The Pezcoller Foundation

# Journal

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- 30th Pezcoller Symposium:
  Program
  Abstracts of oral presentations
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- Call for nomination 2019 Pezcoller Foundation-AACR International Award for Extraordinary Achivement in Cancer Research
- Call for nomination Scholar-In-Training Awards



News from the
Pezcoller Foundation Word

Year 28 – No. 50
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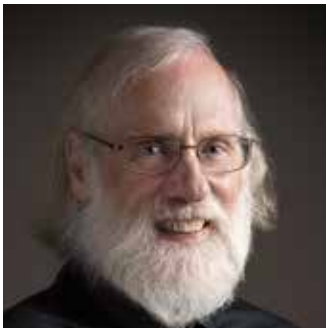
*Picture on front page:*
*2018 Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research in the Teatro Sociale of Trento*

*From the left: President Emeritus Gios Bernardi - Pier Paolo Pandolfi Chairman of the Selection Committee - President Enzo Galligioni - Tony Hunter winner -Margaret Foti CEO AACR and Michael Caligiuri Past President AACR*

# June 2018
# Editorial

It's with a great pleasure that we report that the recipient of the 2018 Pezcoller Foundation - AACR International Award for Extraordinary Achievement in Cancer Research is Anthony Rex Hunter, Fellow of the AACR Academy, American Cancer Society professor, Renato Dulbecco Chair and professor at the Salk Institute for Biological Studies, University of California, San Diego.
The Selection Committee who designated the winner met in Philadelphia on December 1st 2017 and was chaired by Pier Paolo Pandolfi.
Members of the Committee were Pier Paolo Pandolfi MD PhD ( Chair) Director Cancer Center Beth Israel Deaconess Medical Center Boston, Carlos L. Arteaga MD FAACR Director Center for Cancer Targeted Therapies and Breast Cancer Program Vanderbilt-Ingram Cancer Center Nashville, Francis R. Balkwill PhD Centre Lead Barts Cancer Institute London, Michael B. Kastan MD PhD FAACR Executive Director William & Jane Shingleton Duke Cancer Institute Durham NC, Elaine Mardis PhD Co-Director and Professor of Pediatrics Nationwide Children's Hospital Columbus OH, Cornelia Polyak MD PhD Department of Medical Oncology Dana Farber Cancer Institute Boston, Stefano Piccolo PhD Professor of Molecular Biology University of Padua, Verda Rotter PhD Professor and Chair Department of Molecular and Cellular Biology Weizmann Institute of Science Rehovot Israel, Charles Swanton MD The Francis Crick Institute and UCL Cancer Institute London.



*Tony Hunter*

Tony Hunter, PhD, is honored for his significant discovery of tyrosine kinases. This pioneering work has led to proven success in the usage of cancer chemotherapeutics that target disease-causing tyrosine kinases. Furthermore, Dr. Hunter is credited with being the first to demonstrate that dysregulated tyrosine phosphorylation by activated tyrosine kinases, such as the viral Src protein, can cause malignant transformation.
Dr. Hunter's highly regarded work has led to the discovery and development of many effective drugs targeting aberrant kinase signaling in cancer cells.

Dr. Hunter has presented the Pezcoller Lecture "Protein phosphorylation: pancreatic cancer and new frontiers in histidine phosphorylation "at the McCormick Place South Convention Center in Chicago during the AACR Annual Meeting on April 15.
On Thursday May 17 dr. Hunter gave his lecture at the University of Padova Aula Magna "A.Vallisneri" while he gave another lecture at the University of Trento on Friday May 18 at the Centre for Integrative Biology.
The award was solemnly given on May 19 in the Teatro Sociale of Trento introduced by the President of the Pezcoller Foundation Enzo Galligioni with the participation of Michael Caligiuri past President of the AACR, Margaret Foti CEO of the AACR and Gios Bernardi President Emeritus.

In this issue of the Journal we have the pleasure of remembering a few important anniversaries of the Pezcoller Foundation.
For the past thirty years the Pezcoller Foundation has been committed to supporting Cancer research.
In 1988, thirty years ago, in the presence of the founder prof. Alessio Pezcoller, the First Award was given to Vincent de Vita for his studies on the chemotherapy of malignant lymphomas. Also in 1988 the first Pezcoller Symposium was organized in Trento, entitled " Drug Resistance: Mechanisms and Reversal "
Twenty-five years after his death, in January 1993, we remember with gratitude our founder prof. Alessio Pezcoller
We also recall that on April 13, 1987 the agreement between the Pezcoller Foundation and the AACR was stipulated for the assignment of the "Pezcoller Foundation-AACR International Award for Cancer Research" agreement which has been renewed this year also with the modification of the Award title in "Pezcoller Foundation-AACR International Award for Extraordinary Achievements in Cancer Research"
This year we celebrate the 30th Pezcoller Symposium in Trento on June 25th and 26th entitled: "Overcoming the innate resistance of cancer to therapy" with the extraordinary Keynote Enrico Mihich Lecture by Harold Varmus Nobel Laureate 1989.

Gios Bernardi M.D.
Editor and President Emeritus

## 30th Pezcoller Symposium

# OVERCOMING THE INNATE RESISTANCE OF CANCER TO THERAPY

## Trento, Italy • June 25 - 26, 2018

## PROGRAM

### MONDAY JUNE 25, 2018

**8.00 Registration**

**8.35 Enzo Galligioni Welcome**

**8.45 David Livingston Focus & Goals**

08.55 **The Enrico Mihich Lecture**
Harold Varmus
*Pezcoller at 30: Cancer Research, Then and Now*
09.35 Discussion

**Session 1, Pancreas Cancer**
Chairman: Alberto Bardelli

09.50 David Tuveson
*Improving pancreatic cancer response to therapy*
10.15 Christine Iacobuzio-Donahue
*Evolutionary dynamics of cancer*
10.40 Discussion led by Giampaolo Tortora and Davide Melisi
11.20 Coffee Break

**Session 2, Leukemia**
Chairman: Alberto Bardelli

11.30 Brunangelo Falini – Enrico Tiacci
*BRAF inhibition in hairy cell leukemia: biological and clinical aspects*
11.55 Pier Paolo Pandolfi
*Modeling resistance identifies vulnerabilities and effective combination therapy in mIDH AML*
12.20 Discussion led by Pier Paolo Pandolfi
13.00 Lunch

**Session 3, Prostate Cancer**
Chairman: Pier Paolo Pandolfi

14.00 Charles Sawyers
*Cancer drug resistance*
14.25 Massimo Loda
*Effect of the microenvironment on prostate cancer biologic and clinical behavior*
14.50 Discussion led by Francesca Demichelis and Sergio Bracarda
15.30 Poster Session
16.30 Adjourn

18.00 visit to Mezzacorona Winery (shuttle transportation provided)
20.00 Symposium Dinner at Mezzacorona Winery

### TUESDAY JUNE 26, 2018

**Session 4 - Therapeutically resistant Melanoma**
Chairman: Stefano Piccolo

08.30 David Fisher
*Melanoma: clues to therapeutic efficacy from disease pathogenesis*
08.55 Ugur Sahin
*Personalized cancer immunotherapy*
09.20 Discussion led by Alberto Mantovani and Mario Colombo

**Session 5 - Glioblastoma**
Chairman: Stefano Piccolo

10.00 Peter Dirks
*Dissecting brain tumour cell fate with functional approaches*
10.25 Ingo Mellinghoff
*Targeting aberrant signaling in primary brain tumors*
10.50 Discussion led by Felice Giangaspero
11.30 Coffee Break

**Session 6 - Kidney Cancer**
Chairman: Massimo Loda

11.40 William Kaelin
*New models and treatments for clear cell renal cell carcinoma*
12.05 John Josey
*Inhibiting the transcriptional factor HIF-2α:treatment of renal cell carcinoma and VHL disease*
12.30 Discussion led by William Kaelin and John Josey
13.10 Lunch

**Session 7 - Mesothelioma and Small Cell Lung Cancer**
Chairman: Alberto Bardelli

14.10 Anton Berns
*Mouse models of thoracic cancers. Phenotypic consequences of their cell-of-origin*
14.35 John Poirier
*Molecular mechanisms of acquired chemoresistance in small cell lung cancer*
15.00 Discussion led by Alberto Mantovani
15.40 Poster Discussion and Poster Presentation led by Massimo Loda
16.40 David Livingston
Concluding Remarks

## INVITED PARTECIPANTS

### FACULTY

- **Bardelli Alberto** Candiolo Cancer Institute, University of Torino, Italy
- **Berns Anton** Netherland Cancer Institute, Amsterdam, Holland
- **Dirks Peter** The Hospital for Sick Children, Toronto, Ontario, Canada
- **Falini Brunangelo** Institute of Hematology, University of Perugia, Italy
- **Fisher David** Massachusetts General Hospital, Charlestown, MA
- **Iacobuzio-Donahue Christine** Memorial Sloan Kettering Cancer Center, New York, NY
- **Josey John** Peloton Therapeutics Inc, Dallas, TX
- **Kaelin G. William** Dana-Farber Cancer Institute, Boston, MA
- **Loda Massimo** Dana-Farber Cancer Institute, Boston, MA
- **Mellinghoff Ingo** Memorial Sloan Kettering Cancer Center, New York, NY
- **Livingston David** Dana-Farber Cancer Institute, Boston, MA
- **Pandolfi Pier Paolo** Cancer Center, Beth Israel Deaconness Medical Center, Boston, MA
- **Piccolo Stefano** Molecular Medicine Department, University of Padova, Italy
- **Poirier John T.** Memorial Sloan Kettering Cancer Center, New York, NY
- **Sahin Ugur** BioNTech AG, University of Mainz, Germany
- **Sawyers Charles** Memorial Sloan Kettering Cancer Center, New York, NY
- **Tiacci Enrico** Hematology Department, University of Perugia, Italy
- **Tuveson David** Cancer Center Cold Spring Harbor Lab, Cold Spring, NY
- **Varmus Harold** Weill Cornell Medicine Meyer Cancer Center, New York, NY

### DISCUSSANTS

- **Bracarda Sergio** Medical Oncology Department, General Hospital, Arezzo, Italy
- **Colombo Mario** Molecular Immunology, Fondazione Istituto Nazionale dei Tumori, Milan, Italy
- **Demichelis Francesca** Centre for Integrative Biology, University of Trento, Italy
- **Giangaspero Felice** Policlinico Umberto I, Università La Sapienza Roma, Italy
- **Mantovani Alberto** Humanitas University, Milan, Italy
- **Melisi Davide** Medical Oncology, University of Verona, Italy
- **Tortora Giampaolo** Medical Oncology, University of Verona, Italy

30th Pezcoller Symposium

# Overcoming the innate resistance of cancer to therapy

Trento, Italy, June 25-26, 2018

## ABSTRACTS OF ORAL PRESENTATIONS

Enrico Mihich Lecture

### Pezcoller at 30: Cancer Research Then and Now.

*Harold Varmus, MD*

*Lewis Thomas University Professor, Weill Cornell Medicine;*
*Senior Associate Member, New York Genome Center*

To commemorate the long history of the Pezcoller meetings, I plan to take a long view. I will reflect on the manner in which technical advances have enabled the cancer research community to approach old questions in new ways, building on information acquired with technologies that date back to days before the Pezcoller meetings and envisioning answers that are emerging from newer methods to improve our understanding and control of cancer. To illustrate these themes, I will talk briefly about three projects that are currently being pursued in my laboratory:

(i) The search for mutant genes that drive carcinogenesis has been made both easier and more complex by the application of Next Generation Sequencing (NGS) methods. But some of the implicated mutations, such as those affecting genes that encode parts of the RNA splicing machinery, have been very difficult to understand functionally. I will summarize how we have attempted (with only limited success) to address these issues through work with transcriptomes, cancer cell lines, gene editing, and mouse models.

(ii) The complex patterns of mutations observed in individual cancers with NGS methods have some unusual features---such as mutual genetic exclusivity---that invite efforts to understand their functional significance. For instance, we have recently found that mutations in the KRAS and EGFR proto-oncogenes do not appear in the same lung adenocarcinomas because the combination of these two common mutations is cytotoxic. By attempting to understand how mutations in the EGFR-KRAS signaling pathway affect "downstream" kinases, like ERK, and by looking for feedback mechanisms that regulate such kinases, it may be possible to generate new therapeutic approaches for certain cancers.

(iii) Cancer genomics teaches us that mutations In certain genes are more likely to initiate tumors in specific cell lineages, providing insights into neoplasia that were impossible when carcinogenic events were studied mainly in cultured animal fibroblasts. We have, for example, recently learned to induce pulmonary neuro-endocrine cells (PNECs), the proposed precursors to human small cell lung cancers (SCLC), from human embryonic stem cells (hESCs) by inhibiting maturation of NOTCH receptors. We can then make PNECs oncogenic by simulating the most common mutations in SCLC: inactivation of two tumor suppressor genes, RB and TP53. I will discuss how this approach might be applied in several situations to improve our understanding of early events in human carcinogenesis.

### Improving Pancreatic Cancer Responses to Therapy

*David Tuveson MD, PhD*

*Cancer Center Cold Spring Harbor Lab, New York, NY*

Pancreatic ductal adenocarcinoma (PDAC) is almost uniformly lethal, and surgical resection

of localized tumors followed by adjuvant chemotherapy is currently the only curative regimen. Unfortunately, most patients are diagnosed with advanced and surgically unresectable PDAC, due to a lack of early detection methods. Furthermore, such patients oftentimes have a rapid disease course due to the ineffectiveness of therapies. We developed mouse and organoid models of PDAC to explore the biological aggressiveness of PDAC and to address these clinical challenges. Accordingly, we assembled a large collection of patient derived PDAC organoids, and determined that they accurately represented the primary tumors from which they were derived. We find that the combination of therapeutic testing and molecular analyses identifies patients who are more prone to respond to available therapeutics. Furthermore, our chemo-sensitivity signatures predict clinical outcomes in PDAC patients who were retrospectively assessed, prompting a further investigation of organoid profiling as a means to prospectively increase survival in patients.

Tiriac et al, Cancer Discovery 2018, in press

## Evolutionary dynamics of cancer

*Iacobuzio-Donahue Christine*

*Memorial Sloan Kettering Cancer Center, New York, NY*

PDA is the most common neoplasm of the pancreas, and is soon to be the second most common cause of cancer deaths in the United States. Surgical resection in Stage I/II patients provides the only opportunity for cure, yet >80% of patients will recur and die of their disease within 2-3 years. The statistics for Stage III and Stage IV PDA are more dismal, having 12 and 6 month median overall survival times, respectively. Outside of BRCA2 mutations that confer sensitivity to platinum salts or PARP inhibition, or immune checkpoint inhibitors in rare patients with mismatch repair deficiency, there are few actionable targets in the PDA genome. Thus, it is essential that novel strategies are developed to extend survival. To do so it is first imperative that we develop a deeper understanding of PDA evolutionary biology in a stage and context dependent manner. In this presentation we will focus on two questions with clear mechanistic and translational relevance to this goal. First, what are the features of clinically relevant intratumoral heterogeneity at the genetic level? Second, how do PDA therapies influence evolutionary trajectories, and can they be more effectively used within the evolutionary context of a tumor? Second, how to transcriptional subtypes of pancreatic cancer correlate with the genetic or evolutionary features of the same tumor? Our data to be presented relies upon on whole exome or whole genome sequenced samples of primary and metastatic pancreatic cancer tissues, single cell technologies, and computational models. Such questions are of broad interest in cancer biology in general and have a strong likelihood to be relevant to understanding mechanisms of treatment resistance in other tumor types as well.

## Braf inhibition in hairy cell leukemia: biological and clinical aspects

*Enrico Tiacci and Brunangelo Falini*

*Institute of Hematology and Center for Hemato-Oncology Research,*
*University and Hospital of Perugia (Italy)*

Hairy cell leukemia (HCL) is a rare chronic B-cell neoplasm that is initially sensitive to chemotherapy with purine analogs. However, up to 50% of patients relapse and become progressively less responsive to these drugs, which are also myelotoxic and immunosuppressive.
The founding genetic event of HCL is the BRAF-V600E activating kinase mutation, which is present in almost all patients with HCL and in almost no patients with other mature B-cell lymphomas and leukemias (Tiacci et al NEJM, 2011).
HCL relies heavily (and more than BRAF-mutated solid tumors) on the V600E mutation for most of its unique features, including the peculiar hairy morphology, the distinctive transcriptional signature and the characteristic immunophenotype, as well as for its viability (Pettirossi et al., Blood 2015). Thus, BRAF-V600E shapes the hairy cell identity and represents an attractive therapeutic target.
Indeed, the BRAF inhibitor vemurafenib proved impressively active in HCL patients heavily pre-treated with (and often refractory to) purine analogues: an oral, brief treatment with this drug produced almost 100% overall responses and about 40% of complete remissions (CRs), with no myelotoxicity nor immune-suppression (Tiacci et al., NEJM 2015).
However, residual leukemic cells persist in the bone marrow even in complete responders, often showing bypass ERK (re)phosphorylation downstream of BRAF-V600E, and relapses are common after stopping the drug. To improve response depth and duration, these vemurafenib-resistant residual HCL cells should be eliminated, or their emergence prevented altogether, ideally by adding to vemurafenib another targeted, non-myelotoxic drug.

In this regard, the strategies we are currently pursuing in the clinic aim at: i) addressing the MEK-ERK dependent mechanisms of resistance by concomitantly blocking MEK1/2 (the kinases phosphorylated by BRAF) through the addition to vemurafenib of the MEK1/2 inhibitor cobimetinib; and ii) targeting resistant HCL cells, whether still depending on MEK-ERK activation or not, through anti-CD20 immunotherapy in combination with BRAF blockade.

## Personalized cancer Immunotherapy

*Ugur Sahin*

*BioNTech AG, University of Mainz, Germany*

Abstract not received

## Modelling resistance identifies vulnerabilities and effective combination therapy in mIDH AML

*Pier Paolo Pandolfi, MD, PhD*

*Cancer Research Institute, Beth Israel Deaconess Cancer Center, Department of Medicine and Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA*

Although targeted therapies have proven effective and even curative in human leukemia, resistance often ensues. IDH enzymes are mutated in ~20% of human AML, and currently, specific inhibitors of mutant IDH enzymes are actively under clinical investigation as effective treatment for mutant IDH AML. However, resistance to single agent targeted therapies is frequently observed in the clinic. Using mouse models, we characterize leukaemia evolution from mutant IDH2 (mIDH)-dependence to independence, identifying key vulnerabilities for both mIDH-dependent and independent leukemias. By applying high-throughput technologies we identify key vulnerabilities of mIDH leukaemia related to metabolism and signalling. Mechanistically, we identify PIN1 and LSD1 to be mediators of mIDH vulnerabilities. We further demonstrate PIN1 to act as both a key regulator of leukaemia maintenance, and an inhibitor of differentiation in both sensitive (mIDH2 -dependent) and resistant (mIDH2-independent) disease.
Importantly, we confirm our findings for leukemias harboring both mIDH-1 and -2 mutations in general, independent of cooperating genetic events. On the basis of this data we identify a powerful APL-like targeted therapy to effectively treat mIDH mouse and human leukemic models. Thus, our findings pave the way towards the treatment of a sizable fraction of human AML through targeted combinatorial therapies.

## Lineage Plasticity in Prostate Cancer

*Charles L. Sawyers, M.D.*

*Memorial Sloan Kettering Caner Center, New York, NY*

The most common mechanism of acquired resistance to targeted cancer therapies is alteration of the drug target -- typically through mutation or amplification or through bypass of the signaling pathway blockade. There is growing evidence of a conceptually distinct mechanism in which the tumor cells escape growth inhibition by shifting their lineage such that the drug target is no longer essential for the proliferation of that cell type. I will present recent work from our laboratory showing how prostate cancers can escape from hormone therapy by undergoing an identity change from androgen-dependent luminal epithelial cells to more basal-like epithelial cells. This lineage plasticity occurs in the setting of combined loss of function mutations in RB and TP53 and is mediated in part by the reprogramming transcription factor SOX2. In addition, we have found that several prostate cancer driver genes, such as FOXA1, ERF and ERG, also perturb normal basal-luminal differentiation programs. Thus, lineage plasticity can have a role in prostate cancer initiation as well as in acquired resistance, with important clinical implications for the timing and context in which hormone therapy should be used.

References

Watson PA, Arora VK, Sawyers CL. Emerging mechanisms of resistance to androgen receptor inhibitors in prostate cancer. Nat Rev Cancer. 2015 15:701-11. PMCID: 4771416.
Mu P, Zhang Z, Benelli M, Karthaus WR, Hoover E, Chen CC, Wongvipat J, Ku SY, Gao D, Cao Z, Shah N, Adams EJ, Abida W, Watson PA, Prandi D, Huang CH, de Stanchina E, Lowe SW, Ellis L, Beltran H, Rubin MA, Goodrich DW, Demichelis F, Sawyers CL. SOX2 promotes lineage plasticity and antiandrogen resistance in TP53- and RB1-deficient prostate cancer. Science. 2017 355:84-88. PMCID: 5247742.

## Effect of the microenvironment on prostate cancer biologic and clinical behavior

*Hubert Pakula, Svitlana Tyekucheva, Kathryn Penney and Massimo Loda*

*Departments of Oncologic Pathology and Biostatistics & Computational Biology, Dana-Farber Cancer Institute; Departments of Biostatistics and Epidemiology, Harvard T.H. Chan School of Public Health; Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital/Harvard Medical School*

Most human studies have focused on the mutational landscapes in an attempt to predict biologic and clinical behavior of human prostate cancer. In addition, epigenetic and transcriptional epithelial signatures are an important adjunct in predicting aggressive and indolent behavior. While these add additional independent prognostic information, they could be further improved by knowledge of the contribution of stromal elements. Stroma can morphologically and functionally change in the presence of cancer.

Compared to normal stroma, there is a switching of the cellular phenotype, remodeling of the extracellular matrix, increases in expression of growth factors and proteases, increased angiogenesis, and change in the inflammatory infiltrate.

The bidirectional signaling between epithelial cells and stromal constituents during normal prostate homeostasis is disrupted early in tumorigenesis. We performed gene expression profiling of laser capture microdissected normal non-neoplastic prostate epithelial tissue and compared it to non-transformed and neoplastic low and high grade prostate epithelial tissue from radical prostatectomies, each with its immediately surrounding stroma. Whereas benign epithelium in prostates with and without tumor were similar in gene expression space, stroma away from tumor was significantly different from that in prostates without cancer.

When high to low Gleason grade cases were compared, upregulation of frizzled receptors was noted. In addition, a stromal gene signature reflecting bone remodeling was seen. In validation data, the signature discriminated cases that developed metastasis from those that did not.

In order to assess mechanistically interactions between stroma and transformed epithelium, we utilized TMPRSS-ERG (T2E) knock-in mice, and crossed them with PTEN +/- mice. While T2E mice did not show alterations in the epithelium by 3 month of age, histological changes were observed in the stroma. Stromal cells are known to supply Wnt ligands that bind to the Wnt receptors such as Lgr and Frizzled in the epithelium.

We found that there was a progressive increase in Lgr5 positive cells in the epithelium of T2E and T2E/PTEN-/- adjacent to HGPIN, while PORCN was progressively up-regulated in the adjacent tumor stroma of these mice. PORCN acts as a key regulator of the Wnt signaling pathway by mediating the attachment of palmitoleate, a 16-carbon monounsaturated fatty acid, to Wnt proteins. Serine palmitolylation of WNT proteins is required for efficient binding to frizzled receptors. Taken together, these data suggest that the microenvironment influences prostate cancer initiation, maintenance, and metastatic progression and that Wnt signaling plays a significant role in this regard.

## Melanoma: clues to therapeutic efficacy from disease pathogenesis

*David E. Fisher MD, PhD*

*Massachusetts General Hospital, Charlestown, MA*

Cutaneous melanoma is among the few cancers with a well documented carcinogenic etiology (UV) yet with unrelenting increases in incidence.

The melanoma genome typically contains thousands of UV-signature mutations. Many of these lack oncogenic driver activity, but are recognized to contribute to immune recognition as potential neoantigens. This presentation will explore features of cutaneous adaptation to sun/UV, that suggest a powerful set of mechanisms which balance evolutionary requirements for UV (ie vitamin D) against tissue injury inflicted by UV. Carcinogenic features of red/blond pigment will be discussed, as well as manipulation of skin pigmentation, and behavioral signals triggered by UV exposure.

Despite the high mutational load of melanoma, the majority of patients fail to achieve durable clinical benefit from current immune checkpoint therapies.

A genetically defined murine cancer model will be presented, which enables dissection of the role(s) for UV neoantigens in immunotherapy responses and also permitted the development of novel approaches to "rescue" immunotherapy responsiveness in tumors which are deficient in neo-antigens or pre-existing tumor inflammation.

## The Hospital for Sick Children, Toronto, Ontario, Canada

*Peter Dirks MD PhD*

*The Hospital for Sick Children, Toronto, Ontario, Canada*

Brain tumours harbour subpopulations of neoplastic stem cells that drive tumourigenesis. However, the origin of intra-tumoural functional heterogeneity between diverse tumour cells remains poorly understood. We have studied the fate of brain tumour stem cells in mouse medulloblastomas using lineage tracing from Sox2+ cells, revealing that a rare, quiescent stem cell is at the root of tumour growth and regrowth after conventional and targeted therapy. More recently, we have studied the clonal evolution of barcoded human glioblastoma cells in an unbiased way following serial xenotransplantation to define their individual fate behaviours.
Independent of an evolving mutational signature, we show that the growth of GBM clones in vivo is consistent with a remarkably neutral process involving a conserved proliferative hierarchy rooted in glioblastoma stem cells (GSCs). Slow-cycling stem-like cells give rise to a more rapidly cycling progenitor population with extensive self-maintenance capacity, that in turn generates non-proliferative cells. We also identify rare “outlier” clones that deviate from these dynamics, and show that chemotherapy facilitates the expansion of pre-existing drug-resistant GSCs. I will discuss implications of fate mapping approaches to define the patterns of brain tumour growth and resistance.
Our data support that these tumours reflect disturbance of normal developmental hierarchies rooted in subpopulations of cells with stem cell properties.

## Targeting aberrant signaling in primary brain tumors

*Ingo K. Mellinghoff, MD*

*Memorial Sloan Kettering Cancer Center, New York*

Primary Brain Tumors comprise a heterogeneous group of diseases with distinct predilection for certain age groups, locations within the central nervous system, growth patterns, and response to therapy. Molecular characterization of these tumors has uncovered recurrent genetic alterations that allow disease classification into subgroups with distinct clinical outcomes, reflected in the revised World Health Organization (WHO) classification of CNS tumors. Unfortunately, advances in our understanding of the molecular pathogenesis of primary brain tumors has not resulted in better treatment and current therapy still heavily relies on surgery, radiation and chemotherapy. Diffuse tumor growth throughout the brain, uncertain drug delivery, and evolution of the cancer genome during therapy represent formidable challenges for the development of molecularly targeted therapies for brain tumors.
There remains an urgent need for a deeper understanding of the relationship between brain tumor cells and their microenvironment and of brain tumor evolution during therapy.
I will discuss our efforts to address these challenges through sequencing of circulating tumor DNA in cerebrospinal fluid, *in-situ* characterization of the tumor microenvironment, and novel approaches to noninvasive imaging of brain tumors.

## New Models and Treatments for Clear Cell Renal Cell Carcinoma

*William G. Kaelin, Jr., M.D.*

*Howard Hughes Medical Institute, Dana-Farber Cancer Institute and Brigham and Women’s Hospital, Harvard Medical School, Boston, MA 02215*

*william_kaelin@dfci.harvard.edu*

Inactivation of the VHL tumor suppressor protein, which normally serves to target the alpha subunit of the HIF transcription factor for proteasomal degradation, is common in clear cell renal cell carcinoma (ccRCC).
In preclinical studies HIF2α, and not HIF1α, appears to drive ccRCC pathogenesis. HIF2α is a basis helix-loop-helix PAS domain protein with two PAS domains (PAS A and PAS B). Kevin Gardner and Rick Bruick at UTSW identified a potentially druggable pocket in the HIF2α PAS B domain and further identified chemicals that can bind to this pocket and induce an allosteric change that disrupts DNA binding. Medicinal chemistry efforts at Peloton Therapeutics produced the tool compound PT2399 and the clinical compound PT2385.
In preclinical models PT2399 is active against a subset of VHL-/- ccRCC lines and PDXs and PT2385 and has demonstrated activity in a subset of heavily pretreated ccRCC patients. Nonetheless, some ccRCC are insensitive

to genetic and pharmacological inactivation of HIF2α, implying a need for both predictive biomarkers and alternative therapies. With respect to the former, our preliminary data indicate that p53 pathway mutations make ccRCC less dependent on HIF2α.
With respect to alternative therapies, we found that high HIF2α levels make ccRCC hyperdependent on EZH1 because of a HIF-driven increase in H3K27 demethylases. Finally, we are using CRISPR/Cas9 to make immunocompetent models of ccRCC.

## Inhibiting the transcription factor HIF-2α: treatment of renal cell carcinoma and VHL disease

*John A. Josey, Ph.D.*

*Peloton Therapeutics, Inc. Dallas, TX*

Von Hippel-Lindau (VHL) syndrome is a rare inherited autosomal dominant disorder that results from mutations in the VHL tumor suppressor gene on chromosome 3p25.3.
The syndrome is characterized by the formation of visceral cysts and tumors in numerous organ systems. Elegant studies have demonstrated that the von Hippel-Lindau protein (pVHL) acts as a substrate recognition component of an E3 ubiquitin ligase which, under normoxic conditions, tightly regulates the levels of the alpha-subunit of the hypoxia-inducible factors.
VHL patients are prone to develop multifocal and often bilateral clear cell Renal Cell Carcinoma (ccRCC). Both VHL-associated ccRCC and sporadic ccRCC share a common truncal event of loss of pVHL function.
Loss of function of the pVHL tumor suppressor has the immediate downstream consequence of unregulated accumulation of hypoxia-inducible factors and the pleotropic expression of multiple gene products that contribute to tumor angiogenesis, growth, metastasis, and immune evasion. Specifically, in ccRCC tumor cells with pVHL deficiency, HIF-2α upregulates the expression of genes that are important to tumor growth and metastasis, including those that encode cyclin D1 (CCND1), vascular endothelial growth factor A (VEGFA), transforming growth factor α, and C-X-C chemokine receptor 4.
While most transcription factors have historically been considered intractable targets for small molecule modulation, recent efforts have afforded potent, orally bioavailable small molecule antagonists of the transcription factor hypoxia-inducible factor-2α (HIF-2α), a primary driver of ccRCC. One such antagonist, PT2977 binds to a hydrophobic pocket in the Per-Arnt-Sim (PAS)-B domain of HIF-2α, preventing its dimerization with its obligate partner, HIF-1α, thus inhibiting its transcriptional activity. In preclinical models of ccRCC, PT2977 selectively antagonizes HIF-2α resulting in altered gene expression and potent anti-tumor effects.
The preclinical characterization of PT2977 and its performance in patients with ccRCC will be described.

## Mouse models of thoracic cancers. Phenotypic consequences of their cell-of-origin

*Anton Berns[1], Giustina Ferone[1], Ekaterina Semenova[1], Jitendra Badhai[1], Hilde de Vries[1], Ji-Ying Song[3], Rajith Bhaskaran[1], Lorenzo Bombardelli[1], Min chul Kwon[1], and Ivo Huijbers[2]*

*[1]The Oncode Institute, Division of Molecular Genetics, [2]MCCA Transgenic facility, [3]Department of Pathology, The Netherlands Cancer Institute, Amsterdam, The Netherland*

Thoracic cancers belong to the most lethal human malignancies. In particular, patients with small cell lung cancer (SCLC), lung squamous cell carcinoma (LSCC), and malignant mesothelioma (MM) show very poor survival statistics due to the late detection, disseminated spread, and chemo-resistance of the tumors. We have generated multiple mouse models of these tumors and studied how closely they resemble their human counterpart, how these tumors develop over time, from which cells they originate, what genomic alterations are recurrently found and how this influence tumor characteristics and their response to interventions. The mouse tumors induced by the lesions most frequently observed in the specific human tumor subtypes show remarkable similarity to their human counterpart.
This includes their marker profile, their primary site and metastatic spread, their immunophenotype and their response to treatment. We observe substantial inter- and intra-tumor heterogeneity and tumor plasticity, this in spite of the fact that the mouse tumors are driven by the same set of lesions.
The cell-of-origin of these tumors can be surprisingly diverse with the same set of driver mutations giving rise to phenotypically quite different lesions. Clearly, both the specific set of driver lesions as well as the cell-of-origin of a particular tumor subtype are determining factors for the tumor characteristics and response to treatment. The lessons learned from these models will be discussed.

# Molecular Mechanisms of acquired chemoresistance in small cell lung cancer

*J.T. Poirier, MD*

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"Small cell lung cancer is initially highly responsive to cisplatin and etoposide but in almost every case becomes rapidly chemoresistant, leading to death within one year. We modeled acquired chemoresistance in vivo using a series of patient-derived xenografts to generate paired chemosensitive and chemoresistant cancers. Multiple chemoresistant models demonstrated suppression of SLFN11, a factor implicated in sensitivity to a variety of classes of chemotherapeutic agents. In vivo silencing of SLFN11 was associated with marked deposition of H3K27me3, a histone modification placed by EZH2, within the gene body of SLFN11, inducing local chromatin condensation and gene silencing.

Inclusion of an EZH2 inhibitor with standard cytotoxic therapies prevented emergence of acquired resistance and augmented chemotherapeutic efficacy in both chemosensitive and chemoresistant models of small cell lung cancer."

# ABSTRACTS OF POSTERS

## 1. NAMPT is a key element in the acquisition of resistance to BRAF inhibitors becoming an actionable target and a disease marker in metastatic melanoma

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Although recent clinical trials of BRAF inhibitor (BRAFi) combinations have demonstrated improved efficacy in BRAF-mutated melanomas, emergence of acquired resistance limits clinical benefit. In resistant cells tumor metabolism rewires to accommodate increased energy requirements, suggesting that molecules mediating metabolic adaption may become therapeutic targets overcoming drug resistance.
Resistance mechanisms are generally linked to paradoxical activation of the MAPK signaling pathway. We found that this oncogenic signature converges on the overexpression of nicotinamide phosphoribosyltransferase (NAMPT), the main NAD-biosynthetic enzyme, leading to a marked increase in NAD levels. This NAD boost supports metabolic switch toward glycolysis or OXPHOS, both strategies exploited by BRAF-mutated melanomas to adapt to chronic exposure to BRAFi. Treatment of melanoma cells with NAMPT inhibitors (NAMPTi) depleted NAD, inducing mitochondrial stress, cell cycle arrest and ultimately apoptosis. Consistently, NAMPTi were highly effective in the treatment of melanoma xenografts, inducing complete tumor regression in the case of M14 cells.
NAMPT up-regulation upon development of resistance to BRAFi was confirmed in serial biopsies from melanoma patients. Furthermore, NAMPT could be dosed in patient plasma where it correlated with disease burden, response to therapy and overall survival. In conclusion, this work links oncogenic BRAF signaling to metabolic reprogramming through NAD biosynthesis and identifies NAMPT as an actionable target for melanoma patients with BRAF mutations.

## 2. Maternal immunization against alk hinders tumor progression in neuroblastoma-prone offspring

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Introduction and rationale: Neuroblastoma (NB) is the most common pediatric solid tumor, usually diagnosed within the first year of life. One of the most predisposing genetic alterations towards NB development is a somatic mutation in the anaplastic lymphoma kinase (ALK) gene, resulting in a phenylalanine-to-leucine substitution at codon 1174 ($ALKF^{1174L}$). This mutation potentiates the activity of MYCN oncogene, as well documented in the $ALKF^{1174L}$/MYCN preclinical model of spontaneous NB.
Since it has been shown that DNA immunization against ALK oncoantigen is able to induce a

strong specific immune response against different mutated form of ALK, impairing the growth of ALK-positive tumors and enhancing survival in mouse models of non-small cell lung cancer and anaplastic large cell lymphoma, ALK targeting could be exploited against NB. As NB development occurs very early during life or even during fetal life, we sought to evaluate whether maternal immunization (MI) against ALK could be applied to cancer immune-prevention in a model of neonatal NB (ALKF$^{1174L}$/MYCN). Indeed, as a proof of concept of the effect of MI against an oncoantigen in cencer-prone offspring, we previously demonstrated the efficacy of MI against Her-2/neu in hampering tumor progression in a mouse model of Her-2/neu-driven mammary cancer.

Results:Pre-birth immunization against ALK, using a DNA plasmid coding for the extracellular and transmembrane domains of ALK (ALK-ECTM) followed by electroporation, leads to an extended tumor-free survival and a lower tumor growth kinetic in ALK$^{F1174L}$/MYCN offspring born from and fed by ALK-ECTM-vaccinated mothers, as compared to controls born from control empty vector vaccinated mothers. Maternally derived anti-ALK antibodies were successfully transferred from mothers to newborns, as well as immune IgG-ALK protein complexes. Moreover, MI against ALK induces a decrease in ALK expression in tumor harbored in ALKF$^{1174L}$/MYCN offspring born by ALK-ECTM vaccinated mothers.

Conclusions: Overall, these results indicate MI as a possible intervention to hamper NB development in genetically predestined offspring and could be a valuable treatment for this cancer type for which efficient preventive options are currently unavailable.

## 3. Selective killing of T-lymphoblastic leukemia (T-ALL) cells by redox-mediated engagement of the OMA1/OPA1 axis

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Approximately 20% of patients with pediatric acute T-lymphoblastic leukemia (T-ALL) are refractory to current glucocorticoid-based therapies. In the present study we investigated the possibility to selectively sensitize T-ALL cells to apoptosis by increasing their levels of mitochondrial reactive oxygen species (mtROS). For this purpose we employed NS1619, a synthetic benzimidazolone derivative that increases mtROS production by opening the large conductance $Ca^{2+}$-activated $K^+$ (BK) channel and dehydroepiandrosterone (DHEA), which blunts ROS scavenging through inhibition of the pentose phosphate pathway. NS1619 and DHEA increased mtROS and induced death of T-ALL cell lines, patient-derived xenografts (PDX) and primary cells from T-ALL patients (including cases of refractory T-ALL), but did not induce death of normal human thymocytes or PBMC. The importance of depowering ROS-scavenging pathways to increase the efficacy of ROS-producing treatments was underscored by the finding that NS1619 and DHEA activated NRF2, the master regulator of antioxidant pathways. NS1619 and DHEA induced cleavage of OPA1,a mitochondrial protein that controls cristae remodeling and cytochrome c mobilization. OPA1 cleavage and cell death were inhibited by the ROS scavenger N-acetylcysteine and by siRNA-mediated knock-down of the mitochondrial protease OMA1, whose targets include OPA1. Importantly, NS1619 and DHEA sensitized T-ALL cells to death induced by TRAIL or by the glucocorticoid dexamethasone, both of which are known to induce mitochondrial outer membrane permeabilization (MOMP) through the activation of Bid (TRAIL) or Bim (dexamethasone).
Taken together, our findings provide the first evidence for a role of the OMA1-OPA1 axis in sensitizing T-ALL cells to apoptosis, and suggest the feasibility of an integrated pharmacological approach that combines increased mtROS production, inhibition of scavenging pathways and MOMP to treat refractory T-ALL.

## 4. Novel interactions of the von hippel-lindau (pvhl) tumor suppressor with the cdkn1 family of cell cycle inhibitors

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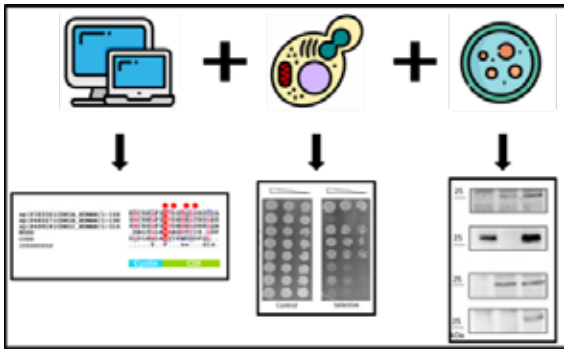
Germline mutations of the von Hippel-Lindau (pVHL) tumor suppressor cause several highly vascularized cancers. pVHL, in complex with Elongin C and B, targets the hypoxia-inducible transcription factor (HIF-1α) for rapid proteasomal degradation, modulating different downstream genes involved in hypoxia response. Prolonged oxygen deprivation is a limiting factor of cell cycle progression, inducing growth arrest and apoptosis. However, the exact molecular details leading to this transition are far from understood. Here, we present a recently published1 novel interaction between pVHL and the cyclin-dependent kinase inhibitor family CDKN1 (p21, p27 and p57). We predicted, dissected and validated their association through bioinformatics analysis, yeast two-hybrid screening and cell co-immunoprecipitation assays. We observe that the N-terminal tails of CDKN1 proteins share a conserved region mimicking the CODD motif of HIF-1α, necessary for pVHL binding. Interestingly, a site-specific p27 pathological mutation (p.Glu40Lys) alters this novel interaction. Our findings suggest a new connection between the pathways regulating hypoxia and cell cycle progression.



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## 5. Cytoreductive Radiotherapy concomitant with Nivolumab beyond progression: a local approach to improve sistemic disease control?

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Background

Immune checkpoint inhibition (CPI) as a therapeutic principle to boost the immune response to tumors has renewed interest in a phenomenon that is known as the "abscopal effect" (eliciting a systemic antitumor response using a local triggering treatment). We present a patient case where systemic progression during CPI treatment was followed by a systemic response after a local radiotherapy treatment.

Material and Methods

In August 2016 a seventy-three year old male patient was referred to our Institute for a treatment of bone metastasis from Non Small Cell Lung Cancer. His oncological history was characterized by previous prostate cancer (status post prostatectomy in 2012), urothelial cancer (s.p. radical radiotherapy in 2015) and a stage IV squamous cell carcinoma of the lung. After first line chemotherapy with partial response the patient experienced disease progression with bilateral lung nodules, a paracardiac lesion, mediastinal nodal and bone metastasis. In April 2016 he started immunotherapy with Nivolumab every two weeks. In July 2017 a shoulder CT scan showed a 4.6 cm osteolytic lesion of the left scapula causing severe pain to left shoulder and omolateral upper arm not well controlled by opioid drugs. Due to progression and pain we decided to submit the patient to cytoreductive/pain relieving radiation therapy.

Results

The patient underwent a planning CT scan, in supine position, with arms along the body. The target lesion was contoured (GTV-CTV-PTV) including almost all of the left scapula. The patient underwent 3D-Conformal Radiotherapy receiving 30 Gy in ten fractions (3gy/die). At the end of the therapy shoulder pain was significantly reduced. The patient was continued on two-weekly Nivolumab and during regular follow up CT scans (February and June 2017) showed a persistent partial response of the lesion treated (scapula) concomitantly with a remarkable reduction of lung nodules and the right paracardiac lesion suggesting a systemic response in line with a possible "abscopal effect"

Conclusions

In most clinical situations, radiation therapy may be safely delivered concomitantly with immunotherapy. This case report suggests the synergistic effects of local treatment with new drugs being able to enhance the response to systemic therapy probably trough immunological pathways (abscopal effect), as also recently suggested by the PEMBRO-RT trial. Further studies are needed to confirm these preliminary observations.

## 6. Redox homeostasis modulation sensitizes DLBCL cell lines to venetoclax

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Diffuse Large B-cell Lymphoma (DLBCL), the most common type of non-Hodgkin's lymphoma, is a very heterogeneous malignancy, divided into two molecular subtypes: germinal center B-cell (GCB) and activated B-cell (ABC). More than 40% of patients (mostly with ABC-DLBCL) are refractory to standard chemotherapy (R-CHOP) or will relapse. Recent combinatorial regimens with bortezomib, lenalidomide and ibrutinib have proven to be more effective against ABC subtype. In addition, BCL-2 inhibitors promise to be effective both in GCB- and in ABC-DLBCLs.
Nevertheless, a significant proportion of DLBCL exhibits primary or secondary resistance to these compounds.
We investigated a novel pharmacological approach aimed at elevating mitochondrial reactive oxygen species (ROS) levels, in association with the BCL-2 inhibitor venetoclax (ABT-199).
Our results indicated that elevation of ROS in DLBCL cell lines (N=7 GCB-type, N=7 ABC-type) strongly increased the pro-apoptotic effect of ABT-199. Interestingly, the magnitude of this effects was higher in cell lines exhibiting an OxPhos metabolic profile compared to glycolytic cell lines that were more resistant.
Current studies are focused on investigating the efficacy of our strategy in samples from patients with resistant/refractory DLBCL.

## 7. mTORC inhibition increases reactive oxygen species and death in T-lymphoblastic leukemia cells

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Approximately 20% of pediatric T-cell acute lymphoblastic leukemia (T-ALL) patients do not respond to standard glucocorticoid-based therapies and have a dismal prognosis.
Hyper-activation of the PI3K/Akt/mTOR oncogenic pathway is a common feature of T-ALL. In addition to controlling cell growth and autophagy, mTORC1 influences mitochondrial activity and oxidative metabolism by controlling the interaction between YY1 and PGC1α.
In previous studies, we showed that T-ALL cells exhibit high levels of mitochondrial reactive oxygen species (ROS). ROS are powerful signalling molecules that can induce apoptosis through p53 activation, but may also increase cancer cell survival through PTEN oxidation, which results in an increased Akt activity. In the present study, we tested the possible cross-talk between mTOR and ROS in T-ALL. As expected, treatment of T-ALL cells with the mTORC1-inhibitor Everolimus induced LC3 lipidation and p62 degradation, events that suggest the activation/induction of autophagy. Importantly, Everolimus also increased ROS levels and induced cell death both in T-ALL cell lines and patient-derived T-ALL xenografts (PDX) but not in primary normal thymocytes. Cell death was reduced by pre-treating cells with the ROS scavenger N-acetyl-cysteine (NAC), indicating that this effect was ROS-dependent.
*In vivo* experiments carried out in NOD/SCID mice inoculated with glucocorticoid-resistant PDX cells showed that Everolimus significantly increased the sensitivity of the cells to the glucocorticoid dexamethasone with resulting increased survival of treated mice.
These studies indicate a connection between mTOR and ROS, and suggest that mTORC1 inhibition may prove to be effective to overcome dexamethasone-resistance in refractory T-ALL patients.

## 8. Generation and Characterization of TRAIL-resistant Glioblastoma Cell Lines

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Glioblastoma (GBM) is the most common and aggressive primary brain tumor. Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) causes apoptosis of target cells and can selectively induce apoptosis in tumor cells. However, many cancer cells are resistant to TRAIL, and mechanisms of resistance are not well understood.
In this study, we generated TRAIL-resistant cell line models, and selected TRAIL-resistant subpopulations of 4 different GBM cell lines (A172, LN18, T98G, and U87MG). Cell viability and caspase assays as well as long-term cell growth assays revealed that the resistant cells exhibited sustained TRAIL-resistance. Genome wide RNA sequencing analysis of the cells revealed major alterations during the transition from TRAIL-sensitive to TRAIL-resistant state and identified PI3K pathway to be markedly changed in all TRAIL-resistant models. In addition, our analysis specifically on the apoptosis regulators demonstrated that Bcl-2 and Bcl-XL expression played a major role in regulating the TRAIL response. Furthermore, functional modulation of Bcl-2 and Bcl-XL through overexpression, shRNA knockdown or BH3 mimetic applications, changed the apoptotic outcome of GBM cell lines. To assess whether the selected TRAIL resistant sub-populations also exhibit resistance to other apoptosis inducers, we utilized Fas-ligand, Staurosporine and Temozolomide and observed that there was heterogeneity for each drugTo then assess the possibility of re-sensitizing the resistant cells to TRAIL, we applied a proteasome inhibitor, Bortezomib, and observed that all resistant subpopulations can be re-sensitized to TRAIL efficiently.
Our future efforts are directed at understanding the roles of commonly de-regulated pathways in resistance mechanisms. Our results will provide further understanding of molecular mechanisms of tumor heterogeneity in GBM cells, and help with the novel treatment strategies.

## 9. ETV7 mediates Doxorubicin resistance in breast cancer cells by repressing DNAJC15

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

## 10. VEGF removal delays the onset of acquired resistance to target therapy and increase the efficacy of immune checkpoint inhibitors in BRAF mutated melanoma

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The introduction of BRAF inhibitors (BRAFi) has improved response rate and overall survival of metastatic melanoma patients compared to standard chemotherapy. However, acquired drug resistance occurs in nearly all patients. The comprehension of cellular and molecular mechanisms underlying BRAFi resistance could help to identify novel actionable pathways in the treatment of BRAF dependent tumors.
VEGFA is an attractive target for combinatorial cancer therapy and we have recently demonstrated in melanoma and CRC xenografts that targeting VEGFA enhanced the antitumor effect of BRAFi by normalizing the tumor vasculature, recruiting M1 macrophages and inducing a remodeling of the extracellular matrix characterized by a reduction in collagen I and in cancer-associated fibroblasts (Comunanza et al EMBO Mol Med 2017).
While the previous proof of concept was obtained within in an immunodeficient model, here we investigated the therapeutic effect of VEGFA targeting in association with PLX4720 (BRAFi) in a dedicated immune-competent model. D4M cells, a $BRAF^{V600E}$-mutant melanoma murine cell line, were subcutaneously injected in syngeneic C57BL/6J mice. We demonstrated that the association of BRAFi with DC101 (antibody anti VEGFR2) had a weak activity while we observed a synergistic antitumor effect when combined with B20 (murine anti-VEGFA neutralizing antibody). Although targeted inhibition of either BRAF or VEGFA delayed the tumor growth, only combined inhibition of both pathways resulted in the regression of initial tumor size, with an evident apoptotic effect, and delayed the onset of acquired resistance to the BRAF inhibition.
Since it has been well characterized the immune suppressive role of VEGFA in tumors we further investigated whether contrasting the VEGF effect along with simultaneous BRAF inhibition can turn into a promotion of both innate and adaptive immunity. Both flow cytometry and immunofluorescence analysis of tumors demonstrated that the combinatorial regimen activated the host immune system, inducing the tumor infiltration by cytotoxic $CD8^+$ lymphocytes, macrophages with tumor suppressive features and NKs. Moreover, the association between BRAF targeting and VEGFA removal reduced the number of circulating $CD11b^+Ly6C^{low}Ly6G^+$ polymorphonuclear MDSCs (PMN-MDSCs).
Moreover, we evaluated whether the therapeutic effect obtained by the simultaneous VEGF blockade and BRAFi could be exploited with immune-checkpoint modulators and we observed that this synergism is further improved by the association with checkpoint inhibitor targeting PD-1. The addition of anti-PD-1 blocking antibody significantly enhanced the antitumor effect and we observed total tumor volume regression in 86% of treated mice.
Our findings provide biological rationale to explore the association of immunotherapy in novel combinatorial approaches which could improve the clinical outcome exerted by oncogene-targeted therapy and further investigation is warranted.

## 11. Detecting cancer biomarkers on polydisperse extracellular vesicles isolated from blood

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Extracellular vesicles (EVs) are heterogeneous membranous particles intensively studied for their potential cargo of diagnostic markers. We developed a new rapid biochemical method to obtain polydisperse EVs in a physiological pH solution, preserving their morphology, dispersity, and stability. We challenged the reproducibility of this method by isolating EVs from different biological fluids. In plasma of healthy donors, the abundance of recovered EV populations, in the range of $10^{10}$ per milliliter, positively correlated with the density of blood erythrocytes, platelets, and leukocytes. Quantitative analyses using specific haematopoietic and epithelial markers demonstrated the unbiased recovering of polydisperse EV lineages. Individual isolated vesicles were used in newly-designed homogeneous assays. We detected a picomolar concentration of PSMA on $10^5$ EVs isolated from plasma of prostate cancer patients and *BRAF* V600E mutation-carrying mRNA in $10^3$ EVs from plasma of colon cancer patients, reaching unprecedented matching with tissue biopsy results.

## 12. A p53/miR-30a/ZEB2 axis controls triple negative breast cancer aggressiveness

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Inactivation of p53 contributes significantly to the dismal prognosis of breast tumors, most notably triple negative breast cancers (TNBC). How the relief from p53 tumor suppressive functions results in tumor cell aggressive behavior is only partially elucidated. In an attempt to shed light on the implication of microRNAs in this context, we discovered a new signaling axis involving p53, miR30a and ZEB2.
By an *in silico* approach we identified miR-30a as a putative p53 target and observed that in breast tumors reduced *miR-30a* expression correlated with p53 inactivation, lymph node positivity and poor prognosis. We demonstrate that p53 binds the MIR30a promoter and induces the transcription of both miRNA strands, 5p and 3p. Both miR-30a-5p and 3p showed the capacity of targeting ZEB2, a transcription factor involved in epithelial-mesenchymal transition (EMT), tumor cell migration and drug resistance. Intriguingly, we found that p53 does restrain ZEB2 expression via miR-30a. Finally, we provide evidence that the new p53/miR-30a/ZEB2 axis controls tumor cell invasion and distal spreading and impinges upon miR-200c expression.
Overall, this study highlights the existence of a novel axis linking p53 to EMT via miR-30a, and adds support to the notion that miRNAs represent key elements of the complex network whereby p53 inactivation affects TNBC clinical behavior.

## 13. Serum micrornas as biomarkers for early diagnosis of non small cell lung cancer

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INTRODUCTION
Lung cancer is the main cause of cancer-related mortality worldwide. Patients with early stage (I-II) non-small cell lung cancer (NSCLC) have a much better prognosis than those diagnosed at late stage. Thus, the development of sensitive and non-invasive methods for screening individuals at high risk for NSCLC is needed. microRNAs (miRNAs) have been suggested as a novel class of tumor biomarkers; their stability in biofluids and their change in levels in disease suggest their potential application as circulating biomarkers.

EXPERIMENTAL MODEL
Upon a critical review of the literature, we selected 8 miRNAs for a two-step screening of early lung cancer, based on their reported sensitivity and specificity and the fact that are not influenced by hemolysis[a]. Since smoking habit or inflammatory conditions may influence miRNA levels in serum, we quantified miRNAs of our panels in three groups of controls (non-smokers, smokers and COPD patients) and in stage I-II NSCLC patients. Droplet digital PCR was applied for quantification of miRNAs.

RESULTS
The two-step screening is composed of a panel of 4 miRNAs endowed with high sensitivity and a second panel with high specificity. The chosen miRNAs should allow to identify true positive patients, that would undergo CT scan. For 3 of the 6 miRNAs analyzed there was no significant difference among control subgroups (non-smokers, smokers, and subjects affected by Chronic Obstructive Pulmonary Disease), whereas miRNA levels were expressed at significantly different levels in tumor and control groups, confirming their possible role as biomarkers previously described in the literature.

CONCLUSIONS
The selected miRNAs may help to identify high risk subjects who need further investigation for the presence of early NSCLC; in particular, miR-223 showed a good accuracy in distinguishing tumor samples from controls.

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## 14. Understanding SLC expression in hematological malignancies

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Solute carriers (SLCs) are membrane proteins responsible for the exchange of various nutrients, vitamins and ions between cells and their environment. Their expression pattern depends both on the genetic program of the respective cell type and on the metabolic state of the cell and its surrounding. To provide enough energy and biomass, various cancers overexpress a number of SLCs, thus enabling continuous growth and proliferation. However, despite their key role in metabolism and growth, not much is known about the drivers of SLC expression. We therefore aim to systematically investigate both genetic and metabolic regulation of a defined set of cancer-relevant SLCs in hematological malignancies. On the one side, genome-wide loss-and gain-of-function CRISPR/Cas9 screens will help to identify genetic drivers and regulators of SLC expression in an unbiased way.
Metabolic perturbations using an established metabolic drug library and a high-content-high-throughput microscopy based readout, will in parallel shed more light on the connection between metabolism and SLC expression.
The identified regulatory dependencies and connections will be validated in primary patient material.
Together, these approaches are expected to contribute important knowledge on the extensive regulatory network of SLCs in cancer. Together with already available essentiality data, these insights might lead to the definition of new metabolic drug targets and potential synthetic lethal interactions.

## 15. Adenocarcinoma-neuroendocrine transition of androgen resistant cancer depends on SPARC down-regulation in stromal accessory cells

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Tumor progression is a multifaceted process in which, complex interactions between tumor and different types of stromal cells and extracellular matrix components, actively contribute to its phenotypic heterogeneity. Among extracellular matrix proteins, secreted protein acidic and rich in cysteine (SPARC) has been deeply studied since conflicting reports have described its expression to be either increased or decreased in different cancer settings, also depending on whether it is produced by the neoplasm or by the neighboring stroma. Nevertheless, the different contribution of tumor- or stromal-derived SPARC in prostate tumor microenvironment has not been addressed yet. Given this evidence, we aimed at providing new insights into the mechanism by which SPARC modulation influences prostate cancer development and progression.
We modeled human disease using TRAMP mice, which spontaneously develop autochthonous prostate tumors following the onset of puberty. Crossing TRAMP mice with Sparc-/- mice, we found the appearance of focal areas of neuroendocrine differentiation within adenocarcinoma. In patients this phenomenon commonly results after androgen ablation therapy and correlates with poor prognosis.
Interestingly, areas of neuroendocrine differentiation in Sparc-/- TRAMP mice were both positive for cytokeratin 8 and synaptophysin (usually expressed by luminal cells within adenocarcinoma or neuroendocrine cells, respectively), further suggesting a differentiation of adenocarcinoma cells to a neuroendocrine-like phenotype. Moreover, immunohistochemistry showed SPARC positivity not only in scattered tumor cells but also in fibroblasts and myeloid cells infiltrating TRAMP prostate. Accordingly, *in vitro* experiments suggested that stromal-derived SPARC limits neuroendocrine differentiation of prostate cancer cells, while they excluded a role of endogenous SPARC in this phenomenon. Indeed, prostate cancer cell lines co-cultured in presence of Sparc-deficient fibroblasts increased or acquired neuroendocrine features. This likely occurs through the effect of IL-6, a cytokine recently discovered to induce neuroendocrine differentiation, and that we found to be released by Sparc-deficient, but not sufficient, fibroblasts.
Data collected so far indicate that stromal SPARC deficiency skews prostate carcinogenesis toward neuroendocrine differentiation. A

deeper understanding of the molecular mechanisms governing the balance between prostate adenocarcinoma and neuroendocrine tumors according to extracellular matrix composition will provide important insights for the development of new therapeutic strategies.

## 16. MYC-mediated enhancer reprogramming favors breast cancers tumorigenesis

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Introduction: Breast cancer (BC) is the most common tumor and the leading cause of death among women cancer patients. Triple negative BC (TNBC) is a particularly aggressive form of BC, which is characterized by the hyper-activation of the MYC oncogene. Importantly, cytotoxic chemotherapy represents the only therapeutical option for TNBC. Consequently, the identification of novel biomarkers and targetable targets in TNBC represents an urgent medical need.

Rationale: In this report, we investigated the role of MYC during TNBC tumorigenesis, by defining its mechanism of action in tumor initiation. In particular, we focused on its activity on the regulation of distal cis-regulatory elements: the enhancers. Indeed, specific enhancer repertoires are critical for cell specification, while their decommissioning is a crucial step towards cell reprogramming. Of importance, dis-regulation of enhancers could favor tumorigenesis, by driving the aberrant activation of oncogenic transcriptional programs.

Results: Specifically, we show that MYC acts as tumor reprogramming factor by reshaping the enhancer landscape in mammary epithelial cells. Indeed, it mediates the transcriptional downregulation of luminal-specific transcription factors, leading to the decommissioning of the enhancers which dictated the luminal mammary epithelial identity. In addition, it favors the acquisition of a stem cell-like fate by driving the activation of de novo enhancers, which control the transcriptional activation of oncogenic pathways. Next, we demonstrated that the enhancer reprogramming occurring *in vitro* is also maintained in in vivo mouse TNBC models. In addition, the genes which are transcriptionally activated by MYC binding on their enhancers, are globally highly expressed in TNBC, in patients with a poor prognosis. Finally, among these genes, we identified MYC downstream factors, which are able to recapitulate its ability to drive the reprogramming of mammary epithelial cells and, potentially, tumorigenesis.

Conclusion: In conclusion, we demonstrate that the MYC-driven epigenetic reprogramming favors the formation of stem cell-like tumor-initiating cells. In addition, we generate a MYC-dependent oncogenic signature for TNBC, identifying downstream targets, which may represent novel therapeutic targets.

## 17. The e3 ubiquitin-protein ligase mdm2 is a novel interactor of the von hippel-lindau tumor suppressor

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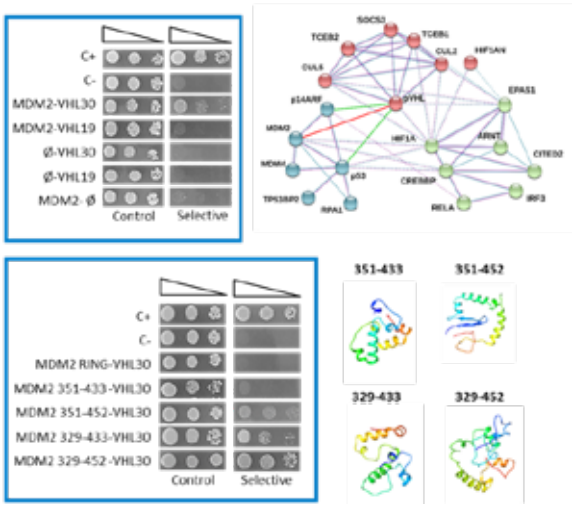
Mutations of the von Hippel-Lindau tumor suppressor (pVHL) are causative of familiar predisposition to develop different cancers. pVHL is mainly known for its role in regulating hypoxia-inducible factor 1-alpha (HIF-1α) degradation, modulating hypoxia response. Previous studies have suggested that isoform-specific specializations can be associated with human pVHL. Here, we present a novel interaction between pVHL and MDM2. Integrating *in silico* predictions with *in vivo* assays, we found that the accessory acidic tail of pVHL30 is required for its association with MDM2. Further, we demonstrate that an intrinsically disordered region upstream of the MDM2 tetramerization domain is responsible for its isoform-specific association with pVHL30. This region is mostly conserved in higher mammals, including human, similarly to what already proposed for the N-terminal tail of pVHL30. Collectively, our data support the idea that the pVHL30

isoform may play a role in MDM2 regulation, suggesting a wider interplay between hypoxia sensing and cell cycle regulation.
This work is supported by Associazione Italiana per la Ricerca sul Cancro (AIRC) grants MFAG12740 and IG17753 to ST.




## 18. Transcriptional addiction in cancer cells is mediated by YAP/TAZ through BRD4

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ABSTRACT
Cancer cells rely on dysregulated gene expression. This establishes specific transcriptional addictions that may be therapeutically exploited. Yet, the mechanisms ultimately responsible for these addictions are poorly understood. Here we show that the transcription factors YAP and TAZ mediate transcriptional dependencies of transformed cells. YAP/TAZ physically engage the general coactivator BRD4, dictating the genome-wide association of BRD4 to chromatin. YAP/TAZ flag a large set of enhancers with super-enhancer-like functional properties.
YAP/TAZ-bound enhancers mediate recruitment of BRD4 and Pol II at YAP/TAZ-regulated promoters, boosting expression of a host of growth-regulating genes. Treatment with small molecule inhibitors of BRD4 blunts YAP/TAZ pro-tumorigenic activity in several cell/tissue contexts, causes regression of pre-established, YAP/TAZ-addicted neoplastic lesions, and reverts drug resistance.
This work sheds light on essential mediators, mechanisms and genome-wide regulatory elements responsible for transcriptional addiction in cancer and lays the groundwork for a rational use of BET inhibitors according to YAP/TAZ biology.

## 19. Uncoupling FoxO3A mitochondrial and nuclear functions in cancer cells undergoing metabolic stress and chemotherapy

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Carcinogenesis is a multistep process by which normal cells evolve to a neoplastic state by acquiring a succession of cancer hallmarks resulting in the ability to survive and proliferate in adverse microenvironmental conditions. Cancer cell homeostasis is sustained by the balance between these newly acquired oncogenic features and pre-existing cellular functions. Paradigmatic in this regard is the reprogramming of energy metabolism, where normal cellular processes providing increased energy production, macromolecular biosynthesis and maintenance of the redox balance are ensured by the preservation of key mitochondrial functions. Consistent with this view, proteins that have been classically considered as tumor suppressors are sometimes required to be functional for full malignant transformation. This is the case for FoxO3A, which can be both friend and foe to cancer cells depending on the cellular context. It is a key determinant of cancer cell homeostasis, playing a dual role in survival/death response to metabolic stress and cancer therapeutics.
We recently described a novel mitochondrial arm of the AMPK-FoxO3A axis in normal cells upon nutrient shortage. We found that in metabolically stressed cancer cells, FoxO3A is recruited to the mitochondria through activation of MEK/ERK and AMPK, which phosphorylate serine 12 and 30, respectively, on FoxO3A N-terminal domain. Subsequently, FoxO3A is imported and cleaved to reach mitochondrial DNA, where it activates expression of the mitochondrial genome to support mitochondrial metabolism. Using FoxO3A−/− cancer cells generated with the CRISPR/Cas9 genome editing system and reconstituted with FoxO3A mutants being impaired in their nuclear or mitochondrial subcellular localization, we show that mitochondrial FoxO3A promotes survival in response to metabolic stress. In cancer cells treated with chemotherapeutic agents, accumulation of FoxO3A into the mitochondria promoted survival in a MEK/ERK-dependent manner, while mitochondrial FoxO3A was required for apoptosis induction by metformin. The interplay between the MEK/ERK and the AMPK cascades, which converge on the N-terminal domain of FoxO3A, represents the first chapter of the mitochondrial tale of the FoxO3A code. Further studies are needed to establish whether other signaling pathways actually target FoxO3A N-terminus to modulate its mitochondrial localization and function and which stimuli do they respond to.
This will be instrumental to devise personalized therapeutic strategies-employing molecularly targeted drugs-aimed at manipulating cellular metabolism to counteract cancer initiation and progression.

## 20. Mechanisms of telomere maintenance in pediatric brain tumors: insights from pre-clinical models

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The up-regulation of a telomere maintenance mechanism (TMM) is a common characteristic of cancer cells and represent a hallmark of neoplasia in 70-80% of cases.
Reliable methods for detecting TMMs in tumor samples are becoming a pre-requisite in the clinic, and for the use of target treatments towards telomerase and/or alternative lengthening of telomeres (ALT) mechanisms. For example, in pediatric brain tumors ALT is found only in a subset of malignant brain tumors, whereas pediatric low grade gliomas rarely exhibit an ALT phenotype.
In our lab, we have developed a juvenile model of glioma in zebrafish, based on the conditional expression of human-relevant oncogenes in a population of brain progenitor cells.
The same model was established also in a background of co-overexpression of TERT and TR. We described how to study TMMs and understand related aspects of telomere biology in tumors derived from dissected tissues.
Each tumor was analyzed by applying techniques for detecting both tumor-derived telomerase and markers of ALT, also if the starting material was little.
Using quantitative TRAP assay, we detected telomerase activity and correlated it with *tert, tr* and *atrx* RNA levels. Telomere length distribution was evaluated by a non-radioactive TRF assay and Q-FISH analysis, in comparison with telomeric content measured with qPCR. C-circles were quantified in genomic tumor DNA. Terra levels were measured by RNA FISH, non-radioactive dot - and northen- blot and correlated with telomere length. Most of these aspects were studied dur-

ing the progression from a single cancer initiating clone of a few cells to a full tumor.
Our data show that this model is ideal to study how the switch between telomere maintenance mechanisms occurs *in vivo*.

## 21. Myeloid Derived Suppressor Cells-Mast Cells Cross-Talk Via CD40-CD40L Mediates Tumor Specific Immunosuppression in Prostate Cancer

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Immunotherapy including checkpoint inhibitors is currently the most potent therapeutic approach for several types of cancers but with limited activity against prostate tumors. Immune suppression instigated by the tumor is thought to be the cause. We indicated mast cells (MCs) as accomplices of adenocarcinoma development within the prostate microenvironment, and their immunosuppressive capacity prompted our investigation on whether they have part in immunosuppression and tolerance to SV40 Large-T antigen, the transforming oncogene in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice.
Crossing TRAMP mice with MCs deficient $Kit^{Wsh}$ mice strongly reduced the incidence of adenocarcinoma. TRAMP mice are generally tolerant to the SV40 Large T antigen otherwise immunogenic in normal syngeneic B6 mice. Genetic ablation of MCs restored the capacity of mounting a tumor-specific cytotoxic T cell response. Notably, in $Kit^{Wsh}$-TRAMP mice the restored T cell immunity correlated with the reduction of polymorphonuclear- myeloid derived suppressor cells (PMN-MDSC) activity, along with reduced expression of Arg1, Nos2 and Stat3. Having found that CD40L-expressing MCs can interact *in vivo* with CD40-expressing PMN-MDSC, we tested whether $Kit^{Wsh}$-TRAMP mice reconstituted with MCs either sufficient or deficient for CD40L can replace or not immunosuppression. Only wild type, CD40L competent, MCs could restore PMN-MDSC suppressive functions, T cell unresponsiveness and adenocarcinoma development. These data unveil an immunoregulatory function of MCs on PMN-MDSC activity through CD40L-CD40 interaction favoring immunosuppression and tumor onset. *In silico* analyses provided correlation with clinical data, showing poor outcome in prostate cancer patients characterized by high expression of MC/PMN-MDSC related-genes.

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

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Abstract
Discovery of novel 'druggable' targets is a primary goal in cancer translational research. Transient Receptor Potential subfamily M member 8 (TRPM8) is a cation channel almost exclusively expressed by the luminal compartment of the prostate epithelium in the human

body. Poorly studied in the context of prostate physiology and homeostasis, the increased expression of TRPM8 in primary and metastatic prostate cancer lesions suggests a proto-oncogenic role of the channel.
Here, by combining a multidisciplinary approach to an in vitro genetic platform modelling the natural history of prostate tumorigenesis, we demonstrate that potent TRPM8 agonists synergize with X-ray treatments to induce massive apoptotic response in radioresistant pre-malignant and malignant prototypes of primary prostate lesions. As well, TRPM8 activation enhance the efficacy of docetaxel or enzalutamide in eradicating hormone naïve metastatic PCa cells.
Overall, we propose TRPM8 activation as a valuable clinical strategy for a more effective treatment of low-risk and high-risk organ confined PCa patients.

## 23. Low-dose radiotherapy as a chemo-enhancer of an induction chemotherapy regimen with GemOx and stereotactic body radiotherapy for locally advanced pancreatic cancer: preliminary results

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Background and Purpose: Results of published phase II trials have indicated that low-dose radiotherapy (LDR) can enhance the effect of chemotherapy and may create an immunological microenvironment that enhances immunological response against tumors; furthermore, phase II studies using stereotactic body radiotherapy (SBRT) demonstrated excellent local control and single radiotherapy doses around 8-10 Gy have also been documented to enhance tumor immunogenicity. We report preliminary safety and effectiveness results of an induction chemotherapy (CHT) regimen combined with LDR used as a chemo-enhancer followed by SBRT in locally advanced pancreatic cancer (LAPC)
Methods: Patients (pts) with non-metastatic inoperable LAPC were enrolled on a prospective single-institution study (NCT02416609). Four CHT cycles with Gemcitabine and Oxaliplatin (GEMOX) (day 1-8 of a 21-day cycle) concurrent with LDR were administered; LDR was delivered on days 1 and 2, 8 and 9 of each CHT cycle, using eight doses of 40 cGy each. If no progression was observed after CHT-LDR, pts received 3 fractions of 8, 10 or 12 Gy (total dose 24-36 Gy) of SBRT based on tumor location in relation to stomach and duodenum. 4D-CT with oral and i.v. contrast was used for treatment planning and IGRT-IMRT for delivery. Seven weeks after SBRT tumour re-staging and evaluation for surgery was performed. Toxicity was scored according to CTCAE v4. Progression free survival (PFS), freedom from locoregional progression (FFLRP), freedom from distant metastasis (FFDM) and overall survival were calculated using the Kaplan-Meier method.
Results: Between February 2014 and December 2017 we enrolled 14 pts. All pts received four CHT cycles, except one because of an intercurrent myocardial infarction. Two pts developed distant metastases after induction CHT, 12 received SBRT. Total SBRT dose was: 36 Gy (2 pts), 30 Gy (3 pts) and 24 Gy (7 pts). At present 3/12 pts underwent resection without complications.
With an overall median follow-up of 17.6 months (range, 3-40), for all patients the locoregional control rate was 57.1% (8/14). Median PFS, FFLRP, FFDM and OS were 12.2, 19, 14, 18.3 months respectively; estimated 1-year PFS, FFLRP, FFDM and OS rate were 64.3%, 92.9%, 64.3% and 78% respectively. Three pts developed acute G3 or greater hematologic toxicity (1 anemia and 2 neutropenia), 1 pt developed acute G3 gastrointestinal pain, no further G3 or greater acute nonhematologic toxicity was observed. One patient developed G2 gastric ulcer and G2 gastric haemorrhage that were medically managed. Late G3 or greater toxicities were not observed.
Conclusion: Induction CHT combined with LDR used as a chemo-enhancer and SBRT in three fractions resulted in excellent local control and seems to improve survival outcomes, with a low rate of side effects. Evaluation upon enrolment conclusion is awaited to provide final results that may provide the rationale to further intensify research into modification/modulation of locoregional treatment strategies for LAPC.

## 24. In silico exploration of von hippel-lindau (pvhl) tumor suppressor molecular functions: correlations between disease mutations, interactors and pathways

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Familiar cancers represent a privileged point of view for studying the complex cellular events inducing tumor transformation. Von Hippel-Lindau syndrome, a familiar predisposition to develop cancer, is a clear example. Here, we present our efforts in deciphering the role of the von Hippel-Lindau tumor suppressor protein (pVHL) in cancer insurgence. We collected high quality information on ca. 1.600 pVHL mutations and ca. 160 interactors from VHLdb, a freely accessible database dedicated to pVHL (1). These were investigated in terms of association between patient phenotypes, mutated surface and impaired interactions. Our data suggest that different phenotypes correlate with localized perturbations of the pVHL structure, with specific cell functions associated to different protein surfaces. We propose five different pVHL interfaces to be selectively involved in modulating proteins regulating gene expression, protein homeostasis as well as to address ECM and ciliogenesis associated functions.
These data were used to drive molecular docking of pVHL with its interactors and guide Petri net simulations of the most promising alterations. We predict that disruption of pVHL association with certain interactors can trigger tumor transformation, inducing metabolism imbalance and ECM remodeling. Collectively taken, our findings provide a novel insight into VHL-associated tumorigenesis. This highly integrated in silico approach may help to elucidate novel treatment paradigms for VHL disease.

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REFERENCE: 1) TABARO F., MINERVINI G., ET AL. VHLDB: A DATABASE OF VON HIPPEL-LINDAU PROTEIN INTERACTORS AND MUTATIONS, SCIENTIFIC REPORTS, 6:31128, 2016.

## 25. The histone methyl transferase DOT1L is a novel epigenetic target for therapy of endocrine-therapy resistant breast cancer

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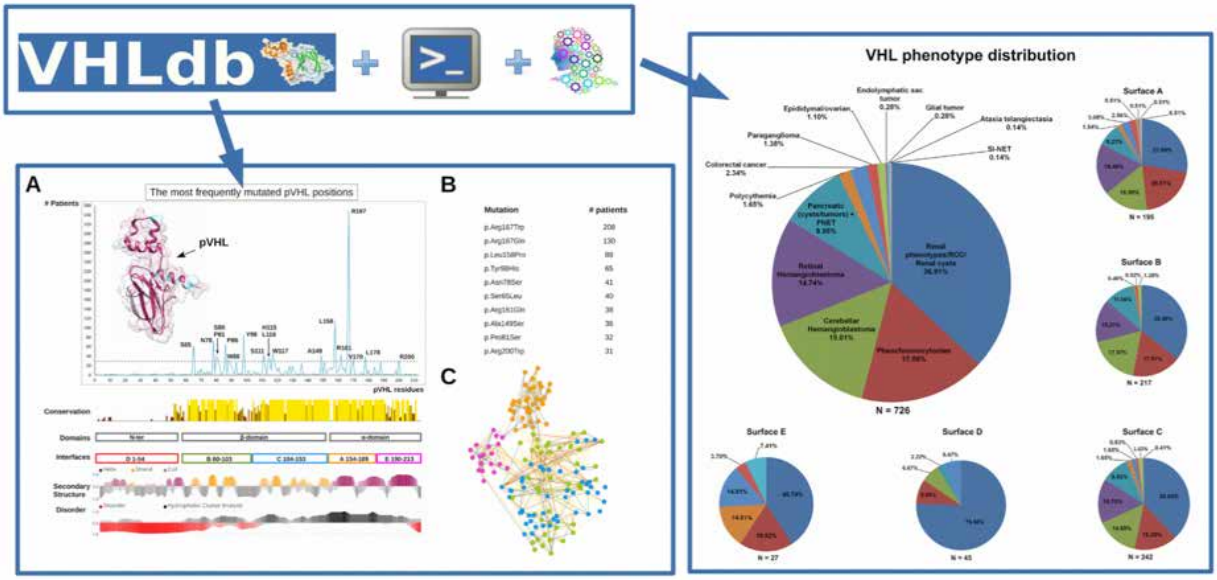
Estrogen Receptor alpha (ERα) is a ligand-inducible transcription factor that mediates estrogen signaling in hormone-responsive breast cancer (BC) and is the primary target of specific anticancer therapies. Although ERα blockade with these drugs is effective, the development of a resistance to treatment represents the key problem in clinical management of patients affected by this disease. Understanding the molecular mechanisms underlying ERα action in BC cells may help the identification of new therapeutic targets for more effective pharmacological treat-



ment of endocrine therapy-resistant tumors. We recently discovered the epigenetic enzyme DOT1L (DOT1 Like Histone Lysine Methyltransferase) as a novel nuclear partner of ERα in BC cells. To investigate the involvement of DOT1L in mediating ERα actions in hormone-responsive and endocrine-resistant BC, physical and functional interaction between these two molecules on chromatin was mapped by Chromatin Immunoprecipitation coupled to Mass Spectrometry (ChIP-MS) and Sequencing (ChIP-Seq). Gene silencing and selective pharmacological inhibition of either protein followed by transcriptome profiling (RNA-Seq) were applied and cellular and functional assays were performed to evaluate the functional impact of this complex in hormone-responsive and antiestrogen-resistant BC cells. ChIP-MS confirmed the co-recruitment of the two factors within a chromatin bound multiprotein complex. Gene expression profiling and Nascent RNA-Seq before and after DOT1L pharmacological inhibition highlighted the involvement of this enzyme in ERα-mediated transcriptional regulation of several estrogen responsive genes, including ERα itself. Global analysis of ERα and DOT1L binding to the genome showed co-recruitment of both proteins on several chromatin sites, including regulatory elements of the ERα gene itself, providing an explanation for the inhibition of cell cycle progression and cell proliferation observed in hormone-responsive and antiestrogen-resistant BC cell models both *in vitro* and *in vivo*. The results obtained reveal that physical and functional interplay between ERα and DOT1L represent a key molecular event of the estrogen-mediated signaling that control BC cell functions, suggesting this enzyme as a new potential therapeutic target against endocrine-resistant cancers.

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## 26. miR-214 overexpressing mice develop more aggressive mammary gland tumors

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MicroRNAs (miRs) are small non-coding RNAs that act as negative regulators of gene expression and control tumor progression. We, and others, demonstrated that miR-214 is upregulated in malignant melanomas and breast tumors. miR-214 coordinates metastasis dissemination via a pathway involving TFAP2C, the adhesion molecule ALCAM and the anti-metastatic miR-148b. Moreover, we showed that the simultaneous inhibition of miR-214 and overexpression of miR-148b led to strong reduction of metastasis, thus suggesting that miR-214 -miR-148b axis can be exploited for combinatorial miR-based therapeutic approaches. In order to study miR-214 function in a model of endogenous tumors, we generated a locus-specific conditional transgenic mouse for miR-214 overexpression and we crossed miR-214 overexpressing animals with a mouse model of breast cancer progression (MMTV-PyMT mice). While we observed a delay in mammary gland tumor onset in the double transgenic animals, carcinomas that developed in these mice were more aggressive than controls and an increased number of lung metastases was observed. Increased aggressiveness can be due, at least in part, to increased mesenchymal traits observed in PyMT-miR-214 overexpressing tumors compared to controls. Moreover, angiogenesis and inflammation resulted to be enhanced in tumors following miR-214 overexpression, thus suggesting a contribution of stromal miR-214 in tumor progression. miR-214 is highly expressed in stroma cells and its overexpression in the stroma compartment is sufficient per se to promote tumor dissemination. Our results suggest that when miR-214 is overexpressed in an established tumor onset it increases metastasis formation acting both on tumor and stromal cells.

## 27. Diagnosis of Glioma Tumors Using Circulating Cell-Free DNA

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Introduction

Gliomas are the most frequent brain tumors, making up about 30% of all brain and central nervous system tumors, and 80% of all malignant

brain tumors. Existing standard diagnostic technique for glioma tumor includes tissue biopsy, which is a highly invasive and hence a risky technique for the patient's survival. 'Liquid biopsy' is a new and recently developed non-invasive cancer diagnostic technique, which includes use of circulating cell-free DNA (cfDNA) fragments for tracing tumor markers. CfDNA fragments are one of those molecular bits that are released into the bloodstream after rapid apoptosis or necrosis of the tumor cells in the cancer patients. Our goal is to do comprehensive study between distinct types of glioma cancer tumors and cfDNA of the respective patients, to elucidate the scope of cfDNA in liquid biopsy technique for glioma diagnosis.

Methods
We collected 8 different glioma patient's tumor tissue and plasma samples and then isolated tumor DNA from glioma tumor tissue and circulating cell-free DNA(cfDNA) from the respective glioma patient's plasma. Isolated tumor DNA and cfDNA then deeply sequenced on Illumina HiSeq 2500 and then NGS data was analyzed to find out single nucleotide variants (SNVs) as well as structural variants on both cfDNA and tumor gDNA.

Results
We have successfully detected glioma specific mutations such as *IDH1, IDH2, PDGFRA, NOTCH1, PIK3R1* and *TP53*, from cfDNA isolated from the plasma of glioma patients and could relate this mutations to the different tumor grades of glioma. We are also studying the dynamics of these mutations in response to glioma drug treatment by collecting blood samples at different time intervals.

Discussion
This study may help in developing liquid biopsy technique for glioma tumor diagnosis and in its prognosis for monitoring the glioma treatment by non-invasive approach, and will eventually help physicians to decide the right treatment on appropriate time while bypassing the existing 'wait-and-see' approach of treatment monitoring.

## 28. The splicing factor PTBP1 promotes expression of oncogenic splice variants and predicts poor prognosis in patients with non-muscle invasive bladder cancer

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Abstract
Non-muscle invasive bladder cancer (NMIBC) is a malignant disease characterized by high heterogeneity, which corresponds to dysregulated gene expression and alternative splicing profiles. Nevertheless, the molecular basis of such aberrant regulation is unknown. Bioinformatics analyses performed by querying public datasets for splicing factors potentially linked to bladder cancer progression highlighted a positive correlation between the heterogeneous nuclear ribonucleoprotein I (i.e. PTBP1) mRNA expression and NMIBC progression. To validate this observation at the protein level, we enrolled a cohort of 152 patients presenting with primary NMIBC (pTa-pT1), for whom detailed follow-up clinical data (>2 years)were available. Immunohistochemistry (IHC) and western blot analyses confirmed that high PTBP1 expression was associated with worse clinical outcome in terms of incidence of tumor relapse and survival in NMIBC patients. Next, we investigated whether PTBP1 was functionally relevant for bladder cancer cells. To this end, we depleted of PTBP1 expression by transfection of PTBP1-specific siRNAs and evaluated its effect on three bladder cancer cell lines. Clonogenic assays, cytoflourimetric analysis of the cell cycle, Annexin V and PI assay and immunofluorescence analysis of caspase-3 cleavage demonstrated that PTBP1 favors bladder cancer cell proliferation and survival. Moreover, silencing of PTBP1 sensitizes bladder cancer cells to mitomycin C-induced death, as indicated by double staining with Annexin V and PI and analysis of caspase 3 cleavage. Mechanistically, we found that PTBP1 modulates the splicing pattern of bladder cancer-related genes, such as *TPM1, FAS, NUMB, MACF1, PKM, CD44, CTNND1, ACTIN1*,by directly binding in proximity of regulated exons and promoting the expression of pro-oncogenic splice variants of these genes. Notably, pro-oncogenic alternative splicing of CD44 correlated with PTBP1 expression also in patient's specimens, suggesting the clinical relevance of these results. Thus, our

study demonstrates an oncogenic role of PTBP1 in bladder cancer and suggests that its expression and/or its splicing signature can represent novel outcome-predictor markers for NMIBC.

## 29. LMW-PTP targeting to improve the effectiveness of chemo- and radiotherapy

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The set point of new effective anticancer strategies to overcome cancer resistance is one the major goals of pharmaceutical companies worldwide. We demonstrated that Low Molecular Weight Protein Tyrosine Phospatase (LMW-PTP) is a new interesting target to revert intrinsic resistance of tumors toward chemo- and radiotherapy. This enzyme is generally overexpressed in aggressive and therapy-resistant tumors and its expression is related to poor prognosis. We demonstrated that LMW-PTP silencing, in many different cell lines, allows the reprogramming of resistant cancer cells toward a phenotype more sensitive to various anticancer drugs, including dacarbazine, docetaxel and 5-FU, or radiotherapy. Furthermore, LMW-PTP silencing strongly impairs self-renewal ability of different types of cancer cells, thereby suggesting that this enzyme sustains survival of staminal cancer cells, promoting relapse. Treating cancer cells with a LMW-PTP inhibitors leads to the same effects of silencing, improving the effectiveness of chemo- and radiotherapy. Interestingly, we observed that LMW-PTP inhibitors are not effective on non-cancerous cells, which express low LMW-PTP levels. This finding reinforce the hypothesis that expression of LMW-PTP contribute to make cancer cells more resistant toward cytotoxic stimuli triggered by traditional anticancer therapies.
Studies conducted on PIRC rats, which spontaneously develop multiple tumours in the colon (a model for both familial adenomatous polyposis and sporadic colorectal cancer) confirmed that treatment with LMW-PTP inhibitors reduces LMW-PTP expression in rat tumors, increasing sensitivity of cancer cells toward chemotherapy. In conclusion, LMW-PTP is a promising target, for improving the outcome of patients affected by cancers refractory to treatments.

## 30. CASP4 gene silencing in epithelial cancer cells leads to impairment of cell migration, cell-matrix adhesion and tissue invasion

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ABSTRACT
Inflammatory caspases, including human caspase-4 (CASP4), play key roles in innate immune and inflammatory responses. The role of inflammatory caspases in cancer cells remains poorly investigated. Here, we explored the consequences of CASP4 expression levels on the migratory behavior of epithelial cancer cell lines. By a gene silencing approach and *in vitro* and *in vivo* studies we show that down-regulation of CASP4 leads to impaired cell migration and cell-matrix adhesion.
This phenotype is accompanied by an increased actin cytoskeleton polymerization, changes in the overall organization of adherens junctions (AJs) and in the number and size of focal adhesions. Interestingly, the cell migration deficit could be reversed by epithelial growth factor treatment, and depletion of calcium ions unveiled a role of CASP4 in the novo assembly of AJs, suggesting that the role of CASP4 is not cell-autonomous. Finally, *CASP4*-silenced A431 cells exhibited a severe reduction in their ability to invade lung tissue, when injected into nude mice. Overall, our data support the emerging evidence that inflammatory caspases can regulate cell migration through actin remodeling and uncover a novel role of CASP4 in cancer cell behavior.

## 31. Proline dehydrogenase expression, regulation and function in non-small cell lung carcinoma

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Introduction.
Non-Small Cell Lung Cancer (NSCLC) is one of the most frequent and deadliest cancers and comprises two main histotypes, adenocarcinoma (ADC) and squamocellular carcinoma (SCC). Identification of markers to better define the diagnosis, prognosis and therapeutic options of NSCLC is needed. We investigated if proline dehydrogenase (PRODH), a mitochondrial flavoenzyme catalyzing the key step in proline degradation, and involved in the regulation of cell survival, autophagy and apoptosis, may be one such marker.

Materials and methods.
We characterized PRODH expression in NSCLC by immunohistochemistry and qPCR and tested if there was correlation between expression of PRODH and clinical features of the tumors or expression of other markers. Phenotypic assays were performed to investigate the cellular influenced by PRODH in lung ADC cell lines. We also tested the regulation of the *PRODH* gene by TTF-1 by means of transfection experiments, expression analyses and luciferase reporter assays.

Results and discussion.
We found PRODH immunostaining in the majority (70%) of lung ADCs. Patients with PRODH positive tumors had better overall survival than those with negative tumors. Protein staining was paralleled by high transcript levels. Clonogenic assays showed that PRODH favors survival of ADC cells. Moreover, TTF-1, a transcription factor essential for thyroid and lung development and physiology, that shows similar expression pattern as PRODH was found to regulate *PRODH* expression. This prompted us to investigate if *PRODH* could be a target of TTF-1. Transfection of a TTF-1 expression construct into ADC cell lines led to an increase in PRODH transcript and luciferase reporter assays showed that a RE was indeed transactivated by TTF-1.

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

## 32. Chemotherapy resistance-associated epithelial to endothelial transition in gastric cancer

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INTRODUCTION
Gastric cancer (GC) is the fifth most common cancer worldwide and the third leading cause of cancer-related deaths. To date, gastrectomy and chemotherapy are the only therapeutic options, but drug resistance is the main cause for treatment failure.
Vasculogenic mimicry (VM) is a new model of neovascularization in aggressive tumors and has been correlated with poor prognosis in GC patients.
Our group has developed chemotherapy-resistant GC cells using the Caucasian adenocarcinoma cell line AGS and three drugs among the most used in clinic (5-fluorouracil, cisplatin and paclitaxel) henceforward denominated 5FUr, CISr, TAXr.
Our study has highlighted phenotypical differences among chemo-sensitive and chemo-resistant cell lines such as acquisition of stem-like phenotype and increased capacity to form vessels.

MATERIALS AND METHODS
Establishment of AGS resistant cell lines exposing cells to increasing dilution of drugs for over 9 months up to dilutions higher than IC50 values initially verified on AGS cells through MTT analysis.
Quantitative RT-PCR, flow cytometry and western blot analysis for stemness and VM markers.
Vasculogenic mimicry assay.

RESULT AND DISCUSSION
AGS cells acquired chemoresistance as indicated by the increase of IC50 values in drug-treated cells with respect to AGS. Furthermore, MTT assay highlighted that there is not cross-resistance among 5FUr, CISr and TAXr. Supportive data is that cells are MDR1 negative.
Resistant cells showed an upregulation of Yamanaka factors either in qPCR and flow cytometer analysis, and particularly interesting is ALDH overexpression in 5FUr.
Twist upregulation suggested the investigation of VM which resulted particularly enhanced in 5FUr cells which demonstrated their ability to form and sustain vessels up to 96 hrs in the tube formation assay.
Laminin γ2 and Ephrin A2 showed an increase in resistant cells and especially in 5FUr.

CONCLUSION
One of the most interesting result is that 5FUr cells acquire stemness properties and are positive to the tube formation assay suggesting

that VM might be one mechanisms adopted by cells to avoid drugs exposure.
These findings suggest that acquisition of chemoresistance could cause a relapse of disease in which tumor cells take advantage of their capability to perform VM in order to self-sustain their growth and that may be cause of poor outcomes.

## 33. ETV7 regulates cancer stem cells content and resistance to 5-FU and radiotherapy in breast cancer

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Cancer stem cells (CSCs) are considered the population of cells within a tumor able to drive tumorigenesis and known to be highly resistant to conventional chemotherapy as well as radiotherapy. ETV7 is a poorly studied transcription factor member of the ETS family, known to be an interferon-stimulated gene. ETV7 has been recently found over-expressed in breast cancer (BC), with higher expression levels in the more aggressive BC subtypes. In this work, we investigated the effects of increased ETV7 expression on breast CSCs population and resistance to chemotherapy and radiotherapy in BC cells.
We generated MCF7 and T47D BC-derived cells stably over-expressing ETV7 and we tested sensitivity of these cells to the chemotherapeutic drug 5-Flouorouracil (5-FU) and to radiotherapy. By viability assays and apoptosis analyses we observed that the over-expression of ETV7 could reduce sensitivity to both 5-FU and radiotherapy in these cell lines. We could also appreciate an increase in ABC transporters and BCL2 anti-apoptotic protein expression following ETV7 over-expression. These effects were also accompanied by the observation that alteration of ETV7 expression could significantly affect the population of breast cancer stem cells (CD44+/CD24low cells) in different BC cell lines. We finally associated higher ETV7 expression with altered proliferation rates and increased colony formation potential in soft agar. We propose a novel role for ETV7 in breast cancer stem cells plasticity and associated resistance to conventional chemotherapy. We finally suggest that an in-depth investigation of this mechanism could lead to the identification of novel breast CSCs vulnerabilities and to the improvement of combinatorial regimens with the aim of avoiding resistance and relapse in breast cancer.

## 34. ANP32E as a putative proto-oncogenic factor in MYC over-expressing cancer cells

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Breast cancer consists of highly heterogeneous tumors, whose driver oncogenes result difficult to be uniquely defined. We have recently reported the central role of MYC in initiating and sustaining a step-wise cell reprogramming process in differentiated mammary luminal epithelial cells (IMEC) toward a stem cell-like condition, which favors cell transformation and tumor initiation. Specifically, over-expression of MYC induces transcriptional repression of lineage-specifying transcription factors, causing decommissioning of luminal-specific enhancers. We also provided evidence that the MYC-driven reprogramming favors the formation of tumor initiating cells endowed with metastatic capacity, once cells have been challenged with the oncogenic activation of the PI3K-pathway. This oncogenic setting permitted the formation of tumors once the IMEC-MYC/PIK3CA$^{H1047R}$ (thereafter named *transformed*-IMEC, t-IMEC) have been transplanted into the mammary gland of NOD/SCID mice. In addition, we demonstrated that *t*-IMEC retained long-term tumorigenic potential as xenograft-derived (XD) cells formed tumors once re-injected in secondary and tertiary recipient mice. Among the different chromatin players, we identified the H2A.Z-specific chaperone ANP32E as a putative cofactor that may synergize with MYC in driving the observed epigenetic reprogramming. By interrogating gene expression pattern of chromatin regulators, we observed that the histone variant H2A.Z and its specific chaperone ANP32E was induced in *t*-IMEC and in XD cells respect to IMEC-MYC. By analyzing the TCGA dataset, we found that ANP32E alteration is specifically enriched among basal-like breast cancer characterized by MYC deregulation and this combination correlates with a worst prognosis. Altogether, these observations suggested a possible cooperative and oncogenic activity of

ANP32E in the tumor onset and maintenance. To asses this aspect, we targeted XD cells with a lentiviral vector expressing ANP32E and assessed effects of ANP32E overexpression on *in vitro* self-renewing capacity and tumorigenic activity. Interestingly, ANP32E overexpression in XD cells caused an increment in the capacity to form single cell-derived mammospheres and to develop cell foci in a semi-solid matrix. Recent studies reported an ANP32E involvement in the DNA damage response (DDR), showing that while H2A.Z incorporation at the damaged chromatin is necessary to allow a correct recruitment of the repair machinery, its subsequent rapid removal by ANP32E is indispensable for the resolution of the damage. In order to assess whether ANP32E overexpression in cancer cells can interfere with the DNA repair machinery, we quantified phospho-H2A.X foci in XD cells +/- exogenous ANP32E and observed that ANP32E overexpressing cells (XD-ANP32E) were characterized by a strong increment in both phospho-H2A.X foci number and dimension.
A critical component of the DDR is represented by ATR, a factor activated by regions of single-stranded DNA, which can occur as a result of oncogene-induced replicative stress. Notably, pharmacological treatment with an ATR inhibitor showed higher sensitivity of XD-ANP32E cells respect to not-transduced cells. This study supports the notion that ANP32E alteration in cancer cells subjected to a MYC-related replicative stress can promote the development and maintenance of a tumorigenic phenotype by interfering with the DDR, therefore favoring the acquisition of oncogenic insults. Of note, the correlation between deregulated expression of ANP32E and increased cell sensitivity to ATR inhibition could establish a therapeutic rational for a targeted treatment of basal-like breast cancers characterized by combined MYC and ANP32E alteration.

## 35. Metabolic pathways promoting colon cancer resistance to 5-Fluorouracil: the role of non-essential amino acids

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Metabolic rearrangements are essential to satisfy the different requirements of cancer cells during tumorigenesis and recent studies have highlighted a role for such metabolic reprogramming in adaptation to therapies and chemo-resistance development.
5-Fluorouracil (5-FU) is the most commonly used drug in colon cancer therapy. However, despite a promising initial response to the therapy, development of drug resistance to 5-FU in human tumor cells is the primary cause of chemotherapy failure. 5-FU exerts its anticancer effects through inhibition of thymidylate synthase (TS) and incorporation of its metabolites into RNA and DNA. TS catalyzes the methylation of dUMP to dTMP using reduced folate as a methyl donor and its over-expression is widely accepted as a major molecular mechanism responsible for 5-FU resistance.
In our study we established a 5-FU-resistant human colon cancer cell line in order to investigate the metabolic adaptation correlated to the resistance development, focusing on one-carbon metabolism and folate cycle.
Real-time PCR analysis revealed increased serine synthesis pathway in resistant cells in accordance with TS overexpression. Serine supplies carbon to the one-carbon pool, which is involved in folate metabolism and supports TS activity. Moreover, folate metabolism also contributes to the generation of S-adenosylmethionine, the methyl donor for both DNA and histone methylation, thus influencing epigenetic control of gene expression.
Radiolabelled metabolic studies and extracellular flux analysis (XF Seahorse analyzer) showed a significant decrease in glucose utilization together with increased glutamic acid metabolism in resistant cells, suggesting that this amino acid represents a valid alternative nutrient to sustain drug resistance.
Both glutamate and serine support several metabolic processes that are crucial for the survival of proliferating cells: they are precursors of the nonessential amino acids, are incorporated into glutathione, a primary cellular antioxidant, and are indispensable for nucleotides biosynthesis.
Together these findings indicate that non-essential amino acid metabolism plays an important role in 5-FU resistance, suggesting new possible approaches to target resistant cells and overcome colon cancer relapse.

## 36. Automated in vivo screen of a zebrafish melanoma model identifies FDA-approved drugs for combinatorial treatments

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Kita:GFP-HRAS fish develop cutaneous melanoma with high frequency (Santoriello et al., PLoS One, 2010). Already at 3 dpf, transgenic kita-GFP-HRAS larvae show a hyperpigmentation phenotype as earliest evidence of abnormal melanocyte growth. Using this transgenic model we performed a chemical screen with the Prestwick library. The screen is based on automated detection of a reduction of melanocyte number caused by any of 1280 FDA or EMA approved drugs of the library. The analysis showed that 55 molecules were able to reduce by 60% or more the number of melanocytes per embryo. Hierarchical clustering showed that the hits clustered in five major classes: anthelmintic, steroids, antisecretory, antifungal and non steroid anti-inflammatory drugs. To generate a dose-response curve we further tested two compounds for each class at six different concentrations alone or in combination with a farnesyltransferase inhibitor (Lonafarnib), that inhibits an essential post-translational modification of HRAS, or two MEK inhibitors (Trametinib and Selumetinib). Combination of Clotrimazole and Lonafarnib showed the most promising results, allowing to reduce the effective dosage of both drugs. We are performing validation of these observations in human melanoma cultures and investigating the mechanisms of action of the anti-fungal compound in blocking the proliferation of HRAS-transformed melanocytes.

## 37. Characterization of epithelial-mesenchymal transition intermediate/hybrid phenotypes associated to resistance to EGFR inhibitors in non-small cell lung cancer cell lines

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Non small cell lung (NSCLC) patients, whose tumors harbor sensitizing and driving mutations in the epidermal growth factor receptor (EGFR), get a meaningful clinical benefit from EGFR tyrosine kinase inhibitor treatments. Unfortunately acquired resistance invariably develops. Increasing evidence points to a key role played by epithelial-mesenchymal transition (EMT) in cancer progression and drug resistance. Importantly, the EMT is not a binary process and cancer cells with intermediate or hybrid epithelial/mesenchymal (E/M) phenotypes characterized by a mixture of epithelial and mesenchymal traits have been described. The effect of target therapy on the selection of intermediated E/M phenotypes in cancer cells is still poorly investigated. In this study, we used wet and in silico approaches to investigate whether intermediate E/M phenotypes are associated to resistance to target therapy in a NSCLC model system harboring activating mutations of the EGFR. The combination of different analysis techniques allowed us to describe intermediate and complete EMT phenotypes respectively in HCC827- and HCC4006-derived drug-resistant human cancer cell lines. Interestingly, intermediate E/M phenotypes, a collective cell migration and increased stem-like ability associate to resistance to the EGFR inhibitor, erlotinib, in HCC827 derived cell lines. Moreover, the use of three complementary approaches for gene expression analysis supported the identification of a small EMT-related gene list, which may have otherwise been overlooked by standard stand-alone methods for gene expression analysis.

## 38. Anti-cspg4 dna vaccination for the treatment of malignant melanoma: a comparative oncology trial for translational medicine

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Abstract
Malignant melanoma (MM) is the sixth most frequently diagnosed cancer and after primary tumor resection, about 30% of patients will experience disease recurrence and metastasis often fatal. In recent years, the development of immune checkpoint inhibitors has changed this poor prognosis, though only in a limited subset of MM patients, while most exhibit innate resistance and disease progression. Therefore, new therapies are needed. In our studies, the feasibility and the anti-tumor potential of targeting the MM-associated oncoantigen chondroitin sulfate proteoglycan (CSPG)4 with DNA vaccines delivered by electroporation (EP) have been evaluated.
Due to the many similarities with its human (Hu) counterpart, canine (Do) MM offers an excellent opportunity for translational clinical investigations, being the CSPG4 an attractive immunotherapy target expressed by both human and canine MM patients. We have previously demonstrated the safety and the clinical relevance of the intramuscular injection of a xenogeneic Hu-CSPG4 plasmid followed by EP in dogs with stage II-III surgically resected CSPG4+ oral MM. Hu-CSPG4 EP caused no side effects and resulted in a significantly longer disease-free (DFI) and overall survival (OS) of vaccinated dogs as compared to controls. However, Hu-CSPG4 vaccine was not effective in activating T cells from human healthy donors *in vitro*. To increase the translational power of our approach, we employed a hybrid plasmid coding for a chimeric HuDo-CSPG4 protein, expected to be effective in both veterinary and human settings.
We tested the immunogenicity and the anti-tumor potential of HuDo-CSPG4 intramuscular DNA EP in mice, in surgically resected CSPG4$^+$, stage II-IV, oral MM canine patients and in an *in vitro* human setting. Chimeric HuDo-CSPG4 EP is immunogenic in mice. In dogs the procedure is safe and induces antibodies (Ab) against both Hu- and Do-CSPG4, with a higher affinity and anti-tumor potential as compared to Hu-CSPG4 vaccine. From the clinical point of view, the hybrid HuDo-CSPG4 is effective in increasing both the DFI and the OS of vaccinated canine MM patients as compared to non-vaccinated controls. Interestingly, data obtained *in vitro* with T cells from human healthy donors also suggest HuDo-CSPG4 is more immunogenic than Hu-CSPG4 plasmid in a human setting.
In conclusion, the HuDo-CSPG4 EP is an effective way to break immune tolerance against the self antigen in dogs and humans and could move this approach to standard therapies for both veterinary and human clinical application.

## 39. Identification of DHX30 as an inhibitor of the translation of pro-apoptotic mRNAs after p53 activation by Nutlin

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Introduction
The transcription factor p53 can be efficiently activated by the small molecule Nutlin-3 without inducing genotoxic stress. Treatment of different cell lines with this small molecule can result in different phenotypes, ranging from cell cycle arrest to apoptosis. HCT116 (colon cancer-derived cells) and SJSA1 (osteosarcoma-derived cells) were used to model these behaviors respectively, by analyzing the transcriptional and translational responses after Nutlin-3 treatment.

Materials and Methods
Total and polysomal-bound mRNAs were collected and sequenced after 12h of 10uM Nutlin-3 treatment. A bionformatics analysis of polysome-enriched mRNAs using Weeder led to identification of a "CG-rich" 3'UTR motif which is enriched in the tranlationally upregulated mRNAs of SJSA1. The effect of this motif on transalation was evaluated after cloning its consensus sequence into the 3'UTR of the b-globin gene, which was put downstream of the luciferase gene. The activity of the construct was evaluated after 12 or 24h of Nutlin-3. The same motif was used for a pull-down experiment followed by mass spectrometry to identify interacting proteins.

Results and Discussion
Our RNA-seq data indicate that HCT116 and SJSA1, although sharing much of the transcriptional program driven by p53, show little to no overlap at the translational level. SJSA1 present different pro-apoptotic translationally-upregulated genes after Nutlin-3, which have one or more instances of a CG-rich motif in their 3'UTRs. The motif is sufficient to enhance ac-

tivity of a luciferase reporter when cloned in two copies flanking the 3'UTR of the b-globin gene, but only in SJSA1. A pull-down experiment using the consensus motif as an RNA bait was used to identify interactors, among which DHX30 was studied further. DHX30 silencing in HCT116 causes: 1) enhanced activity of the reporter construct after Nutlin treatment; 2) enhanced polysomal association of selected mRNAs containing the motif; 3) enhanced induction of apoptosis as assessed by Annexin-V staining. In addition, silencing of DHX30 in U2OS cells decreased their survival after Nutlin-3 treatment.

Conclusions
We show how the transcriptional program dictated by p53 activation can be shaped at a translational level thanks to the action of a cis-acting "CG-rich" motif, which is enriched in the 3'UTR of some pro-apoptotic mRNAs. The motif can be bound by DHX30, acting as a translational repressor.
The exact mechanism of action and the generalization of the model are currently being investigated.

## 40. SMYD3, a novel factor in DNA damage response, is a molecular target to overcome cancer drug resistance

*Paola Sanese[1], Valentina Grossi[1], Valentina Celestini[1], Candida Fasano[2], Alessia Peserico[1], Martina Lepore Signorile[1,3], Giuseppina Caretti[4], Alberto Del Rio[5] and Cristiano Simone[1,2].*

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Human cancers arise from a combination of genetic and epigenetic changes. Drugs that target epigenetic modifiers are a new therapeutic challenge, due to the reversibility of epi-modifications. The SMYD3 histone methyltransferase has an oncogenic role in various cancer types, making it a potential target for drug discovery. Recent studies suggest that its oncogenic activity might also be mediated by its interaction with non-histone proteins. Thus, the identification of novel SMYD3 interactors will be crucial for the full comprehension of its role in cancer. As SMYD3 is involved in cancer growth and cell cycle progression, regulating the S/G2 transition, and considering the impact of cell cycle regulation on resistance to chemotherapeutic drugs (CHTs), we hypothesize that SMYD3 might serve as a molecular target to overcome chemoresistance. We focused on the possibility that BCI-121, which induce S-phase arrest, could improve the effects of conventional chemotherapy by sensitizing cancer cells to S-phase-specific CHTs. In the light of our published data supporting the possibility to classify tumor cell types based on SMYD3 expression levels in order to design an effective SMYD3-targeted epigenetic cancer therapy, we set our experiments in CRC cells with high SMYD3 levels. Our results showed that BCI-121-mediated cell cycle arrest decreases the time needed for CHTs to exert their growth inhibitory effect, confirming that SMYD3 might be used as a molecular target to overcome resistance to CHTs. Through a peptide screening and an *in silico* analysis aimed at identifying SMYD3-interacting proteins, we identified a set of new potential partners involved in DNA damage and S-phase checkpoint, including BRCA2 and ATM. *In vitro* and in breast cancer cell line assays confirmed that SMYD3 interacts with a conserved region of BRCA2, which specifically modulates Homologous Recombination repair on DNA damage.
Our preliminary data indicate that also ATM could interact with SMYD3. In an effort to further characterize the role of SMYD3 in DNA repair, we found that breast cancer cells exposed to DNA damaging agent showed an increase of nuclear SMYD3 in cancer cells, which followed the activation of the repair signals, and an accumulation of unrepaired DNA lesions after SMYD3 genetic ablation.
To exploit the clinical potential of targeting SMYD3 to sensitize cells to chemotherapy, we particularly evaluated the potential of the combined treatment with SMYD3 inhibitor and chemotherapy in Triple Negative Breast Cancer (TNBC), which not usually respond to common therapies. TNBC cell lines expressing high levels of SMYD3 confirmed the efficacy of BCI-121 and S-phase agents combined treatment.
Taken together, our data give further strength to the idea that SMYD3 is a key factor in tumorigenesis, and therefore could be used as a molecular target to counteract cancer development. Furthermore, unveiling the BRCA2 and/or ATM interaction-mediated involvement of SMYD3 in DNA damage response might help finding combined pharmacological approaches to take advantage of SMYD3 inhibition to overcome chemoresistance.

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

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One of the main hallmarks of brain cancer is the formation of an immune-microenvironment infiltrated mainly with tumor associated macrophages (TAMs), but also with other types of leukocytes, which contribute to establish a status of chronic inflammation. Thus, the production of high amount of immune inhibitory cytokines or inflammatory mediators lead to increase tumor cells proliferation and invasion. The most frequent and aggressive form of brain tumor, glioblastoma multiforme (GBM), is resistant to standard of care therapies because of its heterogeneity, infiltration properties and immune suppressive microenvironment, therefore innovative therapies are being investigated, among which immunotherapies, whose aim is to alleviate GBM-associated immune suppression and to boost anti-tumor immune response. In this project we have used a zebrafish model of brain tumor to investigate the immune microenvironment of healthy and tumoral brains and the effect of compounds treatment both on brain tumor development and immune cells, mainly microglia. Leukocytes, including macrophages and microglia are normally present in the brain at homeostatic conditions at larval and adult stages. In the larval tumor model, which expresses the oncogene HRAS under the zic4 enhancer, an increase in the number of immune cells in the telencephalic region was observed; in the adult model changes were detected more in the cellular morphology of immune cells, which dramatically switched to a more spherical and less branched phenotype.
The same larval brain tumor model was used to investigate the effects of immune-suppressive compounds (dexamethasone and an inhibitor of CSF-1R) on the number of microglia cells, which were reduced, but these treatments did not impact on brain size and tumor development.
Given that altered epigenetic landscape is very common in brain tumors, larvae were screened with a library of compounds targeting epigenetic factors and, as a result, 3 hits were found to have an effect in slowing down the development of tumors and in reducing the number of immune cells. In order to characterize changes in the expression pattern of tumor cells after treatment with the 3 hits, we are investigating changes in the translatome as a result of drug treatment.Taken together, these results reinforce the knowledge that zebrafish provides a useful instrument to investigate the interactions between tumor and immune cells in GBM.

## 42. Manipulating MAPK/c-Myc axis to overcome chemoresistance in CRC

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Comprehensive genomic profiling is expected to revolutionize cancer therapy. Recent advances in DNA sequencing technologies provide unprecedented capacity to comprehensively identify the alterations, genes and pathways involved in the tumorigenic process, raising the hope that targeted therapies against the drivers of cancer can be extended from a few successful examples to a broader personalized medicine strategy. Colorectal cancer (CRC) poses a formidable challenge in the form of molecular heterogeneity with involvement of several cancer-related pathways and molecular changes unique to an individual's tumor. Recent discoveries have unveiled an impressive list of the RAS/RAF/MEK/ERK and PI3K/AKT pathway inhibitors, offering a new treatment paradigm for cancer patients. However, despite the initial promise, in 2018 only 15.4% of 610,000 patients with metastatic cancer were eligible for an FDA-approved, genome-guided drug, and just 6.6% of these patients likely benefited, also many patients relapse after a couple of years on the drugs, specially in CRC. The occurrence of chemoresistance is responsible for the limited success of various drugs. Indeed, blocking one pathway, such as RAS, is likely to induce only a cytostatic effect, while inhibiting a crosstalk resistance pathway should induce chemosensitivity and a final cytotoxic effect. These considerations highlight the relevance of molecular profiling and preclinical investigation in order to establish new therapeutic approaches based on the use of specif-

ic drugs targeted against the crucial drivers of cancer-related pathways. Indeed, a paradigm of this view is represented by the p38 MAPK, which is a central node between the Wnt, ERK, AMPK and PI3K cascades. In this work we tested p38α inhibitors in combination with molecularly-targeted drugs and chemotherapeutic agents in CRC xenografted nude mice and the AOM/DSS colitis-associated carcinoma preclinical model. To this aim, animals were treated with p38 inhibitor alone or in combination with the orally administrable MEK1 inhibitor PD0325901, the BRAF inhibitor Sorafenib or the chemotherapeutic agent Cisplatin. The combined use of p38α inhibitor with the indicated compounds significantly reduced tumor growth by inducing cytotoxic effect which is mediated by the apoptotic mechanism, the best method to kill cancer cells. Besides, we show that concomitant inhibition of the p38α and MEK/ERK pathways significantly increases the survival of $APC^{Min/+}$ mice in which tumorigenesis is driven by c-Myc deregulation. Since c-Myc is able to activate cancer stem cells expression profile, which can be considered responsible for chemoresistance mechanism, it becomes clear that found a method to neutralized c-Myc can be the best way to overcome chemoresistance. Moreover we found that p38α and ERK collaborate in c-Myc stabilization by inhibiting its proteasomal degradation in CRC cell lines. We confirmed these results by using Ralimetinib (p38 inhibitor) and Trametinib (MEK inhibitor), which are currently in clinical trials for cancer and inflammatory disease. These data validate that combined inhibition of p38α and ERK pathway or the association with chemotherapy could be a promising approach to overcome chemoresistance in CRC.

## 43. Role of miRNAs in metabolic plasticity correlated with 5-FU resistance in colon cancer cells

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Highly proliferative cells, including cancer cells, respond to their increased energetic and biosynthetic needs primarily through glycolysis. However, this metabolic strategy is not apt to sustain the low proliferative and quiescent state that characterize cancer cells resistant to therapy. Recent studies confirm that acquisition of drug resistance is associated with a metabolic shift toward respiratory metabolism in several cancer models. In this scenario, metabolic plasticity acquires a crucial role in the survival of drug-resistant cells responsible for tumor relapse. In our model, treatment of colon cancer cells with 5-FU selects a resistant subpopulation with mesenchymal stem-like properties that undergo a metabolic reprogramming resulting in addition to OXPHOS.
Our results suggest that these mechanisms can be activated in response to 5-FU in cells that are already resistant to the drug by directly modulating miRNAs expression. miRNAs are small non-coding RNA molecules that function in post-transcriptional regulation of gene expression and have been described as crucial regulators in cancer progression. We identified in resistant cells a set of miRNAs differently expressed in response to the acute treatment with respect to untreated resistant cells. Among them, we selected miR210 as a key player both in DNA damage repair in response to treatment and in metabolic reprogramming. Indeed, downregulation of miR210 in response to 5-FU allows resistant cells to increase DNA damage repair and shift cell metabolism toward OXPHOS addiction.
These adaptations give resistant cells supply of intermediates for nucleotides synthesis and enzyme activity for DNA damage repair. These results demonstrate a crucial role of miR210 in acute response of resistant cells to treatment, suggesting new possible therapeutic approaches for drug resistance.

## 44. Discoidin Domanin Receptor 1 is a therapeutic target in radioiodine refractory thyroid cancer

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Background: Thyroid cancers unresponsive to radioiodine (RAI) treatment are indicated as RAI-refractory and represent a deadly disease with less than 30% survival rate at five years. Overexpression of the insulin receptor isoform

A (IR-A) and its ligand IGF-2 is a relevant feature of these dedifferentiated cancers. Indeed, the IGF-2/IR-A loop has been involved in therapy resistance in several malignancies. We recently identified, in breast cancer, a functional crosstalk between the insulin/IGF axis and discoidin domain receptor 1 (DDR1) a collagen receptor with tyrosine kinase activity, which is implicated in cancer progression and metastasis. Both IGF-2/IR-A and DDR1 may also play a role in cell stemness.

Aims: we aimed at investigating whether DDR1 might affect the biology of RAI-refractory thyroid cancer partially through its functional crosstalk with the IGF-2/IR-A loop. Using various RAI-refractory thyroid cancer cells, we then evaluated the effects of DDR1 in cell differentiation and stem-like phenotype as well as in the modulation of the IGF-2/IR-A axis.

Results: DDR1 silencing, by specific siRNAs, was associated with a more differentiated cell phenotype, characterized by increased thyroid specific differentiation markers and concomitant decrease of invasiveness ability and stemness features. These changes were accompained by decreased IR expression with reduced IR-A:IR-B ratio and downregulation of autocrine IGF-2 production. DDR1 overexpression elicited opposite effects. In turn, activation of the IGF-2/IR-A pathway upregulated DDR1 expression. Interestingly, DDR1 silencing/inhibition enhanced thyroid cancer cells sensitivity to inhibitors of IR/IGF-1R and BRAF in terms of reduced cell viability and invasion.

Conclusions: These data suggest that DDR1 is a valuable molecular target for inducing redifferentiation in RAI-refractory thyroid cancer.

## 45. Identification of Epigenetic Factors That Will Overcome Therapy Resistance in Triple Negative Breast Cancer

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Triple negative breast cancer (TNBC) is a subtype of breast cancer that does not express receptors for estrogen, progesterone and Her2. It has poorer prognosis when compared to other subtypes and lacks targeted therapies.[1] Chemotherapy is the primary established systemic treatment for early stage and advanced TNBC following the surgery.[2] However, developing resistance to chemotherapy is a major obstacle in clinics that usually results in relapse and metastasis. It is known that epigenetic modifications contribute to the development and maintenance of chemotherapy resistant phenotype in different cancers.[3] To date, any comprehensive study has not been conducted to identify the important epigenetic modifiers regulating chemotherapy resistance in TNBC. Therefore, we designed an Epigenetic Knock-out Library (EPIKOL), which utilizes the CRISPR-Cas9 technology to study the functions of wide variety of epigenetic modifiers on chemotherapy-sensitive and resistant TNBC. EPIKOL is composed of 8000 gRNA (10 gRNA per gene) targeting the 5' exons of genes encoding chromatin- and DNA-modifying proteins, such as histone methyltransferases (HMTs), histone demethylases (HDMs), histone acetyl transferases (HATs), histone deacetylases (HDACs), and DNA methyl transferases (DNMTs). gRNAs targeting the essential genes and non-targeting gRNAs were also included to serve as positive and negative controls during the screens, respectively. We utilized MDA-MB-231, SUM159 and SUM149 cell lines to generate chemotherapy-resistant TNBC models. To this end, we exposed these cells to escalating doses of doxorubicin and taxol, the most widely used clinical chemotherapeutics, and selected resistant subpopulations. Comparison of these cell lines will reveal the differences in the epigenetic landscapes that result from transitions from a chemosensitive to chemoresistant state.
Further screens with our EPIKOL gRNA library will allow us to identify novel epigenetic regulators of chemotherapy resistance in TNBC.

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# Pezcoller Foundation–AACR international award for extraordinary achievement in cancer research

## 2019 Program guidelines and nomination instructions

### AWARD SUMMARY

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

- who has made a major scientific discovery in basic cancer research OR who has made significant contributions to translational cancer research;
- who continues to be active in cancer research and has a record of recent, noteworthy publications; and
- whose ongoing work holds promise for continued substantive contributions to progress in the field of cancer.

The award is intended to honor an individual scientist. However, if two candidates are co-nominated to share the Award, curriculum vitae and complete lists of publications for the two candidates must be submitted along with a letter of recommendation that clearly outlines how the work of these individuals is the same or closely related in subject matter and therefore warrants a joint nomination.

The award consists of an unrestricted honorarium of €75,000, a commemorative plaque, and full support to the winner and a guest to attend the AACR Annual Meeting. The winner of the 22nd Pezcoller Foundation–AACR International Award for Extraordinary Achievement in Cancer Research will give an award lecture at the AACR Annual Meeting 2019 in Atlanta, GA, USA (March 30-April 3, 2019).

### ELIGIBILITY

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

## NOMINATION PROCESS

Nominations may be made by any scientist, whether an AACR member or nonmember, who is now or has been affiliated with any institution involved in cancer research, cancer medicine, or cancer-related biomedical science. Candidates may not nominate themselves.
Nominators are asked to maintain the confidentiality of the nomination process and to refrain from informing the candidate about the nomination. There is no restriction on the number of candidates that may be nominated by any institution or individual.
There is no restriction on the number of candidates that may be nominated by any individual scientist. There is no restriction on the number of nominators that may write nomination letters or that may sign a single nomination letter on behalf of a candidate.
Nominations must be submitted online at myaacr.aacr.org no later than 4 p.m. U.S. ET on Wednesday, September 12, 2018. Paper nominations cannot be accepted. Full nomination instructions and program guidelines are available through the link below.
www.aacr.org/ScientificAwards

## NOMINATION MATERIALS

1) Nomination Letter. The letter must:

- be addressed to the Selection Committee; be written in English; and not exceed 1,000 words;
- specify the AACR Award for which the candidate is being nominated;
- contain a concise description of the candidate's major scientific discovery in basic cancer research or significant contributions to translational cancer research, and the impact of these accomplishments on the field, with publications supporting these accomplishments directly referenced within the letter;
- contain a concise description of the candidate's ongoing work which holds promise for continued substantive contributions to progress in the field of cancer; and
- be signed with a handwritten signature by the nominator.

If more than one candidate is nominated to share the Award, the nomination letter must clearly outline how the work of the individuals is closely related in subject matter and warrants a joint nomination.
2) Candidate's CV.
The candidate's curriculum vitae in English, including a complete list of the candidate's publications.
3) Summary Statement.
A statement of no more than 50 words summarizing the candidate's research accomplishments for which he or she is being nominated.

## SELECTION PROCESS

Candidates for the award will be considered by a prestigious international Selection Committee of renowned cancer leaders appointed by the president of the AACR in consultation with the Council of the Pezcoller Foundation. The committee will consider all nominations as they have been submitted; the committee may not combine submitted nominations, add a new candidate to a submitted nomination, or otherwise make alterations to the submitted nominations. After careful deliberations by the committee, its recommendations will be forwarded to the Executive Committee of the AACR and the

Council of the Pezcoller Foundation for final consideration and determination. Selection of the award winner will be made on the basis of the candidate's scientific accomplishments. No regard will be given to race, gender, nationality, or religious or political view.

### RESPONSIBILITIES OF THE AWARD RECIPIENT

The recipient will also present the **Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research Lecture**, both at the University of Padua and at the University of Trento in Italy, just prior to the official Award ceremony in Trento, in early May 2019. Remarks to be made during the ceremony must be delivered to the Pezcoller Foundation at least four weeks prior to allow sufficient time for translation into Italian. Should the recipient be unable to participate in either event, the award must be forfeited and may be presented instead to the alternate.
In the rare event that there are dual winners of the Award, the cash award will be shared equally between them, and the AACR Executive Committee will determine which of the two co-recipients will present the Pezcoller-AACR Award Lecture at the AACR Annual Meeting.

### CHANGES TO THE NOMINATION

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

### INQUIRIES

Questions regarding eligibility of a candidate may be directed to the AACR Scientific Achievement Awards office at awards@aacr.org or by calling (215) 446-7128, prior to submitting an application.

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# 2019 Scholar-In-Training Awards

The AACR is proud to offer Scholar-in-Training Awards to enable the participation of meritorious early-career scientists at the Annual Meeting 2018. Since its inception in 1986, the AACR Annual Meeting Scholar-in-Training Award program has provided more than 4,400 grants to young investigators and has received support from more than 50 cancer research foundations, corporations, individuals and other organizations dedicated to the fight against cancer. This year, twenty organizations or individuals generously provided the funding to support this program.

2019 AACR-PEZCOLLER FOUNDATION SCHOLAR-IN-TRAINING AWARDS
The Pezcoller Foundation supports these awards to enhance participation in the programs and activities of the AACR by early-career investigators residing in Europe and to provide these outstanding Scholar-in-Training Awardees with an opportunity to share their research findings with the international cancer research community at the AACR Annual Meeting.
Selections are made by the criteria from Pezcoller (i.e. European scientists with at least one awardee representing Italy) and based on the meritorious score of the submitted abstract and application.
Any questions can be directed to sita@aacr.org

*Picture:*
*2018 Scholar-In-Training Awardees with President Galligioni in Chicago, April 14, 2018*

# The Pezcoller Foundation

# Journal

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