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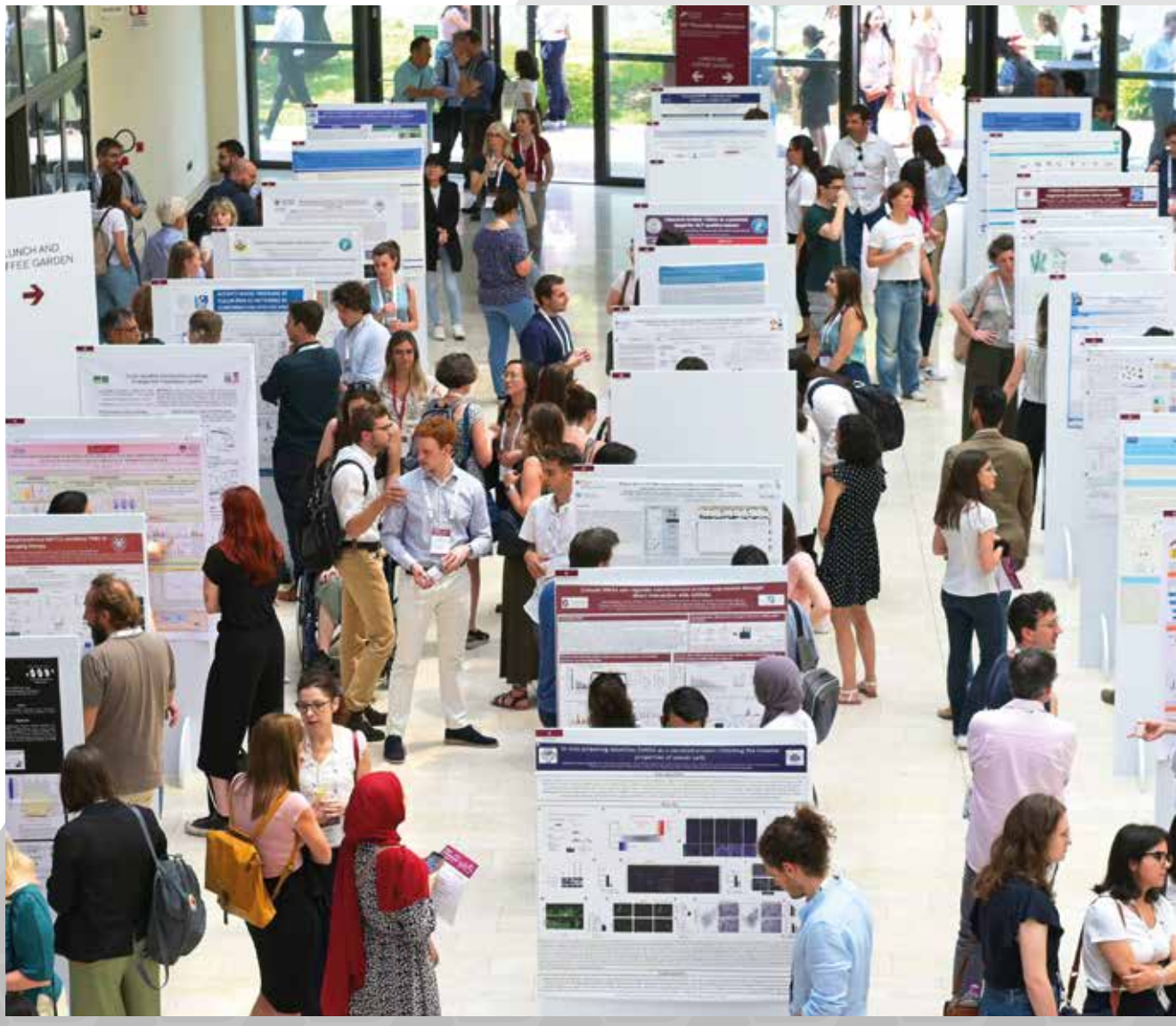

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Picture on front page:
Poster Session at the 34th Pezcoller Symposium,
June 19-20, 2023.

2024 June Editorial

So, we have arrived at the 35th Pezcoller Symposium: an annual event since 1989, with the only interruption in 2020 due to the COVID-19 pandemic. It means that for 35 years the best international scientists have gathered in Trento, to talk about topics that are always at the forefront of cancer research. This was a great insight of Dr. Enrico Mihich, the founder of the Symposia, to associate the Pezcoller International Award for Cancer Research with a major scientific event that would lend credibility and scientific prestige to the Foundation.
Since then, under the leadership of Dr. Enrico Mihich first and Dr. David Livingston after him, the Pezcoller Symposia have become year after year widely appreciated as one of the "not-to-be-missed" yearly meeting on cancer research. This, even more after Dr. Livingston's sudden death and taking over as chairman of Nobel laureate Dr. William Kaelin, thanks to its passionate work and the support of the Scientific Standing Committee (all individually mentioned in this journal).
We have 200 registered participants this year, with the largest number of submitted abstracts (79), of which (53) have been accepted for poster presentation, by the selection committee coordinated by Dr. Massimo Loda, whom we sincerely thank for the heavy work they have done. Moreover, the editors of 2 leading scientific journals (*AACR journal Cancer Discovery* and *EMBO Molecular Medicine*) will attend the Symposium, as in the previous years.
The focus on young people, with the possibility of direct interaction with the world's most authoritative scientists, combined with the fact that the Symposia are held in a small city like Trento, rich in charm and history but still on a human scale, has increased its fame and attractiveness in the scientific community, both in Italy and abroad.
In addition to the traditional formula of 25 minutes for each presentation, followed by a robust discussion, we have maintained this year again the Career Development Panel Discussion, chaired by Dr. Kaelin himself, to emphasize the special focus of the Pezcoller Symposia on young researchers.
Once again we are able to provide 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 of Milan (ESO). They will be presented in an ESO international online event on September 5th 2024.
An important new feature for this year symposium is that for the first time, the American Association for Cancer Research (AACR), the European Association for Cancer Research (EACR), and the Mark Foundation (USA), have agreed to support the participation of young researchers from outside Italy to the symposium, in recognition of its scientific value and the relevant opportunity offered to young people.
We therefore hope that this year Symposium will greatly succeed in crystallizing which new scientific knowledge is likely to inform the topic field and shed light on the mechanisms that affect reciprocal interactions and pitfalls, between tumor cells and their surroundings.
Finally, I can't thank enough all those who made a key contribution to the organization and management of this Symposium: the chairman Dr. William Kaelin, the members of the Scientific Standing Committee and of the Poster Evaluation Committee, the Pezcoller Foundation team, 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 prestigious and comfortable venue.

Enzo Galligioni
President

The Pezcoller Foundation

35th Pezcoller Symposium

June 24–25, 2024
Trento, Italy
Humanities Department, University of Trento
(via Tommaso Gar 14)

Cancer as a systemic disease: interactions between tumor and host

Chairman: Kaelin William G.

Moderators:

Ambrogio Chiara
Bardelli Alberto
Del Sal Giannino
Demichelis Francesca
Draetta Giulio
Loda Massimo
Piccolo Stefano
Schulman Brenda A.

Day 1 – Monday, June 24, 2024

7.45 ***Registration***

8.30 ***Welcome:*** *Enzo Galligioni*

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

8.50 ***Moderator:*** Stefano Piccolo

David Livingston Keynote Lecture

"Investigating tumor-immune interactions using genetically engineered mouse models of cancer"

Tyler E. Jacks, PhD

Koch Institute for Integrative Cancer Research, MIT

9.30 Discussion

9.45 ***Moderator:*** Giannino Del Sal
"Tumor cell as organizer: the tumor microenvironment obeys the cancer cell's directives"

Ben Z. Stanger, MD, PhD

University of Pennsylvania Perelman School of Medicine

10.10 Discussion

10.25 Coffee break and poster exhibition

10.55 ***Moderator:*** Francesca Demichelis

"The role of the intestinal microbiome in cancer immunotherapy"

Marcel R. M. van den Brink, MD, PhD

City of Hope Los Angeles

11.20 Discussion

11.35 ***"Understanding and manipulating immune modulation by the microbiome"***

Michael A. Fischbach, PhD

Stanford University

12.00 Discussion

12.15 ***"Metagenomics of the human microbiome for applications in oncology"***

Nicola Segata, PhD

University of Trento

12.40 Discussion

12.55 Lunch and poster exhibition

14.20 ***EACR-AACR-Mark Foundation Travel Grants -*** Ryan Schoenfeld

14.25 ***Moderator:*** Giulio Draetta

"Leveraging DNA Damage Response to Prevent Evolution of Drug Resistance"

Mariangela Russo, PhD

University of Torino

14.50 Discussion

15.05 ***"Tumor Macro- and Micro-Environment Interactions Remodel Cancer Phenotypes and Drive Therapy Resistance"***

Peter S. Nelson, MD

Fred Hutchinson Cancer Center

15.30 Discussion

15.45 ***Moderator:*** William G. Kaelin

Career Development Panel Discussion *(Panel TBA)*

16.30 END OF DAY 1

19.30 *Symposium Social Dinner at Cantina Storica Rotari Mezzocorona, Trento (transfer provided)*

Day 2 – Tuesday, June 25, 2024

8.30 ***Moderator:*** Alberto Bardelli

Enrico Mihich Keynote Lecture

"Skin Stem Cells: How they Cope with Inflammation and Oncogenic Stress and Retain Memories of These Encounters"

Elaine V. Fuchs, PhD

Rockefeller University

9.10 Discussion

9.25 ***"Targeted Gene-based Delivery of Cytokines Reprograms the Tumor Microenvironment Towards Eliciting Protective Immune Response"***

Luigi Naldini, MD, PhD

Università Vita-Salute San Raffaele Milano

9.50 Discussion

10.05 ***"Systemic and local Immune Dynamics in Cancer Immunotherapy"***

Sophia M. Guldberg

University of California San Francisco

10.30 Discussion

10.45 Coffee break and poster exhibition

11.15 ***Moderator:*** Brenda A. Schulman

"Interorgan communication during cancer cachexia"

Tobias Janowitz, MD, PhD

Cold Spring Harbor Laboratories

11.40 Discussion

11.55 ***"The Ravages of TiME: how the aging microenvironment impacts tumor progression"***

Ashani T. Weeraratna, PhD

Johns Hopkins School of Medicine

12.20 Discussion

12.35 Lunch and poster exhibition

14.05 ***Moderator:*** Massimo Loda

Maria Begnudelli Awards: oral talks of the 3 best poster presenters

14.50 ***Moderator:*** Chiara Ambrogio

"Neuroimmune crosstalk in the gastric cancer microenvironment"

Sandra W. Ryeom, PhD

Columbia College of Physicians and Surgeons

15.15 Discussion

15.30 ***"The neuroscience of brain cancers"***

Michelle Monje-Deisseroth, MD, PhD

Stanford University School of Medicine

15.55 Discussion

16.10 ***"Discovery of a vertebral skeletal stem cell driving spine metastases"***

Matthew B. Greenblatt, MD, PhD

Weill-Cornell Medical School

16.35 Discussion

16.50 *Closing Remarks: William G. Kaelin*

Program

Faculty

- **Michael A. Fischbach, PhD**
  Stanford University, Stanford, CA
- **Elaine V. Fuchs, PhD**
  Howard Hughes Medical Institute, The Rockefeller University, New York, NY
- **Matthew B. Greenblatt, MD, PhD**
  Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY
- **Sophia M. Guldberg**
  University of California, San Francisco, CA
- **Tyler E. Jacks, PhD**
  Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA
- **Tobias Janowitz, MD, PhD**
  Cold Spring Harbor Laboratory, NY
- **Michelle Monje-Deisseroth, MD, PhD**
  Stanford University, Howard Hughes Medical Institute, Stanford, CA
- **Luigi Naldini, MD, PhD**
  San Raffaele Telethon Institute for Gene Therapy, San Raffaele Scientific Institute and Vita-Salute San Raffaele University, Milan, ITA
- **Peter S. Nelson, MD**
  Fred Hutchinson Cancer Center, Seattle, WA
- **Mariangela Russo, PhD**
  Dept. Oncology, University of Torino, ITA
- **Sandra W. Ryeom, PhD**
  Herbert Irving Comprehensive Cancer Center, Department of Surgery, Division of Surgical Sciences, Columbia University Irving Medical Center, New York, NY
- **Nicola Segata, PhD**
  CIBIO Department, University of Trento, ITA
- **Ben Z. Stanger, MD, PhD**
  University of Pennsylvania School of Medicine, Philadelphia, PA
- **Marcel R. M. van den Brink, MD, PhD**
  City of Hope Los Angeles and City of Hope National Medical Center, CA
- **Ashani T. Weeraratna, PhD**
  Johns Hopkins School of Medicine, Baltimore, MD

Discussants

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

35th Pezcoller Symposium

Cancer as a Systemic Disease: Interactions between Tumor and Host

Trento, Italy, June 24-25, 2024

ABSTRACTS OF ORAL PRESENTATIONS

Investigating tumor-immune interactions using genetically engineered mouse models of cancer

Tyler E. Jacks, PhD

Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA

The clinical success of a series of immune-stimulating anti-cancer agents over the past decade has ushered in the current era of immuno-oncology. However, despite this progress, it remains the case that only a subset of patients benefit from these treatments. Expanding the impact of immune-based treatments for cancer will require an improved understanding of the factors that control anti-tumor immune responses. As a tool for probing these questions, we have adapted well-characterized autochthonous mouse models of cancer to express a series of antigens and have examined the dynamic nature of the immune responses using a range of methods. The characterization of these models has led to new insights into the effects of intratumoral heterogeneity on anti-tumor immunity and response to immune checkpoint therapies. Recent work has focused on the effect of CD4 and CD8 antigens in promoting optimal anti-tumor immune responses. These models have also been used to explore a role for the complement system in tumor progression through control of anti-tumor immune responses.

Tumor cell as organizer: the tumor microenvironment obeys the cancer cell's directives

Ben Z. Stanger, MD, PhD

University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

Solid tumor microenvironments have a cellular complexity that is daunting. Each component – fibroblasts, immune cells, endothelial cells, nerves, matrix, and cancer cells – is heterogeneous and capable of signaling to other components, resulting in an overwhelming degree of molecular crosstalk. Despite this complexity, tumor microenvironments follow a logic dictated by the molecular features and signaling competencies of each spatial unit. Cancer cells occupy a privileged position at the apex of such signaling hierarchies, making them the "organizers" of the TME. In this lecture, I will give several examples of how tumor cells establish and/or maintain the phenotype of their associated microenvironments, and the implications for therapy.

The Role of the Intestinal Microbiome in Cancer Immunotherapy

Marcel R. M. van den Brink, M.D., Ph.D.

City of Hope Los Angeles and City of Hope National Medical Center, CA

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative-intent therapy for hematologic and some other malignancies. However, patients can experience life-threatening complications including graft-versus-host disease (GVHD), relapse, and infections. Allogeneic donor T cells attack not only residual tumor cells but also the recipient's tissues and organs during GVHD.

The gut is a primary target of GVHD and plays a role in its development, as allo-HSCT conditioning disrupts host–microbiota equilibrium. The healthy gut is characterized by a diverse, mostly anaerobic community of bacteria, while gut dysbiosis involves expansion of facultative anaerobes such as Enterococci. Microbiota dysbiosis—including decreased bacterial diversity and lower abundance of anaerobic commensal species—is associated with increased mortality rates and other poor outcomes in patients with cancer. Thus far, we have demonstrated a relationship between microbiota composition, clinical outcomes, and the influence of drug exposures and diet on the microbiome. We will briefly highlight some of the main findings of a few of these studies.

Gut bacterial (alpha) diversity decreases during allo-HSCT, owing to cancer treatments, antibiotics, and other medications as well as inflammation and dietary changes. In a study of 8,767 fecal samples obtained from 1,362 patients, we found that certain antibiotics increase the risk of domination by pathobionts (i.e., Enterococcus species), which are associated with worse outcomes, or infection by intestinal pathogens (i.e., Clostridium difficile). We developed a computational method called PARADIGM (parameters associated with dynamics of gut microbiota) to identify medication-microbiome associations and found that several non-antibiotic drugs were associated with reduced alpha diversity and expansion of Enterococcus species (9,167 samples from 1,201 patients). We are now exploring how medical record data on pharmacologic exposures can be used to predict changes in microbiota composition and indicate opportunities to intervene to preserve or increase diversity.

In addition, we demonstrated that commensal species are protective against GVHD and other treatment complications; patients with higher abundance of Blautia species in class Clostridia during the peri-engraftment period had reduced GVHD-related mortality and longer survival after allo-HSCT. Clostridia and other commensals produce short-chain fatty acid (SCFA) metabolites such as butyrate, propionate, and acetate, as a byproduct of carbohydrate fermentation. These bacterial metabolites are important for intestinal gut health and immune regulation.

To further understand how bacterial metabolism affects the immune system, we have studied microbe-derived secondary bile acids (BAs). Our analyses suggest that dysbiosis and loss of these BAs during inflammation may be an important mechanism exacerbating T cell-mediated diseases. However, microbiota-targeted strategies could support therapeutic responses. For instance, some patients receive prophylaxis with ursodeoxycholic acid (UDCA), which is also a microbe-derived BA. While patients with GVHD after allo-HSCT had an altered BA pool in our study, those who received UDCA had better responses, supporting the use of this supplement in the peri-transplant setting (1,301 allo-HCT patients).

We are also analyzing the effects of dietary fiber on the intestinal microbiome. Since shifts in microbiota composition and function occur within days in response to dietary perturbation, we recorded daily dietary data (35,228 food items, days -7 to +30 relative to HCT) and collected fecal samples (1,028 samples) from 173 allo-HCT patients. Our preliminary findings showed positive and significant correlations between fiber intake and overall survival after HCT, microbiome alpha diversity, and fecal butyrate production. We also tested concentrations of cellulose fiber (0%, 6%, 12%, and 40%) in a preclinical GVHD mouse model to assess mechanisms by which dietary fiber could improve the microbiome composition and clinically relevant outcomes. Optimal fiber consumption (12% cellulose) led to increased alpha diversity, decreased pathogen relative abundance, increased T-regs, and decreased GVHD lethality in GVHD mice (3 independent experiments, 180 mice total). These results suggest that dietary fiber could be utilized to prevent GVHD.

We have investigated several strategies to promote intestinal diversity after allo-HSCT, including by limiting the use of broad-spectrum antibiotics, administering dietary interventions, or restoring diversity through fecal microbiota transplantation or the administration of live biotherapeutic products. Our overall goal is to further characterize how the microbiome and its related metabolites influence outcomes for patients receiving immunotherapies.

Understanding and manipulating immune modulation by the microbiome

Michael A. Fischbach, PhD

Stanford University, Stanford, CA

Certain members of the commensal microbiota elicit a potent T cell response upon colonization. In this talk, I will describe two recent projects from my research group that share the goal of characterizing and manipulating anti-commensal immunity. In the first project, we explore the functional properties of colonist-induced T cells by engineering the skin bacterium Staphylococcus epidermidis to express tumor antigens anchored to secreted or cell-surface proteins. Upon colonization, engineered S. epidermidis elicits tumor-specific T cells that circulate, infiltrate local and metastatic lesions, and exert cytotoxic activity, showing that the immune response to a colonist can be redirected against a target of therapeutic interest by expressing a target-derived antigen in a commensal. In the second, we colonize germ-free mice with a complex defined community (>100 bacterial strains) and profile T cell responses to each strain individually. We find that T cell re-

Abstracts

cognition of Firmicutes is focused on a widely conserved cell-surface antigen, opening the door to new therapeutic strategies in which colonist-specific immune responses are rationally altered or redirected.

Metagenomics of the human microbiome for applications in oncology

Nicola Segata, PhD

CIBIO Department, University of Trento, ITA

Metagenomic analyses of the human microbiome have uncovered multiple links between our microbial complement and cancer. In my talk, I will describe some of these links that we contributed to characterize such as the association between the gut microbiome and colorectal cancer, the role of the gut microbiome in response to immunotherapy, and the potential application of fecal microbiota transplantation to improve cancer therapies. I will discuss which are the metagenomic tools that should be further developed to improve the characterization of the host-microbiome interactions in oncology.

Leveraging DNA Damage Response to Prevent Evolution of Drug Resistance

Mariangela Russo, PhD

Dept. Oncology, University of Torino, ITA

Drug resistance remains the main obstacle to the long-lasting effectiveness of anti-cancer therapy. Despite the fact that tumors may initially show clinical responses to chemotherapy and/or targeted agents, achieving minimal residual disease (MRD), the disease inevitably recurs. Indeed, when cancer cells are challenged with anti-cancer agents, a sub-fraction of cells switch to a drug-tolerant persister (DTP) state and survive to the lethal effect of therapies through transient non-genetic mechanisms. However, we recently showed that upon prolonged exposure to drug-induced hostile environment, alike bacteria DTPs, cancer DTPs experience increased DNA damage, impairment of mismatch repair and homologous recombination proficiency, and a shift to errorprone DNA replication process. This in turn leads to a temporary increase of their mutation rate, thus promoting the emergence of resistance. Through genetic and pharmacological screenings, we identified new DTPs' vulnerabilities and propose novel therapeutic approaches to reduce MRD and curb the development of drugresistance. Knowledge that cancer cells alter DNA repair machineries under therapeutic stress exposes a vulnerability that could be exploited to design novel therapeutic strategies to restrain clonal evolution, thus preventing the recurrence of the disease and prolonging the clinical benefit for cancer patients.

Tumor Macro- and Micro-Environment Interactions Remodel Cancer Phenotypes and Drive Therapy Resistance

Peter S. Nelson, MD

Fred Hutchinson Cancer Center, Seattle, WA

There is ample evidence that neoplastic cells interact extensively with components of organ and tissue microenvironments to stimulate and/or repress specific tumor cell characteristics that range from promoting motility and invasion, reception of metastatic cells in distant sites, and evasion of immune system attack. Host macro effects also play key regulatory roles, such as the influence of hormones and other circulating and diffusible factors that exert pleiotropic effects on both tumor cells and microenvironments. This presentation will discuss the consequences of cancer therapeutics - which are often delivered in a systemic manner - with resultant disseminated effects on both tumor and benign cells. The relationships involving this triad of therapeutic agent, tumor, and host organ/tissue may have important implications for the ultimate success of anti-cancer treatments.

Skin Stem Cells: How they Cope with Inflammation and Oncogenic Stress and Retain Memories of These Encounters.

Elaine V. Fuchs, PhD

Howard Hughes Medical Institute, The Rockefeller University, New York, NY

At the interface of our body and the outside world, our epidermis forms the skin's barrier that keeps pathogens out. As such it is subjected to a harsh environment, confronting mechanical stress, pollens and allergens, pollutants, noxious chemicals and both good and bad microbes. My laboratory focuses on the long-lived skin stem cells that are responsible for continuously replenishing and repairing the epidermis and its appendages (hair follicles, sweat and oil glands). After decades of research characterizing these stem cells and learning how they interact with their local tissue microenvironments ('niches') to achieve these tasks, we began to turn to how these stem cells cope with the various stress of the external environment and how their malfunction leads to chronic inflammation and non-melanoma skin cancers. In the laboratory, we use high throughput genetic and genomic approaches to dissect at a molecular level how changes in the skin's external environment influences the behavior of the stem cells and their interactions with immune and other cell types of the skin. In doing so, we've learned that skin stem cells acquire and recall memories of experiences such as inflammation that happened long ago. This ability has profound implications for body health and fitness not only in beneficial but also maladaptive ways, particularly cancer.

A Private Crosstalk Established by Tumor-Targeted Immune Stimulatory Cytokines Rescues CAR-T Activity and Engages Host T Cells against Glioblastoma

Federico Rossari, Giorgia Alvisi, Melania Cusimano, Stefano Beretta, Filippo Birocchi, Deborah I. Ambrosecchia, Ottavia Vitaloni, Tamara Canu, Andrea Annoni, Bernhard Gentner, Ivan Merelli, Nadia Coltella and **Luigi Naldini, MD, PhD**

San Raffaele Telethon Institute for Gene Therapy, San Raffaele Scientific Institute and Vita-Salute San Raffaele University, Milan, Italy

Chimeric antigen receptor T cells (CAR-Ts) represent a novel pillar of immune therapy achieving unprecedented efficacy in several hematological malignancies. However, their therapeutic activity is substantially limited in solid tumors, mostly due to the immunosuppressive tumor microenvironment (TME). While immune stimulatory cytokines can counteract immune-suppression, their systemic administration entails risk of toxicities and counter-regulatory responses. To safely deliver cytokines at the tumor site, we established a gene therapy strategy leveraging on a population of tumor-associated TIE2-expressing monocytes/macrophages (TEMs) as vehicles. Hematopoietic stem cells (HSCs) are engineered with lentiviral vectors (LVs) designed to express the cytokine selectively in their TEM progeny. This strategy allowed TME targeted-cytokine delivery and anti-tumor responses in different tumor models and is currently under clinical testing in a Phase 1/2a trial in unmethylated glioblastoma (GBM) sponsored by Genenta Sciences. Current clinical findings show tolerability and safety, dose-dependent engraftment of engineered cells, targeted interferon activity and immune reprogramming of the TME. To investigate whether the reprogrammed TME could also favor CAR-T function, we generated B7H3-directed mouse CAR-Ts and tested them in an immunocompetent GBM model. While CAR-Ts alone showed poor activity, their combination with IFN gene therapy achieved synergistic anti-tumor activity. Immunophenotypic and transcriptomic analyses showed reduced CAR-T terminal exhaustion and induction of effector/memory states featuring activation of signaling pathways and transcriptional networks boosting anti-tumor activity. We then established a private crosstalk between TEMs and CAR-Ts by targeted delivery of orthogonal IL-2 and co-expression of its cognate receptor together with the CAR. IFN delivery, especially when combined with private oIL2 signaling to CAR-Ts, engaged an endogenous T cell response spreading to tumor-associated antigens beyond B7-H3. Overall, the rescued CAR-T and endogenous T cell function significantly prolonged mice survival, suggesting that the combination of the two gene and cell therapy strategies described here, which are already under clinical testing as monotherapies, could achieve synergistic effects also in GBM patients.

Systemic and local Immune Dynamics in Cancer Immunotherapy

Sophia M. Guldberg

Spitzer Lab, University of California, San Francisco, CA

Immune checkpoint blockade (ICB) has shown remarkable efficacy in a subset of patients, but many cancers remain durably resistant to ICB. A subset of CD8+ T cells in the lymph node (LN) known as progenitor exhausted CD8+ T cells (Tpex; PD1+ TCF1+) have been identified as important responders to immunotherapy. These Tpex traffic to tumors where they must carry out anti-tumor immune responses. Understanding the immunosuppressive, tolerogenic programs that restrict T cell activation in lymph nodes and function in the tumor microenvironment (TME) is key to improving T cell responses during ICB. Peripheral immune cell populations expressing the Autoimmune Regulator (Aire) gene have been suggested to play critical roles in immune self-education, but their roles have not been defined in tumor-draining lymph nodes or in the TME. Here, we demonstrate the presence of Aire-expressing macrophages (aTAMs) across multiple mouse tumor models and characterize them as an immunosuppressive macrophage subtype that restricts T cell responses. The Aire gene is central to the formation of these aTAMs, as Aire-deficient mice exhibit a decrease in the aTAM population and in the gene signature that characterizes this cell population. Ablation of aTAMs (LysM-Cre x Aire-DTR) improves tumor control across multiple immunotherapy-resistant tumor models in a CD8 T cell-dependent manner, resulting in increased CD8 T cell infiltration and inflammatory remodeling of the myeloid compartment. Notably, macrophage-specific loss of Aire (LysM-Cre x $Aire^{fl/fl}$) or aTAM

ablation (Aire-DTR) synergizes with anti-PD-1 to improve anti-tumor immunity. Furthermore, aTAMs can be found in human scRNA-seq tumor datasets across multiple tumor types and share a similar immunosuppressive transcriptional profile. Our results identify a novel macrophage population, aTAMs, and demonstrate that Aire expression in this phenotypically and functionally distinct population plays a critical role in inhibiting anti-tumor T cell responses.

Interorgan communication during cancer cachexia

Tobias Janowitz, MD, PhD

Cold Spring Harbor Laboratory, NY

The systemic effects of cancer can ultimately lead to cachexia, a severe wasting syndrome frequently associated with inflammation. Cachexia precipitates multi-organ dysfunction and is often fatal. Patients with cachexia frequently experience a multiplicity of symptoms and signs, including fatigue, apathy, reduced interest in nutrient intake, disruption in sleep pattern, metabolic changes, neuroendocrine changes, and reduced immunity. This causes significant morbidity and mortality. However, the underlying biological mechanisms of these symptoms and their interconnectivity remain unclear. The seminar will discuss results from cancer models and clinical correlates. Pre-clinical work demonstrates that cachexia triggers apathy-like symptoms through a cytokine-sensing brainstem-to-basal ganglia circuit. This neural circuit detects elevated interleukin-6 (IL-6), an inflammatory cytokine frequently elevated in cachexia in mice and humans, at cachexia onset, and translates it into decreased mesolimbic dopamine, thereby increasing behavioral effort-sensitivity. The resulting reduced nutrient intake during foraging unmasks an IL-6 induced altered hepatic starvation response, ultimately leading to activation of the glucocorticoid stress response that suppresses systemic immunity. The connectivity of the multiorgan response and the fact that cachexia occurs in many non-cancerous inflammatory conditions, suggests an evolved process.

The Ravages of Time: how the aging microenvironment impacts tumor progression

Ashani T. Weeraratna, PhD

Johns Hopkins School of Medicine, Baltimore, MD

Patients 65 and older account for 69% of all new cancer diagnoses. Melanoma is a deadly disease, with chances of survival for patients with stage IV disease totaling only 27%. Systemic age-related changes- both secreted and biophysical-drive metastasis, immune cell recruitment, and changes in the vasculature. We have made the seminal discovery that aged fibroblasts secrete, or stop secreting, key molecules that affect multiple aspects of tumorigenesis. Our current investigations include: the loss of molecules that maintain ECM integrity, resulting in changes in mechanotransduction and increased metastasis; the secretion of molecules that increase resistance to targeted therapy; the secretion of macromolecules such as lipids that are taken up by melanoma cells in the aged microenvironment, affecting tumor cell metabolism; changes in the aged immune tumor microenvironment; the secretion of non-canonical Wnt molecules that affect cell signaling leading to angiogenesis, metastasis, tumor dormancy and therapy resistance. We are beginning to unravel the role of the immune microenvironment in the context of aging, and to explore age-related changes in cancers other than melanoma, specifically pancreatic cancer. We are also attempting to understand the intersectionality of systemic host factors such as biological sex and age, and how those factors compound responses to cancer therapy. We use a variety of techniques from proteomics, to computational modeling, to gene expression profiling, to cellular and in vivo assays. Some of the more exciting 3D models that we use include the construction of artificial skin, the extraction of fibroblast matrices and the development of immune cell 3D trafficking assays, imaged in real time. Because the first few years of my laboratory focused on the Wnt signaling pathway, we have also built up both a sound base of technical and observational knowledge, as well as assembled an array of tools to study Wnt signaling in cells of melanocytic origin. Importantly, we move from our laboratory-based observations to patient samples, and explore the clinical implications of our work both in terms of validation of cell-based observation, and with a view towards optimizing therapy according to patient age. Overall, our studies argue for placing mechanistic studies of cancer biology into the context of aging.

Neuroimmune Crosstalk in the Gastric Cancer Microenvironment

Jin Sun Cho, Jaewon Kim, Jaia S. Wingard, **Sandra Ryeom, PhD**

Herbert Irving Comprehensive Cancer Center, Dept. of Surgery, Division of Surgical Sciences, Columbia University Irving Medical Center, New York, NY

Gastric cancer (GC) is the third leading cause of cancer-related deaths worldwide. Advances in diagnosis and treatment such as the addition of immune checkpoint inhibitors to chemotherapy has improved outcomes but only in a limited subset of GC patients. Understanding changes in the tumor immune microenvironment (TIME) during GC progression will offer insight into new targets. The GC microenvironment is complex consisting of stromal cells, endothelial cells, immune cells and nerves. Studies have shown that cholinergic signaling promotes GC tumor progression in part by inducing expression of nerve growth factor (NGF) by GC cells further increasing innervation by both sympathetic and sensory nerves. NGF is a key regulator of neuronal function, with both activating and inhibitory effects depending on the context and interaction with its high affinity Tropomyosin receptor kinase A (TrkA) receptor or low affinity p75NTR receptor. Utilizing a pan-TRK receptor inhibitor, Larotrectinib, we observe attenuation of tumor progression in an aggressive GC mouse model accompanied by significant changes in the tumor microenvironment, notably restoration of B lymphocyte infiltration in GC tumors. Our data indicate crosstalk between sensory nerves, gastric cancer cells and B cells during tumor progression in the GC tumor microenvironment with B cells in the TIME playing a critical anti-tumor role.

The Neuroscience of Brain Cancers

Michelle Monje, MD PhD

Stanford University; Howard Hughes Medical Institute, Stanford, CA

In the central nervous system, neuronal activity is a critical regulator of development and plasticity. Concordantly, neuronal activity robustly promotes progression of both high-grade and low-grade glioma subtypes in preclinical models. Crucial mechanisms mediating activity-regulated glioma growth include paracrine secretion of BDNF and the synaptic protein neuroligin-3 (NLGN3). NLGN3 induces multiple oncogenic signaling pathways in the cancer cell, and also promotes glutamatergic synapse formation between neurons and glioma cells. Glioma cells integrate into neural circuits through neuron-to-glioma synapses that exhibit mechanisms of malignant synaptic plasticity. This synaptic and electrical integration of glioma into neural circuits is central to tumor progression in preclinical models. Thus, both paracrine and synaptic neuron-glioma interactions play important roles in the pathogenesis of glial cancers, highlighting a number of potential therapeutic opportunities. The subversion of mechanisms of neurodevelopment and plasticity by malignant gliomas underscores the importance of understanding the neuroscience of brain cancer. Similar mechanisms of neuron-cancer interactions are increasingly appreciated in brain metastases and in cancers outside of the CNS; nervous system-cancer interactions are now emerging a new hallmark of cancer.

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Discovery of a vertebral skeletal stem cell driving spine metastases

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Many solid tumors, including breast and prostate cancer, display a marked metastatic predilection for the spine over other skeletal sites. While for many years this was assumed to reflect the distribution of blood flow, using modern techniques to track vascular flux we find that blood flow cannot account for the higher rates of metastasis to the spine. Instead, building upon our recent work that distinct regions of the skeleton are formed by distinct skeletal stem cells, we identify a new skeletal stem cell residing in the endplate cartilage that both forms and maintains vertebral bone. This vertebral skeletal stem cell is intrinsically more pro-metastatic than its long bone counterparts, a phenotype that is due in part to its enhanced secretion of MFGE8, which functions in this context as a secreted bone-derived metastatic trophic factor. In ongoing work, we are extending this concept to examine how additional new stem cells may explain the signature site-specific disease processes of other regions of the skeleton.

Abstracts Of Posters

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Spatial Transcriptomics analysis unveil novel subtype-specific markers driving malignant progression of Intraductal Papillary Mucinous Neoplasms

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Intraductal papillary mucinous neoplasms (IPMN) are ductal pancreatic cystic lesion which may progress to pancreatic ductal adenocarcinoma (PDAC). The number of patients diagnosed with IPMN is increasing and the current IPMN risk stratification is based on clinical and histological features rather than molecular markers. We aimed to improve the current diagnostic criteria of IPMN performing a morphomolecular analysis of a comprehensive IPMN series, comprising all disease stages and morphology of IPMN using spatial transcriptomics (ST) analyses.
We performed Visium ST (10X Genomics) on a discovery cohort of 18 samples from 14 patients: 4 low-grade IPMN (3 low grade dysplasia (LGD), 1 intermediate dysplasia/Borderline) and 9 high-grade IPMN characterized by high-grade dysplasia (HGD) (Including 4 invasive IPMN-associated PDAC and 1 PDAC-associated normal duct). We validated Visium results by GeoMx (Nanostring) ST and Akoya Phenoimager (Akoya Biosciences) on an independent validation cohort of 75 IPMN samples from 58 patients.
By integrated multi-platform ST characterization of IPMN we identified novel subtype-specific markers: HOXB3 and ZNF117 in LGD ; SPDEF, and gastric neck cell markers in Borderline; NKX6-2 and gastric isthmus cell markers in HGD Gastric IPMN.
We utilized the innovative algorithm stLearn to unravel the spatial evolutive trajectory of the progression from Borderline IPMN to Gastric IPMN, highlighting the role of NKX6-2 on the Gastric differentiation of IPMN. We performed spatially-aware GSEA to identify the main oncogenic pathways leading IPMN degeneration to PDAC highlighting the role of TNFα signalling and Myc activation in IPMN progression.
In this project, we have demonstrated the incredible advantages of spatial biology in translational oncology over bulk-techiniques.
With the aid of cutting-edge spatial technologies we identified novel markers associated with IPMN morphology and dysplasia to better delineate progression in patients, improve risk stratification, and clinical management.
These findings pave the way for a better pathological classification of IPMN and provide an unprecendented look into PDAC early stages.

MIR-214-Induced Melanoma Hyperpigmentation And Therapy Resistance: Molecular Insights

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Despite the last decade having witnessed a revolutionary benefit in the care of cutaneous melanoma (CM) patients with the advent of immune-checkpoint inhibitors (ICI), resistance onset urges the development of innovative strategies. Melanin affects the course of melanoma, being hyperpigmentation related to therapy resistance, ICI included. Despite the main melanin function being protecting against damage from light by scavenging reactive oxygen species (ROS), melanin can become a photosensitizer/pro-oxidative agent itself depending on melanin type and redox intracellular state. The melanin ROS-scavenging activity mainly relies on its chelating activity of metal ions including iron, which indeed induces melanogenesis because of its high ROS-generating activity. In the context of melanoma hyperpigmentation and therapy resistance, experimental data suggest a crucial role of miR-214 and this study aims to elucidate the interplay among miR-214, ROS, and iron in hyperpigmented/resistant CM, and eventually identify molecular targets to restore therapy response.
Melanoma cells were forced to stably overexpress miR-214 (miR-214+) through the PiggyBac transposon system. Intracellular melanin content was quantified by spectrophotometry, melanosomes observed at the transmission electron microscope, and key melanogenic proteins by western blot (WB). ROS were detected in flow cytometry (FC), and the intracellular content of iron was quantified by colorimetric assay. The therapy response of melanoma cells was assessed in 2D/3D colorimetric, luminescent, FC, and colony assays. miR-214 levels in plasma samples of CM patients treated with ICI at the Careggi University Hospital in Florence were quantified by droplet digital PCR.
miR-214+ melanoma cells showed increased pigmentation together with deregulated iron metabolism, increased ROS, and a reduced Glutathione S-transferase Zeta 1 (GSTZ1) expression, an anti-oxidant protein also involved in the catabolism of the melanin precursors phenylalanine and tyrosine. miR-214+ hyperpigmented melanoma cells showed less responsiveness to chemo-, target, and radiotherapy in vitro than control. Higher levels of miR-214 were found in plasma samples of ICI-treated non-responder CM patients compared to responders.
miR-214 induces the development of hyperpigmented, resistant melanoma cells. Thereby, a deeper molecular/mechanistic view could be crucial to identify new targets to restore therapy sensitivity in non-responder CM patients

CD73 expression in pancreatic ductal adenocarcinoma: a novel modulator of NK cell pro-tumor polarization

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Posters

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Pancreatic ductal adenocarcinoma (PDAC) tumor microenvironment undergoes dynamic remodeling with tumor-promoting properties. Extracellular adenosine (ADO), a purine metabolite produced from ATP through CD73 enzymatic activities, can inhibit natural killer (NK) cell proliferation, maturation, and cytotoxic function. Our group demonstrated that, in different tumors, NKs undergo a pro-tumor polarization, up-regulating CD9 expression and showing reduced cytotoxicity. Here, we evaluated the frequency of circulating and tissue infiltrating CD9+NK cells in PDAC patients and healthy donors (HC) and the impact of CD73 expression on tumor cells in promoting pro-tumor NKs.
In-silico analysis was used to explore CD73 levels in PDAC patients and its impact on patients' survival. Flow cytometry was used to assess the phenotype of circulating and tumor infiltrating NKs in PDAC patients and HC. Conditioned media (CM) from CD73 expressing or transiently silenced PDAC cell lines (BxPC3 and PANC1), were used to polarize NK cells.
We found that CD73 is up-regulated in PDAC patients compared to HC. CD73high patients have shorter OS compared to CD73low patients, outlining the prognostic role of CD73 as a marker of worse outcomes. In-silico analyses revealed variable levels of CD73 expression in PDAC patients, with distinct immune cell profiles observed between CD73high and CD73low patients. Focusing on the NK cluster, we found an enrichment in the CD9, CD49a, PD1 and TIM3 expression in CD73high patients, while an increase of Perforin and Granzyme was observed in CD73low patients, suggesting a possible contribution of CD73 in NK polarization. CD73- silenced PDAC cell lines showed increased MICA/B NK activation ligand expression. Exposure of HC-derived NKs to CM of PDAC cells increased the frequency of CD9+ NKs and this was limited by CD73 silencing. ADO, was able to induce CD9 expression on HC-derived NKs. Based on the positive correlation between TGFb1 and CD73 expression, observed in TCGA dataset, we found that TGFb1-stimulated PANC1 cells exhibited increased expression of CD73, that was prevented by Galunisertib, a TGFBR1 inhibitor, suggesting a potential regulatory role of TGFb1 on CD73 expression.
Our results suggest the possible role of CD73/ADO axis in promoting the pro-tumor switch of NK cells in PDAC patients, providing the rational for correlating CD73 levels, NK cell subset frequency with clinical parameters to identify high-risk PDAC patients.

Transcriptional and translational modifications of colon adenocarcinoma CT26 ectopic tumor model, by active targeting of vascular endothelial growth factor: beyond angiogenesis modulation.

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Passive targeting of the VEGF-VEGFR2 system has changed the protocols for cancer treatment worldwide. The impact of such therapeutic approaches is known for going beyond modification of angiogenesis by impacting the concentration, senescence, and activation state of systemic and intratumoral immune cells. In this study, we aimed to explore the effect of a VEGF active immunotherapy recently named HEBERSaVax, in the systemic and tumor compartments in BALB/c mice challenged subcutaneously with CT26 syngeneic colon adenocarcinoma cell line.
To this end, mice receive 8 weekly immunizations with HEBERSaVax in Nac-GM3 VSSP (a Very Small Size particle proteoliposome of the outer membrane of Neisseria Meningitides that incorporates the ganglioside N-Acetil-GM3). Between doses four and five, mice were challenged with a lethal dose of 20000 CT26 cells subcutaneously. A week after the last immunization, mice were euthanized, and the tumor and the systemic compartment, including blood, spleen, draining, and contralateral lymph nodes, were analyzed by flow cytometry, IFN-γ ELISPOT, multiplex immunofluorescence-based cytokine and growth factors analysis, and RT-QPCR assays.
HEBERSaVAx in NAc-GM3 VSSP increased systemic VEGF-specific immunity as expected, enhancing the tumor site's humoral and cellular response. Beyond VEGF reduction in mice sera, the treatment also impacts the transcription and translation of other key molecules related to the angiogenic process, the immune response, and the epithelial-mesenchymal transition program (EMT). IFN-γ, IL-2, and GRZ-B were also induced in the tumor microenvironment. The active immunotherapy effectively increased the number of activated non-senescent CD4+ and CD8+ in the tumor and the draining lymph nodes. Such effects were related to the reduction of tumor sizes.
Altogether these results, indicate that HEBERSaVax treatment effects go beyond modulation of VEGF and can be further translated to intratumoral and systemic effects shifting the tumor microenvironment to a "healthier" phenotype with reduced expression of pro-angiogenic molecules and increments on CD8 effector cytokines.

Unravelling the Role of Proline Dehydrogenase (PRODH) in Lung Adenocarcinoma: Implications for Cellular Senescence and the Tumor Microenvironment

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Proline dehydrogenase (PRODH) is a mitochondrial flavoenzyme that catalyzes proline oxidation. The electrons generated from this reaction can be used to produce ATP or ROS, potentially affecting several cellular processes, such as survival, apoptosis, senescence, and inflammation. PRODH is frequently expressed in early-stage lung adenocarcinoma (LUAD), but little is known about its correlation with lung cancer development. Thus, we aim to investigate the effects of PRODH expression in LUAD cell lines.
To investigate how PRODH affects the growth and proliferation of lung cancer cells, we stably transfected the NCI-H1299 LUAD cell line with expression constructs encoding wild-type PRODH or a variant with reduced catalytic activity (p.L441P), as well as the empty vector (control). We compared the survival and proliferation ability of the three types of clones by clonogenic and MTT assays. Furthermore, in wild-type PRODH-expressing clones and controls, ROS production was assayed by 2′,7′-dichlorofluorescin diacetate, and cellular senescence investigated by the senescence-associated-ß-galactosidase assay, analysis of the cell cycle inhibitor p21 and of senescence-associated secretory phenotype (SASP) performed by qPCR and secretome analysis.
Wild-type and p.L441P PRODH-expressing clones had reduced cell viability and impaired proliferation, compared to control clones and the p.L441P variant displayed an intermediate phenotype. PRODH-expressing clones produced higher levels of ROS and had more senescent cells, compared to controls. Moreover, PRODH expression led to higher levels of p21 and cytokines related to the SASP, such as MCP-1, IL-8, and TNF-α. Secretome analysis also revealed that PRODH expression increased the levels of soluble factors involved in monocyte recruitment (MCP-1, MCP-2, MCP-3), monocyte-to-macrophage differentiation (GM-CSF), cytokines involved in M2-like macrophage polarization (IL-10, IL-4, and IL-13) and angiogenesis (IL-8, FGF6, FGF7, and PIGF). These findings suggest that PRODH may influence the tumor microenvironment (TME).
Our study shows that ROS production affects the induction of cellular senescence and LUAD cell proliferation. PRODH may also shape the TME towards an immune-regulatory phenotype. Based on these data, we speculate that, by inducing cellular senescence and cytokine production, PRODH may play a role in the development of lung cancer, in modulating the TME composition, and cancer cells' response to therapy.

Exploring pharmacological vulnerabilities of 9p21-deleted bladder cancer cells

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Loss of the chromosome 9p21 locus is identified in 23% of the TCGA muscle-invasive bladder cancer cohort, making it the most frequent copy

number alteration identified in bladder cancer. This locus encodes for the tumor suppressors CDKN2A/2B and the metabolic gene MTAP, involved in the salvage pathway of methionine and adenine. PRMT5 and MAT2A have been identified as synthetic lethal vulnerabilities of MTAP-deleted cells, stimulating the development of specific inhibitors currently tested in clinical trials. In this context, we explored selective pharmacological vulnerabilities of bladder cancer cells with 9p21 loss. We generated 9p21 locus isogenic clones (WT and CDKN2A/2B/MTAP-null, or 3KO) from 3 bladder cancer cell lines with different genomic backgrounds (HT1197, T24, and TCCSUP) and performed a multi-parametric drug screening using the HT1197 isogenic pair. 2,351 compounds from the Anticancer compound (Selleck) and the MicroSource Spectrum collections were tested.
Cell competition assays demonstrated higher proliferation rates of HT1197 and TCCSUP 3KO clones compared to WT clones, while no difference was identified in T24 clones. We assessed symmetric dimethylarginine (SDMA) marks and found lower levels in 3KO cells with respect to WT and confirmed enhanced dependency on PRMT5 and MAT2A in viability assays. Our multi-parametric drug screening nominated 18 drugs as selectively effective in 3KO cells. Dose-response viability assays performed at different time points and with several WT and 3KO clones confirmed a specific sensitivity of 3KO cells to the antifolates methotrexate and raltitrexed. In order to investigate potential drug interactions, we combined these agents with the MAT2A inhibitor AG270. We also tested two agents, the CDK4/6 inhibitor abemaciclib and an inhibitor of the ATR/CHK1 pathway VX970, reported to be effective in CDKN2A-null cells. Several drug combinations showed synergist or additive effects. Mechanistically, drug combinations preferentially induce replication stress, DNA damage and apoptosis in 9p21-deleted bladder cancer cells.
Our data suggest potential treatments for bladder cancer patients with 9p21 loss. Finally, these therapeutic strategies could be tested in other tumor types with frequent MTAP deletion and in urgent need of new therapies, such as pancreatic cancer and mesothelioma.

Hypoxia-dependent BCR/Abl loss in chronic myeloid leukemia cells is driven by extracellular vesicles release

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Chronic Myeloid Leukemia (CML) is a myeloproliferative disease driven by a singular oncogene, BCR/Abl, which encodes a constitutively active tyrosine kinase. Despite the efficiency of the tyrosine kinase inhibitors (TKi), such therapy does not cure CML. Indeed, the persistence of a subpopulation of TKi-resistant leukemia stem cells (LSCs) sustains the so-called minimal residual disease. The LSCs, along with normal hematopoietic stem cells, are believed to persist in bone marrow stem cell niches, which are sites characterized by severe reduction of oxygen and nutrients, and are likely genomically BCR/Abl-positive but oncoprotein-negative.
CML cells were subjected to severe hypoxia (0.1% O2) for 96 hours in order to downregulate the oncoprotein BCR/Abl expression. Extracellular Vesicles (EVs) were isolated from conditioned media via the ultracentrifugation method and quantified through the Nanosight NS300, which also allowed us to analyze the size distribution of the particles. The isolated EVs morphology was visualized throughout the Transmission Electron Microscope (TEM) and their cargo was analyzed via Western Blot and droplet digital PCR, while the biological effect was evaluated by viability assay and quantitative PCR. The inhibition of EVs biogenesis and secretion was achieved by treating CML cells with Sulfisoxazole.
During the incubation in low oxygen conditions, mimicking in this way the stem cell niche microenvironment, we observed increased secretion of EVs compared to normoxia. Moreover, the EVs isolated in hypoxic conditions resulted loaded with high levels of BCR/Abl mRNA and were capable of transferring their cargo to BCR/Abl-negative cells. Upon uptake of hypoxia-induced EVs, these cells demonstrated to become sensitive to TKi, phosphorylate CrkL, and increase proliferation rate. By inhibiting EVs biogenesis and secretion with Sulfisoxazole, an endothelin receptor antagonist, we were able to maintain high levels of BCR/Abl oncoprotein in CML cells subjected to hypoxia, resulting thereby susceptible to TKi.
EVs are typically secreted by all cells but their biogenesis is commonly enhanced by stress signals such as metabolic limitations. This mechanism was exploited by LSCs to rapidly “get rid” of BCR/Abl expression facilitating entry into a quiescent status. Therefore, the inhibition of EVs biogenesis and secretion prevented BCR/Abl loss and thus the re-sensitization to TKi.

Characterization of circulating and tumor-infiltrating NK cells in patients with renal cancer

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Renal cell carcinoma (RCC) comprises a heterogeneous group of cancers derived from renal tubular epithelial cells and accounts for ~2% of all cancer diagnoses and cancer deaths worldwide, with incidence rates generally higher in developed countries. Inflammation, tumor angiogenesis and altered immune cell phenotypes/functions within the local tissue (micro) and circulating/peripheral (macro) environment, are crucial mediators for insurgence and metastatic tumor progression for several cancer types, including renal cancer.
Natural killer (NK) cells are effector lymphocytes of the innate immune system involved in tumor surveillance and elimination. Decrease in NK cell anti-tumor activities, together with acquisition of pro-tumor/pro angiogenic functions has been observed in diverse cancer types, defining a new NK cell subset, namely decidual-like NK cells.
In our prospective study, we aim at functional and phenotype characterizing circulating and tumor infiltrating NK cell in patients with renal cell cancer, compared to healthy controls, by multicolour flow cytometry. Using a drug repurposing approach, we tested the capability of Pimozide, an anti-psychotic agent able in targeting STAT3 activation, to potentially re-educate circulating NK cells from patients with RCC, in term of degranulation capabilities and production of IFNg.
We observed an enrichment of CD9+, CD49+, CD16- decidual-like NK cell subset in infiltrating NKs from RCC patients, compared to those from circulating NK cells of RCC patients and healthy controls. Circulating NK cells from RCC patients showed increased activation of STAT3, compared to those from control subjects. Finally, following 24 hours of treatment with Pimozide (10 mM) ex vivo, circulating NK cells exhibited reduction of STAT3 activation, increased degranulation capabilities against the K562 target cells, together with augmented production of IFNg.
Our results provide the preliminary rational that tissue-infiltrating NK cells in RCC patients can acquire a decidual-like and anergic phenotypes, potentially in a STAT3-dependent manner and that STAT3 signalling chemical inhibition with Pimozide, can be envisaged as a possible strategy to re-educate NK cells in RCC patients.

TERRA expression from specific chromosomes is upregulated in a zebrafish brain tumor model

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Telomeres are heterochromatic structures at the ends of chromosomes, that are essential for genome stability. Transcription of telomeres results in a long non-coding (lncRNA) called TERRA, which is involved in regulating telomere length, telomerase activity, genome stability and heterochromatin formation at telomeres. TERRA levels and mechanisms of telomere maintenance are currently widely studied in both healthy and cancer cells. Zebrafish are a great model to study both but a characterization of telomeres and TERRA in zebrafish is still missing. In this study, we aim not only at describing the sub-telomeric sequences of each chromosome but also at quantifying chromosome-specific TERRA molecules in a zebrafish model of brain cancer compared with control brain.
The characterization of telomeric ends was done on the zebrafish genome assembly fDanRer4.1 (GCA_944039275.1), which was generated by combining both short and long reads. Wild type zebrafish and a transgenic line developing brain tumors were used in this study. TERRA expression was quantified by qPCRs using chromo-

some-specific primers validated by RNA-Seq in this study. TERRA RNA-FISH was carried out on brain sections.
The presence of the telomeric regions in the fDanRer4.1 assembly allowed us to study the sub-telomeric sequences and identify chromosome-specific regions in TERRA transcripts. Levels of TERRA expression vary throughout development and in different tissues. In brain tumors, TERRA expression increases more than 10 times, with TERRA expressed from Chr18 showing a 10-fold increase compared to control brain. Moreover, increased expression of TERRA from single telomeres in the line developing brain tumors is detected before the development of fully blown tumors.
As the zebrafish brain tumors show features of Alternative Lengthening of Telomeres (ALT). characterized by heterogeneous telomere length, our data suggest that TERRA expression is regulated by telomere shortening/lengthening in a telomere-specific manner and that its upregulation starts prior to the activation of ALT, thus representing the earliest marker of changes in telomere maintenance mechanisms in cancer.

Unveiling interactions between senescent tumor cells and the host immune system - Implications for Senolytic Immunotherapy

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Therapy-induced senescence (TIS) stops tumor growth and improves treatment outcome in many preclinical cancer models but it also alters the tumor biology and remodels the tumor environment. In this way TIS contributes to treatment resistance and tumor relapse. Several publications have described the close and intricate relationship between senescent tumors and the host immune system, but the molecular mechanism of their interaction remain unclear and its impact on treatment efficacy contingent upon cancer type. Consequently, understanding the dynamics between senescent tumor cells and the host immune system emerges as a priority target to improve cancer therapy, for example with new senolytic immunotherapies.
We employed RNA and proteome analyses as well as immunophenotyping by flow cytometry in different tumor mouse models and human tumor cell lines before and after DNA damaging therapy to describe TIS-associated changes in tumor biology. Subsequently, we characterized the interaction of TIS tumor cells and different cells of the host immune system (e.g. macrophages, T cells, Nk cells) in vivo with both immune mass cytometry (IMC) and fluorescence imaging microscopy. Furthermore, we employed genetic and pharmaceutical tools to modulate the interaction of T cells with TIS tumor cells and alter the cytotoxic efficacy of T cells in senolytic therapies.
Upon TIS, various mouse and human cancer cell lines exhibited higher expression of immunologic gene sets and surface markers associated with immune system activation. Investigation of in vivo tumor-host interactions by spatial imaging revealed that TIS promotes the infiltration of both CD4 and CD8 T cells into senescent tumor sites and their direct interaction with TIS cells. Immunophenotyping analyses of these T cells identified overexpression of Fas ligand as an actionable moiety that induces apoptosis in Fas receptor positive TIS cells. Subsequently, we employed genetic and pharmacologic tools, which manipulate the binding of FasL to FasR, to show the crucial impact of this interaction for senolytic therapies and treatment outcome.
This study elucidates the impact of TIS on tumor cell immunogenicity demonstrating enhanced immune system activation and increased susceptibility to T-cell mediated apoptosis by direct interaction of both CD4 and CD8 T cells via the FasL-FasR pathway. These findings identify new actionable moieties to improve the efficacy of senescence-based immunotherapies in different cancers.

Interpreting single-cell messages in normal and aberrant hematopoiesis with the Cell Marker Accordion

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Single-cell technologies offer a unique opportunity to explore cellular heterogeneity in hematopoiesis, reveal malignant hematopoietic cells with clinically significant features and measure gene signatures linked to pathological pathways. However, reliable identification of cell types is a crucial bottleneck in single-cell analysis. Available databases contain dissimilar nomenclature and non-concurrent marker sets, leading to inconsistent annotations and poor interpretability. Furthermore, current tools focus mostly on physiological cell types, lacking extensive applicability in disease.
We developed the Cell Marker Accordion, a user-friendly platform for the automatic annotation and biological interpretation of single-cell populations based on consistency weighted markers. We validated our approach on peripheral blood and bone marrow single-cell datasets, using surface markers and expert-based annotation as the ground truth. In all cases, we significantly improved the accuracy in identifying cell types with respect to any single source database.
The Cell Marker Accordion can identify disease-critical cells and pathological processes, extracting potential biomarkers in a wide variety of contexts in human and murine single-cell datasets. It characterizes leukemia stem cell subtypes, including therapy-resistant cells in acute myeloid leukemia patients; it identifies malignant plasma cells in multiple myeloma samples; it dissects cell type alterations in splicing factor-mutant cells from myelodysplastic syndrome patients; it discovers activation of innate immunity pathways in bone marrow from mice treated with METTL3 inhibitors.
The breadth of these applications elevates the Cell Marker Accordion as a flexible, faithful and standardized tool to annotate and interpret hematopoietic populations in single-cell datasets focused on the study of hematopoietic development and disease.

lncRNA RPPH1 in Melanoma Derived Extracellular Vesicles fuels inflammation through the RLR pathway

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Tumor progression need active communication between cells and the tumor microenvironment (TME). Extracellular vesicles (EVs) as carriers of biomolecules are recognised to be key players in these interactions. In cancer, EVs can influence tumor growth as well as the metastatic process. Recent studies suggested that RNA cargos of tumor EVs can trigger a pro-metastatic inflammation in the TME, by inducing an interferon-mediated inflammatory response mediated by the RNA sensing pathway(s).
RNA sensing pathways are components of the innate immune system that triggers an inflammatory response upon the detection of viral RNAs and pathogenic endogenous RNAs through different receptors. The main pathways are the RIG I like receptor (RLR), the Toll-like receptor (TLR) and the NOD like receptor (NDL) pathways.
In a recent study, we reported a specific accumulation of the lncRNA RPPH1 in zebrafish melanoma derived EVs, an enrichment that we also confirmed in a human cell model of melanoma. The lncRNA RPPH1 is the catalytic component of the ribonucleoprotein complex RNase P, and it's rarely reported to be an EV's cargo.
The injection in healthy zebrafish larvae with melanoma derived EVs or their cargo RPPH1 synthetized in vitro induces sterile inflammation, in a way that suggests the activation of one of the RNA sensing pathways in macrophages.
Treatments with human melanoma cell-derived EVs or synthetic RPPH1 of a human macrophage cell line, are used to investigate if EVs and/or their lncRNA cargo can activate the RNA sensing pathway(s) in macrophages and if it can influence their differentiation.
We designed an in vivo assay to discriminate which pathway is activated by melanoma-derived EVs and/or RPPH1, exploiting the use of specific inhibitors or CRISPR/Cas9 approaches to knock out different players of the RNA sensing pathways in zebrafish larvae.
The expression of inflammatory cytokines and markers for M1/M2 differentiation in macrophages is analysed to evaluate the response to EVs and RPPH1.
In vivo, we assessed the activity of specific key intermediates for each pathway in larvae transfused with melanoma derived EVs and by considering activation of cytokines and Interferon Responsive Genes. Results highlighted the major involvement of the RLRs pathway.
These results may indicate a possible function for melanoma-derived EVs in promoting the formation of metastases by eliciting inflammation through activation of the RLRs in macrophages.

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Rewiring the immune suppressive tumor microenvironment using Antibody-cytokine fusions for the treatment of Pancreatic Ductal Adenocarcinoma

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Despite immunotherapy has shown great potential for the treatment of certain solid tumors, pancreatic ductal adenocarcinoma (PDAC) remains one of the biggest medical challenges. Unfortunately, clinical outcomes achieved with immune checkpoint inhibitors in this context have been disappointing. The presence of a pronounced desmoplasia, accumulation of stroma, and the immunosuppressive environment limit the capacity to evoke a sustained immune anti-cancer response. Hence, there is a need of innovative therapeutic strategies which turn immunological “cold” into “hot” tumors. thereby favoring the antitumor activity of immunotherapy. Antibody-cytokine fusion proteins (ICK) represent an emerging class of biopharmaceuticals, capable of promoting a significant influx of NK and CD8+ T cells into neoplastic masses, thus potentially reverting the barriers of immunological “cold” cancers. Here, we present the pre-clinical results obtained with EDB fibronectin-targeted immunocytokines against orthotopic models of PDAC. EDB is an antigen which is abundantly expressed in the tumor stroma but is virtually undetectable in most healthy tissues.
We used 3D cell-culture murine models KPC06 (low-immunogenic) and KPC12 models (non-immunogenic). The effect of EDB-ICK was evaluated in vitro through immunity-organoid interaction platforms with PBMCs isolated from syngeneic mouse models and 3D models. To test in vivo the efficacy of EDB-ICK, the organoids were injected in the pancreas of C57BL/6 mice. One week later, mice were treated with EDB-ICK, standard chemotherapies administered for two weeks and tumors volume was monitored by ultrasound imaging. Tumor samples collected after the treatments were characterized for tumor infiltrating immune cell components by bulk RNA sequencing and Stereo-seq, an innovative spatial transcriptomics (ST) analysis. The results obtained were confirmed through immunofluorescence analyses.
This innovative targeted therapy could induce long-term complete responses in mice bearing orthotopic cold tumors. Immuno-infiltrate and ST analyses of tumors demonstrated the ability of ICK to dramatically modify the tumor microenvironment when administered alone and in combination with chemotherapy, hence turning “cold” tumors “hot”.
Our results provide a strong rationale to start clinical investigations of EDB-targeted immunocytokines paving the way for new targeted therapies for metastatic PDAC.

The YAP dependent gene NNMT sensitizes castration-resistant prostate cancer cells to NAMPT inhibition

Carreira Ágata

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YAP (Yes-associated protein) is an effector of the Hippo tumor suppressor pathway, whose deregulation results in prostate cancer tumorigenesis. Nicotinamide phosphoribosyltransferase (NAMPT) is a rate-limiting enzyme in nicotinamide adenine dinucleotide (NAD+) biosynthesis pathway which is fundamental for energy metabolism. Increased NAMPT expression is frequently observed in human cancers, unraveling it as a potential target for anti-cancer therapy. We previously showed that YAP ablation in triple-negative breast cancer decreases the sensitivity to the NAMPT inhibitor FK866.
YAP stable silenced cells were obtained by infectitng the prostate cancer cell line PC-3 with lentiviral particules containing pLKO-shYAP. Proteomics was performed on digested samples injected into a Orbitrap Fusion Tribrid mass spectrometer after separation using a reversed-phase C18 column in an Easy-nLC 1200 system. Enrichment analysis of differentially expressed proteins was accomplished using ClusterProfiler function EnrichGO17. 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 cellss to grow in ancorage-independent conditions. Real-Time PCR and Western Blot were performed to determine the expression levels of genes/proteins of interest.
We show that androgen receptor (AR) positive prostate cancer cells (LNCaP) are less sensitive to FK866 than AR null PC-3 cells. Mechanistically, the energy stress induced by FK866 in PC-3, but not in LNCaP cells, promotes YAP dephosphorylation at Ser127, nuclear translocation and activation of YAP-dependent gene expression. Proteomics data reveal increased ER stress-associated gene ontology terms in PC-3 cells exposed to FK866, which is prevented by YAP genetic ablation (PC-3 shYAP). Similarly to LNCaP, the silencing of YAP in PC-3 cells rescues the expression of the ER stress marker CHOP upon FK866 treatment. Interestingly, PC-3 shYAP cells downregulate N-methyl nicotinamide methyltransferase (NNMT), which competes with NAMPT for the substrate nicotinamide. Stable silencing of NNMT in PC-3 cells (PC-3 shNNMT) prevents CHOP expression upon exposure to FK866, which renders them fully resistant to the small molecule.
These findings reveal a cancer cell vulnerability along the YAP-ER stress axis that may improve the clinical treatment options for AR-negative castration resistance prostate cancer treatment.

Immune surveillance is actively impaired by the secretome of mismatch repair proficient colorectal cancers

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Patients affected by colorectal cancer (CRC) with DNA mismatch repair deficiency (MMRd), often respond to immunotherapies based on immune checkpoint inhibitors (ICI), while mismatch repair proficient (MMRp) tumors do not. It is widely accepted that the high mutational burden and the elevated neoantigen levels associated with MMR deficiencies are the main determinants of sensitivity to immune-based therapies. Here, we asked whether factors beyond the neoantigenic landscape could account for the lack of response of MMRp CRC to immune checkpoint blockade.
We developed a model system whereby both MMRd and MMRp tumor cells express equal levels of a given antigen which is recognized by engineered T cells. Using this model, we tested the hypothesis that the lack of T cell-mediate tumor recognition that characterizes MMRp CRC might be due to an intrinsic resistance to T cells attack, and not only to the low mutational burden.
Our results indicate that MMRp CRC cells are capable of actively blocking T cells recognition, activation, and cytotoxic capacity, unveiling a novel mechanism of immune escape.
Furthermore, our data revealed that the tumor secretome plays a key role by influencing tumor-host interaction and immunological recognition dynamics.
Our data provide new transforming knowledge on how tumor cells interact and modulate the immune system of the host, suggesting that the number of mutations is per se not sufficient to overcome the intrinsic resistance of MMRp tumors to immune checkpoint blockade. Combinatorial treatments, designed to elevate neoantigen loads while at the same time neutralizing tumors’ intrinsic resistance should be explored to foster immune surveillance of CRC tumors which are currently not eligible for immunotherapeutic treatment.

Assessment of m6A-readers YTHDF2 inhibitors as Acute Myeloid Leukaemia therapeutics

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YTHDF2 is an RNA-binding protein that recognizes N6-methyladenosine (m6A) residues located in RNA molecules and thus triggers their degradation by recruiting the CCR4-NOT deadeanylase complex. It has been observed that YTHDF2 is upregulated in several subtypes of AML being specifically relevant in the Leukemic Stem Cells (LSCs) compartment: indeed, it has been demonstrated that its stable silencing decreases LSCs proliferation and carcinogenicity. Our main goal is to identify a lead small molecule inhibiting the activity of YTHDF2, thus mimicking the effects of YTHDF2 knock-down.
A virtual high throughput screening (HTS) based on the YTH protein structure was performed. The most promising hits were tested using a secondary screen based on TR-FRET (Time-Resolved Fluoresce Energy Transfer) technology. The recombinant protein (rYTHDF2) was tested against 135 compounds belonging to different chemical classes and supposed to interfer with YTHDF2 binding to target RNA. Hit compounds showed a

residual signal below 30% at 100 μM compared to negative control. REMSAs (RNA ElectroMobility Shift Assays) were used to validate the hit compounds' ability to disrupt the binding between rYTHDF2 and m6A-edit probe.
Both the HTS and further validation experiments led to the selection of a hit molecule m6A71. Treatment with m6A71 reduces both cellular viability and proliferation of the AML cell lines THP1, NOMO and KASUMI, by inducing the activation of the apoptotic process within 24h from administration. Viability assays were performed to assess hit compounds' toxicity, while the activation of the apoptotic process was measured in Incucyte, by incubating treated cells with the CellEvent Caspase-3/7 detection reagent. Finally, mRNA stability assays using actinomycin D were conducted and the treatment with m6a71 sustained increased stability of CREBBP transcript, a well-established YTHDF2 target.
In conclusion, m6A71 is the most promising small molecule we obtained so far, both in terms of potency and activity in leukemic cells. Further medicinal chemistry experiments to improve its pharmacological properties are currently on going, to increase its potency and selectivity to YTHDF2 isoform, and investigate its anti-leukemic properties.

A novel CCDC6/ATF4 signaling cascade controls oxidative stress response, ferroptosis and anticancer immunity in KRAS mutated cancers

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The tumor suppressor CCDC6 is dysregulated in cancer by gene translocations, somatic mutations and increased turnover in a GSK3b/FBXW7 dependent manner. CCDC6 participates in HR DNA repair, and its depletion induces sensitivity to PARP inhibitors. CCDC6 loss is associated with enhanced expression of the cystine antiporter xCT/SLC7A11, a mediator of resistance to ferroptosis, a programmed cell death driven by excessive iron accumulation, lipid peroxidation and plasma membrane damage. Of note, oncogenic ras-mediated transformation requires ferroptosis suppression by inducing xCT upregulation. RAS by activating the ETS1/ATF4 complex, increases xCT transcriptional levels. Importantly, CCDC6 interacts and negatively regulates ATF4 transcriptional activity.
In order to understand if RAS mutants promote CCDC6 degradation to protect cancer cells from oxidative stress by upregulating xCT expression, we investigated if
1. CCDC6 expression levels correlate with KRAS mutational status, in a cohort of human carcinoma surgical samples and in a panel of carcinoma cells analyzed by IHC and wb; 2. KRAS mutations affect CCDC6 protein stability, by using pharmacological inhibition and gene depletion strategies; 3. CCDC6 loss affects the sensitivity of oncogenic KRAS mutant cells to ferroptotic inducers (Erastin, SSZ, RSL3), +/- specific inhibitors (DFO, ferrostatin), by evaluating cells viability, GSH and lipid peroxidation levels; 4. CCDC6, by binding and modulating ATF4 transcriptional activity, affects xCT expression in cells endogenously or ectopically expressing KRAS mutants, by wb, RT-PCR, ChIP and luciferase assays; 5. CCDC6 loss induces a modification of the expression of immunosuppressive molecules in cancer cells and modulates the ability of cancer cells to undergo ferroptosis, by wb and ELISA assay.
We observed:
1. an inverse correlation between KRAS mutations and CCDC6 protein expression levels in human colorectal carcinoma cancers and colon and lung carcinoma cells
2. an increased CCDC6 turnover in cells carrying oncogenic KRAS mutations, in a GSK3b dependent manner
3. a protection from ferroptotic cell death in KRAS-mutated cancer cells, upon CCDC6 loss and consequent increase of the ETS1/ATF4 transcriptional activity on xCT promoter
4. an increase of PDL1 levels in KRAS-mutated cancer cells, upon CCDC6 loss.
In KRAS mutated cancers the CCDC6 increased turnover determines tolerance to oxidative stress and ferroptosis evasion, suggesting novel treatment options.

The TGFßR1 inhibitor Galunisertib re-shapes the PDAC-TME by limiting decidual-like natural killer cells polarization

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Pancreatic ductal adenocarcinoma (PDAC) represents the third leading cause of cancer-related deaths worldwide. Natural Killer cells (NKs), innate immunity effector cells, play a crucial role in immune cancer surveillance. PDAC cells can escape NK elimination, inducing a pro-tumor phenotype, also restraining their cytotoxic abilities.
Here we explored whether the pharmacological modulation of TGFb1/TGFbR1 axis on PDAC cells and Cancer associated Fibroblasts (CAFs) could interfere with the generation of poorly cytotoxic decidual-like NK cells.
In-silico analysis and ELISA assays were used to explore TGFß1 levels in PDAC patients compared with healthy controls (HC), also evaluating their impact on patients' survival. Multicolor flow cytometry was used to assess the phenotype of circulating and tumor-infiltrating NK in PDAC patients, compared to circulating NKs from HC. Conditioned media (CM) from PDAC (BxPC3, MIAPaCa2, PANC-1) cell lines and fibroblasts, treated with GAL, was used to polarize NK cells, and their phenotype and functions were evaluated by multicolor flow cytometry. FC119 murine PDAC cells were orthotopically injected in syngeneic C57BL/6 mice and the effects of GAL treatment on NK polarization and PDAC TME was evaluated.
In silico analysis showed higher levels of TGFß1 in PDAC patients than in HC, and TGFß1high/ TGFßR1high patients showed a worse outcome, in term of OS, compared with TGFß1low/ TGFßR1low patients. The expansion of circulating and tissue-infiltrating CD9+ NKs was observed in PDAC patients, compared to HC. CM of PDAC cell lines and CAFs induced the CD9+ decidual-like NK cells on circulating HC-derived NKs, also dampening their cytotoxic abilities. GAL treatment of PDAC cell lines and CAFs reduce the ability of PDAC cells and CAFs to induce the NK anergic phenotype, decreasing CD9+ NKs and restoring their cytotoxic activities. In line, GAL treatment in-vivo resulted in a decreased frequency of CD9+ NKs, together with the induction of M1 macrophages, and increase in IFNg producing NK and CD8+ T cells.
Our results allowed the identification of decidual-like NKs in PDAC patients and pointed out the relevance of TGFß1/TGFßR1 signaling in promoting the PDAC cell line/fibroblast mediated NK polarization toward the pro-tumor/anergic phenotype. We also provide the rationale to propose GAL as a dual targeting agent, able to both limiting the generation of decidual-like NKs and restoring NK anti-tumor activities in PDAC.

Dissecting the immune microenvironment in a High Grade Serous Ovarian Cancer murine model following Platinum treatment and epigenetic modulation

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High Grade Serous Ovarian Cancer (HGSOC) is still a challenging disease characterized by TP53 mutations in all cases and by defects in DNA repair (dDR) in almost 50% of them. Debulking surgery and platinum-based chemotherapy is the standard of care. The recent introduction of PARP inhibitors (PARPi) as first-line maintenance treatment for patients with dDR has greatly changed patients' prognosis, but eventually resistance occur.
dDRs alter immune response, suggesting that tumor immune microenvironment (TiME) can be exploited in HGSOC therapy. The G9a/GLP his-

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tone methyltransferase, overexpressed in HGSOC, negatively regulate gene expression, promotes DNA damage repair and interferes with the TiME. This study aims to dissect the role of HGSOC TiME in response to PT and PARPi, and the involvement of G9a inhibitors (G9ai) to harness the TiME through combinatorial therapy.
TP53 and PTEN KO ID8 murine ovarian cancer cells were injected i.p. in syngeneic C57BL/6 mice. 4 days after ID8 injection, mice were treated with saline, PT, G9ai and their combo three times/week and sacrificed at 11 or 21 days of treatment. Treated tumor-free mice were used as control. Flow cytometry analysis of ascites, spleen, lymph nodes and bone marrow was performed to assess modulation of myeloid and lymphoid cell populations.
No significant differences were noted in tumor-free mice receiving the different treatments. In tumor-bearing mice, the major changes were observed in ascites, whose volume was reduced in the combo-treated mice. Most alterations in TiME are noticeable at early treatment and are related to the expansion of regulatory T cells (Tregs), mast cells (MCs), and type 1 dendritic cells. Here, Tregs coproduce IL-10 and IL-17. We also found an increase of Ki67+OX40+ CD8+T cells and Granzyme B producing CD49b+ NK, as if these cells are early activated after the combo. No significant changes were observed in the other tissues considered.
Conversely, immune cells are drastically reduced in combo treated mice at late time-point, suggesting that early modifications can shape tumor growth.
In our in-vivo model, the PT-G9ai combo therapy shapes the TiME towards activation. The possible skewing of Tregs into a Th17-like phenotype and the concomitant accumulation of MCs, that can counteract Treg suppressive activity fostering Treg-Th17 differentiation, suggest of deeply investigating the MC-Treg-Th17 network in our setting.
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Concurrent inhibition of oncogene driver and pyruvate dehydrogenase kinases improves tumor response and prevent resistance in NSCLC cells

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In oncogene-driven NSCLC, we previously demonstrated that oncogene inhibition by tyrosine kinase inhibitors (TKIs) causes a reversal of Warburg effect associated with increase of OCR and apoptosis and decrease of ECAR and proliferation. Unfortunately, these targeted therapies led to the development of drug-resistant clones and tumor regrowth. Here, we investigated a combined therapeutic strategy based on targeting both the oncogene and glucose metabolism. Due to their involvement as negative regulator of apoptosis, we selectively targeted pyruvate dehydrogenase kinases (PDKs), cancer overexpressed proteins that favour glycolytic phenotype.
H1993, H1975 and A549 NSCLC cells were treated with TKIs (crizotinib, osimertinib or selumetinib, respectively) alone or in combination with an inhibitor of PDKs, dichloroacetate (DCA). Firstly, we characterized our cells with increasing concentration of DCA to establish the optimal working concentration for combined treatment. Afterwards, to evaluate the potentiate effects of drug combinations compared to TKIs alone, we investigated the expression levels of phosphorylated forms of oncogene signaling pathways and glucose metabolism, metabolic phenotype switch (glucose, ATP or OCR/ECAR), modulation of apoptotic and proliferation markers by mitochondrial membrane potential (MMP) and cell cycle analysis, respectively, along with the effects on cell migration by performing wound healing assay.
Cells were treated with increasing concentrations of DCA to test treatment efficacy through the de-phosphorylation levels of its direct effector (PDH), decrease of glucose consumption and increase of oxidative phenotype. A significant decrease of glucose consumption and a significant increase of ATP production in combined treatment compared to TKI-treated and control cells was observed. Moreover, qualitative and quantitative analysis of MMP indicate a significant increase of apoptosis in combined samples compared to TKI-treated and control cells, data confirmed by expression levels of apoptotic markers. Finally, combined treatment also significantly affects cell cycle and reduces cell migration. Our data demonstrate that combined treatment increases apoptotic response, affects cell cycle and also decreases cell migration ability thus affecting tumor response using half dose of TKI in combined samples. The major translational relevance of this study is to exploit this target for innovative and new therapeutic strategies in NSCLC patients.

miR-223-5p/STAT3 axis predicts response to CDK4/6i and endocrine therapy in luminal breast cancer

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Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) are part of the standard therapy for patients with hormone receptor-positive metastatic breast cancer (MBC). Since their discovery, several challenges regarding their clinical benefit have still to be faced, such as the overcoming of acquired resistance and the need for predictive biomarkers of response. Downregulation of miR-223 is an early step in BC and is also involved in the response to the CDK4/6i palbociclib. To dissect how miR-223 may regulate the response to CDK4/6i and evaluate its role as biomarker to predict response, we evaluated miR-223 levels (3p and 5p strand) in primary tumors from MBC patients who were sensitive or resistant to palbociclib. We generated 5p-overexpressing cells and characterized their growth and response to palbociclib. Finally, we used NSG mice to evaluate tumor growth and response to palbociclib of 5p-overexpressing cells, compared to controls.
We observed that 5p levels were significantly higher in primary tumors of MBC patient sensitive to CDK4/6i. In vitro, 5p cells were more sensitive to palbociclib and to the other CDK4/6i, displaying also downregulation of stemness abilities, EMT features and secretion of inflammatory cytokines, in comparison to control cells. Activation of STAT3 pathway, a common mechanism of palbociclib resistance, was reversed in 5p cells, both at basal level and under palbociclib. Combining STAT3 inhibition with palbociclib led to increased sensitivity and reduction of EMT markers. Moreover, 5p cells were also more sensitive to endocrine therapy and again downregulation of STAT3 was mediating this effect. Inhibition of STAT3 under tamoxifen treatment sensitizes control cells. Finally, we investigated tumor onset, growth and response to palbociclib in vivo. While we did not find differences in terms of onset and growth between control and 5p cells, they displayed a strong difference under treatment, with higher sensitivity of the tumor response by 5p cells.
Our project tries to fill the unmet need for predictive biomarkers of CDK4/6i response. The data presented here demonstrate that miR-223-5p regulates both palbociclib and tamoxifen sensitivity, stemness abilities and EMT in luminal breast cancer cells. These miR-223-5p effects were mediated by STAT3 signaling pathway, suggesting the potential use of a therapeutic combination of CDK4/6i with STAT3i for those patients who experience resistance to CDK4/6i, with high STAT3 activation.

Circulating hallmarks of hyperprogression in NSCLC upon 1st line PD-(L)1 inhibitors alone or in combination with chemotherapy

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Hyperprogressive disease (HPD) has been described in ≅14% of NSCLC patients (pts) upon single-agent PD-1/PD-L1 inhibitors (SA-ICI) and has not been reported upon platinum-based chemotherapy (PCT) and ICI combinations. So far, no predictive biomarkers are available for HPD early detection.
In NSCLC pts treated with 1st line single-agent ICI or PCT-ICI, HPD was defined as delta tumor growth rate (TGR) >50% and/or TGR ratio ≥2. Circulating low density neutrophils (LDNs) were assessed by flow cytometry on PMBCs. LDNs were defined as CD66b+CD15+ cells among CD11b+ PBMCs and immature subtypes as CD10- LDNs. The LDNs predictive role was assessed by Youden's test. Single-cell RNA-sequencing was per-

formed on 14 PBMC samples (6 HPD, 8 PR) using Seurat and harmony R packages. Cell types were identified though AUCell R package and manual curation. The differentiation in trajectories within the HPD cell dataset was described with Monocle3.
144 NSCLC pts were included: 75 treated with SA-ICI, 69 with PCT-ICI. In the SA-ICI cohort, HPD occurred in 8 (11%) pts. Immature circulating CD10- LDNs were significantly higher at baseline in HPD [median: 39.3, interquartile range (IQR): 28.7] versus progressive disease [median: 7.4, IQR: 14.9, p<0.01] or partial response (PR) [median: 3.7, IQR: 12.6, p<0.01]. Circulating CD10- LDNs were associated with HPD [odds ratio (OR): 1.17, 95% CI: 1.06; 1.29], with a good prediction capability [cross-validated AUC 0.97 (95%CI: 0.94;1.00)]. A 30.5% cut-off value for CD10- LDNs was identified to discriminate HPD from others. In the PCT-ICI cohort, 14 pts had circulating CD10- LDNs ≥30.5%, being at high risk of HPD. However, no HPD was observed with PCT-ICI and dynamic evaluation in HPD high risk pts showed 52.3% (IQR: 28.4) reduction in CD10- LDNs upon PCT-ICI, suggesting that PCT prevents HPD by reducing immature LDNs.
At sc-RNA-seq level, LDNs from HPD pts had increased expression of IL-1 receptor type-II (IL1R2) versus PR (log2FC=0.7, p<0.01), in line with an emergency granulopoiesis occurring in HPD. Furthermore, higher proportion of senescent T-cells was observed in HPD versus PR (log2FC=0.65, p<0.01), and genes associated with T-cells senescence were significantly modulated along the pseudotime trajectory (p<0.01).
In HPD an opposite maturation drift occurs in circulating LDNs compared to T-lymphocytes. In particular, immature neutrophils and senescent T-cells identify high risk HPD pts, who should be addressed to 1st line PCT-ICI.

CRISPR/Cas9 screens nominate LIG1 as PARPi sensitizer in castration-resistant prostate cancer

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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 castration-resistant prostate cancer (CRPC) 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. Notably, a subset of biomarker-negative patients benefits from PARPi-based treatments (alone or in combination with AR signaling inhibitors). In this context, we aimed to identify and characterize low-frequency DNA repair gene (DRG) alterations that could represent potential novel biomarkers for CRPC therapy.
We performed CRISPR/Cas9 genotoxic screens in PCa cell lines treated with PARPi, using a custom sgRNA library targeting 356 DRG to identify genes associated with treatment sensitivity and validated nominated hits through survival assays in PCa control and knockout cells. To assess the clinical relevance and prioritize candidates, we examined loss-of-function alterations in primary PCa (TCGA) and CRPC (SU2C-PCF) patient samples. Moreover, we checked for apoptosis induction and activation of specific DNA repair pathways. Finally, we tested a pharmacological inhibition-based approach and we used different preclinical tumor models to confirm treatment sensitization.
We identified 19 gene losses associated with sensitivity to PARPi and validated LIG1, EME1, and FAAP24 as synthetic lethal partners of PARP. We further investigated LIG1, which is found mutated in 6% of CRPC patients. We detected induction of replication stress, DNA double-strand breaks, and apoptosis upon simultaneous inactivation of LIG1 and PARP, and observed a reduction in cell proliferation with their combined pharmacological inhibition. Furthermore, we demonstrated that the LIG1 inactivation sensitizes multiple cancer cell lines, including lung, breast, colorectal, and additional prostate cells, and a PCa mouse xenograft model to PARPi treatment.
Through our work, we identified LIG1 as a novel vulnerability associated with PARPi sensitivity in multiple cancer models and we provided initial evidence for a drug combination-based approach. Moreover, our findings suggest the potential of leveraging low-frequency aberrations in DRG, beyond HRR genes.

New strategies for control Programmed Death Ligand-1 endocytosis to improve cancer checkpoint inhibitor therapy

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Anceschi C, Chillà A, Ruzzolini J, Fibbi G, Del Rosso M, Laurenzana A and Margheri F. - Dipartimento di Scienze Biomediche Sperimentali e Cliniche "Mario Serio".
Immune checkpoint inhibitors (ICI), such antibodies against PD-L1 and PD-1, have shown effectiveness against a large number of cancer types. The therapeutic efficacy of PD-1/PD-L1 inhibitors is high in patients with high PD-L1 expression. Recent data showed that targeting extracellular Plasminogen Activator Inhibitor (PAI-1) by its inhibitor tiplaxtinin (TPX) synergizes with anti-PD-L1 checkpoint blockade in a model of murine melanoma, improving the efficacy of melanoma treatment. PAI-1 induced the internalization of surface-expressed PD-L1, resulting in the reduction of surface PD-L1. Binding of PAI-1 to uPA/uPAR complex results in the recruitment of low-density lipoprotein receptor protein 1 (LRP1), which also mediates PD-L1 internalization. Moreover, PD-L1 on exosomes surface can inhibit antitumor immune responses. We propose to inhibit PDL-1 endocytosis by uPAR/LRP1 complex blockade to maintain high-cell-surface levels of PD-L1 and to reduce the expression of exosomal PD-L1.
A375M6 (metastatic melanoma cells) A549 (non-small cell lung cancer cells) were treated with tiplaxtinin to evaluate the modulation of TPX treatment on exosomal PD-L1. uPAR/LRP1 inhibitors were synthetized starting from the binding site of uPAR. 2D and 3D cultures from A375M6 and A549 were treated with TPX and uPAR/LRP1 inhibitors.
Our results evidenced that in 2D and 3D cultures PAI-1 inhibition by TPX and uPAR/LRP1 inhibitors are able to block the PD-L1 internalization and, consequently, to increase PD-L1 membrane levels. Moreover, we demonstrated that exosomes from treated TPX A375M6, A549 show a decrease of exosomal PD-L1 levels, compared to untreated cancer cells.
These finding demonstrated that in 2D and 3D cultures PAI-1 inhibition by TPX and uPAR/LRP-1 complex inhibition result in a significant increase in surface PD-L1 levels. In parallel, our results evidenced that PAI-1 inhibition showed a decrease of exosomal PD-L1 levels, opening the way for new combined therapeutic strategies with anti-PD-1/PD-L1.

Three-dimensional dynamics of mesothelin-targeted CAR.CIK lymphocytes against ovarian cancer peritoneal carcinomatosis

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Intraperitoneal cellular immunotherapy with CAR-redirected lymphocytes is an intriguing approach to target peritoneal carcinomatosis (PC) from ovarian cancer (OC), which is currently evaluated in clinical trials. PC displays a composite structure with floating tumor cells within ascites and solid-like masses invading the peritoneum. Therefore, a comprehensive experimental model is crucial to optimize CAR-cell therapies in such a peculiar environment.
Here, we explored the activity of cytokine-induced killer lymphocytes (CIK), redirected by CAR against mesothelin (MSLN-CAR.CIK), within reductionistic 3D models resembling the structural complexity of both liquid and solid components of PC.
MSLN-CAR.CIK were confirmed capable of intense OC killing in both settings. In a "floating-like" 3D context with floating OC spheroids, both tumor localization and killing by MSLN-CAR.CIK were significantly boosted by fluid flow. In a "solid-like" context, MSLN-CAR.CIK were recruited through the extracellular matrix on embedded tumor aggregates, with variable kinetics depending on the effector-target distance. Furthermore, MSLN-CAR.CIK penetrated the inner levels of OC spheroids exerting effective tumor killing. Our findings provide currently unknown therapeutically relevant information on intraperitoneal approaches with CAR.CIK, supporting further developments and improvements for clinical studies in the context of locoregional cell-therapy approaches for patients with PC from OC.

Il4i1's Metabolic Mastery: A Key Target for Shaping the Tumor Microenvironment

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In the tumor microenvironment, the metabolism of amino acids, particularly tryptophan, is strongly associated with immune tolerance and unfavorable outcomes. One of the enzymes involved in tryptophan degradation, IL4i1, has recently been identified as a crucial element that shapes the tumor microenvironment to be less resilient. IL4i1 sustains a number of tumor hallmarks, including immune suppression and resistance to ferroptosis. However, the mechanism and biology of IL4i1's action are not fully understood.

Single-cell analysis was performed on a poorly immunogenic fibrosarcoma cell line 10 days after tumor cell inoculation. Metabolite analysis, derived from tryptophan degradation, was conducted using mass spectrometry. To investigate IL4i1's role in the myeloid lineage, Il4i1 flox mice were bred with specific cre mice for enzyme deletion in macrophages and dendritic cells. Lastly, bioinformatic RNA-seq data analysis was carried out on a cohort of 231 patients with various sarcoma subtypes.

Through single-cell RNA sequencing analysis, we demonstrated that in the TME of a transplantable fibrosarcoma cell line, IL4i1 is exclusively expressed in regulatory dendritic cells (mregDCs). IL4i1+ mregDCs are characterized by the expression of mature dendritic cell markers such as CCR7 and Zbtb46. Moreover, we found that the microenvironment is enriched with metabolites derived from tryptophan conversion to I3P and 3-IAld by IL4i1. Il4i1-/- mice, when inoculated with the fibrosarcoma cell line, display reduced tumor growth associated with an increased CD8 T cell-mediated response. Interestingly, deletion of IL4i1 in mregDCs using conditional models results in decreased tumor cell growth and diminished IL4i1 product presence in the TME. Additionally, we demonstrated that IL4i1 metabolites may actively contribute to suppressing CD8 T cell response and conferring resistance to ferroptosis in tumor cells. The recent identification of AhR-expressing target cells responding to IL4i1-produced metabolites expands our understanding of how IL4i1 in select myeloid cell subsets shapes a metabolic network with profound effects on the TME and host anti-tumor response. Moreover, identifying IL4i1 as a negative prognostic factor in specific sarcoma subtypes in the TCGA-SARC dataset further highlights its relevance in cancer contexts.

Our research highlights IL4i1 as a promising therapeutic target in the intricate interplay between metabolism and cancer within the TME.

An in-silico model of EGFR early activation and trafficking

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Epidermal Growth Factor Receptor (EGFR) is one of the most studied tyrosine kinase receptors. The correlation between dysfunctional EGFR signaling and cancer motivates academic interest in this receptor. We propose a mathematical model of EGFR dynamics, focusing on ligand binding, phosphorylation, ubiquitination, and trafficking.

The model extends the one introduced in [PMID: 26264748] and comprises two components. The first one, the extended Early Activation Model, describes the receptors binding to EGF, conformational changes of receptors, recruitment of adaptors, phosphorylation of the nine tyrosine sites, and ubiquitination of the receptor. The second component consists of the trafficking laws describing internalization (via three pathways: constitutive, Clathrin Mediated Endocytosis, and Non-Clathirn Endocytosis), recycling, degradation, and synthesis of receptors.

The overall model is a system of Ordinary Differential Equations governing the evolution of the amount of EGF (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 the various configurations, resulting in about 12k variables.

The model parameters were successfully calibrated and validated to reproduce the dose-response curve of EGFR phosphorylation and ubiquitination [PMID: 26264748], the recycling and degradation rates of EGFR [PMID: 18694561], and the endocytotic rate [PMID: 6279628] in different experimental scenarios.

We then used the model to predict the variation of the endocytotic rate in non-physiological conditions. For instance, the model accurately predicted the experimental outcome in the cases of receptor knock-down and over-expression. Our mathematical model proved to be able to mimic experimental data and predict experimental outcomes. This last aspect positions our model as an essential tool to inform experimental design, helping in deciding which experiments may be of scientific interest. Moreover, the model could also be used to gain insight into the role of receptor configuration in its fate and signaling outcomes. To this aim, we are performing a continuous refinement of the model to support the computation of long-term predictions to achieve biological-relevant predictions.

The Glutamine Addiction Of Multiple Myeloma Cells Shapes A Pro-Tumour Bone Marrow Niche

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Multiple Myeloma (MM) is the proliferation of malignant plasma cells in the bone marrow (BM), which is characterized by bone lesions and increased adiposity. MM is both glutamine (Gln)-addicted and Gln-auxotroph, and the BM of patients presents low-Gln/high-glutamate (Glu) levels compared with pre-malignant stages. These metabolic features force Glutamine Synthetase (GS) expression in Mesenchymal Stromal Cells (MSC) and impair their differentiation into osteoblasts (OB), thus favoring bone lesions. However, the metabolic interaction among MM cells, MSC and the oversized adipocyte population remain to be characterized and is investigated here.

Primary human BM MSC from healthy donors and human MM cell lines were grown in RPMI1640 medium supplemented with 4mM Gln and 10% FBS. MSC were incubated in adipogenic or osteogenic medium for 14 days. Gln secretion was measured by mass spectrometry. 3H-Glu was used to determine EAAT3 transporter activity.

In MM cells more than 50% of Glu directly derives from Gln deamidation. However, MM cells secrete substantial amount of Gln-derived Glu in the extracellular space. MSC, but not OB, display active Glu uptake and a sizable expression of the EAAT3 Glu transporter. Consistently, public transcriptional profiles of BM biopsies of healthy donors or MM patients reveal that the expression of EAAT3 is higher in MSC compared to OB. In Gln-free conditions MSC produce and secrete higher amount of Gln than OB. In MSC, Gln secretion is boosted by extracellular Glu supplementation while it is hindered by either GS or EAAT3 inhibitors. In co-cultures, MSC support MM cell viability under Gln shortage, an effect impaired by either the inhibition or the silencing of GS or EAAT3 in MSC. Lastly, in MSC incubated in adipogenic medium, Gln deprivation induces a nutritional stress response, increasing lipogenesis and the expression of the adipocyte markers PPARG, LEP and ADIPOQ.

These data point to the establishment of a multicellular, MM-driven metabolic pro-tumor BM niche in which MM secrete Glu to sustain their own growth thanks to the ability of MSC to recycle MM-secreted Glu to synthetize Gln, thanks to the activity of EAAT3 and GS. Moreover, MSC differentiation is apparently skewed from osteogenesis to adipogenesis. However, several steps of these deranged pathways are sensitive to pharmacological inhibition and may constitute novel therapeutic approaches to counteract MM.

Role of NSCLC-derived exosomes in inflammation-driven cancer progression and therapeutic resistance

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Exosomes are small lipid vesicles that originate from different cell types and released in the extracellular space as molecular carriers mediating cell-to-cell communication. Tumor derived exosomes (TEXs) may have a relevant role in cancer progression through cross-talk with tumor microenvironment (TME) and the immune system. Our aim was to demonstrate that exosomes derived from sensitive and resistant non-small cell lung cancer (NSCLC) cells may have a pro-metastatic function by carrying proteins related to cancer invasiveness and induce immune suppression by modulation of immune cells pathways. We also planned to assess if these effects are amplified through inflammatory mediators and in resistant cells.
TEXs were isolated from NSCLC H1975, PC9 and PC9 osimertinib Resistant cells (PC9 OR) using differential ultracentrifugation technique and the pro-inflammatory cytokine IL-1β was used for treatment. Western blotting and FACS analysis were performed to detect exosome markers HSP70, CD9 and CD81 as well as the expression of fibronectin and the immune-checkpoint PDL-1. Furthermore, to detect the effect on immune system modulation, we incubated the exosomes with peripheral blood mononuclear cells (PBMCs) derived from NSCLC cancer patients
TEXs showed a mean size of 100-130 nm, were positive for exosomes markers HSP70, CD9 and CD81 and were able to promote cell migration. High levels of fibronectin and PDL-1 were also detected in exosomes and IL-1β treatment contributed to increase such protein levels. However, the highest expression of PDL-1 and fibronectin was found in TEXs derived from PC9 OR cells. Accordingly, we found that exosomes released from PC9 OR cells were able to increase the mRNA expression of PD1, CTLA-4 and FOXP3 in PBMCs, along with an increase of IL-2, INF-γ, TGF-βR and TNF-α mRNA levels. Similar results were also obtained with TEXs derived from IL-1β stimulated cells. In addition, FACS analysis showed that both exosomes isolated from IL-1β treated cells and PC9 OR cells caused a reduction of the CD3+ T cell population in PBMCs.
Our findings indicated that TEXs from resistant cells are able to carry proteins into the extracellular space that may promote metastatic niches formation and induce stronger immune suppression compared to TEXs from sensitive cells. We also demonstrated that IL-1β stimulation may induce release of exosomes with similar properties to those isolated from OR cells.

Effect of anti-inflammatory molecules from food on organoids derived from adenomatous polyps of FAP subjects

Illescas Oscar

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Familial Adenomatous Polyposis (FAP) is a hereditary autosomal-dominant condition caused by germline inactivating mutations in the Adenomatous Polyposis Coli gene (APC). Individuals with FAP develop multiple adenomatous polyps in the colon that, if not treated, progress to cancer (CRC). There are no preventive recommendations for subjects with FAP.
In a non-randomized pilot study on subjects with FAP we demonstrated that a 3-month dietary intervention based on principles and recipes of the Mediterranean diet is effective in improving markers of intestinal and systemic inflammation, and in regulating the expression of miRNAs and genes with inflammatory, oncogenic or tumour-suppressor activities (PMID: 34253565, PMID: 38422953). Bioactive molecules found in food may mediate these effects. Based on food frequency diaries, we found that the dietary intervention increased consumption of the anti-inflammatory molecules Quercetin (QER), Epigallocatechin gallate (EGG) and Fisetin (FIS). To assess whether these molecules may help prevent CRC development by interacting directly with tumour cells, we generated organoids (PDOs) from polyps of FAP patients and tested whether QER, EGG and FIS had an effect on their growth.
Three PDO lines with different APC mutations were developed from polyps obtained from FAP patients after prophylactic colectomy. Cells were isolated from the tissue and grown in culture media containing combinations of growth factors to mimic different niche factors conditions and determine optimal cell culture media composition. PDOs were incubated with different concentrations of QRN, EGG and FIS, and cell viability was determined after 72h.
Three PDO lines with different APC mutations were developed (FAP16, FAP21 and FAP25). All three molecules had a cytotoxic effect on the three PDO lines, which contrasts with their reported low toxicity on normal cells. FIS was the most active, decreasing PDOs viability by 49.9%, 24.8% and 74.0% in FAP16, 21 and 25 respectively, at the lowest concentration tested (20uM).
Considering the cytotoxic activity of QRN, EGG and FIS on PDOs, the dietary intervention has great potential in reducing CRC risk. Moreover, the diet can be safely modified to include larger amounts of these molecules. This highlights how the host can beneficially manipulate the local environment through diet.

Impact of Desmocollin-3 on EMT-Derived Melanomagenesis: Implications for Progression and Patient Outcome

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Malignant melanoma (MM) represents a formidable clinical challenge, necessitating advancements in both diagnostic markers and therapeutic targets. Desmocollin-3 (DSC3), a member of the desmosomal cadherin family, has emerged as a potential player in epithelial-mesenchymal transition (EMT)-driven tumorigenesis. This study aims to elucidate the functional and clinical significance of DSC3 as a diagnostic marker and therapeutic target in MM. Through comprehensive analyses of DSC3 expression in melanoma cell lines and patient samples, coupled with transgenic models for genetic screening and whole-exome sequencing, we seek to uncover the role of DSC3 in melanomagenesis.
We analyzed DSC3 expression in melanoma cell lines and a cohort of 40 melanoma patients to discern its involvement in melanomagenesis. Transgenic models were employed for genome-wide genetic screening to investigate the functional and clinical implications of DSC3 in human melanoma samples. Additionally, whole-exome sequencing was conducted in advanced melanoma cell lines before and after DSC3 knockdown to examine the mutational landscape of DSC3 during melanogenesis. In silico mutation analysis was performed to identify pathogenic mutations affecting DSC3 function.
Our gene and protein analyses consistently revealed DSC3 downregulation in melanoma, particularly in the metastatic stage, with significant implications for disease severity and clinical outcomes. Lower DSC3 levels correlated with poorer overall survival and were associated with key metastatic biomarkers such as NRAS and BRAF. In vitro studies demonstrated that DSC3 downregulation promoted tumor cell scattering, invasion, and migration, thereby enhancing EMT-driven tumor progression and metastasis. Whole-exome sequencing unveiled novel somatic loss-of-function DSC3 mutations, suggesting potential genetic drivers of melanoma pathogenesis, particularly in the TGF-beta pathway.
This study underscores the diagnostic and therapeutic potential of DSC3 in advanced melanoma. Targeting DSC3 mutations holds promise for transcriptional-based therapies, providing new avenues for precision medicine approaches in melanoma treatment. By elucidating the role of DSC3 in melanomagenesis, this research contributes to our understanding of the molecular mechanisms driving melanoma progression and offers insights into novel therapeutic strategies for combating this deadly disease.

Unveiling Nrf2 as Therapeutic Target in the Tumor Microenvironment of Non-Small Cell Lung Cancer

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Lung cancer is one of the most frequently diagnosed malignancies among the population, with non-small cell lung cancer (NSCLC) being its predominant histological type. Clinical and preclinical observations suggest that the progression and aggressiveness of non-small cell lung cancer (NSCLC) depend, at least in part, on the intricate interactions within the tumor microenvironment (TME). Cancer-associated fibroblasts (CAFs) are crucial TME components promoting a fibrotic milieu that shields cancer cells and impedes therapy response. Pirfenidone (PFD) is an antifibrotic drug targeting TGFß signaling, known to inhibit CAFs differentiation and the deposition of extracellular matrix (ECM) proteins. This in vitro study investigates the impact of PFD-altered TME on NSCLC survival markers, including EMT, stemness, redox state, proliferation, and viability, unveiling potential therapeutic targets in tumor-stroma crosstalk
CAFs, isolated from human NSCLC patients, were exposed to PFD (1.5 mg/ml). Subsequently three NSCLC cell lines, A549, H1975 and H1299 were treated with CAFs-conditioned medium for 24 hours, and analyzed using real-time PCR, western blot, immunofluorescence and flow cytometry
i) Cell viability assay showed a decrease in proliferation in response to PFD-CAF-CM compared to CAF-CM and correlates with the modulation of Cyclin D1 and p21, involved in cell cycle regulation; ii) NSCLC cells exposed to PFD-CAF-CM showed a downregulation of Nrf2 transcription factor expression and an increase in the production of reactive oxygen species; iii) Nrf2 showed different subcellular localization in response to CAF-CM; iv) NSCLC cells exposed to PFD-CAF-CM showed a reduced clonogenic ability which was dependent on Nrf2, as confirmed by the use of specific inhibitor
Nrf2 plays a pivotal role in lung cancer progression, with constitutive activation promoting pro-survival genes and cell proliferation. We suggest that PFD exerts a complementary unpredicted anticancer effect targeting Nrf2 pathway perturbating cancer cells-CAFs crosstalk. Thus, Nrf2 will be used as a target for anticancer treatment based on RNA technology in accordance with $NextgenerationUE PNRR 2022 - CN 3 - National Center for Gene Therapy and Drugs based on RNA Technology - Spoke 2 - PhD program: The cross-talk between stroma and cancer cells in the tumor microenvironment as a target in therapies customized RNA

MARK2/MARK3 kinases are catalytic co-dependencies of YAP/TAZ in human cancer

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Human cancer is often caused by dysfunctional developmental pathways, but such mechanisms do not always present clear opportunities for therapeutic intervention. This is exemplified by the Hippo tumor suppressor pathway, which is composed of a kinase module that restrains the transcriptional coactivators YAP/TAZ; a pathway that becomes dysregulated in a wide array of human cancers. Hence, YAP/TAZ hyperactivation is a tumorigenic mechanism and a potential therapeutic target in oncology.
We developed a dual-guide CRISPR screen strategy, that allows for the simultaneous targeting of pre-defined gene pairs. Using basic local alignment, we paired paralogs within functional domain classes and generated combinatoric sgRNA libraries targeting signaling, epigenetic and transcriptional regulators. A total of 22 cancer cell models were screened to robustly identify redundant regulators.
In this study, we used our paralog co-targeting genetic screening strategy to identify the kinases MARK2/MARK3 as co-dependencies of YAP/TAZ across diverse cancer contexts. We use biochemical and epistasis experiments to show that MARK2/3 phosphorylate and inhibit the activity of the Hippo scaffolding component NF2, which leads to indirect upstream control over LATS1/2 activity. In addition, MARK2/3 directly phosphorylate YAP/TAZ to shield these coactivators from LATS1/2-mediated inhibition. The net consequence of this multi-level regulation is that YAP/TAZ-dependent human cancers have an absolute requirement for MARK2/3 catalytic activity to sustain tumor cell proliferation and viability. To simulate therapeutic targeting of MARK2/3 in human organoid models and in vivo, we adapted the EPIYA-repeat region of the CagA protein from H. pylori as a catalytic inhibitor of MARK2/3, which we show exerts anti-tumor activity through modulation of the Hippo pathway. Together, these findings reveal MARK2/3 as a catalytic requirement for YAP/TAZ function in human cancer; targets that may allow for pharmacology that restores Hippo pathway-mediated tumor suppression.

Local antibiotics break microbiota-mediated immune suppression in lung cancer, boosting T memory stem cell response to PD-1 blockade

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Gut microbiota controls the response to immune checkpoint inhibitors (ICIs) and intestinal dysbiosis impairs the immunotherapy effectiveness. Lungs harbor a resident microbiota, which plays a role in regulating pulmonary immune tolerance. Lung microbiota is modified by the presence of tumor, but the effect of tumor-associated microbiota on ICIs blockade efficacy remains largely unexplored. We previously reported that lung microbiota manipulation by antibiotics/probiotics aerosolization reverts immunosuppression and prevents melanoma and breast carcinoma lung metastases. Here, we evaluate if perturbing the tumor-associated microbiota by aerosolized antibiotics can improve ICIs effectiveness in mice bearing experimental lung metastases.
C57BL/6 mice were intravenously (i.v.) injected with LLC1 lung or MC38 colon cancer cells. Mice were aerosolized with vancomycin (50 mg)/neomycin (100 mg) (5 days/week) and/or intraperitoneally administered with anti PD-1 antibody (200 µg/mouse twice a week), starting 7 days after tumor injection. The antitumor immune response was evaluated by measuring cytotoxic activity of lung immune infiltrate and analyzing the expansion/activation of immune cells in the lungs and spleen by flow cytometry and single cell RNA (sc-RNA) sequencing.
Antibiotics aerosol significantly reduced the number of lung tumoral foci in both cancer models. This reduction was associated with an increased cytotoxicity of lung infiltrating immune effector cells and a decrease of Tregs. Myeloid-derived immune subsets were variably modulated in each oncotype. Combination of antibiotics aerosol with the anti PD-1 antibody improved the anti-tumor efficacy of the ICI in mice injected i.v. with LLC1 (p= 0,0255) or MC38 (p= 0,0002) cancer cells compared with monotherapy alone. In LLC1 tumor-bearing mice, both Sc-RNA sequencing () and FACS analyses revealed the reduction of Tregs and the expansion of stem cell memory (SCM) T, iNKT and γδ T cells only in lung tumor tissues from mice treated with antibiotics aerosol plus anti PD-1 antibody. Expansion of SCM T and iNKT cells was also observed in MC38 model and detectable in spleen in both tumor models.
These results reveal that the modulation of lung microbiota by aerosolized antibiotic may represent a strategy to reduce immunosuppression and boost immune activation thus resulting in an improved therapeutic response to ICIs.

Co-culture interactions Induces Morphological Changes and Apoptosis in Cholinergic Cells through Regulation of Inflammation-related Proteins while Sustaining Glioblastoma Cells Progression

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While the interactions between glioblastoma (GBM) and neurons can promote tumour progression, these interactions may affect cholinergic neuronal integrity, leading to dementia-like symptoms. These symptoms often occur early in GBM progression, before first diagnosis of GBM. Although clinical and in vivo studies have supported these observations, the fundamental mechanisms of these interactions remain obscure. This study aims to determine the mechanisms underlying the cellular effects of co-culture between cholinergic and GBM cells.
The retinoic acid-induced cholinergic-differentiated SH-SY5Y cells were co-cultured with LN-18 and T98G at serum-supplemented and serum-reduced conditions, and were further subjected to flow cytometry, JESS Western and LC-MS/MS analysis.
Co-culture with GBM cells resulted in significant reduction of soma diameter, neurite length and arborization in cholinergic cells at both serum conditions. Subsequent cytometry analysis revealed significant increases in mitochondrial membrane depolarisation and apoptosis in cholinergic cells following interactions with GBM cells, but only at serum-reduced condition. Interestingly, this was accompanied by upregulation of autophagy markers in the co-cultured GBM cells, without significant changes in cell cycle and Ki67 level, suggesting that autophagy induction during co-culture interactions supported GBM growth. Shotgun proteomic profiling followed by PANTHER analysis of co-cultured cholinergic cells showed upregulation of proteins such as stress-related 60kDa heat shock protein and apoptosis-related peptidyl-prolyl cis-trans isomerase in cholinergic cells co-cultured with both GBM cells. Furthermore, unique proteins that are associated with apoptosis signalling, cytokine-mediated inflammation and cytoskeletal regulation pathways were enriched in the cholinergic cells co-cultured with LN-18 compared to cells co-cultured with T98G, corroborating our previous data. Meanwhile, proteins involved in cell cycle and PI3K pathways were enriched in both co-cultured GBM cells.
Collectively, the present findings suggested that co-culture at serum-reduced condition elicited autophagic responses in GBM, leading to morphological changes and inflammation-mediated apoptosis in cholinergic SH-SY5Y cells. This consequently sustained GBM cell progression through modulation of cell cycle and PI3K pathway-associated proteins. This study can contribute to future identification of early-detection markers for GBM.

Unveiling the role of cDC1 in shaping Immune hubs in Non-Small Cell Lung Cancer

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In non-small cell lung cancer (NSCLC), unlocking T cell responses by checkpoint inhibitors provides benefits to only one-third of patients, driving the need for innovative combinations to boost response rates. Type 1 dendritic cells (cDC1) are key regulators of anti-cancer T cells and recent data suggest their potential in promoting anti-tumoral immunity in lung cancer (López et al. Nat com 2024). Anti-tumoral immunity in tissues occurs within organized structures called “immune hubs”, whose structure and composition are beginning to emerge. The role of cDC1 in the formation and function of immune hubs in lung cancer remains undefined. In this study we set in place the methodology to address the spatial organization and significance of cDC1-centric hubs in lung cancer tissues across tumor stages, employing transcriptional profiling and spatial analysis techniques.
Here, we generated a unique model of experimental NSCLC, KP-XCR1venus/+ (KP: KrasG12D; P53fl/fl;XCR1Venus/+) that allows the precise identification of the cDC1 compartment and its visualization in tissues during oncogenic progression. Tumor development was induced in KP-XCR1venus by Ad-Cre injection and tissues were harvested at early (4 weeks, dysplasia) and late (8 weeks, adenocarcinoma) stages, non-induced mice were used as controls. Healthy and tumor bearing lung tissues were harvested and processed for flow cytometry analysis, tissue immunofluorescence and RNA seq.
RNA seq analysis unveiled tumor stage-specific transcriptional changes in cDC1. Biological processes indicate upregulation of interferon signaling and cross-presentation at early stages, which faded at late stages. Flow cytometry analysis showed an increase in cDC1 numbers at 4 weeks and a decrease at 8 weeks paralleled by upregulation of maturation markers (MHC-I, CD86, CD40) at early stages and a transition to a more regulatory phenotype at late stages (PD-L1/CCR7). Spatial analysis of tumor tissues showed organized clusters of cDC1 in proximity to T cells and B cells (recapitulating immature and mature TLS) at early stages, which were lost in advanced tumors.
Together these data identify two well-separated stages during cancer evolution, corresponding to “functional” and “dysfunctional “cDC1 states. Incipient tumors trigger an increase in the density of immunogenic cDC1 in lung tissues and spatial organization in clusters. At later stages, the quality and quantity of cDC1 decline and spatial organization into hubs is compromiseed.

The tumor microenvironment supports YAP signaling activation in Uveal Melanoma

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Uveal melanoma (UM) is the most frequent primary intraocular malignancy in adults. More than 50% of the patients develop metastasis, which are fatal within one year from the diagnosis. Despite UM shares the same cells of origin with cutaneous melanoma (CM), there has been no comparable improvement in prognosis and treatment of UM in recent years. Indeed, UM patients carry activating mutations in GNAQ, GNA11 and CYSLTR2, associated with secondary mutations in BAP1 and other genes, which are not reported in CM. GNAQ and GNA11 genes codify for G-protein subunits (Gαq and Gα11) which are associated to CYSLTR2. The mechanisms through which these mutations lead to cancer are not fully understood, but recent reports highlighted the link between mutations in the Gαq and Gα11 subunits and the activation of YAP signaling in UM development. However, the mechanisms of YAP signaling activation are still unknown. Our study aims at investigating if the tumor microenvironment (TME), especially through its influence on CYSLTR2 by the primary ligand leukotriene D4 (LTD4), might lead to the activation of YAP signaling in UM.
We generated four zebrafish UM transgenic/mutant lines which express GNA11Q209L in melanocytes under the mitfa promoter, combined with mutations in Bap1, tp53, APC, or overexpressing YAP and we established a xenograft assay and a slice culture method to study the interaction between tumor cells and the TME.
All four zebrafish UM models are characterized by YAP signaling activation, enabling us to investigate it focusing on the microenvironment’s involvement. We found that treatment with LTD4 leads to increased expression of YAP target genes in zebrafish UM, and nuclear translocation of YAP in human UM cells, while its synthesis inhibitor, Zileuton, decreased YAP target genes expression. Moreover, the transplantation of human UM cells treated with LTD4 in zebrafish embryos supported longer cell survival and metastatic behavior in this in vivo assay. To identify the cell population that in the UM microenvironment produces LTD4, we are using single-cell RNA-sequencing.
Further analysis using the developed zebrafish UM models and transplantation assays, will be essential to clarify the role of the microenvironment in influencing YAP signaling activation, particularly the LTD4-CYSLTR2 axis. Our study underscores the importance of YAP in tumor progression and provides a foundation for future investigations into targeted therapies for UM.

The tyrosine phosphatase SHP2 is a novel druggable target for anaplastic thyroid carcinoma

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The tyrosine phosphatase SHP2 is a key transducer of various proliferative cascades, such as the RAS/ERK pathway, in several types of tumors. SHP2 blockade can affect cancer cell vi-

ability and induce a remodulation of tumor microenvironment boosting anticancer immunity. Moreover, SHP2 inhibition has proven effective in reverting resistance to targeted therapies. Anaplastic thyroid carcinoma (ATC) features hyperactivation of the RAS/ERK pathway and is therefore predicted to be sensitive to SHP2 inhibition. Here, we investigated the anticancer effects of SHP2 blockade in ATC models.
Various ATC cell lines, including 8505c and HTH-7 (human), and T683 (mouse), were used. SHP2 blockade was obtained by using siRNA, or competitive/allosteric inhibitors. SHP099 (allosteric) was selected for its superior activity compared to other drugs. Cell viability was followed by MTT assay; epithelial-mesenchymal transition (EMT) by marker expression, Boyden chamber and scratch assay; stemness by marker expression and spheroid formation. Immunogenicity was defined by the increase of damage associated molecular patterns (DAMPs): eIF2α phosphorylation, calreticulin (CRT) membrane translocation, ATP and HMGB1 secretion. Total ROS production was evaluated by FACS analysis. ML171 was used to block NADPH oxidase complex. The activation of dendritic cells was verified by phagocytosis assays and FACS analysis. T683 tumor growth was induced into 129Sv mice.
SHP2 blockade by SHP099 treatment or SHP2 siRNA significantly impaired ATC cell viability, EMT and stemness features. SHP099, compared to vehicle, significantly increased ATC cell immunogenicity, as shown by DAMPs induction and dendritic cell phagocytosis and activation. These effects were dependent on SHP099-induced ROS production. SHP099 also inhibited the growth of mouse T683 ATC into Sv129 syngeneic mice. The analysis of tumor immune infiltrate showed a significant increase in the cytotoxic/regulatory NK and CD8+/CD4+ T lymphocyte ratio, but also of myeloid-derived suppressor cells (MDSC), in SHP099-treated mice vs controls.
Our data suggest that targeting SHP2 activity inhibits various malignant features of ATC cells and triggers an immunogenic cancer cell death able to evoke an antitumor immune response.

New strategies to improve the response of AML cells to treatments by overcoming the protective effect of bone marrow stromal cells

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In the past decades tremendous progress has been achieved in the development and clinical application of molecular targeted therapies for Acute Myeloid Leukemia (AML). However, drug resistance and relapses are still major issues rendering the rate of cure unsatisfying. This is mostly due to clonal selection and the protective effect of the leukemic bone marrow microenvironment. We previously developed a strategy based on a combination of drugs inducing proteotoxic and oxidative stress. We demonstrated that it efficiently leads to death of AML cell lines and primary leukemic stem cells (LSCs) bearing the mutation FLT3-ITD, both in vitro and in vivo. However, bone marrow stromal cells (BMSCs) protect AML cells by reducing the amount of oxidative stress generated by the treatment in a co-culture system where the cells are in direct contact. Our main focus is to investigate the mechanisms contributing to the protective abilities of the BMSCs. Furthermore, aiming to optimize the combination of drugs to increase its translational potential, we are evaluating the efficacy of combining induction of proteotoxic stress with different drugs that are at the cutting edge in clinical trials for AML, among which the BCL-2 inhibitor Venetoclax.
We tested the sensitivity of FLT3-ITD+ AML cell lines and primary LSCs to different treatments in monoculture or in coculture with BMSCs, in 2D or 3D models. The 3D cultures provide more accurate information into AML-stromal cell interactions upon treatment. In parallel, we evaluated the efficacy of the combination RBA plus Venetoclax in an in vivo orthotopic murine model of AML. The combination of proteotoxic stress and Venetoclax is effective against FLT3-ITD+ AML cells in vitro, overcoming the protection provided by BMSCs in a coculture system, without affecting BMSCs viability, and significantly prolongs the life span of a murine model of FLT3-ITD+ AML. Furthermore, our investigations on the crosstalk between AML cells and BMSCs upon different treatments reveal for the first time the involvement of the transcriptional co-regulator YAP.
We showed the efficacy of a new combined therapeutic strategy based on proteotoxic stress and the inhibition of the antiapoptotic protein BCL-2 and we found that YAP signaling is important for the BMSCs to efficiently protect AML cells from various treatments.

mSWI/SNF-altered hepatocytes fate towards HCC or iCCA is influenced by liver microenvironmental stimuli

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Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA) are among the most common and deadliest types of cancer globally. These cancers are associated with low survival rates due to a lack of reliable biomarkers for early diagnosis and effective treatment.
mSWI/SNF chromatin remodelling complexes, also known as cBAF and pBAF, are large multi-protein complexes that regulate nucleosome organisation and chromatin compaction in an ATP-dependent manner. Several mSWI/SNF subunits are frequently mutated in cancer, including HCC and iCCA. Although HCC and iCCA cases display similar genetic alterations, the reasons for developing one tumor type over the other remain unclear. Previous research suggests that the liver microenvironment influences cell fate. Our work explores the effects of mSWI/SNF subunit loss in adult liver at homeostasis and stress conditions to define novel environmental stimuli impacting cell fate.
We generated tissue-specific, tamoxifen-inducible mSWI/SNF chromatin remodelling (cBAF and pBAF) deficient mice. To investigate the role of these complexes in chronic liver disease we challenged mice with modified diets. Collected tissue samples were characterized by histological and imaging analysis and RNA sequencing.
Under homeostatic conditions, cBAF and pBAF-deficient mice do not show phenotypic alterations of liver architecture. On the contrary, under chronic liver damage, knockout mice show impaired liver regeneration, manifesting large necrotic zones throughout the liver both in short and long-term experiments.
To better understand the effect of necrotic areas on the liver microenvironment, we analyzed the composition of the liver non-parenchymal cell population in cBAF and pBAF-deficient mice. Notably, we observed alterations in immune cell composition and fibroblast populations. Our preliminary data suggest that the co-mutation of cBAF and pBAF complexes might favour the formation of iCCA by forming a necrotic microenvironment and altering the immune landscape.

De Novo Lipogenesis Inhibition Potentiates Androgen Receptor (AR) Signaling Inhibition in metastatic castration-resistant prostate cancer (mCRPC)

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PC exhibits altered lipid metabolism, with elevated expression of Fatty Acid Synthese (FASN) enzyme, the rate-limiting step of de novo lipogenesis. FASN leads to the synthesis of saturated and monounsaturated fatty acids, crucial for fueling cancer cell growth.
AR activates sterol response element-binding proteins (SREBPs), transcription factors that regulate lipid synthesis enzymes, including FASN. Preliminary data show that pharmacologic FASN inhibition decreases AR expression and its splice variant AR-V7. We hypothesize that combining FASN inhibition with AR-targeted therapy, specifically Enzalutamide (Enza), could enhance

CRPC antitumor activity. Pre-clinical data led to the design of a Phase I clinical trial to evaluate TVB-2640 combined with Enza as a new treatment approach for mCRPC.
PC cells (22Rv1 and LNCaP-95 with and without lentiviral-mediated overexpression of AR-V7) and MSK-PCa3 organoids were treated with FASN inhibitor (TVB-2640), Enza, or the combination. Cell growth and AR/AR-V7 expression were measured after treatment. LuCap 35 castrate-resistant PDXs were implanted into 12 castrated SCID mice and treated with Enza, TVB-2640, or the combination. Tumor growth was measured after treatment. FASN, AR, and AR-V7 expression was analyzed in metastatic tissue samples from mCRPC pts using immunohistochemistry (IHC).
Combining the FASN inhibitor with Enza significantly inhibited cell growth compared to either drug alone in PC cells and CRPC organoids. The combination downregulated AR-V7 and FASN. The overexpression of AR-V7 in LNCaP-95 cells partially rescued cell growth inhibition. The drug combination also demonstrated a significant reduction in tumor growth compared to either drug alone in LuCap 35 PDX tumors. Multiplexed fluorescent IHC analysis of 55 mCRPC cases showed co-expression of FASN with AR (87%) and AR-V7 (39%) in metastatic PC lesions. A phase I clinical trial has commenced aimed at determining the optimal and safest dose of TVB-2640 combined with Enza in mCRPC pts.
De novo lipid synthesis inhibitors in combination with AR-targeted therapy is a promising new approach to treating mCRPC.

Natural Killer Cells In Prostate Cancer Patients Acquire The Decidual-Like NK Phenotype/Function And Are Re-Polarized Towards Anti-Tumor Effector Cells, Via Stat3 Chemical Inhibition

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Natural killer cells (NKs) are mediators of the innate immunity involved in tumor elimination. NK cells with altered phenotype and reduced anti-tumor functions, have been found in different solid tumors, including prostate cancer (PCa).
We characterized tumor infiltrating (TINKs) and tumor-associated NKs (TANKs) in PCa patients and seek for molecular pathways potentially involved in the acquisition of the pro-angiogenic/decidual-like phenotype.
PCa TINKs/TANKs were characterized by multicolour flow cytometry (FC) for decidual-like surface markers (CD9, CD49a), compared to circulating NKs from patients with benign prostatic hyperplasia (BPH) and healthy controls. STAT3 activation was determined in circulating NKs of PCa patients, by FC. The plasma of PCa patients and control was profiled for the presence of STAT3 activating cytokines. Using a drug-repurposing approach employing the antipsychotic agent Pimozide, we chemically modulated STAT3 activation in circulating NKs from PCa patients and investigated their secretome changes, degranulation capabilities, and angiogenic activity in vitro and in vivo, using the leech H. verbana, as animal model.
PCa TINKs and TANKs acquire the CD9+CD49a+ decidual-like phenotype. Circulating CD-56brightCD9+CD49a+ decidual-like NKs were also present in the peripheral blood of BPH subjects, but in a lower frequency, compared to those from PCa TANKs. Plasma from PCa patients is enriched in IL-4, IL-6, CXCL8/IL-8 and IL-10, all cytokines able to activate the STAT3 signalling pathway and the exposure of cytolytic NKs to the same cytokines increase the percentage of CD9+ NKs. We detected increased phosphorylation of STAT3 in PCa TANKs, compared to NKs from healthy controls, that was reduced following Pimozide stimulation for 24 hours, together with decreased capabilities of PCa TANKs to secrete pro-angiogenic factors (CXCL8, IL-6), molecules involved in monocytes recruitment/M2-like macrophage polarization (CCL-2, CCL5, GM-CSF, IL-10), increased production of anti-tumor cytokines (IFN-g, TNF-a) and NK augmented degranulation activity. Finally, PCa TANK exposure to Pimozide resulted in decreased angiogenesis in vitro and in vivo.
Our results provided evidence that STAT3 inhibition can be envisaged as a potential strategy to reduce the generation of pro-angiogenic/decidual-like NKs, while contributing to NK cell re-education in PCa.

TERRA- Rig-I-like receptor (RLRs) mediated inflammation in a zebrafish glioblastoma model

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Glioblastoma (GBM) is an aggressive brain tumor with rapid growth, infiltrative behavior, and treatment resistance. Despite being rare (15% of primary brain tumors), it is the most common and aggressive in adults, with poor survival rates (typically 12-15 months). Gaps in understanding GBM biogenesis and progression hinder effective therapeutic options. Tumor heterogeneity complicates treatment strategy development, with diverse cell populations, including abundant microglia, suggesting that inflammation plays an important role. The RNA sensing pathway, a crucial component of the innate immune system, employs cytosolic receptors like RIG-I, MDA5, and LGP2 to detect viral and endogenous RNAs. These receptors belong to the RIG-I-like RNA sensing pathway (RLR) and rely on downstream components, like MAVS, to activate interferon-mediated immune responses. Dysregulated non-coding RNAs (ncRNAs), often present in cancer, can impact RNA sensing, and immune responses. Our Zebrafish glioblastoma model employs the Alternative Lengthening of Telomere (ALT) pathway, similar to 15% of human GBMs, and is classified as ALT-positive. ALT tumors exhibit telomeric abnormalities and elevate expression of telomeric repeat-containing RNA (TERRA). In our model downregulation of TERRA with ASOs reduces microglia infiltration, suggesting TERRA's regulatory role in inflammation. Moreover, RNA-seq analysis indicates upregulation of the RLR pathway and inflammatory markers in the model.
We studied TERRA-RLRs interaction in human cancer cell lines using an innovative Proximity Ligation Assay (PLA). To understand the role of RLRs in brain tumor development we knocked out members of the RLR pathway exploiting CRISPR-Cas9.
We found that in cancer cell lines TERRA interacts with MDA5, mainly in the cytoplasm, suggesting a downstream activation of the signal. Downregulation of MAVS or MDA5 in Crispant larvae reduces microglia infiltration in tumors, supporting the idea that this pathway is involved in the regulation of inflammation in glioblastoma. Besides microglia, KO of RLRs also reduced the number of proliferating cells in the brain.
Further analyses of our model are needed to clarify the role of RLRs and infiltrating microglia in brain tumor development and progression. At the same time, the ability of ncRNAs to activate the RLR pathway in cancer deserves further investigation, as it may be the missing link between cancer development and sterile inflammation.

Gut microbiome associations with colorectal cancer in a pooled analysis of 3,512 individuals from 16 cohorts

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The gut microbiome has been reproducibly associated with colorectal cancer (CRC), but additional large-scale investigations are needed considering microbiome variability across primary tumor location, tumor stages, and tumor-related subspecies differences.
In this study, we sequenced five new international CRC cohorts (n=1,466, 4 from the ONCOBIOME Consortium and one as part of the Nurses' Health Study II). These were integrated with 11 available CRC studies (n=2,046), totalling 3,512 metagenomic samples from controls, adenoma, and CRC individuals. Information on staging and primary tumor location was curated for the CRC cases in the dataset. The microbiome composition and gene-carriage was profiled via the bioBakery tool suite, and analyzed with machine learning and meta-analysis models, and strain-level differences were determined via Anpan.
We improved over previous microbiome-based CRC detection accuracy, reaching an average AUC=0.84 in a leave-one-dataset-out setting, highlighting the importance of newly profiled bacterial species such as Solobacterium SGB6833 and distinct clades of Fusobacterium nucleatum. In addition, we found specific gut species that distinguished early versus late CRC, such as Hungatella hathewayi, which was associated to stage III and IV, and Methanobrevibacter smithii to stage IV, hinting at the presence of heterogeneous microbial targets along the adenoma-carcinoma sequence. The microbiome composition of right-sided tumor patients was enriched with oral-typical microbes and was distinguishable from left-sided cancer communities (AUC=0.67). We further identified strain-specific gene carriage and intra-species genetic differentiation in common gut commensals, such as Ruminococcus bicirculans and Faecalibacterium prausnitzii, which displayed late-stage CRC-associated sub-clades.
This combined analysis of over 3,500 metagenomes from 16 international studies confirms that the gut microbiome – with its recently uncovered taxa and strain-level differences – can be a valuable resource not only for CRC screening but also as a potential clinical target for CRC progression.

Single-cell and functional phenotyping analysis of glioblastoma infiltrative margin

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Glioblastoma (GBM) is the most heterogeneous and invasive brain tumor in adults that almost inevitably recurs, despite radical treatments. As the bulk of the tumor is resected during surgery, the recurrent tumor is driven by cells left behind that are resistant to radio-therapy and chemo-therapy. As a result, only a minority of GBM patients survive 5 years after diagnosis. In previous work, we developed a fluorescence-guided multiple sampling (FGMS) scheme allowing an objective identification of tumor areas, including the surrounding brain microenvironment. In particular, we identified and characterized specific anatomical/functional areas, defining the interface between the tumor and the normal brain parenchyma, namely the infiltrative margin (IM), and the sub-ventricular zone of the lateral ventricles. We showed that these areas represent the source of GBM cells that seed the recurrent tumor, thus they may hold the key to identifying valid therapeutic targets.
By using our FGMS scheme, here we performed single-cell/single-nucleus RNA sequencing and functional phenotyping analysis of the IM in 15 GBM patients and analyzed the cellular composition compared to matched tumor mass and histologically normal brain samples. Specifically, we performed bioinformatic analysis to identify copy number variations, transcription factor regulatory networks, cellular dynamics, differentially expressed genes, and tumor-associated macrophage activation signatures, followed by ligand-receptor predictions, spatial transcriptomics, and experimental work to identify IM-specific therapeutic targets.
Cell type/cell state annotation revealed that the IM is a reservoir of heterogeneous tumor cells and that the IM microenvironment is predominantly characterized by microglia and pro-inflammatory cytokines. Specifically, our results show that: (i) tumor cells of the IM classify as GBMnpc, GBMac, and GBMmes, but the GBMopc state is not represented in this area; and (ii) the IM microenvironment is characterized by tumor supportive/M2-like microglia and by an increased expression of IL7 and IL18.
We built a single-cell/single-nucleus RNA sequencing-based microenvironment landscape of the tumor mass and the IM of 15 GBM patients and used histologically normal brain samples as a control. Single-cell interaction network analysis and functional phenotyping assays are ongoing to dissect the cross-talk between tumor and immune cells in the IM and identify novel therapeutic targets.

Enhancing sensitivity of Triple Negative Breast Cancer to DNA Damaging Therapy through chemical inhibition of the m6A methyltransferase METTL3

Piscopo Fabio

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Among the different types of breast cancer, triple-negative breast cancer (TNBC) displays the most unfavourable prognosis and high risk of recurrence. Conventional chemotherapy and DNA damaging agents are the main treatment for this cancer. Since TNBC presents high m6A methyltransferase METTL3 activity, which correlates with invasiveness and metastasis, we proposed to evaluate the impact of m6A depletion through METTL3 chemical inhibition using STM2457 small molecule.
To assess the therapeutic efficacy of STM2457 in triple-negative breast cancer, we employed both in vitro and in vivo models. Proliferation, migration, and clonogenicity assays were conducted on tumoral cell lines as well as non-tumoral controls. Additionally, the effect of STM2457 was evaluated in organoids to assess viability while we considered migration in a zebrafish xenograft model. Mechanisms underlying the ability of STM2457 to inhibit cell survival were investigated through cell culture, qRT-PCR, Western

blotting, and immunofluorescence analyses.
Here, we show that STM2457, a selective METTL3 catalytic inhibitor, strongly affects TNBC cell proliferation and migration in vitro and in vivo. Moreover, STM2457 sensitizes tumor cells to DNA damaging agents utilized in TNBC therapy, such as platinum-salts and the PARP1/2 inhibitor olaparib. Finally, we show that the catalytic inhibition of METTL3 synergizes with DNA-damaging chemotherapy in TNBC patient-derived organoids with wild-type BRCA1 and BRCA2 genes. Taken together, our data suggests that incorporating small-molecule inhibitors of METTL3 into standard treatment for TNBC holds significant promise, opening avenues for innovative combination targeted therapies. This approach has the potential to enhance anti-cancer efficacy and mitigate the risk of toxicities.

A fiber-rich diet perturbs the microbiota-immune axis and thwarts multiple myeloma progression

Policastro Anna

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Smoldering multiple myeloma (SMM) is a potentially curable but mostly untreated, asymptomatic disease with an overall risk of progression to incurable multiple myeloma (MM) at 10% per year. Thus, a preventive treatment is urgently needed for SMM patients. We previously established a direct link between intestinal microbiota, gut born Th17 cells and MM progression in transgenic Vk*MYC mice that develop de novo mMM. Similarly, bone marrow (BM) levels of IL-17 appeared to predict evolution from SMM to MM in humans. By breaking down dietary fibers, gut commensals produce short-chain fatty acids (SCFAs), which exhibit both local and systemic anti-inflammatory activities, ultimately limiting Th17 cell expansion. We hypothesized that a high-fiber diet beneficially impacts the microbiota-immune axis, eventually preventing disease evolution.
Congenic mice challenged with Vk*MYC-derived mMM cells (t-Vk*MYC) and Vk*MYC mice affected by an asymptomatic disease mimicking SMM (mSMM) that, if untreated, invariably progress to full-blown mMM, were administered either high-fiber or standard diets. Mice were monitored for disease progression and overall survival. Fecal 16S ribosomal RNA sequencing and quantification of SCFAs by nuclear magnetic resonance (NMR) spectroscopy were performed to assess the impact of diets on microbiota composition.
t-Vk*MYC mice fed high-fibers exhibited delayed M-spike appearance and prolonged survival. In mSMM mice, high-fibers increased progression-free survival and prevented evolution to mMM in more than 40% of the mice. Dietary fibers also favored the expansion of SCFA-producing bacteria and increased fecal SCFA concentration. Modulation of the gut microbiota associated with limited expansion in the BM of disease-induced Th17 cells and IL-6-producing dendritic cells and a less immunosuppressive tumor microenvironment as suggested by reduced frequency of monocytic-myeloid-derived suppressor cells and exhausted T cells, and increased IFNg+ T cells. Finally, administration of butyrate to t-Vk*MYC mice reduced BM Th17 cells and delayed tumor progression, thus providing a mechanistic link between high-fiber diet and beneficial effects on plasma cell malignancies.
These findings are the first in vivo demonstration of the favorable effects exerted by plant-based diets in limiting aggressiveness of plasma cell dyscrasias, and strongly support the application of dietary intervention to patients affected by SMM.

Deciphering the role of Pin1 in the interplay between mechanical cues and nuclear integrity in breast cancer

Renzi Anna

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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 cytoskeleton/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. 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, which promotes 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 mouse models, Pin1 KO in cancer 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 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.

Fgfr2 is required for Kras-driven pancreatic tumorigenesis

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Fibroblast growth factor/FGF receptor (FGF/FGFR) signaling plays crucial roles in a multitude of processes during embryonic development and adult tissue homeostasis by regulating cellular lineage commitment, differentiation, proliferation, and apoptosis of various types of cells. The FGFR gene family consists of four members, FGFR1 to FGFR4. FGFR proteins are tyrosine kinase receptors with different ligand affinity and cell lineage-specific expression. The downstream pathways include the Ras/Raf-mitogen-activated protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K)/AKT, phospholipase Cγ (PLCγ), and signal transducer and activator of transcription (STAT).
Dysregulation of the FGF/FGFR signaling pathway has been recognized in a variety of human diseases, including cancer. FGFRs are aberrantly activated through mutations, gene fusions and copy number amplifications in 5–10% of all human cancers and efforts to inhibit their functions have led to the development of selective and non-selective FGFR-targeted therapies.
This study aimed at investigating the contribution of FGF/FGFR signaling to pancreatic ductal adenocarcinoma (PDA) development.
We analyzed FGFRs expression in organoid models and specimens from normal, pre-cancerous and cancerous murine and human pancreas tissues. We employed mouse models of KrasG12D-driven pancreatic tumorigenesis, organoids, including new matched organoid models of pre-cancerous and cancerous cells, and cell lines to investigate the role of Fgfr2 in pancreatic metaplasia and neoplasia.
We found that Fgfr2 was progressively up-regulated in pancreatic metaplasia, pre-neoplasia and neoplasia with classical differentiation

and repressed in basal-like neoplasia by TGFß signaling in both mouse and human. Using genetic mouse models, we showed that Fgfr2 was required for malignant transformation driven by mutant Kras alone or in combination with inflammation, but was dispensable for normal pancreas recovery following injury. Furthermore, we revealed that Fgfr2 inactivation early in pancreatic tumorigenesis extended the survival of these mice and affected the histological differentiation of the tumors.
Together, our data have uncovered a novel role for Fgfr2 in promoting pancreatic tumorigenesis, paving the way for future therapeutic applications of Fgfr2 inhibitors for the treatment of PDAs.

Deciphering the immune landscape of early triple negative breast cancer to improve patient risk stratification

Tosi Anna

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Unlike other breast cancer subtypes, there are no approved targeted therapies available for early-stage triple-negative breast cancer (eTNBC), thus still representing an unmet clinical need. Beside the presence of tumor-infiltrating lymphocytes (TILs), also their activation states and spatial location contribute to clinical outcome in TNBC. Indeed, some patients treated with neoadjuvant chemotherapy (NACT) ultimately relapse despite the presence of high TILs. Spatial profiling of tumor microenvironment (TME) can allow the identification of biomarkers of therapy resistance to tailor treatments in advance and maximize responses.
Formalin-fixed paraffin embedded (FFPE) diagnostic biopsies and residual surgical tumors from eTNBC patients treated with NACT with more than 5-year follow-up were collected. A total of 41 samples (24 diagnostic biopsies and 17 residual diseases) were spatially profiled by the GeoMx Digital Spatial Profiler to characterize the whole transcriptome within T lymphocytes, macrophages and tumor cells. The level of each biomarker in biopsies was correlated with pathologic complete response (pCR) at surgery, while in residual surgical samples with the recurrence of disease.
In pre-treatment biopsies from patients achieving the pCR we observed a positive enrichment of several pathways related to immune activation, PD-1 signalling and collagen degradation in CD3+ T lymphocytes, a negative enrichment of NOTCH signalling in CD68+ macrophages, and a positive enrichment of pathways associated to MHC I Antigen Presentation and Interferon Signalling, while a negative enrichment of IL-7, NOTCH, Mitotic and several Metabolic pathways in cancer cells, as compared to patients not achieving the pCR. In residual diseases from patients without relapse we found a positive enrichment of inflammatory signatures in T lymphocytes, a positive enrichment of oxidation and Metabolic pathways while a negative enrichment of TGF-b and NOTCH signalling in macrophages, and, finally, the expression of genes related to the extracellular matrix reorganization and a negative enrichment of MET pathway, IL-10, IL-4 and L-13 signalling in tumor cells, as compared to relapsed patients.
As the spatial interrogation of the TME has emerged as a potent tool for tumor immunoprofiling, this study will provide a significant contribution to the field, offering critical insights into the complex and heterogeneous immune landscape associated with eTNBC response/resistance to therapy.

Past Symposia

34. Trento, 19 - 20 June 2023
NEW TECHNOLOGIES FOR STUDYING AND TREATING CANCER

33. Trento, 13 - 14 June 2022
WHAT ARE THE OBSTACLES TO CANCER IMMUNOTHERAPY SUCCESS?

32. Virtual, 21 - 22 June 2021
AGING AND CANCER

31. Trento, 17 - 18 June 2019
CANCER AS CORRUPTED TISSUE

30. Trento, 25 - 26 June 2018
OVERCOMING THE INNATE RESISTANCE OF CANCER TO THERAPY

29. Trento, 22 - 23 June 2017
BUILDING NEW BRIDGES BETWEEN BASIC AND CANCER SCIENCE

28. Trento, 20 - 21 June 2016
INITIAL STEPS ON THE ROUTE TO TUMORIGENESIS

27. Trento, 18 - 20 June 2015
CHALLENGING ROADBLOCKS TO CANCER CURES

26. Trento, 19 - 21 June 2014
CANCERS DRIVEN BY HORMONES

25. Trento, 20 - 22 June 2013
METABOLISM AND TUMORIGENESIS

24. Trento, 14 - 16 June 2012
MOLECULAR BASIS FOR RESISTANCE TO TARGETED AGENTS

23. Trento, 16 - 18 June 2011
ENGINEERING AND NANOTECHNOLOGY IN CANCER

22. Trento, 10 - 12 June 2010
RNA BIOLOGY AND CANCER

21. Trento 11 - 13 June 2009
UNCONVENTIONAL THERAPEUTIC TARGETS IN CANCER

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

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

18. Trento: 27 - 29 June 2006
TUMOR MICROENVIRONMENT: HETEROTYPIC INTERACTIONS

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

16. Trento, 10 – 12 June 2004
STEM CELLS AND EPIGENESIS IN CANCER

15. Rovereto, 12 – 14 June 2003
MOLECULAR IN VIVO VISUALISATION OF CANCER CELLS

14. Trento, 30 May – 1 June 2002
THE NOVEL DICHOTOMY OF IMMUNE INTERACTIONS WITH TUMORS

13. Rovereto, 31 May – 2 June 2001
FOCUSING ANALYTICAL TOOLS ON COMPLEXITY IN CANCER

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 2024

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-seven top international scientists have been awarded so far and four of them have been subsequently awarded with the Nobel Prize, for the same motivations.

De Lange is Leon Hess Professor, head of the Laboratory of Cell Biology and Genetics, and director of the Anderson Center for Cancer Research at The Rockefeller University in New York.

MOTIVATION

De Lange is being recognized for discovering the molecular mechanisms by which **telomeres protect chromosome ends**, characterizing the function of the **shelterin** protein complex, and demonstrating how loss of telomere protection results in **aberrant genomic integrity** and **tumorigenesis**.

De Lange's research has brilliantly elucidated the role of telomeres—regions of repetitive nucleotide sequences at the ends of chromosomes—in cancer onset and progression, mainly through work that has defined the role of the shelterin protein complex in protecting telomeric DNA by blocking DNA damage and repair activity.

In 1995, through the use of elegant biochemistry approaches, de Lange identified and cloned the first telomeric mammalian protein of the shelterin complex (TRF1), defining its role in the inhibition of telomeric DNA elongation. Later, de Lange identified four additional shelterin complex proteins (**TRF2, TIN2, Rap1, and TPP1**) that, together with TRF1 and POT1, are responsible for telomere protection. Through a series of groundbreaking experiments using murine knockout models, de Lange and her collaborators characterized the fate of telomeres lacking one or more shelterin complex subunits, demonstrating that cells perceive chromosome ends as damaged DNA when shelterin is compromised, as shelterin is able to inhibit six different DNA damage response mechanisms. De Lange's research showed that in the absence of shelterin, there is aberrant double-stranded DNA damage repair, resulting in the induction of cell death and/or cellular senescence via the ATM and ATR kinase signaling pathway.

In addition, de Lange and her collaborators made a major molecular biology discovery with the identification of the **t-loop structure of telomeres**, whereby a single-stranded overhang is inserted into the double-stranded repeat array of the telomere. This structure protects the telomere end from DNA damage responses, a mechanism orchestrated by TRF2.

De Lange also uncovered a potential cancer-causing mechanism with the observation that telomere shortening leads to genomic instability in cells with mutations in TP53, a gene that is mutated in half of all cancers. Given that telomere lengthening can lead to cancer, de Lange investigated and later found that the POT1, TIN2, and TRF1 shelterin complex proteins may help prevent cancer by blocking the activity of the telomere-lengthening protein telomerase.

Collectively, de Lange's research has had direct and profound clinical implications, highlighted by one of her more recent discoveries that individuals with germline mutations in TIN2 are born with extremely long telomeres and present with an increased risk of developing cancer.

Dr. Titia de Lange delivered the Award Lecture at the AACR Annual Meeting on April 7, 2024 in San Diego, California. From the left: past Award recipients Dr. Tak W. Mak and Dr. Alberto Mantovani, President Dr. Galligioni, Dr. de Lange and Dr. Robert Schreiber, Chairman of the 2023-2024 Selection Committee

Dr. de Lange's Lecture during the Award Ceremony in Trento, Italy, on May 11, 2024

Dr. de Lange officially awarded during the Award Ceremony in Trento, Italy, on May 11, 2024. From the left: Dr. Philip Greenberg 2023-2024 AACR President, Dr. Gios Bernardi Pezcoller Foundation President Emeritus, Dr. Galligioni Pezcoller Foundation President, Dr. de Lange 2024 Award recipient, Dr. Margaret Foti AACR CEO.

Call for Nominations for the 2025 Award

NOMINATION DEADLINE FOR 2025 AWARD: SEPTEMBER 15, 2024

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 on **May 17, 2025**.

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 2024

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 researchers who have demonstrated **academic excellence** and achievements in the field of cancer research and who have, through leadership or by example, furthered the **advancement of women** 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

2024 Translational Cancer Researcher Award:
Elisa Oricchio
Swiss Institute for Experimental Cancer Research (ISREC), EPFL Lausanne, Switzerland.

2024 Women in Cancer Research Award:
Maria Rescigno
Humanitas University e Humanitas Research Hospital, Milan, Italy.

2024 Rising Star Award:
Stamatis Papathanasiou
Institute of Molecular Biology (IMB) Mainz, Germany

The calls for Nominations for the 2025 Awards, are now open. Deadline: 12 September 2024: www.eacr.org

The Pezcoller Foundation – University of Trento PhD Fellowships

The Pezcoller Foundation actively promotes and supports cancer research, with particular attention to the local community of researchers.
The Pezcoller Foundation – University of Trento PhD Fellowships are 3-year fellowships for Italian researchers (€25,000/year), awarded on a competitive basis in collaboration with the University of Trento, Department of Cell, Computational and Integrated Biology (CIBIO), for cancer research projects.
The recipients of the 2023-2026 PhD Fellowships are:

The Pezcoller Foundation - Marina Larcher Fogazzaro PhD Fellowship
Elisa Marmocchi
Project "*Development of immuno-oncology strategies for immunologically "cold" tumors*" coordinated by Prof. Andrea Lunardi (CIBIO Department)

The Pezcoller Foundation - Casse Rurali Trentine PhD Fellowship
Fabio Mazza
Project "*Exploring the complex of interactions between somatic and germline coding variants in cancer*" coordinated by Prof. Alessandro Romanel (CIBIO Department) and Prof. Gianluca Lattanzi (Physics Department, UNITN)

Next call for application will open in 2026.

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, assigned every 2 years.
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

The 7 recipients of the 2025-2026 Pezcoller Foundation - SIC Fellowships will be announced at the 64th SIC Congress, in Milan, on September 25-27, 2024.

#UnNuovoDomani - Today's research, tomorrow's cure

Watch **#UnNuovoDomani!**
This video was created with the aim of spreading the value of **scientific research**, particularly **cancer research**.

We organized a *social experiment* to observe the *reactions* of the people involved, and raise awareness of the important issue of cancer research.

It is a powerful message that we hope will touch the hearts of many.

If you enjoyed the video and would like to help us spread the message, please share it on your social media using the campaign hashtags:
#UnNuovoDomani and **#FondazionePezcoller**

The Pezcoller Foundation

FONDAZIONE CARITRO
CASSA DI RISPARMIO DI TRENTO E ROVERETO

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