The Pezcoller Foundation

# Journal


Summary




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*Picture on front page:*
*David Livingston, chairman of the Pezcoller Symposia*

# 2022 June Editorial



This is the first time of the Pezcoller Symposia without David Livingston, a great scientist and a great friend of the Pezcoller Foundation, who died suddenly last October 17. His death was a huge loss to the entire scientific community, and to the Pezcoller Foundation, for which he spent so much energy, particularly with the Pezcoller Symposia.

After many years of collaboration at the Symposia with Enrico Mihich, David Livingston took over as Chairman and for more than 10 years consistently ensured cutting-edge topics and the best speakers. This symposium itself is primarily the result of his work: his is the topic and largely his are the program and the faculty, all discussed at length with the Standing Committee, up to 3 days before his death. By that time, he had already obtained from most of the speakers the willingness to participate to the symposium, and to come to Trento in person. We'll always miss him, his brilliant mind and incomparable scientific standing, as well as his ability to stimulate discussion with many sharp questions and deep insights. For all these reasons, we decided to remember and honor him with the permanent establishment of the David Livingston Lecture at the Symposia, given this year by the 2019 Nobel laureate William Kaelin, David's great friend.

We strongly wanted this year's symposium in the presence of both participants and speakers, because of the great and widespread need to meet again in person, after so much isolation and so many video calls and virtual meetings. Indeed, we are convinced that the multiple interactions among researchers of various ages and experience, represent a great, unmissable value of our symposia and a reason for their appealing, in addition to the cutting-edge topics and outstanding speakers.

All together, there are more than 180 participants this year. We also received many abstracts, some of outstanding or excellent quality, and 31 have been accepted for poster presentation. It appears to us a reasonable result, considering that this is the first symposium in presence after the pandemic.

In addition, we have the pleasure of hosting this year the editors of 3 leading scientific journals and to report the highlights of the symposium, in collaboration with the CIBIO Department of the University of Trento and the European School of Oncology (ESO). These highlights will be presented in 3 weeks after the symposium, on July 15th, in an ESO international event.

Interestingly, this year's winner of the Pezcoller – AACR international award for extraordinary achievement in cancer research, resulted in Steven Rosenberg the father of modern cancer immunotherapy. This coincidence is quite intriguing because if Rosenberg paved the way for Cancer immunotherapy, much more needs to be done, to understand what are the challenging roadblocks to cure cancer with this modality, just as this symposium seeks to understand.

In this issue of the Journal, we have included some information about the Pezcoller Foundation activities that may potentially interest some participants: the Pezcoller-AACR and Pezcoller EACR International Awards, with the corresponding call for nominations, and the call for the Pezcoller-SIC scholarships.

Finally, I can't thank enough all those who made a key contribution to the realization of this Symposium: the members of the Standing Committee, for the Program and the Faculty, the staff of the Pezcoller Foundation, for all organizational activities with the support of the Orikata agency and Jam Session technical services, and the Humanities Department of the University of Trento, for hosting the Symposium in this so prestigious and comfortable venue.

*Enzo Galligioni*
*President*

## 33rd Pezcoller Symposium

# What are the obstacles to cancer immunotherapy success?

## June 13-14, 2022

Moderators:
Bardelli Alberto, Brisken Cathrin, d'Adda di Fagagna Fabrizio, Del Sal Giannino, Draetta Giulio, Loda Massimo, Mantovani Alberto, Piccolo Stefano, Rescigno Maria

* Central European Time (CEST)

## MONDAY JUNE 13, 2022

**8:00 Registration**

**8:30 Enzo Galligioni** **Welcome**

**8:40 Stefano Piccolo** **Focus & Goals**

**8:50 David M. Livingston Lecture:** William G. Kaelin
(Nobel Laureate, Dana Farber Cancer Institute)

9:35 Discussion led by Stefano Piccolo

**9:50 Keynote Lecture 1:** Laurie H. Glimcher (Dana Farber Cancer Institute)
IL-22 Blockade as a Therapeutic Modality for Blood Disorders

10:35 Discussion led by Massimo Loda

10:50 *Coffee break & poster exhibition*

### 11:05 Session 1: New Immunotherapeutic Approaches

Moderator: Cathrin Brisken 11.05 Chiara Bonini (Università Vita Salute S. Raff aele, Milan)
Genome editing for cancer immunotherapy

11:30 Discussion

**11:45 Philip J. Kranzusch** *(Harvard Medical School; Dana-Farber Cancer Institute)*
*cGAS-like receptors reveal new signals controlling innate immunity*

12:10 Discussion

**12:25 Jamie B. Spangler** *(John's Hopkins University School of Medicine) Mechanism-driven design of antibodies for cancer immunotherapy*

12:50 Discussion

13:05 *Lunch & poster exhibition*

### 14:05 Session 2: New Clinical Observations involving Immunotherapy

Moderator: Giulio F. Draetta

**14:05 JElizabeth M. Jaffee** *(John's Hopkins University School of Medicine)*

*Progress in converting pancreatic cancer into an immunologic disease: Lessons learned from the clinic*17:25 Discussion

14:30 Discussion

**14:45 Emile E. Voest** *(NKI, Amsterdam) Integrating genomics and autologous*

*tumor models to advance precision treatment*

15:10 Discussion

### 15:25 Session 3: Metabolism and Immunotherapeutics

Moderator: Giannino Del Sal

**15:25 Jorge Moscat** *(Weill Cornell Medical College) Targeting the tumor stroma to*

*overcome immunotherapy resistance: The colorectal cancer paradigm rejuvenation*

15:50 Discussion

**16:05 Erika L. Pearce** *John's Hopkins University School of Medicine) Phosphoinositide acyl chain saturation drives CD8+ effector T cell signaling and function*

16:30 Discussion

**16:45 Matthew Vander Heiden (MIT)** *How tissue nutrient availability influences cancer*

*progression and immunity*20:15 Discussion

17:10 Discussion

17:25 End of Day 1

20.00 Symposium dinner

## TUESDAY JUNE 14, 2022

**8:30** Keynote Lecture 2: **Philip D. Greenberg** *(Fred Hutchinson Cancer Research Center)*

*Adoptive therapy with engineered T cells: Designing T cells for success*

9:15 Discussion led by Maria Rescigno

### 9:30 Session 4: Innate Immunity

Moderator: **Fabrizio d'Adda di Fagagna**

**9:30 Caetano Reis e Sousa** *(Francis Crick Institute, UK) Necrophagia, DaNGeRous*

*indigestion and immunity to cancer*

9:55 Discussion

**10:10 Andrea Ablasser** (*EPFL, Lausanne) The cGAS-STING pathway and its impact in cancer*

10:35 Discussion

10:50 *Coffee break and poster exhibition*

## 11:10 Session 5: Bacteria and Cancer Immunity

Moderator: **Alberto Bardelli**

**11:10 Yardena Samuels** *(Weizmann Institute of Science, Israel) Identifying the highly complex intra-tumor heterogeneity in melanoma*

11:35 Discussion

**11:50 Richard A. Flavell** *Department of Immunobiology, Yale*

12:15 Discussion

12:15 Lunch and poster exhibition

**13:30 Poster Session and Maria Begnudelli Awards to the 3 best posters**

*Led by Massimo Loda*

## 14:15 Session 6: Why do Immunotherapies fail

Moderator: **Alberto Mantovani**

**14:15 Antoni Ribas** (*UCLA-Broad Stem cell research center) Mechanisms of response and resistance to PD-1 blockade therapy*

14:40 Discussion

**14:55 Padmanee Sharma** *MD Anderson Cancer Center) From the Clinic to the Lab: Investigating Mechanisms of Response and Resistance to Immune Checkpoint Therapy*

15:20 Discussion

**15:35 Charles L. Sawyers (MSKCC)** *Tumor Microenvironment in Prostate Cancer*

16:00 Discussion

**16.15 Closing Remarks by Alberto Mantovani**

16:30 End of Day 2

## INVITED SPEAKERS

### FACULTY

- **Ablasser Andrea**
  EPFL - Ecole polytechnique fédérale de Lausanne, Lausanne, CH
- **Bonini Chiara**
  Università Vita Salute San Raffaele, Milano, IT
- **Flavell Richard A.**
  Department of Immunobiology, Yale School of Medicine, Yale, CT
- **Glimcher Laurie H.**
  Dana-Farber Cancer Institute, Boston, MA
- **Greenberg Philip D.**
  Fred Hutchinson Cancer Research Center, Seattle, WA
- **Jaffee Elizabeth M.**
  John's Hopkins University School of Medicine, Baltimore, MD
- **Kaelin William G.**
  Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA
- **Kranzusch Philip J.**
  Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA
- **Moscat Jorge**
  Weill Cornell Medical College, New York, NY
- **Pearce Erika L.**
  The Bloomberg~Kimmel Institute for Cancer Immunotherapy - Johns Hopkins University School of Medicine, Baltimore, MD
- **Reis e Sousa Caetano**
  Francis Crick Institute, London, UK
- **Ribas Antoni**
  UCLA-Broad Stem cell research center, Los Angeles, CA
- **Samuels Yardena**
  Weizmann Institute of Science, Rehovot, IL
- **Sawyers Charles L.**
  Memorial Sloan-Kettering Cancer Center, New York, NY
- **Sharma Padmanee**
  University of Texas M. D. Anderson Cancer Center, Houston, TX
- **Spangler Jamie B.**
  Johns Hopkins University School of Medicine, Baltimore, MD
- **Vander Heiden Matthew**
  Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA
- **Voest Emile E.**
  Netherlands Cancer Institute, Amsterdam, NL

33rd Pezcoller Symposium

# What are the obstacles to cancer immunotherapy success?

June 13-14, 2022

## ABSTRACTS OF ORAL PRESENTATIONS

### IL-22 Blockade as a Therapeutic Modality for Blood Disorders: Starring RIOK2

*Laurie H. Glimcher, Shrestha Ghosh and Mahesh Raundhal*
*Dana-Farber Cancer Institute, Harvard Medical School*

Anemia is a major comorbidity in hematologic malignancies, aging, chronic kidney and inflammatory diseases. However, the transcriptomic networks governing hematopoietic differentiation in blood cell development remain incompletely defined. Patients with myelodysplastic syndromes (MDSs) display severe anemia but the mechanisms underlying this phenotype are incompletely understood. Right open-reading-frame kinase 2 (RIOK2) encodes an atypical protein kinase located at 5q15, a region frequently lost in patients with MDS del(5q). Haploinsufficient deletion of Riok2 led to reduced erythroid precursor frequency leading to anemia. Proteomic analysis suggested immune system activation, and transcriptomic analysis revealed an increase in p53-dependent interleukin (IL)-22 and Th22 CD4+ T cells. Blockade of IL-22 signaling alleviated anemia. Serum concentrations of IL-22 were increased in the subset of patients with del(5q) MDS as well as patients with anemia secondary to chronic kidney disease suggesting an opportunity for reversing many stress-induced anemias by targeting IL-22 signaling.
Unexpectedly, we discovered that RIOK2 is also a master transcription factor that not only drives erythroid differentiation, but also simultaneously suppresses myelopoiesis and megakaryopoiesis in primary human stem and progenitor cells. RIOK2 bears a winged helix-turn-helix DNA-binding domain and two transactivation domains that are critical to regulate key hematopoietic transcription factors GATA1, GATA2, SPI1, RUNX3 and KLF. RIOK2 is an integral component of the transcriptional regulatory network governing human hematopoietic differentiation. Importantly, RIOK2 mRNA expression significantly correlates with these TFs and other hematopoietic genes in myelodysplastic syndromes, acute myeloid leukemia and chronic kidney disease. Further investigation of RIOK2-mediated transcriptional pathways should yield therapeutic approaches to correct defective hematopoiesis in hematologic and malignant disorders.

### Genome editing for cancer immunotherapy

*Chiara Bonini*
*Università Vita-Salute San Raffaele and IRCCS Ospedale San Raffaele*

Adoptive T cell therapy for cancer (ACT) relies on the ability of T cells to selectively target tumor antigens. ACT stemmed from two remarkable clinical observations: (i) The magnitude of T cells infiltrating tumor masses often correlates with response to treatment and (ii) Allogeneic donor T cells infused in the context of hematopoietic stem cell transplantation promote clinical response in hematological malignancies. At its dawn, ACT solely relied on tumor-specific T cells isolated from the tumor masses and expanded in vitro, and feasibility was limited. The development of genetic engineering technologies and, more recently, of genome editing tools dramatically changed the landscape of ACT, rapidly making this treatment accessible to an unprecedent number of patients and tumor types. By

inserting a chimeric antigen receptor (CAR), or an exogeneous tumor reactive T cell receptor (TCR) into patient's T cells, the specificity could be precisely redirected toward selected tumor antigens. This new opportunity shifted the research focus and raised up novel questions: the main issue was no more how to harvest a sufficient number of tumor-specific T cells from each single patient, but how to isolate design and combine tools to proficiently generate and expand the most fit engineered T cells for each target disease. The selected tools and protocols should ideally allow T cells to infiltrate the tumor mass, to recognize relevant tumor antigens, to survive and resist the immunosuppressive signals present in the tumor microenvironment and to persist as memory cells, to patrol the organism for recurrence. Challenges and opportunities towards the generation of optimal T cell therapy products will be discussed.

## cGAS-like receptors reveal new signals controlling innate immunity

*Philip J. Kranzusch[1,2]*
*[1]Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute, Boston, MA USA*
*[2]Department of Microbiology, Harvard Medical School, Boston, MA USA*

Human cells use signals called nucleotide second messengers to activate and control the immune response. As one example, the enzyme cyclic GMP–AMP synthase (cGAS) produces the nucleotide signal 2′3′-cGAMP that is a critical component of anti-tumor immunity. Drug analogs developed from 2′3′-cGAMP are rapidly emerging as promising new therapeutics, illustrating the importance of discovery and mechanistic understanding of naturally occurring nucleotide signals. Surprisingly, our work reveals that cGAS is ancient and originated in bacteria as part of a system that defends against phage infection. I will present our latest findings that leverage the direct evolutionary connection between mammalian innate immunity and prokaryotic phage defense to discover new immune signaling pathways and define the molecular rules that allow nucleotide second messengers to control the cellular response to cancer and pathogen infection.

## Engineered multispecific antibodies to empower cancer immunotherapy

*Jamie B. Spangler*
*Johns Hopkins University School of Medicine*

Groundbreaking advances in immunotherapy, the mobilization of a patient's own immune system against disease, have revolutionized the cancer therapy landscape. In particular, there is a growing interest in therapeutics that antagonize T cell-suppressive immune checkpoint pathways in order to unleash anti-tumor immunity. Although immune checkpoint protein-targeted antibodies have seen tremendous success in the clinic, most tumor regressions are partial and the majority of patients (>70%) do not respond to these drugs. There is thus an urgent need to deepen our understanding of immune checkpoint pathways and to design interventions that act through new molecular mechanisms. Whereas all current clinically approved immune checkpoint inhibitor antibodies act through a common approach of inhibiting protein activation by sterically obstructing ligand/receptor interactions, we have devised an innovative strategy designed to downregulate surface presentation of immune checkpoint proteins and thereby prevent the possibility of activation. This approach leverages multispecific antibodies (denoted multiparatopic antibodies) that target two or more epitopes on a single transmembrane protein, biasing protein trafficking to drastically reduce surface presentation. We engineered a panel of multiparatopic antibodies against the immune checkpoint protein programmed death ligand-1 (PD-L1), and demonstrated that they efficiently down-regulated surface expression of the target protein. Our most effective downregulating antibody suppressed immune checkpoint signaling to enhance T cell activation and drastically attenuated availability of immune checkpoint proteins in a mouse tumor model. Overall, our engineered multiparatopic antibodies offer novel insights into immune checkpoint pathways and present a promising new approach for manipulating these pathways to stimulate antitumor immunity.

## Progress in Converting Pancreatic Cancer into an Immunologic Disease: Lessons learned from the clinic

*Elizabeth M. Jaffee, M.D., The Dana and Albert "Cubby" Professor of Oncology, The Sidney Kimmel Cancer Center at Johns Hopkins*

Immune checkpoint inhibitors (ICIs) are providing durable clinical responses in about 20% of cancer patients with tumors that often express high mutational burdens. But these agents, both single and combination therapies, have minimal effect in low mutational burden cancers which lack quality intratumoral T cells. Approaches that turn currently unresponsive cancers into

ones that are more "antigenic" are needed to sensitize tumors to ICIs. Emerging data suggest that it should be possible to develop precision immunology approaches that combine neo-antigen targeting vaccines to activate and expand the limited repertoire of T cells specific for the expressed neo-antigens, with ICIs to induce clinically relevant anti-tumor responses. But challenges to successful immunization include knowledge about the repertoire and functional state of pre-existing anti-tumor T cells, identification of the best adjuvants, and approaches that more precisely predict which expressed neo-antigens are the best T cell targets for immunization. In addition, there are many different immune regulatory signals on T cells, monocytes and other tumor microenvironment cell populations especially cancer associated stromal populations that regulate T cells. This talk will discuss current knowledge of precision immune oncology and novel clinical trial approaches under development that show promise in bypassing one or more of the immune suppressive pathways.

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## Integrating genomics and autologous tumor models to advance precision treatment

*Emile E Voest, Netherlands Cancer Institut*

To make sure that every cancer patient has the best opportunities for a successful treatment we need to make significant changes in the way we organize health care. There is now a growing concern that the gap between science and implementation is widening. Where tumor genomics have unmistakably improved the life of many cancer patients and immunotherapy has revived the hope of being able to cure patients with metastatic cancer, significant over treatment is still present with all its negative consequences for patients and society. Access to DNA diagnostics (e.g. whole genome sequencing on fresh material) is critical to maximize patient identification but it also paves the way to implement novel diagnostics like RNA sequencing and proteomics in daily clinical practice. This is not an insignificant endeavor because it requires changes in pathology workflows. As a consequence of refined molecular profiling, new drugs are tested in single arm studies with limited patient numbers. The golden standard of randomized clinical trials is challenged and presents a significant problem for regulatory authorities to evaluate the value to patient care. Innovative clinical trials are designed to detect early signs of activity, and the use of real-world data is increasingly important as a comparator to determine benefit of a treatment. This means that systematically collecting clinical data of standard of care is critical to interpret findings.
In addition to the relatively static tumor measures such as DNA, RNA and proteomics via single time point biopsies, new, more dynamic, assays are developed to determine sensitivity to treatment. Spheroids and organoids are in the early days of testing but have great potential. Several approaches to miniaturize these assays will be very important for broad clinical use and to perform the validation studies. In summary, we are at a tipping point in how we perform precision treatment but it will require a concerted effort of all medical specialties involved to re-invent our procedures to maximize clinical benefit.

**Reference**

Mateo et al. Nature Medicine, April 19, 2022

## Stromal activation and heterogeneity as a pro-tumorigenic mechanism and a barrier to immunotherapy

*Jorge Moscat and Maria T. Diaz-Meco*
*Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, 1300 York Avenue, New York, NY 10065*

Mounting evidence demonstrates that the tumor

microenvironment plays a critical role in cancer progression and initiation. The recent focus on the stromal fibroblasts has generated confusing data regarding cancer-associated fibroblasts (CAF) pro or anti-tumor role in tumorigenesis. Studies from several laboratories, including our own, demonstrate the importance of stromal fibroblasts as a barrier to immunotherapy in colorectal cancer (CRC). The CRC subtype termed CSM4 is characterized by immune exclusion and a reactive stroma rich in TGFb and includes approximately one-third of the CRC patient population. Therefore, identifying the precise role that the stroma plays in desmoplastic and immunosuppressed CRC evolution and therapy is a clear unmet medical need. Our laboratory has recently found that the expression of atypical protein kinase Cs, PKCz, and PKCl/i, is reduced in human CRC with features of serrated carcinoma and desmoplasia that transcriptionally conform to the CMS4 subtype (Nakanishi et al., 2019; Nakanishi et al., 2018). The simultaneous inactivation of both kinases in the mouse intestinal epithelium resulted in spontaneous serrated tumorigenesis that progressed to advanced cancer with a strongly reactive and immunosuppressive stroma. Interestingly, these tumors were resistant to anti-PD-L1 therapy, in keeping with the characteristics of human CMS4 CRC. However, the treatment with a TGFb receptor inhibitor enabled the anti-PD-L1 checkpoint blockade to manifest therapeutic activity. Although inhibiting TGFb therapeutically is not exempt from associated toxicities, these findings highlight the importance of stromal fibroblasts in the induction of the stem/mesenchymal phenotype and the response to immunotherapy. The stroma, therefore, is a barrier to immunotherapy in this type of desmoplastic tumor. Importantly, our unpublished data demonstrate that these serrated mouse tumors and human specimens also show a dramatic enrichment of hyaluronan (HA), a glycosaminoglycan that accumulates in the stroma of highly aggressive tumors and that is the ligand of the stem cell receptor CD44. Furthermore, CD44 is expressed in several immunosuppressive myeloid cell types, which suggests that interfering with the CD44-HA interaction might reduce immunosuppression and boost the cancer cytotoxic activity of the immune system. I will present new data demonstrating that the treatment of mice harboring CMS4 CRC tumors with PEGPH20 results in a dramatic reduction in tumor burden and cancer incidence. Interestingly, our recent results also demonstrate that the actions of PEGPH20 are pleiotropic, acting directly on the tumor epithelium and stromal fibroblasts but, significantly, also in the immune system by boosting its anti-cancer activity. This effect of PEGPH20 does not require co-treatment with anti-PD-L1 or anti-PD1, in contrast to therapies aimed at blocking TGFb signaling. In addition, our unpublished single-cell transcriptomic analysis of these tumors brought to light unexpected connections in the tumor microenvironment that allow us to identify new therapeutic targets in cancer by modulating the barrier imposed by the stroma to ICB. Our most recent data identified the master transcriptional regulators of CAF activation in mesenchymal CRC, which will also help design new, more targeted therapies to reprogram the stroma to block its role as a barrier to immunotherapy.

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Nakanishi, Y., Duran, A., L'Hermitte, A., Shelton, P.M., Nakanishi, N., Reina-Campos, M., Huang, J., Soldevila, F., Baaten, B.J.G., Tauriello, D.V.F., et al. (2018). Simultaneous Loss of Both Atypical Protein Kinase C Genes in the Intestinal Epithelium Drives Serrated Intestinal Cancer by Impairing Immunosurveillance. Immunity 49, 1132-1147.

## Phosphoinositide acyl chain saturation drives CD8+ effector T cell signaling and function

*Erika L. Pearce*
*Bloomberg~Kimmel Institute for Cancer Immunotherapy - Johns Hopkins University School of Medicine*

Rapid interconversion and salvage of phosphoinositides (PIPn) following TCR ligation and co-stimulation is critical for initiation of signaling cascades that mediate early T cell activation. However, whether different PIPn pools with specific functions exist, and how PIPn signaling is maintained during differentiation into effector T cells (TE) with increased metabolic, biosynthetic, and functional demands is unknown. We carried out a comprehensive analysis of the lipidome of CD8+ TE and discovered a unique PIPn pool, marked by more saturated

acyl chains - findings that were reproducible in mouse and human CD8+ TE in vivo. The introduction of 'new' PIPn that increases the net PIPn pool occurs through de novo synthesis of unphosphorylated PI. Pharmacological inhibition or genetic deletion of CDIPT, the final enzymatic step in de novo PI synthesis, specifically depleted the saturated PIPn pool, while the polyunsaturated PIPn pool was maintained. Decreased saturated PIP2 was linked to reduced CD8+ TE fitness and cytotoxic function in vitro and in vivo in models of infection and cancer. Strikingly, the necessity for saturated PIP2 was restricted to fully differentiated TE, as polyunsaturated PIP2 was sufficient to mediate early TCR signaling events. Stable-isotope tracing with U-13C-glucose revealed glucose as a predominant substrate for PIPn synthesis, thus directly linking the nutrient microenvironment with TE signaling. Finally, we found that immunotherapy-boosted TE cells synthesize more saturated PI in models of mouse and human melanoma, indicating the importance of our findings in a clinical setting. In summary, these findings uncover a novel role for PIPn acyl chain saturation in CD8+ T cells, unveiling that specific T cell programs utilize differing PIP2 pools to drive key signaling events, and directly linking enhanced CD8+ TE glycolytic metabolism to CD8+ TE signaling.

## How tissue nutrient availability influences cancer progression and immunity

*Matthew G. Vander Heiden*
*Koch Institute for Cancer Research at MIT*

Complex regulatory mechanisms enable cell metabolism to match physiological state. How specific cancers use metabolism to support proliferation is determined both by cell intrinsic factors and the nutrients available to cells within the tumor, and nutrient availability can also affect the function of immune cells. Accumulating evidence suggests that nutrient availability in tissues is heavily influenced by non-cancer cells in the tissue, such that tissue location is a major determinant of nutrient availability leading to each tissue having a unique nutrient environment. We also find that the nutrients available in lymph nodes changes if those lymph nodes contain cancer cells. This has implications for understanding how cancer and immune cells use nutrients to function, and one what might limit metastasis and response to cancer therapy. Specifically, we find that to metastasize, cancer cells have to adapt to the nutrient conditions found within a particular organ. These topics, and their implications for cancer biology will be discussed in my presentation.

## Adoptive therapy with engineered T cells: Designing T cells for success

*Philip D. Greenberg, Kristin G. Anderson, Jihoon W. Lee, Tijana Martinov, Shannon K. Oda, Leah Schmidt, Yapeng Su, Ashley M. Thelen, E. Gabriela Chiorean, Tom M. Schmitt, and Aude G. Chapuis*

*Fred Hutchinson Cancer Research Center and University of Washington*
*1100 Fairview Ave North, Mail Stop D3-100, Seattle, WA 98109, USA*

We have been exploring, in both preclinical models and clinical trials, methods to reproducibly provide therapeutic T cell responses to cancer patients by transfer of genetically engineered T cells. Our clinical experiences treating human Acute Myelogenous Leukemia (AML) with CD8 T cells engineered to express a high affinity TCR specific for WT1, a protein associated with promoting leukemic transformation that is over-expressed in human leukemic stem cells, and more recently treating human pancreatic cancer with CD8 T cells engineered to express a high affinity TCR specific for mesothelin, a protein associated with tumor invasiveness, have provided evidence of therapeutic activity but also exposed obstacles that need to be addressed to achieve eradication of advanced tumors. Ongoing studies are using high dimensional multi-omics analyses to provide further insights into the mechanisms mediating acquired T cell dysfunction so that more effective therapies can be designed. We have been using preclinical models to develop and test strategies to enhance efficacy, and will discuss some of the insights that are becoming ready for clinical translation. These approaches have included blocking inhibitory pathways, which has revealed potential benefits but also limitations of targeting multiple checkpoints concurrent with T cell therapy. Therefore, we have pursued modifying the epigenetic state of the T cells at the time of infusion by altering the conditions employed during therapeutic T cell generation, as well as by further genetic modification of the T cells incorporating synthetic biology to create engineered immune responses with properties that can potentially sustain responses in tumor microenvironments (TMEs). These strategies include creating a coordinated T cell response by engineering CD4 T cells to be Class I-restricted and recognize the same tumor target as engineered CD8 T cells. Additionally, as most chronically stimulated T cells undergo apoptosis as a consequence of

Fas-mediated signaling, we have expressed a synthetic Fas receptor in T cells that both serves as a dominant negative preventing caspase activation and provides costimulatory, proliferative, and survival signals. Finally, we have also created synthetic receptors that allow T cells to interpret distinct inhibitory signals and cytokines encountered in the TME as stimulatory signals.

## Necrophagia, DaNGeRous indigestion and immunity to cancer

*Caetano Reis e Sousa*
*Immunobiology Laboratory, The Francis Crick Institute, 1 Midland Road, London NW1 1AT, United Kingdom.*

Innate and adaptive immunity work concertedly in vertebrates to restore homeostasis following pathogen invasion or other insults. Like all homeostatic circuits, immunity relies on an integrated system of sensors, transducers and effectors that can be analysed in cellular or molecular terms. At the cellular level, T and B lymphocytes act as an effector arm of immunity that is mobilised in response to signals transduced by innate immune cells that detect a given insult. These innate cells are spread around the body and include dendritic cells (DCs), the chief immune sensors of pathogen invasion and tumour growth. At the molecular level, DCs possess receptors that directly sense pathogen presence and tissue damage and that signal to control antigen presentation or to regulate a plethora of genes encoding effector proteins that regulate immunity. The lecture will focus on understanding how DCs integrate environmental signals to drive immunity to cancer, with applications in immunotherapy.

## Sensing DNA as a danger signal through the cGAS-STING pathway

*Andrea Ablasser*[1,2]
1 Global Health Institute, Swiss Federal Institute of Technology Lausanne (EPFL), Switzerland
2 Swiss Institute for Experimental Cancer Research, Swiss Federal Institute of Technology (EPFL), Switzerland

The life of any organism depends on the ability of cells to detect and to respond to pathogens. In order to detect the immense variety of pathogenic entities, the innate immune system of mammals has evolved a range of distinct sensing strategies. One major mechanism is based on the recognition of microbial DNA - an invariant and highly immunogenic pathogen-associated molecular pattern. Host cells, however, contain abundant sources of self-DNA. In the context of cellular damage or metabolic derangement, "out-of-the-context" self-DNA can elicit potentially damaging inflammatory responses. Our research focuses on the so-called cGAS-STING system - an evolutionary highly conserved innate DNA sensing system. On DNA binding, cGAS is activated to produce a second messenger cyclic dinucleotide (cyclic GMP-AMP), which stimulates the adaptor protein STING to induce innate immune responses. While this process was originally discovered as a crucial component of the immune defense against pathogens, recent work has elucidated a pathogenic role for innate DNA sensing in a variety of sterile inflammatory diseases. In this talk I will discuss recent findings on cellular mechanisms that regulate STING activity and present work on the pharmacological manipulation of aberrant cGASSTING signaling in the context of ageing-associated inflammation.

## Immunotherapy has sparked new hope for oncology in recent years

*Yardena Samuels*
*Weizmann Institute of Science*

Immunotherapy has sparked new hope for oncology in recent years, due to its remarkable ability to induce long-term tumor regression of metastatic cancer. Accumulating evidence suggest that tumor regression observed with immunotherapy are driven by targeted elimination of antigen-bearing tumor cells that are explicitly recognized by T-lymphocytes. Our systematic analysis of melanoma tumors for HLA-presented peptides using HLA peptidomics has allowed us to identify cancer/melanoma antigens, neo-antigens, intracellular microbial antigens and aberrant peptides. Our studies reveal that the landscape of melanoma-presented HLA-peptides is highly complex. We will discuss relevant mechanisms, effects on immune recognition and therapeutic implications.

## Personalized modeling of human cancer in humanized mice

*Richard A. Dr. Flavell*
*Department of Immunobiology, Yale School of Medicine*

ABSTRACT Personalized modeling of human cancer in humanized mice: To date, humanized mouse models have required large numbers of adult hematopoietic stem cells (HSCs) for robust engraftment, limiting their applicability

to patient-specific experiments. By replacing the mouse IL-6 coding region with human IL-6, we generated the MISTRG6 strain which allows efficient engraftment of adult HSCs from bone marrow (BM) aspirates. When co-engrafted with patient-derived xenografts (PDXs) from cancer patients, autologous PDX mice recapitulate the tumor microenvironment in a genetically matched system.

## Mechanisms of response and resistance to PD-1 blockade therapy

*Antoni Ribas, MD, PhD*
*UCLA-Broad Stem cell research center*

The main reason for primary resistance to PD-1 blockade therapy (defined as lack of response to upfront therapy) in patients with cancer, is the absence of a pre-existing intratumoral cancer-specific T cell infiltration that were being kept in check by the expression of PD-L1. In a minority of cases with primary resistance, there may be genetic mechanisms of primary resistance to PD-1 blockade therapy that are a result of strong immunoediting of the cancer genome under continuous pressure by the immune system. This is particularly evident in cancers with a high mutational load (high antigenicity), resulting in the genetic inactivation of key molecules in the antigen presentation pathway (beta-2 microglobulin – B2M- or HLAs) or in the interferon gamma signaling pathway (JAK1 or JAK2). Approximately one third of patients with metastatic melanoma who initially had an objective tumor response to PD-1 or CTLA-4 blockade therapy develop acquired resistance, with a delayed tumor relapse. The full range of mechanisms are currently poorly understood. In some instances, acquired resistance is induced by a genetic immunoediting mechanism resulting in loss of function mutations in the interferon gamma receptor or the antigen presentation pathway. Work in the clinic to overcome resistance has focused on combination immunotherapies. The intratumoral injection of oncolytic viruses or Toll-like receptor (TLR) agonists can trigger a local type I interferon response, given together with anti-PD-1 therapy to result in a systemic response. Early clinical trials with such combinations suggest a higher response upfront with objective responses in patients without a pre-existing T cell infiltrate, as well as responses in patients who had previously progressed on prior anti-PD-1 therapy. These and other combinations can increase response rates or reverse resistance to anti-PD-1 therapy.

## From the Clinic to the Lab: Investigating Mechanisms of Response and Resistance to Immune Checkpoint Therapy.

*Padmanee Sharma*
*MD Anderson Cancer Center*

Immune checkpoint therapy has revolutionized cancer treatment, but there are many unanswered questions pertaining to mechanisms of response and resistance. In an attempt to address some of these questions, Dr. Sharma focuses her effort on a “reverse translation” process. She studies human immune responses to generate hypotheses related to mechanisms of tumor rejection, which she tests in appropriately designed pre-clinical models, and subsequently uses the new data to design novel clinical trials to improve outcomes for patients with cancer. For example, Dr. Sharma designed and conducted the first neoadjuvant (pre-surgical) trial with immune checkpoint therapy (anti-CTLA-4) in 2006. These studies were conducted in patients with localized bladder cancer and led to translational research projects in pre-clinical models, which identified the ICOS/ICOSL pathway as a novel target for cancer immunotherapy strategies. This initial clinical trial also provided the first safety data for administration of immune checkpoint therapy in the neoadjuvant setting, which led to many other neoadjuvant studies. The clinical trial also led to the first anti-tumor responses with immune checkpoint therapy in patients with bladder cancer, which led to many other clinical trials in patients with bladder cancer, including clinical trials with anti-PD-1 (nivolumab) that were led by Dr. Sharma and her colleagues to enable FDA-approval of nivolumab for patients with metastatic bladder cancer. In addition, Dr. Sharma demonstrated for the first time that human tumors express VISTA as an immunosuppressive pathway, which acts as a resistance mechanism to immune checkpoint therapy. Clinical trials targeting VISTA are now ongoing. Dr. Sharma was also the first to demonstrate that anti-CTLA-4 plus inhibition of the EZH2 epigenetic pathway can improve anti-tumor responses, which led her to design a new clinical trial with this combination. This clinical trial is currently accruing patients. In addition, Dr. Sharma demonstrated for the first time that the unique immunologic niche within a specific organ, such as bone, impacts response to treatment with immune checkpoint therapy. Dr. Sharma will present data from many of the clinical

trials and the associated pre-clinical studies investigating mechanism of response and resistance to immune checkpoint therapy.

## Inflammatory Signaling in Prostate Cancer Progression

*Charles Sawyers, MD*
*Investigator, Howard Hughes Medical Institute*
*Memorial Sloan Kettering Cancer Center*

Inflammation has long been associated with early precursor lesions in prostate cancer but mechanistic evidence for a causal role has been lacking. Single cell characterization of a genetically engineered mouse prostate cancer model (Hi-Myc) which progresses histologically from a precursor stage (prostatic intraepithelial neoplasia or PIN, age 6 months) to invasive adenocarcinoma (age 8-9 months) revealed infiltration of myeloid cells coinciding precisely with the timing of this stage transition. Depletion of macrophages using an anti-CSF1R antibody profoundly delayed or prevented invasion if initiated during the initial stages of the PIN-to-invasive cancer transition (age 6 months), but not in mice with established tumors (age 8 months). Transcriptional profiling of these tumor associated macrophages (TAMs) revealed markedly elevated expression of the inflammatory cytokine interleukin 1-beta (IL1b), which has been implicated as a therapeutic target in human lung adenocarcinoma based on recent clinical data with an IL1b neutralizing antibody. Treatment of Hi-Myc mice with a murine IL1b neutralizing antibody blocked the transition from PIN to invasive prostate adenocarcinoma to a similar magnitude seen following TAM depletion, thereby implicating IL1b as the primary mechanism by which TAMs promote tumor progression. Furthermore, Myc transgene-positive tumor cells robust express IL1R and display elevated MAPK (p38) activation, which is downregulated following IL1b neutralizing antibody treatment. Single cell analysis of human prostate cancer samples also reveals infiltration of IL1b-expressing macrophages in localized tumors, as well as bone and soft tissue metastatic sites. Collectively, these findings, coupled with additional evidence of inflammatory pathway activation in late stage prostate cancer, underscore the importance of the tumor microenvironment in prostate cancer initiation and progression.

# ABSTRACTS OF POSTERS

## STAT3 is a master regulator of CAFs pro-oncogenic functions, acting via the induction of secreted proteins including ANGPTL4, MMP13 and STC1.

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In the tumor microenvironment of both primary tumors and metastatic lesions, Cancer Associated Fibroblasts (CAFs) actively contribute to cancer progression via production and remodeling of extracellular matrix components, secreted factors and exosomes, influencing tumor cells proliferation, invasion and dissemination to distant organs, immune responses and drug resistance. The pro-oncogenic transcription factor STAT3 is frequently constitutively activated in breast cancer (BC), acting with the pro-inflammatory cytokine IL-6 in a positive feedback loop that maintains high levels of IL-6 secretion and STAT3 activation in both tumor and stroma cells. Here, we demonstrate that STAT3 is essential for the pro-tumorigenic functions of murine BC CAFs both in vitro and in vivo, with STAT3 silencing significantly blunting CAF-induced primary tumor growth and metastasis. Interestingly, the CAF-induced increase in metastatic burden is likely to occur via impairing the physiological anti-tumor immune response, since it cannot be recapitulated in immuno-compromised NSG mice. We identified a CAF-STAT3 signature significantly enriched for genes encoding for secreted proteins, among which ANGPTL4, MMP13 and STC-1 were functionally validated as STAT3-dependent mediators of CAFs pro-tumorigenic functions. Moreover, both in vitro and in vivo CAFs activities were impaired by MMP13 inhibition or anti-IL6R monoclonal antibodies, supporting the feasibility of a therapeutic approach based on inhibiting STAT3-induced CAF-secreted proteins. To investigate the clinical relevance of these findings, we generated a human CAF-STAT3 signature and assessed its expression in publicly available datasets of BC in which the stromal content was concomitantly determined. In agreement with STAT3 orchestrating the activities of tumor stroma, STAT3 signature's expression correlated positively with stromal content, and was highly expressed in stromal versus epithelial cells in single cell analysis of triple negative BC. Importantly, disease-specific survival was significantly reduced in BC patients with high expression of the signature, independently of their molecular subtypes. In conclusion, these evidences establish a pivotal role for STAT3 in the CAF-tumor crosstalk through the regulation of secreted proteins, paving the way to their therapeutic inhibition, bypassing the hurdles of STAT3 in vivo targeting.

## Characterization of the immune regulatory functions of the promyelocytic leukemia protein PML in triple-negative breast cancer

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Triple-negative breast cancer (TNBC) is an aggressive tumor subtype that lacks expression of actionable estrogen, progesterone and HER2 receptors and represents an urgent clinical need. We found that the promyelocytic leukemia protein PML is overexpressed in TNBC, where it promotes tumor growth and metastasis. To fully characterize PML-dependent TNBC responses, we performed RNA-sequencing upon PML silencing and found that an important part of the PML-regulated transcriptome is involved in immune regulation. Thus, we evaluated in vivo the intra-tumor immune cell representation in TNBC murine models upon PML silencing by flow cytometry and single cell RNA-sequencing (scRNA-seq) and investigated the cytotoxicity of CD8+ T from OT-I mice co-cultured with EO771-OVA cells expressing different PML levels. We found that PML regulates the expression of numerous genes participating to interferon signalling, antigen presentation and immune checkpoint inhibition in TNBC. This includes positive regulation of the immune checkpoint inhibitor PD-L1 and suppression of proteins involved in antigen processing and presentation. Consistently, flow cytometry and scRNA-seq analyses of CD45+ cells showed that PML silencing in tumor cells caused a major reorganization of the immune environment, with a decrease in CD4+ T cells and Tregs and increased representation of cytotoxic populations of CD8+ effector T and NK cells. Also, reduced PML expression in mouse TNBC cells improved tumor cell killing by OVA-directed CD8+ T cells. These preliminary results allow us to propose that PML is an important modulator of the immune microenvironment, and indicate that its inhibition can remodel the immune infiltrate by increasing cytotoxic lymphocytes, reducing immunosuppressive Tregs and promoting immune rejection, suggesting that PML may represent a marker of immune activation and a promising therapeutic target for TNBC.

## Mutant p53 promotes a specific amino acid metabolic program to foster breast cancer growth

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**Abstract**
Amino acids (AAs) are crucial nutrients for cancer cells since they provide plenty of metabolic and energetic intermediates and promote their survival in challenging environments, enabling proliferation, dissemination, and metastases formation. Tumors are indeed avid for amino acids and, consequently, they aberrantly increase AAs intake, biosynthesis, and catabolism, leading to metabolic reprogramming, a key feature of cancer cells. For this reason, unveiling the oncogenic drivers that reprogram amino acid metabolism in cancer is fundamental to understand disease progression and to find therapeutic opportunities. In this work, we disclosed a new role of mutp53 in regulating amino acid metabolism in breast cancer cells. We demonstrated that mutp53 promotes synthesis of aspartate, serine, and glycine through the upregulation of amino acids biosynthetic enzymes and increases the expression of specific amino acids transporters. Our findings indicate that mutp53, unleashing this metabolic program, supports metabolic adaptation to TME-related stresses, such as nutrient starvation.
Indeed, in conditions of amino acids scarcity, mutp53 sustained cancer cells survival and proliferation via upregulation of serine synthesis and BCAAs/bulky amino acids intake. Furthermore, we showed that a stiff ECM cooperates with mutp53 in the induction of such genes, unveiling a novel branch of amino acid metabolism regulated in response to mechanical inputs and fostered by mutp53.

## The role of mSWI/SNF complex in preserving liver tissue homeostasis and preventing hepatocellular carcinoma formation

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*University of Trento*

**Abstract**
mSWI/SNF complexes are a class of ATP-dependent chromatin remodelers whose role is to slide, insert, or evict nucleosomes from the DNA. They promote chromatin accessibility thus regulating gene expression. Although mSWIS/SNF subunits are mutated in around 25% of human cancers, little is known about their impact. Hepatocellular carcinoma (HCC) is the third cause of cancer-related deaths worldwide and the sixth most frequent cancer type. Given the lack of pharmacological treatments there is a dire need for novel molecular targets. Recent studies have identified specific mutations in HCC patients, including ARID1A, a major subunit of the mSWI/SNF complex. The way mSWI/SNF maintains liver homeostasis and contributes to HCC development is still under debate. In this work, we provide in vivo evidence that ARID1A is required for preserving genome integrity but dispensable for the maintenance of tissue-specific enhancers. Furthermore, we show that ARID1B is essential to preserve the tissue transcriptional program by compensating for the absence of ARID1A. Early events associated with ARID1A loss are the accumulation of DNA damage, followed by the activation of the innate immune response that eventually leads to chronic inflammation. Upon stimulation with a non-mutagenic compound, ARID1A deficient mice developed aggressive tumours that synergise with β-catenin mutations and ultimately form metastasis. Overall, these results confirm ARID1A as a tumour suppressor gene and open the possibility to novel therapeutic approaches exploiting the mutual exclusivity with ARID1B.

## Zebrafish Melanoma-Derived Interstitial EVs Are Carriers of ncRNAs That Induce Inflammation

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**Abstract**
Extracellular vesicles (EVs) are membranous particles released by all cell types. Their role as functional carrier of bioactive molecules is boosted by cells that actively secrete them in biological fluids or in the intercellular space (interstitial EVs, iEVs). Here we have optimised a method for the isolation and characterization of zebrafish iEVs from whole melanoma tissues. Zebrafish melanoma iEVs are around 140 nm in diameter, as determined by nanoparticle tracking and transmission electron microscopy (TEM) analysis. Western blot analysis shows enrichment for CD63 and Alix in the iEV fraction, but not in melanoma cell lysates. Super resolution and confocal microscopy reveal that purified zebrafish iEVs are green fluorescent protein positive (GFP+), indicating that they integrate the oncogene GFP-HRASV12G used to induce melanoma in this model within their vesicular membrane or luminal content. Analysis of RNA-Seq data found 118 non-coding (nc)RNAs differentially distributed between zebrafish melanoma and their iEVs, with only 17 of them being selectively enriched in iEVs. Among these, the RNA components of RNAses P and MRP, which process ribosomal RNA precursors, mitochondrial RNAs, and some mRNAs, were enriched in zebrafish and human melanoma EVs, but not in iEVs extracted from brain tumours. We found that melanoma iEVs induce an inflammatory response when injected in larvae, with increased expression of interferon responsive genes, and this effect is reproduced by MRP- or P-RNAs injected into circulation. This suggests that zebrafish melanoma iEVs are a source of MRP- and P-RNAs that can trigger inflammation in cells of the innate immune system.

## Genome-wide CRISPR Activation Screens Revealed Novel Resistance Factors to CAR-T Cells

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The chimeric antigen receptor (CAR) T cell is a revolutionary gene- and cell-based cancer immunotherapy. The initial clinical successes resulted in the rapid approval of anti-CD19 CAR-T cell products for B cell hematological malignancies, such as acute lymphoblastic leukemia (B-ALL) and diffuse large B cell lymphoma (DLBCL). Despite the unprecedented responses induced by CAR-T cells, lack of primary response, T cell transcriptional profiles and cancer developing resistance have hindered their full anti-tumor potential and impeded durable responses.
Intense research prompted to transfer the paradigm to solid tumors, which accounts for ca.

90% of all malignancies. However, the use of CAR-T cells in solid tumors - e.g. in hepatocellular carcinoma (HCC) and glioblastoma (GBM) - has faced considerable obstacles, which can be primarily ascribed to the physical impediment of the tumor stroma and its immunosuppressive microenvironment. Nonetheless, there are additional cancer cell-intrinsic resistance mechanisms that enable their persistence whose molecular underpinnings are not yet completely understood, in both liquid and solid tumors.
Forward CRISPR/Cas-based genetic screens allow for the unbiased interrogation of gene function at a genome-wide scale. Here, to uncover unique as well as shared resistance factors to CAR-T cells, we performed in vitro genome-wide gain-of-function CRISPR activation screens in cell lines of different cancer etiologies (CD19+ JeKo-1 mantle cell lymphoma, GPC3+ Huh-7 hepatocellular carcinoma and IL13Ra2+ U-343MG malignant glioblastoma), using cognate CAR constructs. Several uncovered genetic factors were concordant with published studies and clinical data, whereas others were novel and previously undescribed. Interestingly, a previously uncharacterized E3-ubiquitin ligase emerged as a shared resistance factor across the three screens. With our established LC-MS/MS pipeline and with ultra-high-throughput single-cell RNA sequencing and perturbation screens, we further investigate the molecular mechanism of resistance in bona fide factors and better define the transcriptional resistance states more broadly, respectively. We believe that our work will have enormous basic and translational implications that would ultimately help to overcome the current obstacles, and to harness the full potential and widespread clinical use of this highly effective treatment modality.

## Tumor warm-up with editopes: RNA editing for immunotherapy

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Over the last two decades immunotherapy revolutionized cancer medicine. However, not all cancer types nor patients benefit from it. One factor associated with responsiveness to such therapies is a high tumor mutational burden (TMB)1, which is one of the features of the so called “hot” tumors. On the other side, poorly mutated tumors, also called “cold” tumors, are often resistant to immunotherapy. These observations are consistent with the hypothesis that a high TMB is associated with a higher amount of immunogenic neoepitopes presented by cancer cells that can induce an anti-tumor immune response. Recently, it has been shown that RNA editing mediated by Adenosine Deaminases Acting on RNA (ADARs) can also be a source of proteomic diversity within tumors2 and lead to the formation of immunogenic neoepitopes3. Based on this study, we aim at applying targeted ADAR-mediated RNA editing to generate neoepitopes, which we now call “editopes”, in cancer cells to make cold tumors turn hot and thus favor a T cellmediated anti-tumor immune response. Here, as proof of principle, we genetically modified a well-known tumor antigen, Melan-A, such that its processing would result into a peptide (Melanoma antigen recognized by T-cells-1 or MART-1) no longer recognized by T cells. We then used targeted RNA editing to restore the original MART-1 sequence and therefore rescue antigen-specific T-cell activation. Currently, we are validating the same approach in a mouse model of melanoma, where tumor progression is dependent on the presentation of specific epitopes. At the same time, we aim to apply targeted RNA editing to generate “editopes” in a poorly immunogenic pancreatic cancer model, to increase its immunogenicity and tumor responsiveness to immunotherapy. The results obtained so far and the promise of the upcoming experiments pave the way to the application of targeted RNA editing to favor responsiveness to immunotherapy in otherwise resistant tumors.

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## Immunogenic cell death is required for anticancer effect of high-dose Vitamin C in mouse colorectal and breast tumors

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Unleashing of immune system by immune checkpoint inhibitors can induce long-term disease control or even cure cancer in a limited number of cases. However, most patients are primarily refractory or develop resistance to this type of immunotherapy. Hence, there is a need to identify additional agents that could stimulate anticancer immune response either alone or in combination with immunotherapy. Immunogenic cell death (ICD) is an important mechanism of antitumor agents mediated tumor cell-kill, where anticancer drugs can induce antigen release, immune priming and activation, which all trigger an anti-cancer immune response. ICD is mediated by release of factors such as High-mobility group box 1 (HMGB1) protein, Calreticulin (CALR), annexin A1 (ANX A1) and extracellular ATP. Intravenous high dose Vitamin C (VitC) can reach plasmatic millimolar concentration and may have anticancer activity in murine and human tumors by induction of oxidative stress. We have previously shown that VitC can promote cytotoxic T cell activity and cooperate with anticancer immune checkpoint therapy in immunocompetent mice. We hypothesize that VitC could act as an ICD inducer. In vitro we found that high dose VitC can foster ICD markers in three different mouse cancer models, including BRAF mutant MMR proficient colorectal and breast cancers. In vivo we found that administration of high dose VitC could delay tumor growth in syngeneic immunocompetent mice, but not in immunocompromised hosts. The ex-vivo analyses performed on tumors explanted from immunocompetent mice revealed that VitC could foster ICD markers also in vivo. Indeed, we observed a statistically significant increase of CALR translocation and HMGB1 release in breast and colon tumors treated with VitC. The immune profiling performed on colorectal cancer bearing mice revealed that VitC enhanced the infiltration and activation of the NK and T lymphocytes. To functionally characterize the role of ICD in mediating the anticancer effects of VitC in vivo we employed a CALR blocking antibody. The administration of this antibody was able to prevent CALR translocation and HMGB1 release in mouse tumor tissues. Notably, preventing ICD by anti-CALR antibody was able to impair the anticancer effect of VitC in breast and colon mouse tumors.

In conclusion, our data indicate that high dose VitC as monotherapy could foster ICD in several murine cancer models and that this effect is required for unleashing an anticancer immune response.

## Targeting Toll-like Receptor 2 to tackle breast cancer resistance to therapy

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**Introduction**

Toll-Like Receptor (TLR)2 acts as a double-edge sword in cancer. Besides its role in immune responses, TLR2 is expressed on breast cancer (BC) cells and is associated with poor prognosis in patients. TLR2 is overexpressed in breast cancer stem cells (CSCs), a rare population of cells responsible for cancer progression, therapy resistance and invasion, and promotes their self-renewal through an autocrine loop initiated by high mobility group box (HMGB)1. The dual role of TLR2 in BC needs to be dissected to develop new anti-cancer therapies.

**Material and Methods**

Rat HER2-neu transgenic mice developing BC were crossed with TLR2 WT or KO mice (TLR-2WT-neuT and TLR2KO-neuT). Tumor progression, CSCs and immune cells were compared. TLR2-mediated cancer cell intrinsic and extrinsic pro-tumor activities and the effect of TLR2 inhibitors in combination with chemotherapy on BC cells were analyzed both in vitro and in vivo.

**RESULTS and Discussion**

TLR2KO-neuT mice showed a delayed tumor onset, increased survival, reduction of CSCs and T regulatory cells as compared to TLR2WT mice. Transplantation experiments showed that TLR2 acts mainly through cancer cell-intrinsic mechanisms, although TLR2 expressed on immune cells also contributed to tumor promotion. TLR2 increased cancer cell proliferation and CSC self-renewal, and conferred resistance to chemotherapy. This was mediated by chemo-

therapy-induced release of TLR2 ligands, such as HMGB1. Treatment with TLR2 inhibitors impaired viability and induced apoptosis of BC cells, and a synergistic effect was observed when they were administered with chemotherapy.

**Conclusions**

We demonstrated that TLR2 promotes BC progression and represents a mechanism of chemoresistance, since chemotherapy induces the release of its activatory ligands. TLR2 silencing or inhibition impair BC progression and restores sensitivity to chemotherapy. Therefore, the use of TLR2 inhibitors in association with chemotherapy opens new perspectives in the treatment of BC patients, while questioning the possibility to administer TLR2 agonists as adjuvants in anti-cancer immunotherapy.

## Oncogenic control of cancer cell communication with the tumor microenvironment.

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**Abstract**

Tumors are complex ecosystems composed by a heterogeneous population of tumor cells embedded in a dynamic tumor microenvironment (TME). Communication of tumor cells with the TME displays both local and systemic tumor-promoting effects, including angiogenesis, ECM remodeling and modulation of immune/inflammatory cells to support tumor growth and escape from immune-surveillance. Cancer cells are prone to DNA damage, which triggers the activation of the DNA-sensing cGAS-STING pathway and consequent expression of type-I IFNs and other immune-stimulated genes, thus engaging anti-tumor immune surveillance. Accumulating evidence established that attenuation of the cGAS-STING-IFN pathway by oncogenic drivers is critical for tumor evolution and immune evasion. Recently, our research group highlighted an oncogenic axis affecting tumor-stroma crosstalk. We discovered that miR-30d, a secreted onco-miRNA cooperatively induced by HIF1α and mutp53 oncoproteins, regulates targets involved in the secretory pathway, causing tubulo-vesiculation of the Golgi apparatus (GA). This increases the release of a pro-malignant secretome, which alters the TME fostering tumor growth and metastatic colonization.

Alterations of GA structure can attenuate the cGAS/STING/IFN-I DNA sensing pathway, which regulates anti-tumor immunity. We postulated that regulation of ER-Golgi trafficking by miR-30d may prevent the type-I interferon response by impacting on the cGAS/STING pathway in cancer cells, an hypothesis that is supported by transcriptomic data.

We demonstrated that miR30d prevents type-I IFN response by impacting on STING activation at the GA in metastatic breast cancer (BC) cell lines; moreover, downregulation of miR30d stimulates also cGAS activation in breast tumors and cell lines.

Our results suggest a putative role of miR-30d in inducing an immunosuppressive TME in BC through attenuation of the cGAS/STING/IFN-I pathway. We aim to explore the possibility that miR-30d, by dampening innate immune signaling in both cancer and stromal cells through paracrine effects, could represent a potential target whose inhibition may improve the efficacy of current chemo- and immune-therapies.

## Targeting the crosstalk between TLR2 and the cystine/glutamate antiporter xCT to design a new strategy for breast cancer treatment

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Breast cancer is still the leading cause of cancer death in women, due to relapses and metastases. Therefore, the development of combination therapies able to target key cancer-inducing or cellsustaining pathways is needed. We have previously demonstrated that mammary cancer stem cells (CSCs) overexpress the cystine-glutamate antiporter xCT and Toll-Like Receptor (TLR)2, which play a key role in their self-renewal and resistance to chemotherapy. Both xCT and TLR2 are promising targets for breast cancer therapy, and a deeper characterization of their crosstalk may lead to the setup of effective combination therapies for breast cancer. The effects exerted by TLR2 activation on xCT expression and function were analyzed in vitro on mouse and human breast cancer cell lines in which TLR2 was either activated with endogenous or bacteria-derived ligands or silenced using specific siRNA. Moreover, xCT expression was analyzed in a mouse model of HER2-neu-induced mammary carcinogenesis on a TLR2 WT or KO background (TLR2WT-neuT and TLR2KO-neuT mice) and on cell lines derived from their tumors. The efficacy of a combined inhibition of TLR2 and xCT, in association or not with chemotherapy, was tested in vitro on these cell lines.

TLR2 promoted CSC self-renewal and its deletion impaired mammary carcinogenesis in vivo.

TLR2 induced the upregulation of xCT in breast cancer cells, and its silencing or deletion decreased xCT both in vitro and in mouse models of mammary cancer. Since xCT controls intracellular redox balance, TLR2 downregulation induced an increase in intracellular reactive oxygen species in breast cancer cells. TLR2 inhibitors synergized with xCT inhibitors in hindering breast cancer cell viability and inducing their apoptosis, and these results were further increased by their association with doxorubicin. We demonstrated that TLR2 promotes breast CSC self-renewal, cancer progression and that it upregulates xCT expression in breast cancer cells, protecting them from oxidative stress. The use of TLR2 inhibitors in association with xCT inhibition (or immunotargeting) and chemotherapy may lead to the setup of more effective combination therapies for breast cancer.

## Baseline circulating immature neutrophils anticipate hyperprogressive disease (HPD) upon 1st-line PD-1/PD-L1 inhibitors (ICI) in non-small cell lung cancer (NSCLC) patients (pts) and are reduced by platinum-based chemotherapy (PCT) and ICI combinations.

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**Background.** HPD has been described in ~14-26% of pretreated NSCLC pts upon single-agent (SA) ICI and has not been reported upon PCT-ICI. So far, no predictive biomarkers are available for HPD early detection.
**Methods.** NSCLC pts treated with 1st line SA-ICI or PCT-ICI were assessed for HPD and circulating neutrophils. HPD was defined as delta tumor growth rate (TGR) [TGR upon ICI – TGR prior ICI) >50% and/or TGR ratio (TGR upon ICI/ TGR prior ICI) ≥2. Circulating low density neutrophils (LDNs) were assessed by flow cytometry on peripheral blood mononuclear cells (PMBCs). LDNs were defined as CD66b+CD15+ cells among CD11b+ PBMCs and immature subtypes as % of CD10- LDNs. The association between LDNs and outcome and the LDNs predictive role were assessed by non parametric and penalized model-based tests. The LDNs' sensitivity to cisplatin induced cell death were tested in-vitro.
**Results.** 141 NSCLC pts were included: 75 treated with SA-ICI and 66 with PCT-ICI. In the SA-ICI cohort, PD and HPD occurred in 31 (41%) and 6 (8%) pts. CD10- LDNs were significantly higher (p=0.011) in HPD [median (Me): 43.55, interquartile range (IQR): 46.5] vs PD [Me: 9.44, IQR: 15.44]. CD10- LDNs were associated with HPD [odds ratio (OR): 2.88, 90% CI: 1.61; 5.16] showing a good HPD prediction capability [cross-validated ROC AUC: 0.77, 90% CI: 0.51;1.00].
A 29.5% cut-off value was identified by Youden index to discriminate HPD from others. In the PCT-ICI cohort, 10 pts had CD10- LDNs >29.5% being at high HPD risk, however none of them experienced it. In 5 of these 10 HPD high-risk pts with available dynamic LDNs evaluation upon PCT-ICI, a 52.2% decrease in median CD10- LDNs occurred, while in 6 pts with HPD upon SA-ICI, only a 2.8% decrease was observed, suggesting that PCT prevents HPD by reducing immature CD10- LDNs. In vitro experiments also showed that cisplatin increased necrotic cell death preferentially among immature CD10- LDNs vs mature CD10+ LDNs.
**Conclusion** Baseline immature CD10- LDNs is a simple cost-effective HPD predictor upon 1st line SA-ICI and could select NSCLC pts to be addressed to PCT-ICI.

## Combinations of Local Radio- and Immuno- therapy to Induce Systemic (abscopal) Therapeutic Effects in Different Cancer Models

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**Abstract**
In the last years immunotherapy has considerably improved cancer therapy. However, many patients still do not respond to it and new approaches are required. It is known that radiotherapy has immunomodulatory functions. Particularly, after radiotherapy exposure there is a local and systemic expansion of immunosuppressive cells but also a release of tumour antigens

and increase of cytosolic dsDNA that stimulate the immune response against the tumour. The activation of antitumour immunity can also mediate the regression of untreated metastases, an effect called "abscopal". In order to encourage this response, combining radio- and immunotherapy could be a promising therapeutic strategy. In this project we are evaluating the efficacy of local administration of two different Toll-like receptors (TLRs) agonists and fractionated radiotherapy in murine models of solid tumours. Tumour cells are injected in both flanks of mice but only one side is treated in order to evaluate the abscopal effect. Preliminary data showed that in a head and neck cancer model TLR-9 and TLR-3 agonists, both alone or combined with radiotherapy, were able to reduce the growth of treated and, slightly, of nottreated tumour lesions. Similar effects were observed in a prostate cancer model, with the exception that the TLR-9 agonist alone did not induce an abscopal effect. Despite similar readouts in terms of volume, tumour histology and microenvironment composition were different after radiotherapy, immunotherapy or combo, in both treated and untreated lesions. Therapeutic effects correlated with modifications of the tumour-infiltrating immune-milieu in both models. Particularly, with TLR-9 agonist we observed an increase of CD8+ cells. Moreover, in the head & neck model, it caused a reduction of immunosuppressive cells together with an expansion of pro-inflammatory ones. Interestingly, different subtypes of immune cells expressed higher levels of PD-1 or PD-L1 after TLR-9 agonist treatment.
These preliminary data support the hypothesis that combining radiotherapy with TLR-agonists could improve the anti-tumour immune response, inducing an abscopal effect that could lead to regression of untreated lesions. Further studies are required to validate the results and to investigate the molecular mechanisms behind the observed therapeutic effects.

## Therapy-induced senescence triggers viral mimicry and promotes anti-tumour immunity in acute myeloid leukaemia

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**BACKGROUND** – Acute myeloid leukaemia (AML) is an aggressive haematological malignancy currently treated with high-dose chemotherapy. Nevertheless, treatment failure is frequent and mainly due to the development of chemo-resistant tumours, thus underscoring the need to investigate the molecular events occurring immediately after chemotherapy. Therapy-induced senescence (TIS) is a state of irreversible growth arrest triggered by several stressors including chemotherapeutic agents. Senescent cancer cells may on one hand elicit immune-mediated responses that contribute to senescent cell eradication or alternatively favour tumour relapse. Our work aims at functionally interrogate the crosstalk between senescent leukemic cells and the immune system with the final longterm goal to unveil mechanistic insights into the factors dictating AML response to conventional chemotherapy and immunotherapy.
**MATERIAL & METHOD** – Multiparametric flow cytometry analysis integrated with quantitative imaging was used to measure several senescent markers and human leukocyte antigen (HLA) expression in a longitudinal cohort of primary AML samples at diagnosis and upon ex-vivo therapy. Dissection of senescence players was achieved by means of RNA interference or chemical inhibition. Mixed lymphocyte reaction (MLR) assay was adopted to evaluate activation of T cells in response to senescent blasts. Immunological synapses formation was analysed by ImageStreamX technology. Viral mimicry via transposable elements (TEs) reactivation was identified by RNA-seq and validated by qPCR.
**RESULTS** – We established ex-vivo cultures of primary AML samples or AML cell lines undergoing senescence by chemotherapy or Ibrance (a CDK4/6 inhibitor) treatment. RNA sequencing and flow cytometry analyses of treated samples revealed upregulation of senescence-associated genes and immune-related interferon gene categories with concomitant induction of several HLA class I and class II molecules, suggesting a causal link between TIS and blasts immunogenicity. Mechanistically, we discovered that TIS cells activate distinct endogenous retroviral elements (TEs) families converging on NFkB or IFN-related gene programs depending on the initial senescence inducer. TIS blasts triggered enhanced CD4+ and CD8+ T cell proliferation and activation and promoted an increased formation of immunological synapses, ultimately leading to leukaemia eradication. In vivo profiling of T cell subsets in senescent-competent AML prima-

ry samples revealed increased percentage of T cells with an activated phenotype and reduced exhaustion markers upon chemotherapy compared to diagnosis.
**CONCLUSIONS** - Our findings uncover a novel link between senescence induction and leukemia immune recognition via viral mimicry, which may rapidly translate into innovative senescence-based strategies to cure or prevent AML post-therapy relapse.

## Polarization of circulating and tumour infiltrating NK cells in prostate cancer: role of STAT3.

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Natural killer (NK) cells are mediators of the innate immunity involved in tumour recognition and elimination. Altered NK phenotype and functions have been found in tumours, including prostate cancer (PCa). We recently demonstrated that PCa circulating NK cells (TANKs) acquire the proangiogenic/ decidual-like CD56bright CD9+ CD49a+ phenotype, release CXCL8 and MMP-9, functionally support endothelial cells activation and secrete monocyte-recruiting and M2-like macrophage-polarizing factors. Here, we characterized tumour infiltrating NKs (TINKs) and tumour-associated (TANKs) in PCa patients and seek for molecular pathways potentially involved in the acquisition of the proangiogenic/ decidual-like NK phenotype. PCa TINKs and TANKs were characterized by multicolour flow cytometry (FC) for decidual-like surface markers (CD9, CD49a). Detection of STAT3 activation, that we previously reported in TANKs from colorectal cancer patents, was determined in circulating PCa NK cells, by FC. Using a drug-repurposing approach employing the antipsychotic agent Pimozide, we chemically modulated STAT3 activation, ex vivo, in circulating NK cells from PCa patients and investigated their secretome changes, by commercially protein membrane arrays. We found that PCa TINKs acquire the same CD9+ CD49a+ decidual-like NK cell phenotype, as observed in PCa TANKs. We detected the presence of C56brightCD9+ CD49a+ decidual-like NK cell also in the peripheral blood of subjects with benign prostatic hyperplasia (BPH), but in a lower frequency, compared to those from PCa TANKs. We observed that sera from PCa patients are enriched in IL-4, IL-6, CXCL8/IL-8 and IL-10, all cytokines able to activate the STAT3 signaling pathway. Based on this evidence, we detected increased phosphorylation of STAT3 in PCa TANKs, as compared to NK cells from healthy controls, that was reduced following 24 hours of stimulation by Pimozide. This treatment resulted in decreased capabilities of PCa TANKs to secrete proangiogenic factors (CXCL8, IL-6), molecules involved in monocytes recruitment/ M2-like macrophage polarization (CCL-2, CCL5, GM-CSF, IL-10), together with increased production of anti-tumour cytokines (IFN-g, TNF-a). Our results provided preliminary 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.

## Aryl hydrocarbon receptor activation in type 1 conventional dendritic cells suppresses anti-tumor immunity

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The immune system has a key role in controlling tumor initiation and growth. Cancer immunotherapies aim at harnessing the immune system to eradicate cancer cells and control tumor growth. Unfortunately, only a small subset of patients responds to immune checkpoint therapy. Conventional dendritic cells (cDCs) are immune cells critical for innate and adaptive immune responses, and include cDC1, cDC2 subtypes. cDC1s are critical for CD8+ T-cell priming early in an antitumor response, and this function is affected by environmental signals. The transcription factor Aryl hydrocarbon Receptor

(AhR) is an environmental "sensor" of specific metabolites, both endogenous and exogenous in nature. Emerging data have shed light on an unexpected role of AhR in fostering tumor escape mechanisms. Thus, we assessed the impact of AhR deletion in cDC1 on the immune response to tumors. Whole genome analysis revealed that AhR is expressed in mature cDC1 to greater extent than in cDC2. Moreover, AhR deletion in cDC1 strongly potentiated IL-12 and TNF-a productions. Therefore, in a cDC1–T CD8+ co-culture system, we demonstrated that Ahr deletion in cDC1 greatly increases proliferation and IFN-g production in OTI transgenic T cells. Notably, in an in vivo fibrosarcoma mouse model, single-cell RNA-seq analysis showed that AhR is highly expressed in cDC1 tumor infiltrate. Accordingly, selective AhR deletion in XCRI expressing cDC1 accelerated spontaneous immune rejection of an otherwise progressive fibrosarcoma. Further, to translate our observations to human tumors, we have bioinformatically analyzed TCGA sarcoma samples, and observed that patients with soft tissue sarcomas (n=231) could be stratified for prognosis based on AHR gene expression levels, with AHRhigh subset carrying a poorer disease-specific survival (compared to AHRlow subset, p-value<0.05). We then deconvolve the immune contexture of malignant sarcomas by applying xCell digital cell sorting to TCGA sarcoma samples stratified by AHR gene expression, and have found that i) AHR positively correlates with AHR circuitry activity (AHR signature) and tumor-tissue cDC1 cell abundance, and ii) AHRhigh sarcomas exhibit cellular (Tregs and myeloid-derived suppressor cells infiltration of the TME) and molecular features (profound downregulation of IL-2 expression and increased expression of TGF-beta related gene signature) of tumor immunosuppression. Our data demonstrate that i) AhR is a metabolic gatekeeper able to govern immunoregulatory functions of cross-presenting cDC1; ii) AhR have an impact on the prognosis of patients with malignant soft-tissue sarcomas and might represent an actionable immune-switch in this high-risk clinical setting. Overall, these data point to AhR as a new immune inhibitory target in cDC1, which can be targeted pharmacologically to overcome immune tolerance and resistance to immunotherapy.

# The role of human-specific genes in medulloblastoma.

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Medulloblastoma (MB) is the most frequent pediatric brain tumor, accounting for 20% of all childhood brain tumors. Late diagnosis, tumor aggressiveness, and the lack of specific therapies are the reasons why the 5 years percentage of survival can reach 50% [1], [2]. Thus, it is necessary to develop a reliable model and better understand this disease. The empirical observation of an absence in the occurrence of spontaneous pediatric brain tumors in mice made us speculate about the importance of human-specific genes (HSGs) for the tumor initiation or progression. I focused on NOTCH2NL, a family of HSGs derived from the partial duplication of the NOTCH2 gene. NOTCH2NL was reported to activate the Notch pathway in cortical progenitors, by repressing the cis inhibition between Delta and Notch receptor [3]. Notably, Notch pathway activation has already been shown to be necessary for cerebellar progenitors to be competent in generating group 3 MB [4]. Through gain-of-function experiments, tested in both in-vivo and in-vitro models, I was able to observe over-proliferating cells in human iPSCs-derived cerebellar organoids after overexpressing MYC+NOTCH2NLB. Moreover, the engraftment of the engineered organoids into immunocompromised mice resulted in tumor formation. In parallel, with two different in-vitro approaches, I'm investigating the role of NOTCH2NL in development and cell fate determination, in order to get some insights into its cellular and molecular function in the cerebellum. Taken together, this project aims to determine the role of evolution in the development of medulloblastoma and pediatric cancer in general.

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## Liver cancer organoids reveal human liver cancer cellular plasticity as a potential relapse mechanism

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Liver epithelium is characterized by an exquisite cellular plasticity which endows liver cells with the potential to change between cellular fates upon external cues. However, whether this plasticity is conserved in human tissues and whether it plays a role in diseases states, like human cancer, remains unexplored. This is mainly due to the lack of good human models that recapitulate human liver health and disease states in vitro. We recently developed an organoid in vitro culture model that recapitulates many aspects of three of the most common subtypes of primary liver cancer in a dish (hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA) and mixed subtype (CHC)), including the genetic, transcriptional and histological changes observed in the patient's tumour tissue, in a patient-specific manner (Broutier et al. Nature Medicine 2017). Here, by using scRNAseq analysis, in vitro organoid cultures and in vivo xenotransplantation assays we show that our organoids retain the phenotypic heterogeneity present in human tumours. We identify sub-populations of highly proliferative tumorigenic cells residing at the apex of the hierarchy of the tumour pool while other sub-populations more closely resemble terminally differentiated cells and are unable to start tumours. Notably, the differentiated progeny exhibits a significant degree of plasticity as this otherwise non-tumorigenic sub-population, is capable of re-initiating tumours, both in vitro and in vivo, upon environmental changes. Our observations whereby differentiated cells can re-acquire tumour initiating potential upon certain environmental cues have critical implications for the management of cancer patients, and could help the design of therapies to fight tumour relapse.

## Regulatory CD4+ T cell landscape in human cancer and in response to checkpoint blockade immunotherapy

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Regulatory CD4+ T cells (Tregs) are required to maintain tolerance to self and prevent autoimmunity via inhibition of immune responses. Yet, in cancer, the chronic activation of Tregs limits anti-tumor immunity and the effectiveness of checkpoint blockade immunotherapy. Indeed, Treg depletion, or interference with Treg activity results in the control of tumor growth. In several human solid tumors, we have recently identified a subpopulation of CCR8+ ICOS+ Tregs whose superior suppressive activity is controlled by the transcription factor IRF4 (1). We have now used high-dimensional flow cytometry to characterise the T cell compartment of intrahepatic cholangiocarcinoma (iCCA), a rare, yet aggressive tumor of the biliary tract with no therapeutic options. Besides the poor infiltration of putative tumor-specific CD39+ CD8+ T cells, iCCA is characterized by the abundant infiltration of hyperactivated Tregs (2). Single-cell RNA-sequencing identified an altered network of transcription factors in iCCA-infiltrating compared to peritumoral T cells, overall suggesting reduced effector functions by tumor-infiltrating CD8+ T cells and enhanced immunosuppression by Tregs. Specifically, we found that expression of MEOX1, a transcription factor novel to Treg biology, was highly enriched in tumor-infiltrating Tregs, and demonstrated that MEOX1 overexpression is sufficient to reprogram circulating Tregs to acquire the transcriptional and epigenetic landscape of tumor-infiltrating Tregs. Accordingly, abundance of the MEOX1-dependent gene program in Tregs was strongly associated with poor prognosis in a large cohort of iCCA patients. I will additionally show the spatial distribution of Tregs in relation to other immune and stromal cell types in tumors by the means of high-dimensional tissue profiling at single cell resolution, and discuss cell:cell and molecular interactions that are important to sustain hyperactive Tregs in response to checkpoint blockade.

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## The orphan nuclear receptor nr2f6 a novel regulator of cancer immune Responses

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**BACKGROUND:** The development of antibodies blocking the "inhibitory immune checkpoints" has emerged as key strategy in cancer therapies. Recently, NR2F6 (Nuclear Receptor Subfamily-2 Group-F Member-6), an orphan nuclear receptor, whose expression is highest in immune cells, has been suggested as a novel immune checkpoint, acting as a transcriptional repressor of specific cytokines relevant for tumor rejection. The exact mode of action of Nr2f6 in regulating immune responses against tumors remains still unclear. Although the role of Nr2f6 in CD8+and CD4+ T cells has been documented in cancer murine models, information is still lacking about the role of Nr2f6 in other immune cells, like dendritic cells (DCs), crucial for initiating immune responses. Based on this evidence this study aimed at evaluating the role of Nr2f6 in DCs and to identify new small molecules targeting this orphan nuclear receptor.
**METHODS:** Dendritic cells were isolated from C57BL/6 wild-type or Nr2f6-/- mice and differentiated in vitro for 9 days. Conventional dendritic cells cDC1 and cDC2 were purified from dendritic cells culture by magnetic sorted column after incubation with a mix of biotin conjugated antibodies plus Streptavidin. Mouse CD4+ and CD8+ T cells were prepared from spleen of OT.II and OT.I mice respectively and used for in vitro crosspresentation assay. Small molecule binding to Nr2f6 was identified at TES Pharma and label-free binding assay (Enspire) has been setup as NR2F6 primary screening.
**RESULTS:** Our data clearly show that Nr2f6 is highly expressed in conventional DCs (cDCs) and mostly in the cDC1, involved in cross-presentation of tumor-associated antigens to effector cytotoxic CD8+T cells. Surprisingly, in DC/T coculture systems we found that Nr2f6 deficient cDC1 induce greater T cells proliferation than wild-type cDC1, confirming the hypothesis that the absence of Nr2f6 in cDCs reprogram these cells towards a better APC functional phenotype. Additionally, we have recently identified new small molecule inhibitor that can specifically bind to Nr2f6 protein in cell-free assays and in cell lines overexpressing Nr2f6. In particular, this new compound can increase the production of specific cytokines including IL2 and INFg, when added in vitro during CD4+T cells and CD8+ cytotoxic T cells stimulation. It is worth noting that, the cDC1 pretreatment with these compounds can promote T lymphocytes to produce high levels of the cytokine IL2.
**CONCLUSIONS:** Overall, these data reveal that Nr2f6 may have critical implications in regulating the function not only of T cells but also of selected DC subsets. Moreover, these data suggest that innovative small molecules, targeting Nr2f6 may be capable to boost both T and DCs functions, resulting in potentiation of anticancer immune responses.

## Linoleic acid regulates CD8+ T cell metabolic fitness and anti-tumor immunity.

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Adoptive T cell therapy (ACT) has achieved unprecedented clinical results in the treatment of cancer, but scarce intra-tumor infiltration, persistence and function of adoptively transferred T cells limit its efficacy, especially in solid tumors. Metabolic constrains imposed by the tumor microenvironment (TME) greatly influence the success of immune-based therapies. A common metabolic alteration in the TME is lipid accumulation, a feature often associated with defective anti-tumor responses. We had previously demonstrated that specific lipids harm CD8 T cell mitochondrial integrity and function, leading to exhaustion and defective anti-tumors responses. These results suggested that lipids may play a previously unappreciated role in CD8 T cell biology. However, whether all lipids are detrimental for T functions and how they regulate different fate decision remains poorly understood. Here, we identified Linoleic Acid (LA) as a major positive regulator of CTL activity.
LA endows CTL with an improved metabolic fitness and redirects them away from exhaustion and towards a memory-like phenotype with superior effector functions. Mechanistically, LA treatment fosters the formation of ER-mitochondria contacts and mitochondrial-associated-membranes (MAMs), which in turn promotes calcium (Ca2+) signalling, mitochondrial energetics and

CTL effector functions. As a result, LA-instructed CD8 T cells mediate superior control towards different tumour types both in vitro and in vivo following ACT on mouse models, overcoming the hustle of a highly immunosuppressive TME. Moreover, LA-instructed T cells retain a memory-like phenotype within the TME, with enduring PD-1 downregulation, improved mitochondrial function and polyfunctionality, providing evidence that LA treatment shapes CTL fate and supports a molecular switch to fine-tune memory formation and metabolic fitness, linking lipid metabolism to anti-tumour surveillance.
Our results pave the way for a new generation of adoptive T cell-based therapies, where LA can be used to during ex vivo CAR- and TCR- T cell manufacturing as novel approach to achieve metabolic reprogramming and long-term functionality, broadening the therapeutic efficacy of ACT to a wide range of malignancies (Patent No. EP 212079941).

## Novel immunogenic therapeutic combinations in anaplastic thyroid carcinoma.

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**Abstract**
Immunogenic cell death (ICD) is a form of regulated cell death triggered by different type of stressors, able to evoke tumour regression through a durable antitumor immune response. Single compounds or combination therapies have been shown to induce ICD in different tumour types.
Anaplastic thyroid carcinoma (ATC) is an aggressive malignancy of the thyroid follicular epithelium with poor overall survival despite extensive combinatorial therapies. ATC is characterized by elevated tumour mutational burden and high Programmed death ligand-1 (PD-L1) levels. Preclinical and clinical studies indicate synergism between PD-L1 monoclonal antibodies and antiangiogenic tyrosine kinase inhibitors.
We recently found that ATC cells express, besides PD-L1, Programmed death-1 (PD-1). The PD-1/PD-L1 autocrine circuit, by activating a SHP2/RAS/ERK signaling cascade, stimulates ATC cell proliferation, migration and xenograft growth; accordingly, PD-1 blockade by Nivolumab, or SHP2 inhibition by SHP099, reverted these effects. We also showed that ATC is characterized by expression of the interleukin-8 (IL-8)/CXCR1/2 circuit, that promotes proliferation, survival, stemness, motility, and tumorigenicity of tumoral cells. Reparixin, a CXCR1/2 inhibitor, repressed these IL-8-mediated activities.
Here, we wish to identify novel therapies capable of inducing ICD in ATC. To this aim, we tried different combinations of Reparixin, Nivolumab and SHP099, and we found that these treatments significantly reduced human and mouse ATC cell viability and induced apoptosis. Notably, cell death was characterized by ICD hallmarks, including, eif2α phosphorylation, cell surface calreticulin (CRT) exposure, ANXA1 and CXCL10 protein increase, ATP and HMGB1 cell release in the extracellular space. We plan to use syngeneic mice models of ATC to verify if these drug combinations can induce in vivo ICD, tumor regression and durable immunity against ATC.

## ETV7 – a novel regulator of breast cancer immunity

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ETV7 is an oncogene known to be up-regulated in different types of cancer. In breast cancer (BC), increased expression of ETV7 correlates with the aggressiveness of cancer. Previously, we demonstrated that in BC, ETV7 is up-regulated upon the treatment with various DNA damaging drugs and that this increased expression promotes resistance to chemotherapy. Interestingly, ETV7 is also an interferon-stimulated gene (ISG). Recently, it has been demonstrated that ETV7 represses several ISGs and hence negatively regulates the IFN-mediated control of influenza viruses, confirming that ETV7 is a suppressor of the type I IFN response in mammalian cells. Type I interferons are critical regulators of tumor-immune system interaction; hence, in this study, we are investigating the role of ETV7 in BC immunity and propose ETV7 as a novel reg-

ulator of the immune response. Gene ontology analyses of the RNA-seq data from MCF7 and T47D BC cells over-expressing ETV7 vs. Empty vector showed that ETV7 represses pro-inflammatory and Interferon-stimulated genes. After confirming the down-regulation of these putative ETV7 target genes, we focused on unveiling the potential biological consequences. Firstly, we focused on the Interferon signaling pathway, and we demonstrated that the ETV7-mediated repression of the ISGs could lead to an increased subpopulation of breast cancer stem cells (BCSC). We confirmed that upon ETV7 over-expression, there was a significant increase in the population of CD44+/CD24- cells and in the protein levels of EpCAM (representing typical markers of BCSC). Noteworthy, we were able to consistently observe these effects both in MCF7 and T47D cells over-expressing ETV7. Furthermore, we demonstrated a significant increase in the mammosphere formation efficiency of MCF7 cells over-expressing ETV7, which even further confirmed that ETV7 regulates BCSC features. In the second part of this study, we focused on the roles of ETV7 in the inflammatory processes. We demonstrated that ETV7 directly represses TNFRSF1A (encoding for TNFR1) by binding to its Intron I. This ETV7-mediated repression of TNFRSF1A leads to reduced activation of the NF-κB pathway and consecutively the reduced expression of pro-inflammatory genes. The repression of ISGs, as well as the reduced activation of NF-κB, is known to cause the down-regulation of antigen-presenting – one of the primary mechanisms of immune evasion in cancer.

Here we present two novel ETV7-regulated molecular mechanisms involved in immune response in BC. Firstly, we propose an unknown role for ETV7 as a regulator of breast cancer stem cell-like plasticity, which is mediated by the repression of a panel of ISGs. Secondly, we show that ETV7 directly down-regulates TNFRSF1A, reducing the activation of NF-$_{\kappa}$B signaling. Both mechanisms suggest that ETV7 can modulate tumor immunity and potentially promote immune evasion.

## Interferon αβ-receptor subunit 1 (IFNAR1) stabilization at hepatic metastatic site

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**Background**

Liver metastases from colorectal cancer (CRC) are the second leading cause of cancer-related deaths worldwide. Therefore, new therapeutic approaches are highly needed. Type I IFNs contribute to anti-tumor immunity by stimulating specific CD8+ DCs to cross present antigens to cytotoxic CD8+ T cells (CTLs) and by providing a "third signal" to stimulate clonal expansion of tumor-specific CTLs. However, tumors in mice and humans activate immune escape mechanisms that target the type I IFNs signaling pathway. Indeed, down regulation of IFNAR1 by components of CRC TME reduces the viability and accumulation of CTLs within CRC tumors, establishing an immune-privileged niche. Mechanistically, degradation of IFNAR1 on the cell surface follows ubiquitination by a specific E3 ligase that binds to phosphorylated Ser526 IFNAR1 in mice, or Ser535 in humans. Importantly, a genetic variant of IFNAR1 with a single Ala substitution of Ser526 or Ser535 (IFNAR1SA), renders cell surface IFNAR1 undegradable and restores tumor-specific CTL viability, accumulation within CRC tumors and efficacy of ACT with TCR redirected and CAR-T cells. It is currently unknown whether this pathway is also deregulated in liver CRC metastases and what are the cellular and molecular drivers of this deregulation.

**Methods**

Using human and murine models of CRC liver metastases as well as human liver CRC metastasis samples, we tested the hypothesis that cells of the hepatic CRC metastatic microenvironment deregulate IFNAR1 and that its stabilization may restore tumor specific CTL viability, accumulation within CRC tumors and efficacy of adoptive cell therapies (ACT).

**Results**

We first confirmed that IFNAR1 is downregulated in primary human CRC lesions and in the corresponding synchronous liver lesions of a cohort of patients undergoing combined surgery for CRC and liver metastases.

To define the liver microenvironmental clues associated with IFNAR1 downregulation in the liver we monitored a panel of different genes associated with immune-privileged niches in murine or human CRC tumor organoids with increasing volume. We found that several type I IFN subtypes are upregulated in metastatic liver lesions and this is associated with increased

numbers of interferon-regulated genes (IRGs), checkpoint inhibitors, inflammatory cytokines, and genes associated with the IFNAR1 degradation machinery, which is typical of an immunosuppressive microenvironment. To elucidate the cellular source of type I interferons, we examined various MSI and MSS CRC mouse cell lines and tumor organoids (MTO) and found that CRCs express the same type I IFN subtypes in vitro.
We then obtained data showing that pharmacological stabilization of IFNAR1 by p38/PDK inhibitors reduced tumor burden and improved survival in mice with CRC liver metastases. Independent experiments using anti-tumor T cells showed that CD8+ T cells with a non-degradable IFNAR1 significantly reduced tumor burden. Mechanistically, IFNAR1 stabilization reversed the immune deregulation associated with intrahepatic tumor growth by promoting infiltration, persistence, and anti-tumor effector functions of CD8+ T cells and reducing multiple populations of tumor-infiltrating leukocytes with immunoregulatory properties. Finally, we developed a continuous delivery strategy to administer recombinant type I IFN molecules with different affinities for IFNAR1 (IFNα1 and IFNα11) to mice with established intrahepatic CRC tumors. While the use of these molecules reduces intrahepatic tumor growth to some degree, it does not significantly improve survival. Finally, we are in the process of defining whether continuous IFNα therapy and IFNAR1 stabilization strategies will improve therapeutic outcome, a necessary step toward clinical translation.

**Conclusions**

Stabilization of IFNAR1 in liver CRC metastases represents a promising new therapeutic approach to improve immunotherapies.

## GZMK-CD8+ T effector memory cells are associated with CD15high neutrophil abundance in early-stage colorectal tumors and predict poor clinical outcome.

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**Equal contribution.*

Tumor contexture has emerged as a major prognostic determinant and tumor infiltrating CD8+ T cells have been associated with a better prognosis in several solid tumors, including early-stage colorectal cancer (CRC). However, the tumor immune infiltrate is highly heterogeneous and understanding how the interplay between different immune cells impacts on the clinical outcome is still in its infancy. Here, we describe on a prospective cohort the interaction between a CD8+ T effector memory (TEM) population and tumor infiltrating neutrophils expressing high level of CD15 (CD15high). The interaction with neutrophils promotes Granzyme K (GZMK) production in TEM and a gene signature defining GZMK-TEM associates with worse prognosis on a larger independent cohort of CRC and lung cancers from TCGA. Thus, we provide both in vitro and in vivo evidence of the role of stromal cell-derived factor 1 (CXCL12/SDF-1) in driving functional changes on neutrophils at the tumor site, promoting their retention and increasing the crosstalk with CD8+ T cells. Mechanistically, as a consequence of their interaction with neutrophils, CD8+ T cells are skewed to produce high levels of GZMK, which in turn decreases E-cadherin pathway leading to tumor progression both in mice and CRC patients. Overall, our results highlight the emergence of GZMK-TEM in resectable not-metastatic CRC tumors as a hallmark driven by the interaction with neutrophils, which could implement current patient stratification and be targeted by novel immune-based therapeutics.

## Circulating and tumour infiltrating NK cells acquire the decidual-like CD9+CD49a+ phenotype in pancreatic ductal adenocarcinoma: reversion by anti-fibrotic treatments.

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Pancreatic ductal adenocarcinoma (PDAC), representing the 90% of pancreatic cancers, accounts as the fourth leading cause of cancer-related deaths worldwide. During PDAC progression, the microenvironment (TME) undergoes a dynamic remodelling at both cellular and molecular level, acquiring tumour-promoting properties. A fibrotic/desmoplastic stroma represents a peculiar hallmark of cancer that affects therapy efficacy and immune cell infiltration/anti-tumor functions. Here, we have characterized circulating and tumour infiltrating NK cells in PDAC patients and murine models and evaluated the effects of the anti-fibrotic agent Galunisertib (GAL) on PDAC TME and NK cell polarization. NK polarization was characterized by multicolor flow cytometry comparing healthy donor-derived NK cells exposed to PDAC conditioned medium (CM) or control medium, NK cells isolated from peripheral blood or tumour tissues of PDAC patients, as compared to those from age-matched healthy subjects. FC1199 cells derived from LSL-KrasG12D/+;LSLTrp53R172H/+; Pdx-1-Cre mice were orthotopically transplanted in C57BL/6 mice. The effect of GAL treatment (75mg/Kg twice daily p.o. starting from day 11) on tumour growth, fibrosis and NK cell polarization was investigated. We found that both circulating, and tissue-infiltrating NK cells acquire the anergic and proangiogenic/ decidual like CD56britghtCD9+CD49a+ phenotype. The same polarization was observed in circulating NK cells from healthy donors, exposed for 72 hours to tumour CM. We also found that NK cell isolated from the peripheral blood and tumour tissue of FC1199 bearing mice had increased expression of CD9 and CD49a surface antigens. Also, NK cells isolated from FC1199 bearing mice had higher expression of the pro angiogenic factors VEGF, PlGF, MMP-10 and IL-10, than NK cells from control mice. GAL treatment reduced the expression of pro-inflammatory/pro angiogenic cytokines (IL-1b, IL-6, CXCL8/IL-8), pro fibrotic factors (TGFb and IL-17a) and Stat3, while increasing the expression of anti-tumour factors (Granzyme-B and IFNg) in the tumour bulk. GAL was effective in reducing the frequency of CD9+CD49a+ NK cells. Our results show that circulating and tumour infiltrating NK cells acquire a decidual-like phenotype in PDAC patients and in vivo models of PDAC and that anti-fibrotic treatment could be envisaged as a potential strategy to restore an anti-tumours PDAC TME, also acting of NK cell polarization state.

## Macrophage-mediated melanoma reduction after HP-NAP treatment in a zebrafish xenograft model

*Nicole Papa (IRCCS Istituto Oncologico Veneto), Padova*

The Helicobacter Pylori Neutrophil Activating Protein (HP-NAP) is endowed with immunomodulatory properties that make it a potential candidate for anticancer therapeutic applications. By activating cytotoxic Th1 responses, HP-NAP inhibits the growth of bladder cancer and enhances the anti-tumor activity of oncolytic viruses in the treatment of metastatic breast cancer and neuroendocrine tumors. The possibility that HP-NAP exerts its anti-tumor effect also by modulating the activity of innate immune cells has not yet been explored. Taking advantage of the zebrafish model, we examined the therapeutic efficacy of HP-NAP against metastatic human melanoma, limiting the observational window to 9 days post-fertilization, well before the maturation of the adaptive immunity. Human melanoma cells were xenotransplanted into zebrafish embryos and tracked in the presence or absence of HP-NAP. The behavior and phenotype of macrophages and the impact of their drug-induced depletion were analyzed exploiting macrophage-expressed transgenes. HP-NAP administration efficiently inhibited tumor growth and metastasis and this was accompanied by strong recruitment of macrophages with a proinflammatory profile at the tumor site. The depletion of macrophages almost completely abrogated the ability of HP-NAP to counteract tumor growth. Our findings highlight the pivotal role of

activated macrophages in counteracting melanoma growth and support the notion that HP-NAP might become a new biological therapeutic agent for the treatment of metastatic melanomas.

## Teneurin-4 as valuable immune-target and biomarker in triple negative breast cancer.

*Giulia Peppino[1], Roberto Ruiu[1], Maddalena Arigoni[1], Federica Riccardo[1], Elisabetta Bolli[1], Laura Conti[1], Giuseppina Barutello[1]*, Elena Quaglino[1]**

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Triple negative breast cancer (TNBC) is one of the most aggressive types of human breast cancer with a high incidence of recurrences and metastasis. This is attributable to the lack of targetable molecules and to the low efficacy of current therapies to eliminate cancer stem cells (CSC). Therefore, the aim of this work is to find a novel therapeutic target associated to TNBC stem cells suitable for immunotherapy.
Teneurin-4 (TENM4) was selected among the overexpressed transmembrane proteins in murine and human TNBC stem cells-enriched tumorspheres. Its silencing, through specific siRNA, significantly impairs the tumorsphere forming potential and cell ability to migrate, accompanied by a significant decrease in FAK, ERK and AKT phosphorylation. Similar results were also observed by using TENM4-KO and TENM4-Sh cells. This suggests a role of TENM4 in cancer stem-like features and metastatic potential. Indeed, while no differences in the tumor take and growth was observed between TENM4-KO and TENM4-WT cells, a significantly lower number of lung metastasis was found in TENM4-KO tumor-bearing mice.
Interestingly, we detected a higher amount of TENM4 in the blood of tumor-bearing mice and of some breast cancer patients, as well as in the exosomes from cancer cell supernatants and from breast cancer patients' plasma, as compared to healthy donors, suggesting its potential use also as biomarker. Finally, DNA-based vaccination of BALB/c mice with plasmids coding for either mouse or human TENM4 resulted safe and effective in the induction of an anti-TENM4 antibody response as well as a specific T-cell response. Future experiments will be held to evaluate the impact of anti-TENM4 vaccination on TNBC tumorigenesis.
Overall, these data identify TENM4 as overexpressed in TNBC stem cells with a role in stemness and metastatic potential. Moreover, the presence of TENM4 in the supernatant of TNBC cells and in the sera of breast cancer patients, suggest its potential use as biomarker. Furthermore, the safety and the immunogenicity of the vaccination against TENM4 point out the possibility to use TENM4 as a valuable immune-target in TNBC.

## Insight of the role of epigenetically regulated endogenous retroviruses (ERVs) in senescence

*Liliana Ranzino, E. Di Giorgio, E. Dalla, C. Brancolini*
*Department of Medicine, University of Udine.*

In several models of cellular senescence, we observed upregulation of endogenous retroviruses (ERVs) expression, which are mainly folded as dsRNA, as shown by immunofluorescence assay. We also found that genomic regions undergoing H3K27me3 demethylation during a model of oncogene-induced senescence in leiomyosarcoma cells promote ERVs expression. The same ERVs are also regulated during RAS -induced senescence and during replicative senescence.
Retinoic acid-inducible gene I (RIG -I)-like receptors (RLRs) are cytoplasmic RNA-sensing molecules that play a key role in the cellular antiviral immune response against invading viral RNAs. This class of pattern recognition receptors (PRRs) mainly includes RIG -I and MDA5 (melanoma differentiation-associated gene 5), which can recognize viral double-stranded (ds) RNA. In the RLR signaling axis, a common adaptor protein is the mitochondrial antiviral signaling protein (MAVS), which initiates the type I antiviral interferon response. These cytosolic immunosensors can also be activated by ERVs. Their activation can promote proliferation arrest, senescence, and apoptosis. To better understand the involvement of ERVs in senescence, we focused on the RLRs pathway. To this end, we knocked-down the expression of endogenous RIG-1, MDA5, or MAVS in RAS-induced senescence cell models using siRNA. Interestingly, we found that knockdown of MDA5 or MAVS greatly reduced SA-β-gal positivity and promoted an increase in BrdU-positive cells. On the other hand, depletion of RIG-1 was unable to counteract senescence in the RAS-dependent model of OIS, as shown by the SA-β-gal and BrdU assays. These preliminary data revealed a different biological response between MDA5 and RIG-1, which

might be related to the altered sensitivity of the two intracellular receptors to different types of ERVs.
To better clarify this point, we are conducting further experiments using RAS cellular inducible models of senescence in which we will stably knock-down the expression of RIG-1, MAVS and MDA5. An exciting new prospect arising from these findings is the use of combination therapies, such as senolytics plus dsRNA mimics, for the treatment of early-stage malignancies.

## The alarmin-like RNASET2 proteins impacts on prostate cancer (PCa) cell proliferation and inflammatory properties and shape macrophage polarization in a prostate cancer (PCa) in vivo model

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Molecules enabling tumour cells to become visible by the immune system represent a promising tool in tumour immunology. In this context, alarmins (stress-induced secreted molecules of endogenous origins acting as early warning signals for the immune system) represent a relevant example. RNASET2, a highly conserved human extracellular RNase endowed with a powerful oncosuppressive activity, has been reported to behave as an alarmin-like factor. Here, we tested the activity of RNASET2 overexpression in vitro and in vivo, together with the subsequent readout on macrophage polarization, in PCa models. RNASET2-overexpressing PC- 3 and 22Rv1 prostate cancer cell lines, generated by transfection with a RNASET2 expression vector or a control empty plasmid, were investigated to assess cell proliferation, colony formation, adhesion, and migration, coupled to FACS analysis for factors involved in migration/invasion (CXCR4, CXCL12), angiogenesis (VEGF, CXCL8) and inflammation (TNFa, IFNg). In vivo studies were also performed in nude mice, subcutaneously injected with RNASET2-overexpressing PC-3 or 22Rv1 cells, to evaluate tumour cell growth, tumour weight and M1/M2-like macrophage infiltration. We found that RNASET2 differentially impact on PCa cell proliferation and ability to generate colonies in vitro, being more effective in 22Rv1, compared to PC-3 cells. RNASET2 expression did not impact on 22Rv1 and PC-3 cells ability to express factors involved in migration/invasion (CXCR4, CXCL12), or angiogenesis (VEGF, CXCL8); however, it induced a marked decrease in their production of pro-inflammatory cytokines (TNFa, IFNg). Moreover, RNASET2 overexpression affected the cytoskeleton organization: clustering of actin bundles was found at the cell periphery in RNASET2-overexpressing 22Rv1 cells, whereas in PC-3 cells overexpression of RNASET2 triggered a change in cell morphology characterized by a marked spreading of the cells. Finally, mice injected with RNASET2-overepressing 22Rv1 cells exhibited increased intra-tumor M1-like macrophages, while reducing M2-like macrophages, as compared to those receiving control 22Rv1 cells. The same effect was not observed with PC-3 cells, in vivo. Our results underline a cell line-specific role of RNASET2 in different PCa models as a molecule able to act both in a cell autonomous and non-cell autonomous mechanism

## Continuous sensing of IFNα by hepatic endothelial cells shapes a vascular antimetastatic barrier

*Giovanni Sitia[1], Ngoc Lan Tran[1]*, Lorena Maria Ferreira[1]*, Blanca Alvarez-Moya[1]*, Valentina Buttiglione[1], Barbara Ferrini[1], Paola Zordan[1,3], Andrea Monestiroli[1], Claudio Fagioli[1], Eugenia Bezzecchi[2], Giulia Maria Scotti[2], Antonio Esposito[3,4], Riccardo Leone[3,4], Chiara Gnasso[3,4], Andrea Brendolan[5] and Luca G. Guidotti[1,3]*

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** These authors contributed equally to this work*

**Background:** The liver is the most common site of colorectal cancer (CRC) metastases and liver CRC metastatization is a leading cause of cancer-related deaths in CRC. Thus, strategies aimed at reducing the risk of hepatic CRC colonization and minimal residual disease after surgery are of strong interest.
**Methods:** Using mouse models of CRC metastatic spreading to the liver, either through intravascular seeding in the mesenteric vein of CT26 or MC38 CRC cell lines or by orthotopically implanting in the cecal wall CT26LM3 cells that spontaneously metastasize to the liver, we tested the efficacy and mode of action of continuous IFNα therapy.
**Results:** We show that the continuous infusion of therapeutic doses of interferon-alpha (IFNα) controls CRC invasion by acting on hepatic endothelial cells (HECs). Mechanistically, IFNα promoted the development of a vascular antimetastatic niche characterized by liver sinusoidal endothelial cells (LSECs) defenestration extracellular matrix and glycocalyx deposition, thus strengthening the liver vascular barrier impairing CRC trans-sinusoidal migration, without requiring a direct action on tumor cells, hepatic stellate cells, hepatocytes, or liver dendritic cells (DCs), Kupffer cells (KCs) and liver capsular macrophages (LCMs). Moreover, IFNα endowed LSECs with efficient cross-priming potential that, along with the early intravascular tumor burden reduction, supported the generation of antitumor CD8+ T cells and ultimately led to the establishment of a protective long-term memory T cell response.
**Conclusions:** These findings provide a rationale for the use

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**32. Trento, 21-22 June 2021**
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**12. Trento, 1 - 3 June 2000**
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**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

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**2. Rovereto, 11 - 13 June 1990**
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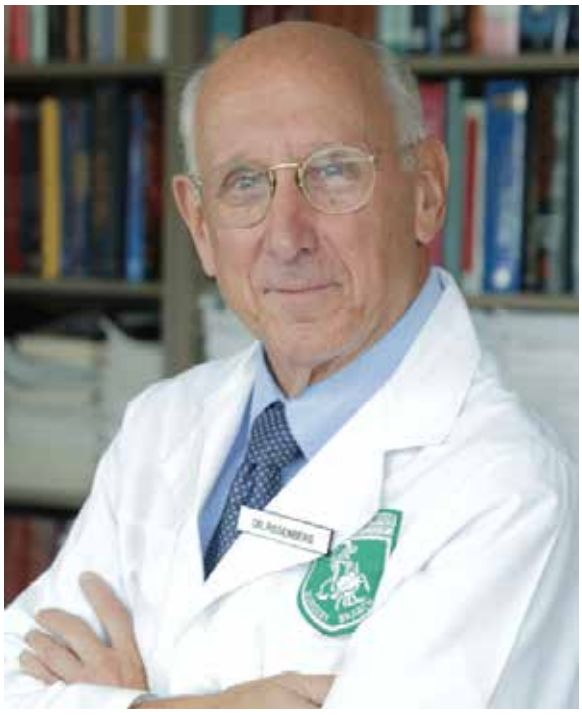
DRUG RESISTANCE: MECHANISMS AND REVERSAL

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

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

## 2022 Winner



Steven A. Rosenberg, MD, PHD

Rosenberg is a senior investigator in the Center for Cancer Research at the National Cancer Institute (NCI); Chief of the NCI Surgery Branch; and professor of surgery at the George Washington University School of Medicine and Health Sciences.

Rosenberg earned his undergraduate and medical degrees from Johns Hopkins University in 1961 and 1964, respectively. He also earned a doctorate in biophysics from Harvard University. Rosenberg completed his surgical residency at Peter Bent Brigham Hospital (now Brigham and Women's Hospital), while simultaneously completing his research fellowship in immunology. Over the course of his illustrious career, he has authored or co-authored more than 30 books and more than 1,000 peer-reviewed publications.
Rosenberg is being honored for his discovery and development of the first U.S. Food and Drug Administration (FDA)-approved immunotherapy for patients with cancer and for his contributions to effective cellular immunotherapies, including chimeric antigen receptor (CAR) T-cell therapy for patients with hematologic malignancies.


Steven Rosenberg presented an Award lecture at the AACR Annual Meeting, on April 10, 2022 in New Orleans.


Steven Rosenberg participated in an official Award ceremony in Trento, Italy, on May 14 and presented a scientific lecture at the Universities of Trento and the University of Padova (University online).

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

## Call for Nominations for the 2023 Award

**NOMINATION DEADLINE FOR 2023 AWARD**
September 15, 2022

**NOMINATION PROCESS**
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, and all nominations must be submitted electronically to https://myaacr.aacr.org. Paper nominations will not be accepted.
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 cancer field. Letter must specifically outline the candidate's current research activity and indicate how their research holds promise for continued substantive contributions to the 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
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 plaque, and present a featured scientific lecture in conjunction with the AACR Annual Meeting immediately following their selection. The award recipient will also be invited to present a featured scientific lecture at the University of Trento, in conjunction with the official award ceremony to be held in Trento, Italy in May 2023.

**ELIGIBILITY CRITERIA**
Cancer researchers affiliated with any institution involved in cancer research, cancer medicine, or cancer-related science 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.

**AWARD SELECTION**
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: www.aacr.org

# Pezcoller Foundation – EACR Awards

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

- The annual Pezcoller Foundation - EACR Translational Cancer Researcher Award, to Young European researchers
- The annual Pezcoller - Marina Larcher Fogazzaro - EACR Women in Cancer Research Award, to European Women working in cancer research
- The biannual Pezcoller Foundation - EACR Rising Star Award, to very promising, early career cancer researchers.

## Winners of the Pezcoller Foundation – EACR Awards


### 2022 Translational Cancer Researcher Award:

**Nicholas Turner**

Molecular Oncology Team at The Institute of Cancer Research, London.

### 2022 Women in Cancer Research Award:

**María S. Soengas**

Leader of the Melanoma Group at Spanish National Cancer Research Center (CNIO), Madrid.

### 2021 biannual Rising Star Award:

**Sam Behjati**

Wellcome Sanger Institute, Cambridge, UK.

The call for Nominations for the 2023 Awards, will open in Autumn 2022:
www.eacr.org

# The Pezcoller - SIC Fellowships

The Pezcoller Foundation actively promotes and supports cancer research, with particular attention to young researchers, through the Pezcoller Foundation – SIC Fellowships.

These are two-year fellowships, € 25,000/year, for Italian researchers, working in Italian institutions, awarded on a competitive basis in collaboration with the Italian Cancer Society (SIC).

The call for application, for the 2023-24 period, is open until July 1, 2022: www.pezcoller.it

Fondazione Pezcoller

SOCIETA' ITALIANA DI CANCEROLOGIA

# Fondazione Pezcoller - SIC

## Bando di concorso per 7 borse di studio

La **Fondazione Pezcoller,** con la partecipazione organizzativa della Società Italiana di Cancerologia (SIC) bandisce 7 borse di studio per giovani ricercatori con cittadinanza italiana, che abbiano conseguito una laurea specialistica in discipline biomediche e affini e che desiderino approfondire la loro già acquisita e documentabile esperienza nella ricerca oncologica. Le borse, denominate Borse **Fondazione Pezcoller-SIC**, saranno dedicate al ricordo dei singoli donatori che di volta in volta provvedono al rispettivo finanziamento.Le borse sono finalizzate al supporto di progetti di ricerca da svolgersi in Italia, presso istituti scientifici, istituti universitari o centri ospedalieri dedicati alla ricerca oncologica, che siano disponibili ad ospitare i borsisti e sostenere le spese della ricerca e che ne assicurino un'adeguata formazione professionale. L'ammontare di ogni borsa è di € 25.000,00/anno al lordo delle ritenute fiscali di legge, per la durata di un anno a decorrere dal 1° Gennaio 2023; esse saranno rinnovabili per un secondo anno previa riconfermata disponibilità dell'istituzione ospitante e valutazione dell'attività svolta, da presentare due mesi prima della scadenza annuale alla Società Italiana di Cancerologia. Le borse non sono cumulabili con alcuna forma di retribuzione continuativa, salvo eventuali integrazioni degli istituti ospitanti, a loro carico. Sono ammessi al concorso i candidati in possesso di una laurea specialistica in Biotecnologie, Chimica, Chimica e tecnologie farmaceutiche, Farmacia, Medicina e chirurgia, Scienze biologiche, Veterinaria e Discipline affini, in possesso dei seguenti requisiti:

- Età non superiore ai 35 anni (**che non abbiano compiuto i 36 anni alla data di scadenza del bando**);
- Avere conseguito un Dottorato di ricerca o una Specializzazione oppure un PhD, o avere svolto attività di ricerca documentata per almeno cinque anni dopo la laurea;
- Almeno 3 pubblicazioni scientifiche su riviste internazionali censite, di cui almeno una come primo o ultimo autore;
- Essere soci SIC, in regola con l'associazione per l'anno 2022 e impegnarsi a partecipare al Congresso SIC che eventualmente li vede coinvolti come vincitori, nel primo e nel secondo anno.

La domanda (disponibile sui siti **www.cancerologia.it** e **www.pezcoller.it**) dovrà contenere:

1. Progetto di ricerca a valenza oncologica, da cui si evinca l'impegno preponderante del proponente, previamente concordato con la struttura ospitante (massimo 6 pagine, compreso un breve riassunto, in italiano o inglese);
2. Curriculum Vitae, con relazione dell'attività scientifica svolta (massimo 2 pagine) ed elenco delle pubblicazioni, di cui almeno una come primo o ultimo autore, indicando l'Impact Factor (JCR 2021);
3. Certificato attestante il conseguimento della laurea specialistica;
4. Documentazione attestante il conseguimento del dottorato di ricerca o della specializzazione o del PhD o che certifichi l'attività di ricerca pregressa *post-lauream;*
5. Fotocopia documento attestante l'età e la cittadinanza italiana del candidato;
6. Certificazione che attesti l'accettazione del candidato e il suo inserimento nell'attività di ricerca oncologica da parte della struttura ospitante;
7. Non più di 3 pubblicazioni in extenso,utili al fine della valutazione del progetto, di cui almeno una come primo o ultimo autore (non devono essere acclusi *abstract* congressuali).

Ogni responsabile di gruppo di ricerca potrà presentare un unico candidato. La trasmissione della documentazione dovrà avvenire entro il **1° luglio 2022** con la seguente modalità:

- Invio all'indirizzo mail sic@istitutotumori.mi.it di un file in formato pdf contenente la domanda e gli allegati dall'1 al 6 e un file formato pdf contenente non più di 3 pubblicazioni utili al fine della valutazione (allegato 7);

Le domande dei candidati saranno vagliate da un'apposita Commissione il cui giudizio è insindacabile. Ai candidati vincenti verrà data comunicazione entro il **17 ottobre 2022.**
Le borse saranno assegnate ufficialmente nel corso del 62° Congresso della Società Italiana di Cancerologia, che si terrà a Venezia nei giorni 16-18 novembre 2022, al quale i vincitori dovranno essere presenti pena la rinuncia alla borsa.
Al termine del biennio i ricercatori saranno invitati a presentare, nel corso di un Congresso SIC, una relazione sul lavoro svolto.

***Fondazione Pezcoller***
Il Presidente
*Dr. Enzo Galligioni*

***Società Italiana Cancerologia***
Il Presidente
*Dr. Nicola Normanno*

*Trento, 6 aprile 2022*

Fondazione Pezcoller

SOCIETA' ITALIANA DI CANCEROLOGIA

La **FONDAZIONE PROF. DOTT. ALESSIO PEZCOLLER** - ente senza fini di lucro voluta dal Prof. Alessio Pezcoller (1896 - 1993) già Primario dell'Ospedale S. Chiara di Trento - ha come fine istituzionale la promozione della ricerca scientifica nella lotta alle malattie che affliggono l'umanità e specificatamente al cancro.
La Fondazione Pezcoller provvede al perseguimento dei propri fini mediante lo svolgimento di varie attività

- **Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research**
  Premio annuale internazionale alla ricerca sul cancro, in collaborazione con l'Associazione Americana per la Ricerca sul Cancro (AACR), di € 75.000. Il vincitore viene presentato all'Annual Meeting AACR, dove tiene la Pezcoller Award Lecture.
- **Pezcoller Foundation-EACR Awards**
  - **Translational Cancer Researcher Award,** premio annuale di € 10.000.
  - **Women in Cancer Research Award,** premio annuale di € 10.000.
  - **Rising Star Award**, premio biennale di € 5.000.

  I vincitori di ciascun premio verranno presentati al congresso annuale della Associazione Europea per la Ricerca sul Cancro (EACR) dove terranno la Pezcoller Award Lecture.
- **Pezcoller Symposia**
  Serie ininterrotta da 33 anni di incontri scientifici con ricercatori di fama internazionale su temi all'avanguardia della ricerca sul cancro.
- **Seminari Pezcoller**
  Incontri di aggiornamento professionale per clinici o ricercatori di base, in ambito prevalentemente regionale o macroregionale.
- **Borse di Studio/di Ricerca**
  Per il finanziamento, su base competitiva, di programmi di ricerca biennali per giovani ricercatori italiani.
- **Borse di Aggiornamento**
  per il supporto all'aggiornamento in campo oncologico.

## Per maggiori informazioni rivolgersi a:

SIC Società Italiana Cancerologia
Via Venezian 1 - 20133 Milano
Tel. 02 2666895 – Fax 02 2664342
sic@istitutotumori.mi.it
www.cancerologia.it

The Pezcoller Foundation

FONDAZIONE CARITRO
CASSA DI RISPARMIO DI TRENTO E ROVERETO

# Journal

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