization of HuR is observed in several inflammatory and tumoral diseases.
We have developed a class of synthetic small molecules called tanshinone mimics (TMs) that disrupt HuR-mRNA interaction and, here, we show their anticancer efficacy in triple-negative breast cancer cell lines. TMs inhibited cancer cell growth, proliferation and affected energetic metabolism. We also investigated the modulation of M1 polarization markers of a macrophage cell line induced by the conditioned medium of the triple-negative cell line MDA-MB-231. We observed a decreased M1 polarization, represented by a reduction in CXCL10, IL1β, and IL6 expression.
To show the relevance of HuR in this model, we generated a HuR Knock Out MDA-MB-231 cell model by CRISPR-Cas9 technology and characterized the relative phenotype. Interestingly, we did not observe major differences among wild-type and KO populations in 2D culturing, but HuR KO cells xenografted into nude mice produced a significantly lower tumoral mass than wild-type cells. Collectively, these data indicate that TMs are endowed with biological activity in cancer

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cells and that HuR has a strong impact on tumor formation in vivo.

miR-223 levels dictate taxol sensitivity in breast cancer

Favero Andrea

miR-223 is a well-known regulator of the immune response, playing a central role in myeloid differentiation and granulocyte function. In solid tumors, miR-223 function is highly context-dependent, but in breast cancer (BC), its expression is consistently downregulated, suggesting a tumor suppressive role in the mammary gland. Taxol is an antineoplastic drug that represents a pillar in the therapeutic plan of many BC patients. We observed that taxol systematically induced miR-223 upregulation in BC cells, both in vitro and in vivo. We asked whether miR-223 could represent a predictive biomarker of taxol response in BC. In vitro, we generated miR-223/low and miR-223/high BC cells using lentiviral transduction of shRNA particles and overexpression strategies. These models were used to assess taxol sensitivity, in dose-response curves, and gene expression, in microarray followed by pathway enrichment analyses with the GSEA software. Intracellular modulation of potential miR-223 targets was investigated by immunoblotting and immunofluorescence analyses, while miR-223 levels were evaluated by qRT-PCR. In vivo, we employed the MMTV-Δ16HER2/miR-223 WT and KO animals, which develop multifocal mammary tumors with a 100% penetrance at 15 weeks of age, and treat them with taxol, twice a week for 8 weeks. We observed that, not only BC cells upregulated miR-223 upon treatment with taxol, but also that miR-223 overexpressing cells were more sensitive to taxol compared to their control counterpart, suggesting that miR-223 might be might be necessary for the cytotoxic activity of this drug. To investigate this point, we exploited MMTV-Δ16HER2/miR-223 WT and KO mice, demonstrating that tumors from miR-223 KO mice were more resistant to taxol, compared to the WT counterpart. Gene expression analysis followed by GSEA, comparing taxol-treated BC cells displaying miR-223/low or miR-223/high expression levels, showed that the WNT5-ROR1 axis was among the top-enriched pathways in miR-223/low BC cells. On the opposite, miR-223 overexpressing cells displayed a global downregulation of the WNT5A-ROR1 pathway and, as expected, ROR1-silenced cells showed a higher sensitivity to taxol than control cells. Collectively, our data demonstrated that miR-223 modulates taxol sensitivity in BC, both in vitro and in vivo. The WNT5-ROR1 pathway, known to be involved in BC metastasis and drug resistance, was hyperactivated in miR-223/low taxol-resistant BC cells. Further studies are in progress to assess whether the identified targets or pathways might be druggable, alone or in combination, to improve the efficacy of taxol and overcome resistance in BC patients.

Cyclic disulfide redox probes and bioreductive prodrugs: applications to seco-duocarmycins targeting the thioredoxin system

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Specialised cellular networks of oxidoreductases coordinate the dithiol/disulfide homeostasis. To design probes and prodrugs selective for distinct physiological redox states, they must resist nonspecific activation by cellular monothiols. Recently, we presented a rational design of unique dichalcogenide structures, integrated them into modular fluorogenic probes, screened for biological selectivity and identified the first dichalcogenide motifs with excellent stability to monothiols, yet high selectivity for each the key redox-active protein, thioredoxin (Trx) or its upstream enzyme thioredoxin reductase (TrxR). Herein, we present a novel series of activatable prodrugs of the duocarmycin class of DNA-alkylating agents (#200carmycin). The structural elements essential for activity were masked by conjugation. Prodrugs were designed for activation by members of the thioredoxin system, such that they liberate the agent's strong cytotoxic effect after reduction primarily inside solid tumours. We mechanistically controlled both activation in cells and profiled the genetic markers that differentiate reduction vs. hydrolysis between different cancer cell types. We applied these prodrugs for in vivo studies to characterise their antitumour activity. Exemplarily showing how selective reduction sensing units are modularly utilized from imaging towards drug delivery opens new strategies to freely combine enzyme-triggered activators and bioactive cargos towards fine-tuned and custom-oriented therapeutics.

CRISPR/Cas9 screenings and in silico investigations nominate low-frequency alterations in DNA repair genes as biomarkers for castration-resistant prostate cancers.

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PARP inhibitors (PARPi) have received regulatory approval for the treatment of several tumors, including prostate cancer (PCa), and demonstrated remarkable therapeutic potential for metastatic castration-resistant prostate cancer (mCRPC) patients characterized by defects in homologous recombination repair (HRR) genes. Yet, clinical trial results suggest that further improvements are possible, for instance, in patients' enrolment criteria. Indeed, about 50% of BRCA1/2-deficient patients do not respond to PARPi, sensitivity to the treatment is heterogeneous, and a limited number of marker genes have been tested for PARPi. We aimed to identify and characterize low-frequency DNA repair gene (DRG) alterations that could represent potential novel biomarkers for mCRPC therapy.
To characterize the full spectrum of DRG aberrations, germline variants and somatic alterations of 302 DRGs involved in the principal repair pathways were analyzed at the allele-specific level in the SU2C-PCF dataset (N=429 mCRPC patients). Survival analyses were performed to correlate the identified DRG defects with patients' outcome and response to androgen receptor-signaling inhibitors (ARSI). This approach identified concomitant germline and somatic alterations in base and nucleotide excision repair pathways (joint frequency of aberration of about 10% for each pathway) as significantly associated with poor response to ARSI and worse prognosis (p=0.0071 and p=0.04, respectively).
In parallel, 11 CRISPR/Cas9 genotoxic screens were performed on PCa cell lines using a custom sgRNA pooled library targeting 356 DRGs combined with the administration of several DNA damaging agents, most of which are currently used in the clinic. Based on the validation experiments and the frequency of aberrations in the SU2C-PCF cohort, we further investigated LIG1 loss (aberrant in 6% of mCRPC patients) as a vulnerability in the context of PARPi treatment. We confirmed the synthetic lethality between the two DRGs in multiple PCa models. We observed that concomitant inactivation of LIG1 and PARP increased DNA damage and led to the induction of apoptosis. Altogether, these results show that low-frequency DRG defects are relevant for mCRPC patient stratification and that they can be exploited through a synthetic lethal approach (as for LIG1 loss and PARPi) to improve the therapeutic options of CRPC patients.

Non-invasive detection of neuroendocrine prostate cancer through targeted cell-free DNA methylation

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Metastatic castration-resistant prostate cancer (CRPC) is a heterogeneous disease in which treatment resistance can arise through multiple mechanisms. While most CRPCs are driven by androgen receptor (AR) signaling, up to 20% develop AR-independence[1]. AR-independence has been associated with aggressive clinical features and changes in tumor phenotype, including histologic transformation from castration-resistant adenocarcinoma (CRPC-Adeno) to neuroendocrine prostate cancer (CRPC-NE) arising through divergent clonal evolution[2]. The current diagnosis of CRPC-NE remains challenging due to the need for metastatic biopsy as well as to intra-patient tumor heterogeneity. Sequencing of plasma cell-free DNA is an ideal tool to overcome those limitations[3], [4]. Still, cancer-specific mutations or copy number changes are only modestly enriched in CRPC-NE compared to CRPC-Adeno.
Conversely, we and others have observed extensive DNA methylation changes associated with CRPC-NE [2]. In principle, whole-genome bisulfite cfDNA sequencing (WGBS) offers a comprehensive picture of the patient's disease status with optimal information on methylation content. Given the cost and computational burden of high-depth whole genome sequencing, only coarse low-pass variants of WGBS are suitable for large-scale clinical studies. As most CpG sites in the genome are non-informative or highly redundant, we aimed to dramatically reduce the sequencing space to a minimal set of CpGs that can accurately probe the CRPC phenotypes of interest.
We present the NEMO assay (NEuroendocrine detection and MOnitoring), a targeted DNA methylation sequencing platform for CRPC disease monitoring and neuroendocrine phenotype detection. We grounded the selection of informative regions upon an extensive analysis of published datasets from CRPC metastatic tumors, white blood cells (WBC), and plasma cfDNA from healthy individuals. First, we demonstrated that a few dozens of CpG-rich regions are sufficient to accurately estimate the fraction of

cancer-derived DNA in cfDNA (tumor content, TC), which we orthogonally validated through genomic-based tumor content estimation and in vitro dilutions. Next, we leveraged the DNA methylation of the collection of genomic regions that can distinguish CRPC-NE from CRPC-Adeno, accounting for the variable tumor content in cfDNA. We showcase the capability of the NEMO design to detect CRPC-NE on independent data, including cell lines, organoids, and patient-derived xenografts. The clinical utility of NEMO is demonstrated in its application to multiple clinical cohorts from the Italian the PRIME consortium, Dana Farber Cancer Institute, Weill Cornell Medicine, and two Phase 2 clinical trials of alisertib [5] and chemotherapy for aggressive variant and neuroendocrine prostate cancer [6]. Finally, a binary classifier to detect CRPC-NE cases achieves an AUC of 0.94 (CI:0.88-0.99) across plasmas with any detectable tumor content and 0.98 (CI: 0.93-1) with at least 50% of tumor content, with as few as 25kbp of genomic space and ~1500 CpGs, highlighting the robustness of the approach.

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Mathematical Modeling of EGFR Trafficking

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Epidermal Growth Factor Receptors (EGFRs) are among the most studied tyrosine kinase receptors. Academic interest in this particular receptor is motivated by the correlation between dysfunctional EGFR signaling and cancer. EGFR signaling is regulated by different mechanisms, the most important one being endocytosis. The receptors can be internalized via multiple pathways, usually grouped in two distinct mechanisms: the Clathrin-Mediated Endocytosis (CME) and the Non-Clathrin Endocytosis (NCE). CME is active both at low and high doses of ligand, and receptors internalized via CME tend to be recycled. On the other hand, NCE is active only at high EGF doses, and receptors internalized via NCE are mainly degraded.
We propose a mathematical model of EGFR dynamics to describe the trafficking of these receptors. The goal of the model is to make predictions and test hypothesis on the mechanisms, such as receptor phosphorylation and ubiquitination, regulating the two pathways.
The model consists of two major components. The first component consists of an extension of the Early Activation Model (EAM) [1]. This component describes the binding of the receptor to its ligand, the conformational changes of the receptor, the recruitment of adaptors, the phosphorylation of the tyrosine sites, and the ubiquitination of the receptor. The second component consists of the trafficking laws that describe the internalization of receptors, as well as the recycling and the degradation.
The overall model consists of an autonomous Ordinary Differential Equation (ODE) governing the evolution of the amount of ligand (both in the medium and in the endosomes), the amount of two molecular players involved in receptor ubiquitination (Cbl and Grb2), and the number of receptors in various configurations.
The model is able to reproduce the phosphorylation and ubiquiti-nation signals. Moreover, as it can be seen in the preliminary results presented in Figure 1, it is also able to correctly estimate the internal-ization rate Ke of EGF. This is the average rate of change of the ratio between internalized EGF and EGF bounded to receptors at the Plasma Membrane (PM). It is a crucial value since it defines the probability of an occupied receptor being internalized in one minute and can be used to determine other important cellular rate constants [2]. This model allows us to simulate different experimental scenarios (e.g., knock downs of endocytic pathways and receptor recycling) and can be used to make predictions to suggest which experiments may be of interest in validating hypothesis. Moreover, the model can help in obtaining insights on the role of the receptor configuration in its fate and signaling outcome.

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Characterization of novel second-generation small molecules for the improvement of cancer immunotherapy

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INTRODUCTION: The current immunotherapy, based on inhibiting immunological checkpoints by mAbs, represents a breakthrough in cancer treatment. Unfortunately, the effectiveness of mAbs cannot be extended to all tumor types. Moreover, they often cause autoimmune reactions in treated patients, and about 15% of them have to stop the treatment due to adverse effects. Histone acetylation and histone deacetylation, performed by histone acetyltransferases (HATs) and histone deacetylases (HDACs), are essential epigenetic modifications that remodel chromatin structure and alter gene expression. Aberrant epi-modifications in specific genome regions, such as oncogenes or tumor suppressor genes, and the mistargeting of specific non- histone proteins, can alter cell proliferation and motility, leading to carcinogenesis. Specifically, HDAC6, which is mostly localized in the cytoplasm, has many functions in different biological pathways, through its deacetylase activity of several substrates such as α-tubulin, HSP90 chaperone, FOXP3, HIF1α and p53. It plays a crucial role in oncogenic transformation, progression and metastasization, which makes HDAC6 a relevant target for treating cancer. This study focuses on characterizing new second-generation selective HDAC6 inhibitors, including ITF3756, which shows epigenetic activity and potential antitumor properties.

METHODS AND RESULTS: ITF3756 was tested in a nanomolar range on human T cell leukemia Jurkat cells and human breast cancer HCC1806 cells. A significative increase in α-Tubulin acetylation was observed in treated samples analyzed by immunofluorescence staining and capillary electrophoresis. While ITF3756-treated samples showed a slight or no decrease in total HDAC activity, unexpectedly, a significative increase in total HAT activity was observed, with a consequent increase in H3K9 and H3K27 acetylation levels. Similar results were observed in murine melanoma cells B16F10, where CRISPR/Cas9 silenced HDAC6. It was therefore hypothesized that ITF3756 could interfere with the interaction between HDAC6 and the HAT p300. CO-IP experiments showed a decreased p300-HDAC6 interaction upon ITF3756 treatment. Furthermore, multi-omic analysis (ATAC-seq, ChIP-Seq, and RNA-seq) demonstrated that HDAC6 inhibition/inactivation led to chromatin remodeling, with consequent gene expression modulation in the biological processes, cellular components, and molecular function pathways.

CONCLUSIONS: Our results provide evidence that HDAC6 inhibition/inactivation might interfere with epigenetic complexes formation/stabilization, and it is associated with increased histone acetylation, leading to changes in chromatin structure, gene expression, protein synthesis, and cellular function associated with cancer progression. Our data suggest that a combined-therapeutic strategy, epi-immunotherapy, might synergistically potentiate cancer treatment.

LINE-1 hypomethylation is associated with poor outcomes in locoregionally advanced oropharyngeal squamous cell carcinoma

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BACKGROUND: Human papillomavirus (HPV) positivity represents a strong prognostic factor for both reduced risk of relapse and improved survival in patients with oropharyngeal squamous cell carcinoma (OPSCC). However, a subset of HPV-positive OPSCC patients still experience poor outcomes. Furthermore, HPV-negative OPSCC patients are still lacking suitable prognostic biomarkers. Here, we evaluated the prognostic value of LINE-1 methylation level in OPSCC patients, and further addressed the relationship between LINE-1 methylation status and p53 protein expression as well as genome-wide/gene-specific DNA methylation. Results. DNA was extracted from 163 formalin-fixed paraffin-embedded tissue samples retrospectively collected from stage III-IVB OPSCC patients managed with curative intent with up-front treatment. Quantitative methylation-specific PCR revealed that LINE-1 hypomethylation was directly associated with poor prognosis (5-year overall survival - OS: 28.1% for LINE-1 methylation<35% versus 69.1% for ≥55%; p<0.0001). When LINE-1 methylation was dichotomized as <55% vs. ≥55%, interaction with HPV16 emerged: compared with hypermethylated HPV-16 positive patients, subjects with hypomethylated HPV16-negative OPSCC reported an adjusted higher risk of death (HR: 4.83, 95% CI: 2.24-10.38) and progression (HR: 4.54, 95% CI: 2.18-9.48). Tumor protein p53 (TP53) gene is often mutated and overexpressed in HPV-negative OPSCC. Since p53 has been reported to repress LINE-1 promoter, we then analyzed the association between p53 protein expression and LINE-1 methylation levels. Following p53 immunohistochemistry, results indicated that among HPV16-negative patients with p53≥50%, LINE-1 methylation levels declined and remained stable at approximately 43%; any HPV16-positive patient reported p53≥50%. Finally, DNA methylation analysis demonstrated that genome-wide average methylation level was significantly lower in HPV16-negative OPSCC patients who relapsed within two years. The subsequent integrative analysis of gene expression and DNA methylation identified 20 up-regulated/hypomethylated genes in relapsed patients, and most of them contained LINE-1 elements in their promoter sequences. Conclusions. Evaluation of the methylation level of LINE-1 may help in identifying the subset of OPSCC patients with bad prognosis regardless of their HPV status. Aberrant LINE-1 hypomethylation might occur along with TP53 mutations, and lead to altered gene expression in OPSCC.

Activity-based profiling of cullin-RING E3 networks by conformation-specific probes

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The cullin-RING E3 ligase (CRL) network comprises over 300 unique complexes that switch from inactive to activated conformations upon site-specific cullin modification by the ubiquitin-like protein NEDD8. Assessing cellular repertoires of activated CRL complexes is critical for understanding eukaryotic regulation. However, probes surveying networks controlled by site-specific ubiquitin-like protein modifications are lacking. We developed a synthetic antibody recognizing the active conformation of NEDD8-linked cullins. Implementing the probe to profile cellular networks of activated CUL1-, CUL2-, CUL3- and CUL4-containing CRLs revealed the complexes responding to stimuli. Profiling several cell types showed their baseline neddylated CRL repertoires vary, and prime efficiency of targeted protein degradation. Our probe also unveiled differential rewiring of CRL networks across distinct primary cell activation pathways. Thus, conformation-specific probes can permit nonenzymatic activity-based profiling across a system of numerous multiprotein complexes, which in the case of neddylated CRLs reveals widespread regulation and could facilitate development of degrader drugs.

Intracellular osteopontin stimulates the release of cytokines by mast cells to restrain neuroendocrine prostate cancer

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Fatal neuroendocrine prostate cancer (NEPC) often emerges in patients relapsing after hormone therapies. Besides, de novo NEPC can rarely occur in treatment-naïve patients. The processes which guide NEPC onset and development are still poorly characterized, making challenging the identification of predictive/prognostic biomarkers and of effective therapies. Tumor cell plasticity known to drive the trans-differentiation from adenocarcinoma to NEPC, can be influenced also by tumor microenvironment (TME)-derived stimuli.
Investigating the TME, we recently found that mast cells (MCs) accumulate within hormone-sensitive prostate cancer favoring its growth, whereas are excluded by NEPC both in patients and in the transgenic TRAMP spontaneous mouse model. TRAMP mice backcrossed with MC-deficient KitWsh mice showed increased frequency of de-novo NEPC. The frequency of de-novo NEPC similarly raised also in TRAMP mice deficient for the matricellular protein osteopontin (OPN). Reconstituting KitWsh -TRAMP mice with wild type, but not with OPN-deficient MCs, lowered the frequency of NEPC to that of untreated TRAMP mice.
We found that MCs stain positive for OPN in human and murine tumor sections and in vitro cultures, but release a tiny amount of OPN in supernatants if compared to NEPC cells. Notably, OPN has both secreted (sOPN) and intracellular (iOPN) forms; the latter can bind to MyD88 and regulate the signaling downstream toll-like receptors (TLRs) toward the production of cytokines. In vitro, wild type, but not OPN-/- or MyD88-/-MCs, inhibited the growth of NEPC cells

through the production of a cytotoxic cytokine which induces apoptosis, verified by detection of activated caspase-3. Also, in silico analyses showed that genes related to inflammatory response and TLRs signaling is down regulated in human and murine NEPC. Looking for candidate ligands able to trigger the TLR pathway, we discovered that the protein SDC1 is over-expressed by NEPC cells.
Our data suggest that SDC1 could activate the TLRs/MyD88/iOPN axis in MCs causing the release of cytotoxic cytokine restraining NEPC growth. This pathway could be exploited to set up innovative MC-based therapies efficiently improving NEPC targeting.

The FOXP3+ pro-inflammatory T cell: A Potential Therapeutic Target in Crohn's Disease

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Intestinal inflammation displays a major risk factor for cancer, however, the contribution of CD4+ cell populations remains to be elucidated. We provide an in-depth transcriptional assessment of CD4+ cells driving chronic inflammation in ileal biopsies isolated from Crohn's disease (CD) patients compared to healthy individuals using single cell RNA-sequencing. We identified five distinct FOXP3+ regulatory T (Treg) subpopulations. Tregs isolated from healthy controls represent the origin of pseudotemporal development into inflammation-associated subtypes. These pro-inflammatory Tregs displayed a unique responsiveness to TNFα signaling and an impaired suppressive activity in vitro as well as an elevated cytokine response in an organoid co-culture system. As predicted in silico, the histone deacetylase inhibitor Vorinostat normalized gene expression patterns, rescuing the suppressive function of FOXP3+ cells in vitro. Together, we identified a novel, pro-inflammatory FOXP3+ T cell subpopulation in CD patients and developed a pipeline to specifically target these cells using the FDA-approved drug Vorinostat.

ANP32E drives vulnerability to ATR inhibitors through R-loops formation in Triple Negative Breast Cancer.

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Triple Negative Breast Cancer (TNBC) is often characterized by MYC oncogene overactivation, causing dysregulation of gene expression and uncontrolled proliferation. We found that the expression of ANP32E chaperone protein is increased in dependency of MYC in human mammary epithelial cells, with associated induction of genome instability. TCGA data confirmed that ANP32E is overexpressed in TNBCs with respect to the other BC subtypes. TNBC patients show higher frequency of genomic mutations, correlating with higher ANP32E expression. Investigation of the DNA damage response (DDR) pathways showed an upregulation of several genes in correlation with ANP32E overexpression. We demonstrated that inhibiting the DDR master regulator ATR triggers vulnerability of TNBC overexpressing ANP32E in vivo and leads to Double-Strand Breaks and micronuclei accumulation in vitro. We hypothesized the involvement of R-loops (DNA:RNA hybrids) in DDR triggering, due to their accumulation after ANP32E overexpression and the generation of R-loop dependent Transcription Replication Conflicts. To clarify the underlying mechanism, we tested several DDR players, showing that ANP32E overexpressing cells have: i) sustained stimulation of DDR measured as phRPA32, phChk1, γH2A.X and 53BP1 activation; ii) enhanced recruitment of DDR players to specific chromatin loci; iii) R-loops accumulation at ANP32E chromatin bound sites; ii) elongating RNApolII stalled at R-loops sites, resulting in reduced nascent RNA. With the advantage of Next Generation Sequencing technologies, we mapped R-loops genome wide and observed an accumulation of longer, thus more stable, R-loops in dependency to ANP32E overexpression. Those sites also showed an increased phosphorylation of RPA32, a surrogate marker for Transcription Replication Conflicts. The consequent dependency of cancers with such biological background from a constant activation of DDR puts the bases for a successful therapy based on ATR inhibitor drugs. Moreover, we observed an empowering of ATR inhibitor effect thanks to the synergism with anti-proliferative molecules such as Navitoclax.
In summary, dissection of the molecular mechanism linking ANP32E overexpression to the dependency on DDR specific players and fragile sites will help to rationally design patient-specific therapies and identify novel biomarkers for TNBC diagnosis.

Hijacking integrin/Hippo signaling in prostate cancer blocks neuroendocrine progression

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Introduction
Neuroendocrine (NE) differentiation in prostate cancer (PC) associates with metastasis and poor prognosis. While NEPC arises by trans-differentiation of castration-refractory adenocarcinoma, rare pre-existing castration-resistant (CR)PC-like cells exist in untreated primary PC, some of which display NE features. The mechanism by which the presence of androgens confines NEPC precursors and inhibit their ability to exert full pathogenic potential remain to be fully elucidated. We hypothesized that the loss of more differentiated, androgen-sensitive PC cells caused by castration provides the opportunity for novel interactions between androgen-independent cancer stem/like cells (CSCs), and between CSCs and the extracellular matrix (ECM), eventually supporting NEPC aggressiveness.
Material and method
Taking advantage of well-defined lines of mouse prostate CSCs with exocrine (PAC-SCs) or neuroendocrine features (PNE-SCs) and decellularized extracellular matrix (ECM), we conducted an in-depth investigation of the interactions between neuroendocrine and exocrine PC cells, and of the interactions between these cells and the ECM in the contexts of androgen-sufficiency and ablation. We interfered with identified molecular pathways in vitro by pharmacologic inhibition and gene knock out. We assessed the in vivo therapeutic efficacy of inhibitors in primary metastatic models of NEPC and in vitro on human CRPC organoids.

Results and discussion
Without androgens, PAC-SCs promoted integrin α2 upregulation and YAP activation in PNE-SCs, supporting proliferation and invasion, whereas differentiated PC cells did not. PC-derived ECM, which was stiffer than wild type prostate, also supported PNE-SC proliferation and α2 upregulation. RANK/RANKL and NF-kB inhibition prevented a2 upregulation in PNE-SCs, indicating a cell-to-cell and cell-to-matrix contact-driven process. Integrin α2β1 or YAP inhibition also restrained PNE-SC invasion in vitro. While microenvironment-conditioned PNE-SCs showed metastatic behavior in vivo, YAP inhibition almost completely blocked NEPC in castrated mice and prevented metastasis. YAP inhibitors also restrained growth of human organoids from CRPC.

Conclusion
Our findings highlight how interacting and sensing the surrounding environment is pivotal for PC development and progression; in fact, molecular and cellular composition, organization and remodeling of the lesion may have profound influence on tumor development and progression. Moreover, this work suggests the possibility to act on the integrin α2-YAP axis as a potential strategy for PC patients to improve standard-of-care treatment and prevent therapy-related NEPC development.

Oncogenic enhancers prime quiescent metastatic cells to escape NK immune surveillance by eliciting a transcriptional memory

Michelatti Daniela

Metastasis arises from disseminated tumour cells (DTCs) that are characterized by intrinsic phenotypic plasticity and the capability of seeding to secondary organs. DTCs can remain latent for years before giving rise to symptomatic overt metastasis. In this context, DTCs fluctuate between a quiescent and proliferative state in response to systemic and microenvironmental signals including immune-mediated sur-

veillance. Despite its relevance, how intrinsic mechanisms sustain DTCs plasticity has not been addressed. By interrogating the epigenetic state of metastatic cells, we found that tumour progression is coupled with a re-arrangement of the spatial chromatin context of oncogenic enhancers, that leads to the activation of a robust transcriptional response upon repeated exposure to retinoic acid (RA). We showed that this adaptive mechanism sustains the quiescence of DTCs through the activation of the master regulator SOX9, and increases the fitness of metastatic cells by supporting the escape of quiescent DTCs from NK-mediated immune surveillance. Overall, these findings highlight the contribution of oncogenic enhancers in establishing transcriptional memories as an adaptive mechanism to reinforce cancer dormancy and immune escape, thus amenable for therapeutic intervention.

HiCONA: Hi-C Organization with Network Analysis

Morelli Leonardo

Chromosome conformation capture (3C) techniques exploit digestion and subsequent relegation of cross-linked chromatin in cell nuclei, allowing the identification of spatial proximity between DNA sequences. The output of 3C techniques is a matrix, representing the chromatin interactome of a population of cells. In order to better understand intrinsic relationships between chromatin interactions, we decided to take advantage of network analysis: contact maps are interpreted as distance matrices and easily transformed into adjacency matrices of chromatin networks.
We developed HiCONA, a python package which is able to perform several network analysis tasks, starting from standard 3C inputs. In particular, HiCONA generates chromatin network starting from files in cool format. Subsequently, it performs network sparsification, in order to increase the speed of analysis and to improve network handling. Starting from the sparse network, we are able to weight the contribution of some standard chromatin annotation (ChIP-peaks, ChromHMM states) to global network measures. On the other hand, it is possible to associate local network measures to chromatin annotations. In addition, HiCONA enables the cluster analysis of chromatin networks, exploiting the Nested Stochastic Block Model: which allows the comparison between different experiments. Finally, through the network motifs analysis, it is possible to scan chromatin network, searching for enriched chromatin interaction conformations. With HiCONA we dissected the major rules of chromatin dynamics and regulation, by comparing different 3C-derived datasets.

Telomeric lncRNA TERRA as a potential target for ALT-positive tumors

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Telomeres are nucleoprotein structures found at the end of eukaryotic chromosomes and their role is to prevent chromosome degradation and fusions. Due to the “End Replication Problem”, telomeres in somatic cells shorten after each cell division, leading to cell cycle arrest, cellular senescence, and ultimately cell death. In order to achieve replicative immortality, cancer cells elongate telomeres using two mutually exclusive mechanisms: via the reactivation of the holoenzyme telomerase, detected in 85-90% of human tumors, or via the Alternative Lengthening of Telomeres (ALT), relying on the homologous recombination (HR) among telomeres, used by 10-15% of human cancers. The ALT telomere maintenance mechanism is prevalent in specific cancer types. For example, 45% of pediatric glioblastomas show ALT features. Notably, in response to anti-telomerase treatments, tumors are capable of activating a switch in the telomere maintenance mechanism known as Telomerase-to-ALT transition, enabling cells to survive the treatment. Very little is known about both the Telomerase-to-ALT transition and the mechanisms that trigger ALT remain to be defined. Telomeres also give rise to a class of lncRNAs called TERRA (telomeric-repeat containing RNA) which play a variety of roles in the cell, mainly related to telomere homeostasis. As TERRA has been shown to promote HR in ALT-positive tumors and its expression is induced in ALT-positive tumors, as compared to ALT-negative tumors, we hypothesized that TERRA may act as a trigger of ALT and its downregulation impair the ALT onset and the telomerase-to-ALT transition, potentially, causing the death of tumor cells unable to maintain telomere homeostasis.
To test this hypothesis, we are currently working on a zebrafish model that, through the overexpression of the human oncogene RAS, generates a brain tumor that resembles a pediatric glioblastoma. These tumors have been characterized to be ALT-positive and to express increased levels of TERRA. We are currently performing a downregulation of TERRA through the micro-injection of an antisense oligonucleotide which promotes TERRA degradation (TERRA-ASO) in single cell embryos of our RAS model. We are planning to confirm TERRA downregulation and measure whether TERRA-ASO drives a change in the ALT status of the tumor at a molecular level and investigate its effects on tumor growth and development. Our findings will help define the use TERRA a possible novel therapeutic target to treat ALT-positive tumors.

Development of PROTACs for Cancer Treatment

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Bromodomain Adjacent To Zinc Finger Domain 2A (BAZ2A), also known as TIP5, is a large multi-domain protein. BAZ2A is part of the nucleolar remodeling complex containing SNF2H (NoRC) and is involved in silencing a fraction of mammalian rRNAs. The key event in this mechanism consists of recognizing the acetylated lysine (Kac)16 at histone H4, mediated by the BAZ2A bromodomain. Recently, BAZ2A was found overexpressed in late-stage prostate cancer. In particular, TIP5 establishes epigenetic alterations favoring aggressive phenotype and promoting stem cell-like features. Thus, blocking the recognition of Kac exerted by BAZ2A bromodomain can represent a valid strategy for the treatment of prostate cancer.
Here, we show a successful example of structure-based drug design approach, leading to the discovery of two potent BAZ2A bromodomain inhibitors. However, cell-based assays revealed that simple inhibition is insufficient to exert an antiproliferative effect.
Consequently, we exploited inhibitors to develop PROTAC degraders. PROTAC technology exploits a heterobifunctional molecule binding to a protein of interest (POI) and E3 ubiquitin ligase. By forcing the contact between a BAZ2A bromodomain and E3 ligase, developed degraders can mediate target ubiquitination and consequent degradation through the 26S proteasome. Obtained molecules will be tested on cancer cell lines, and degradation will be verified by western blotting. In addition, ternary complexes will be structurally characterized by X-ray crystallographic analysis.
In conclusion, we developed the first BAZ2A PROTAC degrader that represent a valid alternative to reduce metastatic potential and avoid disease reoccurrence in late-stage prostate cancer.

High-resolution molecular atlas of a lung tumor in 3D

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Solid tumors are complex, three-dimensional (3D) tissues shaped by the crosstalk between malignant, stromal and immune cells. However, while cells live and interact in 3D cellular neighborhoods, histology and spatial omics mostly focus on 2D tissue sections.
Addressing this technological limitation, we present the first 3D molecular reconstruction of a human tumor (non-small cell lung cancer) by combining the in situ quantification of 960 cancer-related genes across ~340,000 cells (CosMx) with measurements of tissue-mechanical components. Through the unbiased analysis of 3D cellular neighborhoods in the tumor microenvironment, we assigned 18 different cell types to 10 distinct tumor, stromal, and immune multicellular niches. In this way, we identified tumor cells infiltrating beyond the tumor surface and went on to analyze the spatio-temporal dynamics of tumor invasion. Interestingly, pseudotime revealed that pro-invasive epithelial-to-mesenchymal (EMT) already occurred in one region at the tumor surface. We then leveraged our multimodal atlas to investigate the mechanical, cellular and molecular make-up of such EMT niche. Importantly, myofibroblasts and M2 macrophages specifically co-localized with pre-invasive tumor cells and their combined molecular signature predicted poor patient survival. On the other hand, cytotoxic T-cells did not infiltrate this niche but colocalized with inhibitory dendritic and regulatory T cells. Leveraging the sensitive detection of receptor and ligand expression, we systematically scored cell-cell interactions in 3D neighborhoods and identified which signalling axes orchestrated tumor invasion, T cell recruitment and immune escape. Compared to 2D, 3D neighborhoods improved the characterization of immune niches by identifying dendritic niches, capturing the 3D extension of T-cell niches and boosting the quantification of niche-specific cell-cell interactions, including druggable immune checkpoints. Overall, we provide the proof-of-principle for the computational reconstruction and systematic molecular exploration of 3D cellular neighborhoods, leading to the identification of targetable molecular mechanisms active in the patient under study (e.g. with anti-VEGF and anti-CTLA4 agents). We envision that 3D molecular pathology will inform large-scale clinical studies aimed at assessing the benefit of mechanism-based, personalized, combination therapies in the immuno-oncology era.

In vitro implication of an oleocanthal-enriched evoo extract-associated ros production in the Treatment of resistant gastric cancer

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INTRODUCTION
Gastric Cancer (GC) remains a critical problem for health system being the fifth malignancy for incidence and the fourth for mortality globally. GC has no specific symptoms, therefore the diagnosis of the majority of GC is at advanced stages when surgery, the only curative approach, is substituted or supported by chemotherapy (CT). However frequently CT fails due to patients' resistance. The possibility to use nutraceuticals in association with CT in a "complementary therapy" to enhance efficacy of treatment and eventually limits side effects reducing dosage could represent a breakthrough in tumor therapy. The use of extra-virgin olive oil (EVOO) has gained the interest of many scientists thanks to its multiple biological activities and its extremely low toxicity for the organism. In particular, Oleocanthal (OC), an EVOO compound, characterized by an ibuprofen-like chemical action, shows effects in many types of cancer. The aim of this work is to verify whether an OC-enriched EVOO extract (OCF) might be useful to overcome GC resistance.

METHODS
We used the AGS gastric adenocarcinoma cell line and its resistant subpopulations obtained in our laboratory through chronic exposure to 5-Fluorouracil (5FUr), Cisplatin (CISr) or Paclitaxel (TAXr). Cells treated with OCF were analyzed through MTT, Annexin V-PI cytofluorimetric assay, cell cloning ability, ROS evaluation, Western Blot and Real-Time PCR.

RESULTS
We found that 60 μM OCF promotes the apoptotic death of 25-50% wild type AGS, 5FUr and TAXr, but not CISr cells, which needs at least 240 μM. We suggest that OCF efficacy may be due to cell cycle inhibition in accordance with its ability to promote ROS production, driving a p21 up-regulation mediated by p53 increase. CISr's OCF higher resistance seems to be dependent to greater levels of antioxidants enzymes counteracting OCF-induced intracellular ROS production. Treating GC resistant cells with 60 μM OCF plus 5-Fluorouracil, Paclitaxel or Cisplatin, we found a potentiating effect respect to mono-treatment in all resistant GC cells, including CISr. This last finding is of particular importance in view of the therapeutic protocol represented by FLOT regimen where these drugs are used.

CONCLUSIONS
These preliminary results open-up the possibility to evaluate efficacy of OCF in vivo, as valuable adjuvant in resistant GC treatment.

NAT so perfect: Limitations of normal adjacent tissue as a standard control in colorectal cancer research

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Normal adjacent tissue (NAT), characterized by histological normalcy, is utilized as a "healthy" control to be compared to tumor tissue in most cancer studies. Previous work has already detected transcriptome-wide differences between NAT and tissue isolated from healthy individuals by bulk RNA-sequencing. However, which cell populations contribute to the difference between NAT and healthy tissues and how they differ at the gene expression levels remains unknown. This knowledge could be used to identify biomarkers to differentiate between healthy tissue and NAT.
Here, we analyzed datasets of state-of-the-art next-generation sequencing techniques including single-cell RNA sequencing (scRNAseq) and spatial transcriptomics of colorectal cancer (CRC) samples, corresponding NAT as well as colorectal biopsies isolated from healthy individuals. In general, NAT displayed a substantial infiltration of immune regulatory cells, especially B and T cells. In fact, most of these cells were highly distinct at the gene expression level when comparing NAT to healthy samples and, as expected, NAT displayed a significant enrichment of inflammation-associated pathways. By incorporating scRNAseq and spatial transcriptomic profiling of CRC sections, we identified nine marker genes for differentiation between healthy, NAT, and tumor samples. The differential regulation of these nine markers was validated in bulk RNAseq datasets as well as in immunohistochemically stained human CRC and healthy tissue. Finally, to evaluate how to revert the gene expression pattern of NAT to the healthy situation, we employed A Single-cell Guided pipeline to Aid Repurposing of Drugs (ASGARD) analysis, a bioinformatics tool that identifies treatment options based on transcriptomic changes. As suggested by our in silico findings, treating human CRC cell lines with histone deacetylase inhibitors in vitro reversed the expression patterns of the nine markers we found. Together, our findings will lead to a better selection of control tissue, enable the identification of the healthy/NAT tissue margin in CRC patients, and establish a pipeline for the research community to use suitable controls in their studies. In addition, we identified a treatment option to shift the transcriptome of NAT and tumor into the direction of healthy tissue. This treatment regimen opens up a new view on cancer treatment and post-surgical care to allow for the complete removal of cancer-like features within the colorectum.

Deciphering the role of Pin1 in the interplay between nuclear mechanotransduction and innate immunity in breast cancer

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Biomechanical alterations characterize most tumors and elicit cell responses contributing to tumor progression. Transduction of mechanical signals from the ECM to chromatin by cytoskel-

eton/Lamin nucleoskeleton connection allows adaptation of nuclear envelope (NE) structure, chromatin
organization, and gene expression to mechanical cues. In mechanically challenged cells, lack of key mechanosensors causes NE/DNA damage, with cytosolic leakage of DNA, triggering cGAS/STING innate immunity pathway. We recently found that in normal cells, the prolyl isomerase Pin1 plays a key role in the maintenance of NE and heterochromatin (HC), in response to mechanical stress (Napoletano et al., Cell Reports 2021). Pin1 loss/inhibition led to NE malformations, HC relaxation, causing mobilization of transposable elements (TEs) and DNA damage, which led to IFN-I induction and cell death. Cancer cells experience mechanical challenges during tumor progression and mount a nuclear mechano-protective response, whose failure causes NE/genome damage, leading to cGAS/STING/IFN-I pathway activation and promoting immune surveillance. In cancer, Pin1 is upregulated and amplifies tumorigenic pathways, while its depletion/inhibition curbs tumor
growth, sensitizing to therapies. We posit that in cancer cells, Pin1 could maintain NE/genome integrity, and that Pin1 loss/inhibition could cause NE/DNA damage and HC relaxation leading to TE hyperactivity, thus activating cGAS/STING/IFN-I. We assessed the role of Pin1 in mechanical response of cancer cells, using breast cancer (BC) cells 3D-cultured in matrices with defined composition and mechanical properties. Also, we assessed the role of Pin1 in maintaining NE/genome integrity, generating Pin1 CRISPR KO BC cells and mouse models, in which Pin1 can be specifically knocked-out in cancer cells. In mechanically challenged BC cells, Pin1 was recruited to the NE and required to maintain NE structure and HC condensation. Pin1 KO caused NE ruptures,
HC relaxation, TE upregulation, DNA damage, and cGAS/STING/IFN-I activation. In vivo, using the syngeneic 4T1/Balb/c BC lung metastasis model, Pin1 knockdown in metastatic cells led to cGAS/STING activation and immune cells infiltration, with reduction of tumor mass. Importantly, similar effects were obtained with Pin1 inhibitors, which also synergized with Immune checkpoint blockade (ICB). We have provided evidence that Pin1 could be a key regulator of mechanoresponse in cancer cells and treatment with Pin1 inhibitors could sensitize BC cells to ICB.

Generating patient-specific networks to unveil mechanisms of cancer drug resistance

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Cancer is primarily a signaling disease: genetic and epigenetic alterations drastically impact key pathways, leading to tumor onset and progression. Most of targeted approaches are directed against signaling molecules. However, the success of targeted therapies is often limited and drug resistance mechanisms arise, leading to therapy failure and dismal patient prognosis. To address this issue, a comprehensive, patient-specific characterization of signaling network rewiring can offer the unprecedented opportunity to identify novel promising, effective combinatorial treatments. Here, to identify genotype-driven anti-cancer strategies, we developed “SignalingProfiler”, a strategy supporting the integration of high-sensitive mass spectrometry-based (phospho)proteomics, RNA sequencing datasets with literature-derived signaling networks. We applied this approach to propose novel combinatorial treatments increasing the drug sensitivity of acute myeloid leukaemia patients, carrying internal tandem duplications (ITDs) in the FLT3 gene.
Our approach enabled to generate FLT3-ITD genotype-specific predictive models and revealed a novel and conserved role of the WEE1-CDK1 axis in drug resistance. Remarkably, pharmacological suppression of WEE1 kinase synergizes and strengthens the pro-apoptotic effect of TKIs therapy in cell lines and patient-derived primary blasts. Finally, our work demonstrates how unbiased, system-level studies have the potential to accelerate the discovery of more granular, patient-specific mechanisms of disease and chemoresistance toward the identification of more effective therapeutic approaches.

Inhibition of mitochondrial translation by streptogramin antibiotics suppresses glioblastoma stem cell growth

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IDH wild-type glioblastoma (GBM) is the most common central nervous system malignancy in adults. It is highly aggressive and inevitably lethal, having a median survival of 12-15 months after diagnosis and a 5-year survival of less than 5%. New therapeutic strategies for glioblastoma treatment, especially tackling the tumour’s glioblastoma stem cell (GSC) component, are an urgent medical need. GSCs rely highly on oxidative phosphorylation (OXPHOS), whose function requires mitochondrial translation. Here we explore the therapeutic potential of targeting mitochondrial translation and report the results of high-content screening with putative blockers of mitochondrial ribosomes. We identify the streptogramin antibiotic combination quinupristin/dalfopristin (Q/D) as an effective suppressor of GSC growth. Q/D also decreases the clonogenicity of GSCs in vitro, consequently dysregulating the cell cycle and inducing apoptosis. Cryoelectron microscopy reveals that Q/D binds to the large mitoribosomal subunit, inhibiting mitochondrial protein synthesis and functionally dysregulating OXPHOS complexes. Guided by docking calculations we have synthesized a series of twenty-two streptogramin A derivatives and we performed a structure-activity relationship refinement to evaluate the capability of these compounds to suppress GSC growth and inhibit mitochondrial translation, either alone or in combination with quinupristin. Among all, the fluorine derivatives and in particular flopristin resulted in being more potent than the corresponding lead compounds and penetrating to a greater extent into the cells. Taken together these data suggest that targeting mitochondrial translation could be explored to therapeutically suppress GSC growth in GBM and that Q/D or the flopristin derivative could potentially be repurposed for cancer treatment and are worthy of further in vivo evaluation.

Adoptive cell therapy with Cytokine-Induced Killer cells retargeted with immunotools against HER-2 expressing breast cancer.

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BACKGROUND: Cytokine-Induced Killer (CIK) cells are a heterogeneous population of T lymphocytes expressing CD3 and CD56, expanded and activated ex vivo from peripheral blood mononuclear cells (PBMCs) or cord blood, with the addition of recombinant human interferon-γ (rhIFN- γ), anti-CD3 monoclonal antibody (mAb), and recombinant human interleukin-2 (rhIL-2).
CIK cells are achieving considerable clinical relevance, due to the low risk of acute graft versus host disease (GvHD) in both autologous and allogeneic settings, their feasibility, and the limited costs of production. In this study we combined CIK cells with both the mAb Trastuzumab (TRS), due to the CD16a expression, or with the engineered mAb Trastuzumab (TRS) V90Lec13, which bears two amino acid substitutions (S239D/I332E) and lacks Fc fucosilation, or with the bispecific single chain Fragment variable (bsscFv) Her2xCD3.

MATERIALS & METHODS: CIK cells were obtained from PBMCs of both healthy donors and breast Her2+ cancer patients, by the addition of IFNγ, OKT3 and IL-2. The effector cell cytotoxicity and the dose-dependent activity of HER2xCD3 and TRS V90lec13 were evaluated with a 4-hours Calcein- AM assay or with a 72-hours real-time cell analysis (XCELLigence) against HER-2-expressing breast cancer cell lines. The concentration of cytokines released upon the co-colture of CIK cells with target cells was assessed with a multiplex assay (MACSPlex). The biodistribution of the bsAb was evaluated in NSG mice upon the chemical conjugation of HER2xCD3 with a fluorophore.

RESULTS: CIK cells from patients cultured in GMP condition are able to expand in clinically relevant number. The bsAb HER2xCD3 showed to bind exclusively to HER-2 target antigen and CD3 complex on the effector cells and the combination with CIK cells resulted in a significant improvement of the antigen-specific cytotoxic activity against breast cancer cell lines, as the combination of CIK cells with TRS or the (TRS) V90Lec13. In particular, in a real time analysis showed that even at a very low effector/target (E/T) ratio, such as 0.1:1 E/T ratio, CIK cells combined with HER2xCD3 had a remarkable cytotoxicity, that completely kill target cells. Interestingly, TRS-resistant tumor cell lines showed to be sensitive to HER2xCD3-redirected CIK cell lytic activity. Moreover, bsAb

resulted to be effective also at very low concentrations, and the cytokines released from CIK cells matched with a proinflammatory profile, with no significant concentration of cytokines correlated with Cytokines Release Syndrome (CRS). The analysis of the in vivo biodistribution showed that the bsAb arrives efficiently at the tumor site where accumulates and reaches the maximum concentration 8 hours after i.v. injection.

CONCLUSIONS: Taken together, these results highlight the potentiality of using recombinant immunotools to improve the antigen-specific cytotoxic activity of CIK cells against HER-2 positive tumor cells.

The ATG5 autophagy-independent interactome in epithelial to mesenchymal transition

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Autophagy is a highly conserved catabolic process used by cells to degrade and recycle non-functional cellular components such as proteins and organelles and to maintain cellular homeostasis. During autophagy, autophagosomes, double-membraned vesicles enclosing intracellular substrates, deliver their content to lysosomes for recycling. Autophagy addiction is a common feature in advanced and drug-resistant tumors and it is often associated with activation of epithelial-mesenchymal transition (EMT), a molecular program that boosts tumor cell aggressiveness. ATG5 is a key autophagic molecule that interacts with ATG12 and ATG16 to induce autophagic vesicle elongation. Recent studies indicate that ATG5 affects EMT in both autophagy-dependent and -independent manner. Data collected in our laboratory suggest that ATG5 plays an autophagy-independent role in modulating EMT, but the mechanism of action remains to be elucidated. To identify the molecular mechanisms behind the proposed autophagy-independent activity of ATG5 and determine whether this activity may be involved in breast cancer subtypes characterized by mesenchymal features (basal-like and triple negative breast cancer, TNBC), we interrogated the data from the TCGA-BRCA project. This analysis revealed that ATG5 expression was increased in TNBC, compared to other BC subtypes and associated with poor prognosis. Modulation of ATG5 in BC cell lines affected the transcription of both epithelial (E-cadherin) and mesenchymal markers (ZEB1, vimentin). Since ATG5 is not a transcription factor, we hypothesized that it may participate to EMT by acting as a cofactor. Therefore, we profiled the ATG5 interactome in TNBC cell lines overexpressing either ATG5 or an autophagy-deficient ATG5 allele (ATG5Φdef). Liquid chromatography mass spectrometry (LC-MS/MS) of affinity purified ATG5 interactors identified a number of positive hits. These included a group of known autophagic genes (validating the approach) but also a number of non-autophagic proteins we are currently investigating in deeper detail.

Dedifferentiated liposarcoma with rhabodmyoblastic differentiation: molecular dissection of a particularly aggressive sarcoma variant

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Dedifferentiated liposarcomas (DDLS) are rare and aggressive adipogenic tumors arising in the limbs and in the retroperitoneum. The gain of myogenic features, namely rhabdomyoblastic dedifferentiation, marked by the activation of myogenin (MYOG), correlates with poor outcome. To shed light on the pathobiological characteristics of this aggressive DDLS variant, we investigated the transcriptome of 42 retroperitoneal DDLS (9 MYOG+ and 33 MYOG-). Unsupervised analysis of RNA-sequencing data highlighted a net separation between MYOG+ and MYOG- DDLS. Functional annotation of differentially expressed genes, besides enrichment of myogenic signaling (with activation of numerous genes involved both in early and late skeletal muscle differentiation, besides MYOG), showed enrichment of proliferation-related pathways (e.g. MYC, E2F) in MYOG+ vs MYOG- DDLS. Conversely, an enrichment for immune-related signatures was observed in MYOG- DDLS. Both in silico and in situ analyses indicated that MYOG+ DDLS are less infiltrated by immune elements that MYOG- tumors. This reduced infiltration correlated with a lower expression of genes of the antigen presenting machinery. Our data suggest that rhabdomyoblastic DDLS are characterized by a profound transcriptional reprogramming, with activation of a whole set of genes involved in skeletal muscle differentiation and cell proliferation, and a reduced immune infiltration. The hyperactivation of the MYC signaling is known to induce phenomena of immune exclusion. Thus, this pathway could contribute, at least in part, to the aggressive clinical course of this variant by promoting both unfueled cell proliferation and evasion from immune surveillance.

Bcl-2 family inhibitors sensitize human cancer models to therapy

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Cancer patients often show intrinsic/innate or acquired resistance to treatments that ultimately results in death, thus, managing these patients represents a challenge, and new therapeutic options are needed. One attractive strategy is the combination of currently available therapies with inhibitors of anti-apoptotic proteins from the Bcl-2 family, which are often expressed at high levels in various types of cancers. Thus, favouring apoptosis could represent a valuable approach overcoming resistance.BH3 mimetics, targeting the Bcl-2 family anti-apoptotic proteins, represent a promising therapeutic opportunity in cancers. In 2016, venetoclax/ABT-199, a specific Bcl-2 inhibitor, was approved by the FDA for the treatment of several kinds of leukemia and lymphoma, thus validating the relevance of targeting apoptotic machinery in onco-hematological neoplasms. In addition, the first clinical study analyzing the efficacy of venetoclax in solid tumors evidenced a good activity in metastatic breast cancer. Experimental findings support the use of anti-apoptotic protein inhibitors in combination therapy both in melanoma and ovarian carcinoma. Inhibition of BRAF and/or MEK/ERK pathways, which together with immunotherapy represent the standard-of-care for melanoma treatment, has been reported to modulate the expression of some members of the Bcl-2 family. We have recently identified IS21, a pan-inhibitor of anti-apoptotic proteins with antitumor activity in different histotypes, including melanoma. Based on these data, the aim of this project was to evaluate the efficacy of IS21 and other BH3 mimetics, both as single agents and combined with the currently used antineoplastic agents in T-cell acute lymphoblastic leukemia, ovarian cancer and melanoma. Our results showei) IS21 was active in T-cell acute lymphoblastic leukemia, melanoma, lung, pancreatic, and ovarian cancer cell lines; ii)Bcl-xL and Mcl-1 protein levels predicted IS21 sensitivity in melanoma and ovarian cancer, respectively. In combination experiments, BH3 mimetics sensitized leukemia cells to chemotherapy, ovarian cancer cells to PARP inhibitors and melanoma models to MAPK inhibitors.We showed that this enhancing effect was related to the potentiation of the apoptotic pathway, both in hematologic and solid tumors. In conclusion, our data suggest the use of inhibitors of anti-apoptotic proteins as a therapeutic strategy to enhance the efficacy of anticancer treatment.

The transcription factors NFATc1 and NFATc2 control glucocorticoid resistance in paediatric T-cell acute lymphoblastic leukemia

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Resistance to Glucocorticoids (GCs), such as prednisone and dexamethasone, represents the major obstacle in the treatment of pediatric T-cell Acute Lymphoblastic Leukemia (T-ALL) patients. Indeed, for those patients that display GC resistance (~25%), no novel therapeutic options are available, and the prognosis is dismal. Therefore, the identification of new mechanisms underlying GC resistance can suggest alternative therapeutic approaches to overcome GC resistance clinical issue. In a previous study we demonstrated that LCK kinase contributes to GC resistance and that its inhibition, both in vitro and in vivo, sensibilizes T-ALL cells to GC. Recently, we uncovered the involvement of the NFAT family of transcription factors, downstream LCK, in GC resistance in T-ALL cells. Specifically, we observed that NFATc1 and NFATc2 are more expressed in GC resistant T-ALL patients at the diagnosis and that their specific gene silencing in T-ALL GC resistant cell line models increases dexamethasone response, by restoring the Glucocorticoid Receptor transcriptional activity. Conversely, NFATc1 or NFATc2 overexpression in a murine T-ALL GC sensitive cell line confers resistance to dexamethasone treatment. Additionally, we observed that NFATc1 gene silencing in T-ALL GC resistant cells results in a decreased amount of intracellular cholesterol, as well as a reduced number of plasma membrane lipid rafts (LRs), and consequently a decreased expression/activation of LCK kinase, anchored in LRs. Furthermore, we revealed that NFATc1 modulates cholesterol biosynthesis in T-ALL GC resistant cells by directly regulating the transcription of the HMGCS1, DHCR7 and EBP enzymes. Overall, these results indicate the existence of a positive feedback loop between LCK and NFATc1 to modulate GC response in T-ALL cells, in which cholesterol is a key player. In agreement, the addiction of exogenous cholesterol to T-ALL NFATc1 knock-down cells, rebuilds GC resistance. Otherwise, NFATc2 gene silencing leads to a downregulation of the Wnt/β-catenin signalling pathway and increases GC resistant T-ALL cell differentiation. In agreement, the induction of Wnt/β-catenin signaling activation, by Wnt3a stimulation, restores the resistance to dexamethasone treatment in NFATc2 knock-down T-ALL cells. Finally, the in vitro inhibition of cholesterol biosynthesis by simvastatin or of Wnt/β-catenin by ICG-001, increases GC sensitivity in T-ALL GC resistant cells. Altogether these results suggest that NFATc1 and NFATc2 guide GC resistance in T-ALL cells regulating different well-known cellular biological processes involved in chemotherapy resistance, such as cholesterol biosynthesis and Wnt/β-catenin signaling. Therefore, the pharmacological inhibition of these signaling pathways can represent new therapeutic options for T-ALL GC resistant patients to overcome GC resistance, ameliorating their outcome.

Synthesis, Characterization and Evaluation of Arsenicin A Related Polyarsenicals as Inhibitors of Glioblastoma Stem Cell Growth

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BACKGROUND: Glioblastoma is a highly aggressive type of brain tumor that represents a significant medical challenge due to its aggressiveness and resistance to current therapies. Developing novel drugs that target this tumor is a urgent priority. One promising chance is represented by organic polyarsenicals related to the marine metabolite Arsenicin A (Figure 1a) isolated from a New Caledonian sponge [1], that has already shown remarkable antitumor activities in NCI-US screening. [2]

OBJECTIVE: Chemical synthesis of Arsenicin A analogs and evaluation of their properties in the inhibition of glioblastoma stem cells (GSCs).

RESULTS: We recently reported the efficient and selective synthesis of new polyarsenical compounds. [3] These analogs share the peculiar adamantane-like arsenicin A cage with different alkyl substituents or a arsenic-sulfur related core structures (Figure 1b). The latter thio-analogs were unambiguous characterized with the support of simulated NMR spectra. The alkyl compounds 1-3 have the best chemico-physical properties and were for this selected for biological investigation against GSCs. The inhibition results were compared with current available chemotherapy drug (Temozolomide) and arsenic trioxide (ATO), a compound approved by FDA for the treatment of acute promyelocytic leukemia but characterized by not so favorable chemico-physical properties. The synthesized compounds inhibited the growth of nine GSC lines potently, with GI50 values in the submicromolar range, both under normoxic and hypoxic conditions, and presented high selectivity toward non-tumor cell lines (Figure 1c). Moreover, we found that these molecules can strongly induce apoptosis in COMI cells in a dose-dependent manner at concentration around their GI50 values.

CONCLUSION: We have efficiently produced, purified and structurally characterized synthetic alkyl polyarsenicals able to inhibit the growth of GSCs in a potent and selective way.

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Extracellular matrix-targeted PSMA-CAR T cell therapy: a next-generation approach to treat Prostate Cancer.

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BACKGROUND: Adoptive cell therapy (ACT) with T lymphocytes expressing Chimeric Antigen Receptors (CAR-T) has emerged as a promising immunotherapeutic strategy to treat haematological malignancies. However, CAR-T cell therapy in solid tumor remains challenging, mainly due to the immunosuppressive tumor microenvironment (TME). The high-density extracellular matrix (ECM) contributes to tumor immunosuppression by blocking immune cell infiltration, hence it has recently emerged as a novel therapeutic target. Among others, the prostate cancer (PCa) ECM is particularly rich in collagen and hyaluronic acid. Our group have successfully developed CAR-T cells against the prostate-specific membrane antigen (PSMA), but the immunosuppressive TME still remains a primary challenge.

MATERIALS AND METHODS: Herein, we developed a next-generation PSMA CAR-T cells engineered to coexpress either collagenase (MMPs) or hyaluronidase (PH20), to overcome the poor invasion and trafficking of CAR-T cells in PCa. We tested the co-expression of the transgenes by Western blotting and flow cytometry analysis in Jurkat cells and primary T cells. The PSMA-CAR cytotoxicity was assessed in-vitro by co-culturing engineered T cells and either LNCaP or PC3-PiP cells, which constitutively express the PSMA antigen. PH20 functionality was assessed by performing particle exclusion assay to measure in vitro the pericellular matrix formation and subsequent degradation. While, MMP14 collagenase activity was evaluated by transwell cell invasion assay in Matrigel.

RESULTS: Firstly, we cloned either collagenase (MMPs) or hyaluronidase (PH20) downstream the PSMACAR transgene in a clinically relevant lentiviral vector. We then infected Jurkat (PSMA CAR.E-J) and primary T (PSMA CAR.E-T) cells, and we observed a high expression of both PSMA-CAR and enzyme proteins. Next, the PSMA CAR.E-T cells showed a higher cytotoxic activity when co-cultured with PC3-PiP and LNCaP cells, when compared to the PSMA CAR.E-T cells co-cultured with PSMA-negative PC3 cells. Lastly, preliminary results demonstrated that PSMA CAR.E-J cells are able to successfully degrade both HA- and collagen-based ECM mimicking matrix, favouring effector cells migration towards tumor cells.

CONCLUSIONS: Our preliminary data indicate that we successfully obtained cytotoxic PSMA CAR.E-T cells, which are able to deliver genetically encoded payloads such as ECM-degrading enzymes. In this work, we propose a promising strategy to enhance CAR-T cells infiltration, invasion and finally therapeutic efficacy on PCa, which can be easily applied to other ACT and other tumor types.

IL-1ß$^{+}$ macrophages fuel pathogenic inflammation in pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with high resistance to therapies.

Inflammatory and immunomodulatory signals co-exist in the tumor microenvironment (TME), leading to dysregulated reparative and cytotoxic responses. Tumor-associated macrophages (TAMs) control immune dynamics in the TME, but their heterogeneity and plasticity have hampered our understanding of the underlying mechanisms. Here, we combined single-cell and spatial genomics with functional experiments to elucidate macrophage functions in PDAC. We uncovered an inflammatory cross-talk between tumor cells and TAMs that fuels disease progression. In particular, scRNAseq analysis of human PDAC and of mouse models of pancreatic cancer uncovered IL-1ß+ TAMs, a subset co-expressing inflammatory and reparative genes. Virtually undetectable in the healthy pancreas, IL-1ß+ TAMs accumulated during PDAC progression in descrete inflamed area of the tumor stroma and were elicited by a local synergy between prostaglandin E2 (PGE2) and tumor necrosis factor (TNF)-a. IL-1ß release in the TME induced an inflammatory reprogramming of tumor cells that, in turn, released increasing levels of PGE2 and TNFa, thus supporting a positive feedback loop sustaining the IL-1ß TAM state. Interfering with the PGE2-IL-1ß axis elicited TAMs reprogramming and antagonized tumor cell-intrinsic and -extrinsic inflammation, leading to PDAC control in vivo. IL-1ß+ TAMs are conserved across human cancers and correlate with patient survival in a context-dependent manner.
In conclusion, our data highlight a key role of the PGE2-IL-1ß axis in driving pathogenic inflammation and fueling cancer progression. Thus, targeting IL-1ß+ TAMs may represent a powerful therapeutic strategy to reprogram immune dynamics in cancer.

Cardiac extracellular matrix composition and stiffness in adult mice contribute to the inhibition of cancer cell migration

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Cardiac metastases are uncommon and the ectopic injection of cancer cells into the myocardium results in their poor local invasion and infiltration, and the formation of small and avascular tumors. Extracellular matrix (ECM) composition and stiffness are recognized to play an essential role in the control of tumor migration, invasiveness, and metastases by multiple mechanisms, including regulating the activity of transcription factors and stimulating angiogenesis. Accordingly, cancer cell migration depends on the protein composition and the specific structure of the ECM. On the other hand, low ECM stiffness decreases epithelial, endothelial, and cancer cell proliferation and migration. An intriguing possibility is that the cardiac ECM composition and stiffness contribute to the migration suppression of cancer cells in the adult heart. To experimentally explore this possibility, we compared the composition and mechanical properties of cardiac ECM with those of lung ECM, as the lung is often colonized by cancer cells. We used two complementary approaches to obtain and study organotypic ECM: first, we decellularized the ECM from the two organs and, second, we purified both cardiac and lung primary fibroblasts and let them produce ECM ex vivo for a total of seven to eleven days. In both cases, the obtained ECM was characterized by proteomics, JPK II atomic force microscopy, and live cell time-lapse imaging assay assessing the capacity to support the migration of cancer cell lines including LG1233 lung adenocarcinoma cells expressing Green Fluorescent Protein (LG-GFP), C57BL/6-derived B16 melanoma cells (B16), murine colorectal carcinoma cells (CT26), and Lewis lung carcinoma cells (LLC).
We observed that 90 proteins were upregulated in the cardiac ECM, resulting in a significant difference in the cardiac ECM architecture and a 2-fold lower stiffness compared to the lung ECM. Functionally, the migration of LG-GFP, B16, CT26, and LLC cells on cardiac ECM decreased significantly compared to those on the lung ECM. Taken together, these results indicate that cardiac ECM composition and stiffness suppress the migration of cancer cells, possibly justifying the low incidence of local and distal metastasis in the heart.
Keywords: migration, extracellular matrix, protein, stiffness, cancer cell.

Truncated FGFR2 – a clinically actionable oncogene in multiple cancers

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Human cancers frequently bear driver alterations in genes encoding receptor tyrosine kinases (RTKs), which has led to effective therapeutics targeting oncogenic signaling of RTK variants. Somatic hotspot mutations and structural amplifications and fusions affecting fibroblast growth factor receptor 2 (FGFR2) likewise occur in multiple tumor types including breast cancer. However, clinical responses to FGFR inhibitors have remained variable, emphasizing a need to better understand which FGFR2 alterations are oncogenic and therapeutically targetable. We applied transposon-based screening and tumor modeling in the mouse mammary gland to uncover truncation of the last exon (E18) of Fgfr2 as a potent driver mutation. Mouse and human FGFR2-E18 encodes the C-terminus of this RTK. Human oncogenomic datasets revealed a plethora of somatic FGFR2 alterations potentially causing transcription of E18-truncated FGFR2. These alterations were comprised of canonical in-frame fusions as well as diverse FGFR2 variants of unknown significance (VUS), which included non-canonical rearrangements, E1-E17 partial amplifications, and E18 nonsense and frameshift mutations. Functional in vitro and in vivo interrogation of a compendium of E18-truncated and full-length Fgfr2 variants pinpointed FGFR2 E18-truncation as a potent single-driver alteration in cancer. The FGFR2 C-terminus is crucial to fine-tune FGFR2 signaling. However, permutation of previously annotated C-terminal FGFR motifs did not recapitulate the pro-tumorigenic signaling and in vivo tumorigenicity of E18-truncated Fgfr2. Conversely, our functional annotation efforts led to the discovery of a phenylalanine–serine motif in the FGFR2 C-terminus that mediates binding to the kinase domain and therewith directly suppressed kinase domain activity. Consequently, permutation of the phenylalanine–serine kinase domain binding and suppression (KDBS) motif in conjunction with other FGFR2-regulatory C-terminal sites fully phenocopied the oncogenic driver competence of E18-truncated Fgfr2 and delineated how the C-terminus prevents FGFR2 from aberrant oncogenic activation. Together, these data suggest that genomic alterations that generate stable E18-truncated FGFR2 variants are actionable therapeutic targets, which we confirmed in preclinical mouse and human tumor models, and in a clinical trial. Thus, we uncovered a novel paradigm in oncogenic FGFR2 signaling and propose that breast and other cancers harboring any FGFR2 variant that truncates E18 should be considered for FGFR-targeted therapies.

Anti-hPSCA NK92 cell immunotherapy for the treatment of pancreatic cancer

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BACKGROUND
Pancreatic cancer (PC) is one of the leading causes of malignancy-related death, with almost half-million new diagnosis every year. Aberrant over-expression of the human prostate specific membrane antigen (hPSCA) has been detected in the vast majority of patients diagnosed with PC. This molecule can be used as potential target for treatments, in particular for ready-to-use immunotherapy and adoptive cell therapy (ACT). NK92 cells are a new strategy among ACTs, able to avoid the immunosuppressive tumor microenvironment, due to the absence of most inhibitory killer-cell immunoglobulin-like receptors (KIRs), with well-defined expansion kinetics and high transduction efficiency. Furthermore, this cell line is already approved for the clinical use towards different tumor types and shows high cytotoxic activity, even after irradiation required by authorities.

METHODS
We designed a new CAR anti-hPSCA to transduce NK92 cells, in order to create a ready-to-use therapy and to avoid problems related to other ACTs. We analyzed the expression of the CAR molecule and the phenotype of NK92 cells, before and after the transduction. Firstly, we tested anti-hPSCA CAR-NK92 cells for cytotoxic activity, cytokine release ability and degranulation in presence of target cells. Moreover, we have evaluated the therapeutic activity of CAR-NK92 in vivo in an orthotopic xenograft mouse models of human pancreatic cancer.

RESULTS
After 72h from the transduction of NK92 cells, the CAR anti-hPSCA and the eGFP, as reporter gene, under the control of a bi-directional promoter, were already detectable. The phenotypic profile of activated NK cells was stable before and after the transduction. Cytotoxic activity of CAR-NK92 cells was evaluated after 4h-co-incubation with target cells, showing a high and specific lysis of the antigen expressing tumor cells, as well as specific cytokines release ability and the activation of degranulation after antigen engagement. In vivo, in a disseminated and orthotopic tumor mouse models, the CAR-NK-92 cell therapy has shown a control of the tumor growth and a significant improvement of survival of the mice treated.

CONCLUSIONS
Pancreatic cancer is still a hurdle in terms of effective treatments. NK-92 cells are a promising strategy in the Adoptive cell therapy landscape, concerning the economic aspect, the high-scalable manufacturing, and the manageable handling procedure. Overall, the preliminary data of this work demonstrated that this CAR NK-92 therapy paves the way of a new therapeutic approach for PC, based on the development of an Off-the-Shelf, renewable, effective and low-cost product that can be advanced as a ready-to-use drug.

A multiplexed spatial profiling of the tumor microenvironment of extensive-stage Small Cell Lung Cancer to find predictive biomarkers of benefit from carboplatin-etoposide plus atezolizumab first-line treatment

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BACKGROUND: First-line systemic treatment with carboplatin-etoposide plus atezolizumab (CEA) is a new standard of care for extensive-stage small cell lung cancer (ES-SCLC). No predictive biomarkers for patient selection have been identified so far.

METHODS: This is a single-center translational study on ES-SCLC patients receiving first-line CEA, investigating the predictive value of tissue biomarkers. Gene expression profiling (GEP) was performed analyzing 770 immune/cancer-related genes, while two 9-color multiplex immunofluorescence (mIF) panels were used to assess immune checkpoint expression, tumor-infiltrating immune cell populations and their spatial relationships. Moreover, the expression of ASCL1, NeuroD1 and POU2F3 transcription factors was investigated to define ES-SCLC subtypes. The level of each biomarker was correlated with clinical endpoints.

RESULTS: Forty-two patients were included; median follow-up was 7.7 months. Overall response rate was 66%, median time to treatment failure (TTF), progression free survival (PFS), duration of response (DoR) and overall survival (OS) were 5.3 (95%CI 4.8-5.7), 5.3 (95%CI 3.9-6.6), 3.6 (95%CI 1.5-5.8) and 7.8 months (95%CI 2.9-12.6), respectively. Responding patients and those with a longer duration of response had a higher cell proliferation, DNA damage repair and epigenetic regulation scores. Non-responding patients had a higher density of intratumoral CD163+ M2-polarized tumor-associated macrophages as compared to responders. Moreover, a higher CD163+/CD8+ cell ratio was associated with a shorter DoR, TTF and OS, as well as a higher percentage of CD8+ cells in close proximity to CD163+ macrophages. Patients with a higher density of tumor-infiltrating lymphocytes, and those with a higher percentage of CTL close to tumor cells had a better outcome. Furthermore, higher expression of exhaustion CD8 markers, and higher density of PD-1+ or PD-L1+ cells were associated with a better prognosis, as well as a higher percentage of PD-1+ T cells in close proximity to PD-L1+ tumor cells or macrophages. NeuroD1+ tumors had different molecular and phenotypical characteristics as compared to the other subtypes, that were associated to a more aggressive phenotype and worst patient outcome.

CONCLUSIONS: We identified predictive and prognostic immune signatures, immune cell populations and cell-to-cell interactions in ES-SCLC patients receiving chemo-immunotherapy through a multiplexed spatial profiling. These results highlight the importance of the tumor microenvironment and spatial interactions in tumor response and survival.

Super-resolution genetics in haploid stem cells: decoding Hedgehog signaling in development and cancer

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All somatic cells in mammals carry two sets of chromosomes, while haploid cells are restricted only to gametes and occasionally found in tumors with genome instability. Advances in the generation of haploid embryonic stem cells (ESCs), capable of self-renewal and differentiation, have laid the groundwork for numerous biomedical applications in developmental biology and reproductive medicine. Our group was the first to derive mammalian haploid embryonic stem cells and deeply contributed to explore their developmental potential. Haploid cells possess one copy of each gene, facilitating the generation of loss-of-function mutations in a single step and enabling the development of efficient functional genomic strategies. In these years, the methodology reached a maturity that allows us to challenge complex phenotypes with a sensitivity unreachable with any other screening approach. Using this cellular system, we explored the role of the Hedgehog (HH) pathway during development and diseases. The HH pathway is one of the signaling cascades crucially involved in shaping embryo development. We used genetic screens in haploid ESCs to identify novel modulators of the HH pathway, and we discovered an unexplored function of the Golgi compartment in signaling activation. Additionally, we aim to understand the molecular events leading to HH signaling deregulation in multiple tumors. For this purpose, we take advantage of the plasticity of haploid ESCs to generate tumor models reflecting the earliest events of cellular transformation. Deep mutagenesis of their haploid genome will allow us to detect so far missed connections unveiling oncogenic-induced lethal interactions highly relevant for the development of new cancer therapies.

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12. Trento, 1 -3 June 2000
SIGNALING CROSS-TALKS IN CANCER CELLS

11. Rovereto, 5- 7 June 1999
MOLECULAR HORIZONS IN CANCER THERAPEUTICS

10. Trento, 29 June - 1 July 1998
THE GENETICS OF CANCER SUSCEPTIBILITY

9. Rovereto, 4 – 7 June 1997
THE BIOLOGY OF TUMORS

8. Trento, 17 – 19 June 1996
GENOMIC INSTABILITY AND IMMORTALITY IN CANCER

7. Trento, 14 – 16 June 1995
CANCER GENES. FUNCTIONAL ASPECTS

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

5. Trento, 9 – 11 June 1993
APOPTOSIS

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

3. Trento, 5 – 7 June 1991
TUMOR SUPPRESSOR GENES

2. Rovereto, 11 – 13 June 1990
THE THERAPEUTIC IMPLICATIONS OF THE MOLECULAR BIOLOGY OF BREAST CANCER

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

Pezcoller Foundation – AACR International Award for Extraordinary Achievement in Cancer Research 2023

This International Award was established in 1997 to annually recognize a scientist who has made a major scientific discovery in basic or translational cancer research, continues to be active in cancer research, has a record of recent noteworthy publications and holds promise for substantive contributions to progress in the field of cancer. Twenty-six top international scientists have been awarded so far and four of them have been subsequently awarded with the Nobel Prize, for the same motivations.

Tak W. Mak is senior scientist at the Princess Margaret Cancer Centre, University Health Network, Professor in the departments of medical biophysics and immunology at the Temerty Faculty of Medicine at the University of Toronto and Professor in the department of pathology at the University of Hong Kong

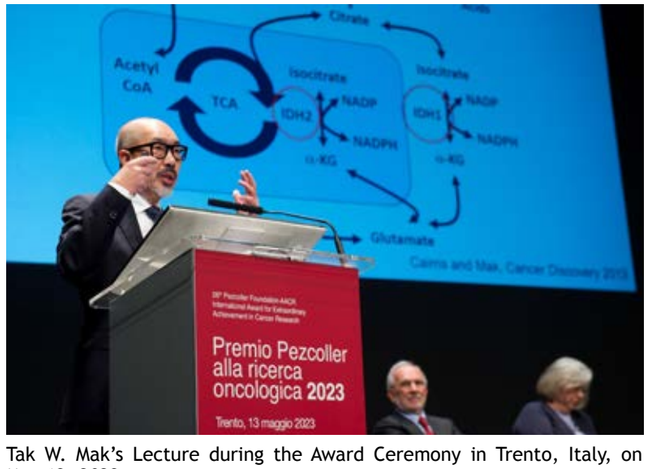
MOTIVATION

He is being recognized for leading the group that cloned the human T-cell receptor beta chain, a key component of the immune response, which has helped stimulate a remarkable series of advances in cancer immunology research.

Mak's group was among the first to generate genetically modified mouse models to study the molecular mechanisms driving immune system development and control and how perturbations to such processes contribute to tumorigenesis. His examinations of these mouse models helped elucidate critical intracellular signaling pathways that govern immune responses, malignant cell transformation, cellular survival, and programmed cell death.His discoveries have had substantial translational impacts - his team demonstrated that CTLA-4 is a negative regulator of T-cell activation, a finding that James P. Allison, PhD, later leveraged to conceptualize and design the first immune checkpoint inhibitor employed as a cancer therapeutic. Mak's characterization of T-cell receptors has also paved the way for the development of CAR T cell technology, a treatment option now approved for certain leukemias and lymphomas. Mak's work on oncogenic IDH enzymes also led to the development of IDH1/2 inhibitors and their subsequent approval for the treatment of acute myeloid leukemia. His group is currently investigating how various IDH mutations are capable of driving brain and blood cancer malignancies and confer the ability of cancer cells to survive under harsh environmental conditions that would normally result in cell death. He has also recently bolstered the concept that immune responses are connected directly to the nervous system through effector T cell secretion of the neurotransmitter acetylcholine.

Tak W. Mak presented an Award lecture at the AACR Annual Meeting, on April 16, 2023 in Orlando, Florida.

Tak W. Mak officially awarded during the Ceremony in Trento, Italy, on May 13, 2023.



Tak W. Mak's Lecture during the Award Ceremony in Trento, Italy, on May 13, 2023

Call for Nominations for the 2024 Award

NOMINATION DEADLINE FOR 2024 AWARD

September 15, 2023

DESCRIPTION

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 Award 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.

The Award recipient will receive an unrestricted grant, a commemorative award, and present a scientific lecture in conjunction with the AACR Annual Meeting immediately following their selection. The Award recipient will also present scientific lectures at the University of Padua and at the University of Trento in Italy, just prior to the official Award ceremony in Trento, Italy in May 2023.

ELIGIBILITY CRITERIA

Cancer researchers affiliated with any institution involved in cancer research, cancer medicine, or cancer-related sciences anywhere in the world may be nominated. Such institutions include those in academia, industry, or government.

Individuals who have previously been awarded the Nobel Prize in any category are ineligible to receive this Award.

Institutions and/or organizations are not eligible to receive the Award.

NOMINATION CRITERIA

Nominations may be submitted by any individual, 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 sciences.

Self-nominations are prohibited.

Nominators must maintain strict confidentiality of their nominations.

Eligible nominations must include the following:

- A nomination letter written in English (Max: 1,000 words), which comprehensively describes the candidate's major scientific discovery in basic cancer research or significant contributions to translational cancer research, and the impact of these accomplishments on the field. Letter must specifically outline the candidate's current research activity and indicate how their research holds promise for continued substantive contributions to the cancer field. All publications that directly support the mentioned research accomplishments must be referenced within the provided letter.
- A brief scientific citation (Max: 50 words) highlighting the major scientific contribution(s) justifying the award candidate's nomination.

SELECTION PROCESS

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 recipient shall be made on the basis of the candidate's scientific accomplishments without regard to race, gender, nationality, geographic location, or religious or political views.

For all information:

Pezcoller Foundation EACR Awards

Since 2012, the Pezcoller Foundation and the European Association for Cancer Research, EACR, have collaborated to support excellence in cancer research. Presently, three Pezcoller Foundation – EACR Cancer Research Awards are made jointly by the two organizations, to celebrate academic excellence and achievements in the field of cancer research. They are

- The Pezcoller Foundation – EACR Translational Cancer Researcher Award, to Young European researchers
- The Pezcoller – Marina Larcher Fogazzaro – EACR Women in Cancer Research Award, to European Women working in cancer research
- The EACR – Mark Foundation – Pezcoller Foundation Rising Star Award, to very promising, early career cancer researchers (established in 2023 thanks to the new collaboration with the Mark Foundation, USA)

Winners of the Pezcoller Foundation – EACR Awards

2023 Translational Cancer Researcher Award:
Nicola Aceto
ETH Zurich

2023 Women in Cancer Research Award:
Johanna A. Joyce
Ludwig Institute for Cancer Research, University of Lausanne

2023 Rising Star Award:
Isidro Cortes-Ciriano
EMBL-EBI Cambridge, UK

The call for Nominations for the 2024 Awards, is now open. Deadline: 14 September 2023: www.eacr.org

The Pezcoller Foundation SIC Fellowships

The Pezcoller Foundation actively promotes and supports cancer research, with particular attention to Italian young researchers, through the Pezcoller Foundation – SIC Fellowships.
These are two-year fellowships, € 30,000/year, for researchers working in Italian institutions, awarded on a competitive basis in collaboration with the Italian Cancer Society.
These are the recipients of the 2023-2024 Pezcoller Foundation – SIC Fellowships

Alessio Biagioni, Università di Firenze, Dipartimento di Medicina Sperimentale e Clinica, with the research project: Hypoxia-induced lipid metabolism supports tumor progression and angiogenesis in liquid and solid cancers

Andrea David Re Cecconi, Istituto Mario Negri, Milano, with the research project: Musclin as a promising therapeutic option for cancer cachexia

Beatrice Foglia, University of Torino, with the cancer research project: Oncostatin M and tumor inflammatory signature as prognostic markers of Nash-related HCC

Fabiana Conciatori, Istituto Nazionale Tumori Regina Elena Roma IRCCS, with the research project: Characterizing IL-8/CXCR1-2 axis in tumor stroma-interactions in genetically/molecularly defined CRC models

Federica Portale, Humanitas Mirasole Milano, with the research project: Dissection of Natural killer cells dynamics and functions in prostate cancer

Luigi Ippolito, University of Firenze, with the cancer research project: Dissecting the lactate-driven Discoidin Domain Receptor-1 activation in supporting prostate cancer cells dissemination

Martina Di Modica, Fondazione IRCCS Istituto Nazionale Tumori Milano, with the research project: Role of gut microbiota in Her2-positive breast cancer recurrence

Next call for application will open in 2024, for the 2025-2026 period

The Pezcoller Foundation

FONDAZIONE CARITRO
CASSA DI RISPARMIO DI TRENTO E ROVERETO

Journal

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