

Virtual edition Tumor Heterogeneity, Plasticity and Therapy

5-6 MAY 2021

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#THPT21

UPCOMING 2021 VIB CONFERENCES & EVENTS

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24
Jun 2021

Interdisciplinary Symposium on COVID-19 (virtual edition)

28-10
Jun/Jul 2021

GCC2021 (Virtual edition)
#UseGalaxy

08-10
Sep 2021

Nanobodies (hybrid 2nd edition)
#Nanobodies21

15-17
Sep 2021

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Leuven - Belgium #TRP2021

22-24
Sep 2021

Genome Engineering and Synthetic Biology (virtual 4th Edition)
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27-28
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Leuven - Belgium #NHAD21

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Nov 2021

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Ghent - Belgium #TransImmu21

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ABOUT

VIB is a non-profit research institute in life sciences.

Basic research in life sciences is VIB's raison d'être. VIB is an independent research institute where some 1,500 top scientists from Belgium and abroad conduct pioneering basic research. As such, they are pushing the boundaries of what we know about molecular mechanisms and how they rule living organisms such as human beings, animals, plants and microorganisms.

Based on a close partnership with five Flemish universities – Ghent University, KU Leuven, University of Antwerp, Vrije Universiteit Brussel and Hasselt University – and supported by a solid funding program, VIB unites the expertise of all its collaborators and research groups in a single institute.

VIB's technology transfer activities translate research results into concrete benefits for society such as new diagnostics and therapies and agricultural innovations. These applications are often developed by young start-ups from VIB or through collaborations with other companies. This also leads to additional employment and bridges the gap between scientific research and entrepreneurship.

VIB also engages actively in the public debate on biotechnology by developing and disseminating a wide range of science-based information. More info can be found on www.vib.be

ABOUT VIB CONFERENCES

VIB offers a program of international conferences on various VIB research areas where world-renowned scientists and companies whose discoveries are landmarks in life sciences are brought together. The conferences facilitate networking and collaborations between scientists. There are two types of international meetings:

Science conferences

VIB's Science conferences focus on modern molecular and cellular biology fields, which relate strongly to some of VIB's research areas: cancer, cardiovascular biology, immunology, microbiology, neuroscience, structural and plant biology. The aim of VIB Science conferences is to bring together scientists from all over the world to discuss topics at the forefront of biological research.

Tools and Technologies

VIB's Tools and Technologies meeting series aims to provide a forum for both academic top speakers and scientists from emerging life science companies to present their latest scientific achievements and exciting technological solutions. Catalyzed by VIB's Technology Scouting team, the Tools and Technologies meetings focus on the front end of innovation and bring together key innovators in selected life sciences research fields.

ABOUT VIB SCIENCE EVENTS

In addition to the conferences that are part of the structured VIB conference series program, there are a lot of science events initiatives that arise within the centers. Those events are organized by VIB scientists in collaboration with external parties or VIB scientists are hosting a recurring international event. VIB conferences is happy to support such science events.

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WELCOME

Dear colleagues,

On behalf of the organizing committee, it is our pleasure to welcome all of you to the virtual first edition of Tumor Heterogeneity, Plasticity and Therapy.

Tumors are complex and evolving ecosystems composed of a wide variety of cell types, in addition to the cancer cells themselves. Increasing evidence indicates that the bi-directional interplay between tumor cells and their tumor microenvironment shape tumor evolution and therapy resistance. Within a tumor, many cancer cells differ from each other at the (epi-)genomic, transcriptional and proteomic level, as well as phenotypically and functionally. Tumor heterogeneity represents a major hurdle for therapy, which may only target a subset of tumor cells. In addition, cancer cell plasticity permits rapid adaptation to therapeutics and development of tolerance and resistance, presenting additional challenges for both targeted and immunotherapies.

During this conference 18 internationally renowned experts will discuss the latest developments in basic research, translational and clinical research and therapeutic avenues during 4 plenary sessions.

To support junior scientists and provide them with the opportunity to present their research, we have included eight shorter talks in the program, which were selected from submitted abstracts, as well as offer the opportunity for further interactions at poster sessions.

Although the conference is virtual, there are plenty of opportunities to interact and discuss with colleague-attendees. Plenary speaker presentations will be followed by a short Q&A session on stage and during the breaks you have the opportunity to join the 'meet the speaker' sessions for further discussion. In addition, over 100 researchers have a poster booth which you can visit and join via video chat.

Finally, we would like to thank our sponsors for supporting this event. You can visit their virtual booths during the entire event.

We wish you all an inspiring and productive conference.

Organizing Committee

Jean-Christophe Marine, VIB-KU Leuven Center for Cancer Biology, BE
Diether Lambrechts, VIB-KU Leuven Center for Cancer Biology, BE
Cédric Blanpain, VIB-KU Leuven Center for Cancer Biology, BE
Barbara Marte, Senior Editor Nature, UK
Evy Vierstraete, Science Events Manager, VIB, BE

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The 2021 VIB Conference Series is kindly supported by the following corporate partners:



11:00 - 12:00 Virtual coffee

12:00 - 12:10 Welcome by Dlether Lambrechts

Session 1 Stage

PLASTICITY & INITIATION/PROGRESSION AND METASTASIS (INCLUDING CTCs)

Chair: Cédric Blanpain

12:10 - 12:40 Small Cell Lung Cancer and Vasculogenic Mimicry - a route to metastasis
Caroline Dive, CRUK Manchester Institute, The University of Manchester, UK

12:40 - 13:10 Dissecting the metastatic microenvironment
Ilaria Malanchi, Francis Crick Institute, UK

13:10 - 13:20 *Selected talk:* Mitochondrial translation drives metabolic reprogramming required for metastasis
Sylvain Delaunay, German Cancer Research Center (DKFZ), DE

13:20 - 13:30 Short break

13:30 - 14:00 Metabolic intra-tumor heterogeneity driving metastasis formation
Sarah-Maria Fendt, VIB-KU Leuven Center for Cancer Biology, BE

14:00 - 14:30 Targeting Regenerative Plasticity in Metastasis
Karuna Ganesh, Memorial Sloan Kettering Cancer Center, US

14:30 - 14:40 *Selected talk:* Single-cell profiling of myeloid cells in glioblastoma across species and disease stage reveals macrophage competition and specialization
Kiavash Movahedi, VIB Center for Inflammation Research & VUB, BE

14:40 - 14:45 *Sponsored talk:* How VectorBuilder can support your oncology research
Justin Mirus, Sr. BD Manager, Central Europe, VectorBuilder GmbH, DE

14:45 - 15:15 Meet the speaker session 1

15:15 - 16:00 Poster and booth session

Session 2 Stage

HETEROGENEITY & REPROGRAMMING OF THE TUMOR (IMMUNE) MICROENVIRONMENT

Chair: Barbara Marte

16:00 - 16:30 Distinct properties of tumor-infiltrating immune cells across multiple cancer types
Zemin Zhang, BIOPIC, College of Life Sciences, and Beijing Advanced Innovation Center for Genomics, Peking University, CN

16:30 - 17:00 Exploring and Exploiting the Tumor Microenvironment
Johanna Joyce, Department of Oncology, University of Lausanne, Ludwig Institute for Cancer Research, CH

17:00 - 17:10 *Selected talk:* Delayed immune pressure in never-smoker lung cancer sculpts tumour evolution
Clare E Weeden, The Walter and Eliza Hall Institute of Medical Research & Department of Medical Biology, University of Melbourne, AU

17:10 - 17:20 Short break

17:20 - 17:50 Neuronal activity promotes the progression of glial malignancies
Michelle Monje, Stanford University, US

17:50 - 18:20 Immune contexture and tumor evolution in the era of immunotherapy
Jérôme Galon, INSERM & Laboratory of Integrative Cancer Immunology, Sorbonne Université, FR

18:20 - 18:30 *Selected talk:* Mass spectrometry imaging for the study of intra-tumor heterogeneity
Benjamin Balluff, Maastricht University, NL

18:30 - 19:00 Closing talk: Epigenetic influence of our (fatty) diet on metastatic-initiating cells
Salvador Aznar-Benitah, Stem Cells and Cancer Lab, Institute for Research in Biomedicine (IRB Barcelona), ES

19:00 - 19:30 Meet the speaker session 2

19:30 - 20:30 Networking

11:00 - 12:00 Virtual Coffee

Session 3

Stage

ADAPTATION AND RESISTANCE TO IMMUNOTHERAPIES

Chair: Diether Lambrechts

- 12:00 - 12:30** Cancer evolution, immune evasion and metastasis
Charles Swanton, *The Francis Crick Institute, UK*
- 12:30 - 13:00** Epigenetic mechanisms of malignant clonal dominance and immune evasion
Mark Dawson, *University of Melbourne, AU*
- 13:00 - 13:10** A Single-cell Map of the Dynamic Changes during Immune-checkpoint Blockade in Early Breast Cancer
Diether Lambrechts, *VIB-KU Leuven Center for Cancer Biology, BE*
- 13:10 - 13:20** *Selected talk:* Immunological response and resistance of human cancers to PD-1 blockade
Lisanne Roelofsen, *Netherlands Cancer Institute, NL*
- 13:20 - 13:30** Short break
- 13:30 - 14:00** Primary and secondary resistance to cancer immunotherapy
Thomas Gajewski, *University of Chicago, US*
- 14:00 - 14:30** Harnessing the potential of myeloid cells in brain and liver cancer treatment: a matter of genetics, time and combination
Leila Akkari, *Onco Institute. Netherlands Cancer Institute (NKI), NL*
- 14:30 - 14:40** *Selected talk:* Identification of Chitinase 3-like 1 as a soluble immune checkpoint paralyzing the cytotoxic granule machinery
Abbass Darwich, *Humanitas research hospital, IT*
- 14:40 - 14:45** *Sponsored talk:* Accelerating (immuno)oncology research and therapies
Matthieu Pesant, *NGS product manager, Takara Bio Europe, FR*
- 14:45 - 15:15** Meet the speaker session 3
- 15:15 - 16:00** Poster and booth session

Session 4

Stage

ADAPTATION AND RESISTANCE TO TARGETED THERAPIES

Chair: Chris Marine

- 16:00 - 16:30** Understanding the molecular evolution of tumors during therapy
Anna C Obenauf, *Research Institute of Molecular Pathology (IMP), AT*
- 16:30 - 17:00** Starvation and pseudo-starvation as drivers of phenotypic plasticity
Colin Goding, *Ludwig Institute for Cancer Research, University of Oxford, UK*
- 17:00 - 17:10** *Selected talk:* THEM6-mediated lipid remodelling sustains stress resistance in cancer
Arnaud Blomme, *CRUK Beatson Institute, UK*
- 17:10 - 17:20** Short break
- 17:20 - 17:50** Tumor cell plasticity as a challenge for targeted therapies in colorectal cancer
Fred de Sauvage, *Vice President and Staff Scientist, Genentech, US*
- 17:50 - 18:20** Heterogeneity and plasticity in head and neck cancer
Itay Tirosh, *Weizmann Institute of Science, IL*
- 18:20 - 18:30** *Selected talk:* Therapy-induced epigenetic inactivation of MCT1 renders tumor cells vulnerable for MCT4 inhibition
Catherine Vander Linden, *Pole of Pharmacology and Therapeutics (FATH), Institut de Recherche Expérimentale et Clinique (IREC), UCLouvain, BE*
- 18:30 - 19:00** Closing talk: Phosphatidylinositol saturation drives CD8+ effector T cell signaling and anti-tumor function
Erika Pearce, *Johns Hopkins University & Bloomberg-Kimmel Institute for Cancer Immunotherapy, US*
- 19:00 - 19:10** Closing remarks by Chris Marine & Nature Poster Prize announcement
- 19:00 - 19:30** Meet the speaker session 4
- 19:30 - 20:30** Networking

SPEAKER OVERVIEW



Leila Akkari
Netherlands Cancer
Institute (NKI), NL

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**Salvador
Aznar-Benitah**
Institute for Research in
Biomedicine, ES

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Mark Dawson
University of
Melbourne, AU

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Fred de Sauvage
Vice President & Staff
Scientist, Genentech, US

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Caroline Dive
The University of
Manchester, UK

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Fendt**
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INSERM & Sorbonne
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UK

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Johanna Joyce
Ludwig Institute for
Cancer Research, CH

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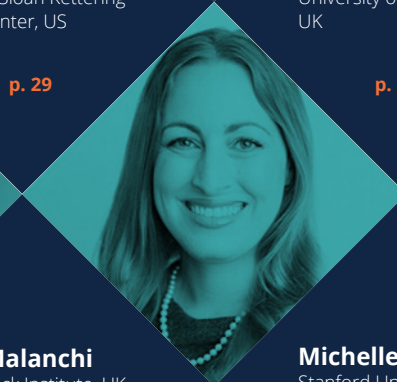
Diether Lambrechts
VIB-KU Leuven Center for
Cancer Biology, BE

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Weizmann Institute of
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BIOPIC & Peking
University, CN

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HARNESSING THE POTENTIAL OF MYELOID CELLS IN BRAIN AND LIVER CANCER TREATMENT: A MATTER OF GENETICS, TIME AND COMBINATION

Cancers evolve within complex tissue microenvironments that can influence multiple stages of the disease. Thus, to impact therapeutic outcome, our understanding of cancer dynamics and diversity must not solely view cancer cells as the fundamental unit, but as part of an integrated and reactive system centered around heterotypic communication with their environment.

We have investigated how microenvironment components such as tumor-associated macrophages (TAMs) and their molecular mediators fuel cancer progression and recurrence in glioblastoma radiotherapy treatment and targeted these cells in combination trials.

This revealed a timely regulated adaptation of their education that support recurrence in GBM. We additionally examined the dynamics of these cells and their monocytic precursors in genetically distinct hepatocellular carcinoma mouse models and found that the genetic make-up of cancer affects not only the composition of the immune landscape, but also the activation phenotype of myeloid cells infiltrating HCC.

Our studies provide mechanistical insights into myeloid-cell dependent immunosuppression and pro-tumorigenic features that impede response to treatment and favor tumor progression, which can be exploited to rationally design novel combination therapies.

LEILA AKKARI

Division of Tumor Biology & Immunology, Oncode Institute. Netherlands Cancer Institute (NKI), NL

My PhD in health sciences at the Molecular Genetics Institute of Montpellier, CNRS, was focused on studying the effects of HCV viral proteins on cancer initiation through alteration of hepatocyte polarity and cell shape. These years triggered my interest in understanding the mechanisms by which extracellular cues regulate cell transformation. Once my PhD finished,

I wanted to develop my focus in the tumor microenvironment field in rare tumors, and I joined Prof. Johanna Joyce's laboratory at Memorial Sloan Kettering Cancer Center in NYC. During this time, I began to work with mouse models of brain and pancreatic cancer, primarily focusing on tumor associated macrophages and their pro-tumorigenic roles in multiple tumor microenvironment.

In 2017, I became an Assistant Professor at the NKI, where I lead a research group at the Netherlands Cancer Institute in Amsterdam that is interested in the microenvironment-mediated mechanisms of tumor maintenance and therapeutic resistance to therapy in brain and liver malignancies.

The main focus of our research is to understand and target the dynamic changes in the tumor microenvironment that are associated with cancer malignancy, with a particular interest in macrophages, a highly plastic and heterogenous immune cell type in solid cancers. My lab uses a plethora of murine models of cancers to develop and test microenvironment-targeted drugs in a stage-dependent and population-dependent manner.

EPIGENETIC INFLUENCE OF OUR (FATTY) DIET ON METASTATIC-INITIATING CELLS

We have recently identified the cells uniquely responsible for the formation of metastasis in different types of human tumors. These cells have intriguing characteristics: i) they form primary lesions as efficiently as their tumor-initiating counterparts, but are exclusive in their ability to generate metastases; ii) they express the fatty acid translocase CD36, and are characterized by a unique lipid metabolic signature; iii) they are exquisitely sensitive to the levels of fat in circulation, and consequently, they relate the predisposition of metastasis directly to the content of dietary fat; iv) they are highly sensitive to CD36 inhibition, which almost completely abolishes their metastatic potential in preclinical models.

In my talk, I will present our new data on the molecular characterization of metastatic-initiating cells. I will place special emphasis on unpublished data showing how specific dietary lipids exert a striking long-term effect over metastatic-initiating cells related to promoting tumor innervation, putting forward a novel concept of “metastatic epigenetic memory” elicited by our diet.



SALVADOR AZNAR-BENITAH

Stem Cells and Cancer Lab, Institute for Research in Biomedicine (IRB Barcelona), ES

Salvador Aznar Benitah obtained his Honours BSc in Biochemistry and Molecular Biology at McGill University in 1998. In 2007, after a postdoctoral work in the laboratory of Fiona Watt at the London Research Institute (Cancer Research UK), he established his own lab at the Center for Genomic Regulation (CRG) as a Junior ICREA researcher. In 2014 Salvador was promoted to ICREA Research Professor, one of the most prestigious awards in research in Spain, and moved to the Institute for Biomedical Research (IRB) in Barcelona as a senior researcher. In 2015, he was also appointed as a Foundation Botín Researcher.

His lab aims at understanding the molecular mechanisms underlying adult stem cell function during homeostasis, ageing and cancer, with special interests in epigenetics, spatiotemporal regulation of stem cells (circadian rhythms), and the link between metastatic-initiating-cells and the content of fat in our diet.

He is the recipient of the Banc Sabadell Award in Biomedicine (2015), Doctores Diz Pintado Award in Biomedicine (2016), Beug Foundation Metastasis Award (2015), City of Barcelona Award in life sciences (2017), and National Award on life sciences (2017). In 2018, he has

been appointed as an EMBO member and has received an Advanced ERC grant.

EPIGENETIC MECHANISMS OF MALIGNANT CLONAL DOMINANCE AND IMMUNE EVASION

A hallmark of acute myeloid leukaemia (AML) is epigenetic dysregulation, which is initiated by recurrent translocations and/or mutations in transcription factors and chromatin regulators. This manifests as a block in myeloid differentiation and an increase in malignant self-renewal. Whilst AML is a malignancy with a very low mutational burden, the poor outcome that is pervasive in this disease is underpinned by marked genetic and transcriptional heterogeneity.

Using a novel strategy that couples cell fate determination with transcriptional and chromatin profiles we have studied the effect of clonal competition, the consequences of immune surveillance and therapeutic pressure in AML.

These findings provide new insights into the molecular pathogenesis of the disease and enable a greater understanding of clonal dominance and evasion of therapeutic pressure.

A portrait of Mark Dawson, a man with a beard and glasses, smiling. The image is overlaid with a teal gradient.

MARK DAWSON

Sir Peter MacCallum Department of Oncology & Centre for Cancer Research, University of Melbourne, AU

Professor Dawson is a clinician-scientist at the Peter MacCallum Cancer Centre and Centre for Cancer Research University of Melbourne. He is the program head of the Translational Haematology Program, Group leader of the Cancer Epigenetics Laboratory and Consultant Haematologist in the Department of Haematology. His research interest is studying the role of epigenetic regulators in the initiation, maintenance and progression of cancer. He is a fellow of the Royal Australasian College of Physicians and Royal College of Pathologists of Australasia.

After completing his clinical training in Melbourne, Australia he was awarded the prestigious General Sir John Monash Fellowship and Cambridge Commonwealth

Trust Fellowship, which he used to complete his PhD at the University of Cambridge. Following his PhD, he was awarded the inaugural Wellcome Trust Beit Prize Fellowship to pursue his research into epigenetic regulation of leukaemia stem cells.

His research has identified new therapeutic strategies for a range of cancers and has helped set the platform for clinical trials with first-in-class epigenetic therapies. He is currently a Professor in the Sir Peter MacCallum Department of Oncology and Centre of Cancer Research at the University of Melbourne. He is the Sir Edward Dunlop Fellow for the Cancer Council of Victoria and a Howard Hughes Medical Institute International Research Scholar.

TUMOR CELL PLASTICITY AS A CHALLENGE FOR TARGETED THERAPIES IN COLORECTAL CANCER

Colorectal cancer (CRC) is a leading cause of cancer related death and is largely thought to progress through the acquisition of specific genetic alterations, including functional loss of the tumor suppressors APC, TP53, and SMAD4 as well as activating mutations in oncogenes such as KRAS or BRAF. Despite extensive biological, molecular and clinical knowledge, CRC remains a high unmet medical need. This is especially the case once the tumor has disseminated beyond their primary site. Indeed, patients that present with metastatic disease, in particular to the liver, have a poor prognosis.

Hence, a better understanding of the processes that drive CRC progression and metastatic dissemination are key to enable the development of novel and effective therapies. To do so, pre-clinical *in vivo* models that faithfully recapitulate the human disease are critically needed. Despite the availability of xenograft, chemical-induced and genetically-engineered mouse models of CRC, these models fail to recapitulate full disease progression, including dissemination to the liver, the most relevant site of metastasis of human CRC.

To overcome these limitations, we have

developed orthotopic implantations of engineered intestinal organoids. Introduction of organoids with increasing number of mutations lead to a corresponding increase in tumor growth rate and to spontaneous metastatic dissemination to the liver and lung.

This approach enables the study of each and every step of the metastatic process as well as the testing of therapeutic modalities. Targeting these oncogenic pathways or the stem cells compartment driving the tumorigenic process represent therapeutic opportunities for the treatment of these tumor types.

Through this approach, we also have also uncovered that tumor cell plasticity poses new challenges for the efficacy of these therapeutic approaches in cancer.

A portrait of Fred de Sauvage, a middle-aged man with short, wavy brown hair, wearing a dark suit, white shirt, and a patterned tie. He is smiling slightly and looking towards the camera. The background is blurred, showing other people in a social setting.

FRED DE SAUVAGE

Vice President and Staff Scientist, Genentech, US

Frederic de Sauvage is Vice President of Research-Molecular Oncology at Genentech. He obtained his PhD *summa cum laude* from the Catholic University of Louvain in Belgium. He joined the laboratory of David Goeddel at Genentech as a postdoctoral fellow in 1990 and was hired as a Scientist in 1992.

In 1994, Dr. de Sauvage's team at Genentech discovered Thrombopoietin (TPO), the long sought physiological regulator of platelet production. His laboratory made many contributions to the field of hematopoiesis before switching its focus to the Hedgehog pathway in the late 1990s. His work led to

the development of vismodegib, the first Hedgehog Pathway Inhibitor approved for the treatment of metastatic or locally advanced basal cell carcinoma (BCC). Dr. de Sauvage has recently turned his attention to studying the role of intestinal stem cells in tumorigenesis.

In 2011 he received the Achievement in Advancing Targeted Therapies for Cancer & Melanoma Award from the American Skin Association in recognition of his work for patients with BCC. He was elected a fellow of the American Association for the Advancement of Science in 2016.

SMALL CELL LUNG CANCER AND VASCULOGENIC MIMICRY - A ROUTE TO METASTASIS

Small cell lung cancer (SCLC) is an aggressive, incurable neuroendocrine (NE) tumour with poor prognosis, characterised by early metastatic spread and a high burden of circulating tumour cells (CTCs). Most patients respond initially to standard of care chemotherapy (a platinum/etoposide doublet) but acquired chemotherapy resistance is rapid and relapse almost universal. Vascular mimicry (VM), the trans-differentiation of tumor cells endowing endothelial-like characteristics that support *de novo* vessel formation, is associated with poor prognosis in several tumour types including SCLC. In genetically engineered mouse models (GEMMs) of SCLC, NOTCH and MYC cooperate to drive a NE to non-NE cell phenotypic switch and co-operation between NE and non-NE cells is required for metastasis.

We sought to identify the molecular mechanisms underpinning SCLC VM and the identity of VM competent cell phenotypes using circulating tumour cell-patient derived explant models (CDX) and GEMMs. We show that VM supports tumour growth and that only non-NE cells are VM-competent, expressing signatures consistent with pseudohypoxia, blood vessel development and extracellular matrix (ECM) organization. Non-NE cells were also relatively

chemoresistant *ex vivo* compared to NE cells. On Matrigel, VM-primed non-NE cells re-model their ECM, forming VM surrogate hollow tubular networks. Network formation requires integrin $\beta 1$ which undergoes VM specific glycosylation.

Our data identify VM as an exemplar of functional heterogeneity in SCLC and support therapeutic co-targeting of NE and non-NE cells to curtail SCLC progression.

CAROLINE DIVE

CRUK Manchester Institute, The University of Manchester, UK

Upon completing her PhD studies in Cambridge, Professor Caroline Dive established her own research group at Aston University's School of Pharmaceutical Sciences in Birmingham studying mechanisms of drug induced tumour cell death, before moving to The University of Manchester to continue this research. Caroline was awarded a Lister Institute of Preventative Medicine Research Fellowship before joining the Cancer Research UK Manchester Institute (CRUK MI) in 2003. Currently, she is Interim Director of the Institute and Director of the CRUK MI Cancer Biomarker Centre, a multidisciplinary centre with expertise spanning tumour biology, preclinical pharmacology, biomarker discovery, biomarker assay validation and clinical qualification to regulatory standards, bioinformatics, biostatistics and most recently digital clinical trials.

Throughout her career, Caroline has attracted several prizes and awards, most notably she

was awarded the Pasteur-Weizmann/Servier International Prize in 2012 for her Biomarker Research, the AstraZeneca Prize for Women in Pharmacology in 2016 and in 2019, the Heine H. Hansen Lectureship Award by the International Association for the Study of Lung Cancer (IASLC). She is an elected Fellow of the Academy of Medical Sciences (2015), Fellow of the British Pharmacological Society (2012) and Fellow of the European Academy of Cancer Sciences (2011). In 2017, Caroline was awarded Commander of the Order of the British Empire (CBE) for her services to cancer research. Most recently, she became an elected member of EMBO (2020), received the first inaugural Johann Anton Merck Award in recognition for exceptional contributions to the field of preclinical oncology (2020), and was the recipient of the Mary J. Matthews Pathology/Translational Distinguished Service Award by IASLC (2021). Caroline is the current President of the European Association for Cancer Research (2020 – 2022).

METABOLIC INTRA-TUMOR HETEROGENEITY DRIVING METASTASIS FORMATION

Cancer metastasis requires the transient activation of cellular programs enabling dissemination and seeding in distant organs. Genetic, transcriptional and translational intra-tumor heterogeneity contributes to this dynamic process. Beyond this, metabolic intra-tumor heterogeneity has also been observed, yet its role for cancer progression remains largely elusive.

We discovered that intra-tumor heterogeneity in the expression of particular metabolic enzymes drives breast cancer cell dissemination and metastasis formation. Thus, this highlights the importance of metabolic intra-tumor heterogeneity for cancer progression.

A portrait of Sarah-Maria Fendt, a woman with dark hair, smiling. The image is overlaid with a teal color filter.

SARAH-MARIA FENDT

VIB-KU Leuven Center for Cancer Biology, BE

Sarah-Maria Fendt is a Principal Investigator at the VIB Center for Cancer Biology and Assistant Professor of Oncology at KU Leuven, Belgium.

Sarah has a Master degree of Science in Biochemistry from TU Munich and a PhD in Molecular Systems Biology from the ETH Zurich. Sarah worked as postdoc at the MIT, before joining VIB as an independent VIB group leader in 2013.

Sarah's lab is specifically interested in elucidating general regulatory principles

in metabolism, and understanding cancer metabolism during metastasis formation as well as during altered nutrient availability. To perform novel research in her fields of interest her group exploits their expertise in metabolomics and fluxomics.

The research of Sarah's lab is currently funded by multiple (inter)national grants and industry, which include an ERC consolidator grant. Sarah received several awards such as the Conquer-Cancer-Now and the Grants4Targets award.

PRIMARY AND SECONDARY RESISTANCE TO CANCER IMMUNOTHERAPY

Checkpoint blockade immunotherapy has revolutionized cancer treatment paradigms, but only a subset of patients with each approved cancer indication responds clinically. Clinical activity of anti-PD-1 Ab therapy is favored in patients having a T cell-inflamed tumor microenvironment at baseline.

The composition and magnitude of endogenous immune infiltration into tumors is regulated by at least 3 parameters: tumor cell-intrinsic oncogenic events that regulate immune responses, germline polymorphisms in immune regulatory genes, and the composition of the gut microbiome. Secondary resistance following initial clinical activity is dominantly impacted by tumor cell-intrinsic events and is favored by clonal heterogeneity.

Recent data have indicated that secondary resistance can occur upon immune selection not only for antigen-loss variants but also for tumor cells harboring additional immune-evasive oncogenic events. Genome-wide CRISPR screens are identifying new mechanisms of resistance, and recent evidence has implicated alterations in the ferroptosis pathway of cell death as being critical.



THOMAS GAJEWSKI

University of Chicago, US

Thomas Gajewski, MD, PhD is Professor in the Departments of Pathology and Medicine. He oversees the melanoma oncology clinic, is Leader of the Immunology and Cancer Program at the University of Chicago Comprehensive Cancer Center, and directs the Human Immunologic Monitoring core facility.

His laboratory studies the molecular and cellular regulation of T lymphocyte activation and differentiation, and in turn applies this information to preclinical and clinical efforts to promote anti-tumor immunity *in vivo*. Dr. Gajewski is committed to investigating and developing new treatments for patients with melanoma, with a special interest in the development of immunotherapies against the disease. He also leads development of immune-based therapies for other cancers, using new laboratory data on how the immune system is regulated to develop novel clinical trials.

Dr. Gajewski has published more than 200 papers on these subjects, has served on numerous NIH grant review panels, is an editor for Cancer Research and Journal for Immunotherapy of Cancer, is on the Program Committees for the American Society for Clinical

Oncology (ASCO) and the American Association for Cancer Research (AACR), and is past president of the Society for Immunotherapy of Cancer. He was recently awarded the first American Cancer Society-Jules L. Plangere Jr. Family Foundation Professorship in Cancer Immunotherapy.

As his laboratory work leads to new potential immunotherapies for cancer, Dr. Gajewski is involved with several early biotech companies. He is scientific co-founder of Jounce Therapeutics, and has licensing arrangements with Aduro Biotech and Evelo, a new company focused on the microbiome and cancer.

IMMUNE CONTEXTURE AND TUMOR EVOLUTION IN THE ERA OF IMMUNOTHERAPY

To-date, the evaluation of the prognosis of cancer patients relied on the anatomic extent of tumor (TNM-classification). However, this classification provides limited prognostic information and does not predict response to therapy. We redefined cancer by integrating the immune system to transfer cutting-edge medicine to the patients. We have previously shown that tumors from human colorectal cancer with a high-density of infiltrating memory and effector-memory T-cells are less likely to disseminate to lympho-vascular and perineural structures and to regional lymph-nodes. We also demonstrated the critical tumor-microenvironment parameters determining the dissemination to distant metastasis. We found that the combination of immune parameters associating the nature, the density, the functional immune orientation and the location of immune cells within the tumor was essential to accurately define the impact of the local host-immune reaction on patients' prognosis. We defined these parameters as the "immune contexture". We characterized the immune landscape within human tumors, and showed the importance of several adaptive immune cells. We described the immunophenotype and antigenome associated with immune escape mechanisms and demonstrated mechanisms associated with pre-existing and proliferating intratumoral T-cells.

Based on the immune contexture, a standardized, simple and powerful digital-pathology-based immune stratification-system, termed "Immunoscore", was delineated having a prognostic power superior to that of the currently used cancer staging-system. Tumor invasion parameters were statistically dependent on the host-immune reaction. A worldwide consortium validated the prognostic value of the consensus Immunoscore, using a standardized-assay.

We have demonstrated the significant role of Immunoscore and immunoediting in affecting metastatic dissemination in space and time. We hence proposed a "parallel immune selection model" of tumor evolution incorporating the effects of the immune system in shaping and driving metastatic spread. Further analyses revealed a large inter- and intra-metastatic immune heterogeneity. Immunoscore and T and B cell score (TB score) from the least-infiltrated metastasis are the most associated with survival. Thus, tumor progression, invasion and recurrence are dependent on pre-existing immunity and on Immunoscore. Finally, novel concepts underpinning tumor evolution at the pre-cancerous stages, and predictive and mechanistic immune signatures of response to cancer therapies will be discussed.

JÉRÔME GALON

INSERM & Laboratory of Integrative Cancer Immunology, Sorbonne Université, FR

Dr. Jérôme Galon is Director of Research at INSERM (French NIH), and Head of the laboratory of Integrative Cancer Immunology, in Paris, France.

Dr. Galon was trained as an immunologist at the Pasteur Institute and at the Curie Institute (Paris, France). He holds a Ph.D. degree in Immunology (Jussieu University, Paris, France, 1996). Between 1997 and 2001 he worked at the NIH (National Institute of Health, Bethesda, USA). Since his full-tenured position at INSERM in 2001, he directs interdisciplinary research programs on tumor-Immunology. He is associate Director and co-founder of European Academy of Tumor Immunology (EATI), board Director of the Society for Immunotherapy of Cancer (SITC).

His work on the comprehensive analysis

of the tumor-microenvironment and the role of T-cells in human cancer led to the demonstration of the importance of adaptive pre-existing immunity in human cancer, and the concept of cancer immune-contexture. He pioneered the Immunoscore.

He is the co-founder of HalioDx company and the Chairman of its scientific council. His contributions have been recognized with numerous awards, including the William B. Coley Award, an international prize which honors the best scientists in the fundamental and cancer immunology, the Award from the National Academy of Science and from the National Academy of Medicine. In 2019, he was the winner of the prestigious European Inventor Award from the European Patent Office.

TARGETING REGENERATIVE PLASTICITY IN METASTASIS

Metastatic cancers invariably relapse due to the emergence of therapy resistant subclones that are capable of self-renewal, slow cell-cycling, tumor re-initiation and therapy resistance. Emerging evidence suggests that advanced human cancers must dynamically change their gene expression programs in order to enter into regenerative cell states (termed metastasis initiating cells (MICs)) that enable them to survive the stresses of dissemination and distant organ colonization while evading immune surveillance. However, the molecular mechanisms that underpin the phenotypic plasticity of metastasis stem cells, their relationship to tumor initiating cancer stem cells and macrometastasis, and the co-evolution of the immune response to dynamically emerging tumor regenerative states are not understood. Insight into the evolution of metastatic cell states has been limited by the lack of representative preclinical models that reproduce the dynamic cell state plasticity and heterogeneity of advanced human cancer. We have established protocols to generate 3-dimensional organoid cultures from normal colons, primary tumors and metastases of patients with metastatic colorectal cancer (CRC). Using organoid systems, we recently defined metastasis-stem cells (MICs) as a regenerative entity distinct from tumor-initiating cancer stem cells in patients, and identified L1CAM as

a clinically actionable marker and mediator of MetSCs.

We are adopting a patient-derived functional biospecimen approach combining (1) hypothesis-generation using transcriptomics, epigenomics and spatial/histological analysis of patient samples of primary and metastatic gastrointestinal (GI) cancer with (2) mechanistic dissection in cutting-edge patient-derived organoid models, including co-cultures with immune/stromal cells, and (3) genetically engineered and orthotopic transplantation models of metastatic GI cancer. By profiling, lineage tracing and genetically interrogating the evolving transcriptomic and chromatin landscapes of regenerative metastatic states, we seek to define the molecular mechanisms that underpin the phenotypic plasticity of metastatic cancer, and the co-evolution of the tumor microenvironmental response to dynamically emerging tumor regenerative states. Our goal is to identify crucial signaling nodes required for metastatic plasticity that can be therapeutically targeted to improve outcomes for patients with advanced cancer.

KARUNA GANESH

Memorial Sloan Kettering Cancer Center, US

Karuna Ganesh, MD, PhD, is a physician-scientist focused on investigating and treating metastatic gastrointestinal cancers at Memorial Sloan Kettering Cancer Center, New York, USA. Dr. Ganesh leads a research laboratory in the Molecular Pharmacology Program, is an Attending Physician in the Gastrointestinal Oncology Service, and Director of Metastasis Research in the Center for Colorectal Cancer at Memorial Sloan Kettering. She established an institutional program to generate organoids from primary and metastatic colorectal cancer, defined metastasis-stem cells (MetSCs) as a regenerative entity distinct from tumor-initiating cancer stem cells in patients, and identified L1CAM as a marker and mediator of MetSCs.

The Ganesh laboratory employs (1) hypothesis-generation using transcriptomics,

epigenomics and spatial/histological analysis of patient samples of primary and metastatic gastrointestinal (GI) cancer with (2) mechanistic dissection in cutting-edge patient-derived organoid models, including co-cultures with immune/stromal cells (3) genetically engineered and orthotopic transplantation models of metastatic GI cancer. By combining murine and clinically relevant patient-derived models, we seek to define the molecular mechanisms that underpin the phenotypic plasticity of metastatic cancer, and the co-evolution of the tumor microenvironmental response to dynamically emerging tumor regenerative states.

Our goal is to identify crucial signaling nodes required for metastatic plasticity that can be therapeutically target to improve outcomes for patients with advanced cancer.

STARVATION AND PSEUDO-STARVATION AS DRIVERS OF PHENOTYPIC PLASTICITY

Phenotypic heterogeneity in cancer arising as a consequence of bi-directional interactions between cancer cells and their microenvironment drives cancer progression and is increasingly recognized as a major barrier to effective therapy. Considerable progress has been made in identifying the host of microenvironmental signals that effect reversible phenotypic transitions underpinning the early steps in the metastatic cascade. However, although the general principles underlying metastatic dissemination have been broadly outlined, a common theme that unifies many of the triggers of invasive behaviour in tumours is only beginning to emerge.

Using melanoma as a model, we reveal how microenvironmental influences including nutrient limitation and inflammatory signalling converge on reprogramming of protein translation via phosphorylation of eIF2 α , a hallmark of the starvation response. As a consequence of the p-eIF2 α -mediated translation switch, melanoma cells dedifferentiate and reprogram their metabolism leading to stabilization of an invasive state with increased capacity for tumour initiation.

The results highlight how multiple cell extrinsic microenvironmental signals, as well as cell intrinsic events such as oncogene activation, trigger translation reprogramming as an evolutionarily conserved survival strategy that leads to phenotypic heterogeneity, increased metastatic dissemination and therapy resistance.



COLIN GODING

Ludwig Institute for Cancer Research, University of Oxford, UK

Colin Goding completed a PhD in virology at the National Institute for Medical Research, London, UK, working on adenovirus DNA replication. He then moved as a Post-doc to Pierre Chambon's lab in Strasbourg, France, where his interest in gene regulation developed. At the Marie Curie Research Institute he continued working on how genes are regulated, both in *S. cerevisiae*, as well as in melanocytes and melanoma. His work revealed how transcription factors could discriminate between different classes of binding motif, and identified the basis for regulation of melanocyte-specific genes. In 2008 he moved to the Ludwig Institute for Cancer Research, University of Oxford, UK to take up the position of Professor of Oncology where he continues to examine the role of signaling and transcription in melanoma biology.

He has made a major contribution to understanding the molecular mechanisms underpinning melanoma cell plasticity and developed the concept of phenotype-switching that drives metastatic dissemination and therapy resistance. More recently he uncovered the critical role of translation reprogramming in cancer progression, and has identified a key regulator of lipid homeostasis

as a major determinant of phenotypic identity.

He was elected EMBO member in 2008 and Director of the Oxford Stem Cell Institute in 2015.

EXPLORING AND EXPLOITING THE TUMOR MICROENVIRONMENT

Cancers do not arise within a vacuum; rather they develop and grow within complex organs and tissue environments that critically regulate the fate of tumor cells at each sequential step of malignant progression. The tumor microenvironment (TME) can be viewed as an intricate ecosystem populated by diverse innate and adaptive immune cell types, stromal cells, extracellular matrix, blood and lymphatic vessel networks that are embedded along with the cancer cells. While bidirectional communication between cells and their microenvironment is critical for normal tissue homeostasis, this active dialog can become subverted in cancer leading to tumor initiation and progression.

Through their exposure to tumor-derived molecules, normal cells can become “educated” to actually promote cancer development. As a consequence of this tumor-mediated education, TME cells produce a plethora of growth factors, chemokines, and matrix-degrading enzymes that together enhance the proliferation and invasion of the tumor. Moreover, these conscripted normal cells also provide a support system for cancer cells to fall back on following traditional therapies such as chemotherapy and radiation, and

additionally contribute to a general immune-suppressive state, thus limiting the efficacy of immunotherapies.

Consequently, multi-targeted approaches in which co-opted cells in the microenvironment are “re-educated” to actively fight the cancer represent a promising strategy for the effective long-term treatment of this devastating disease

JOHANNA JOYCE

Department of Oncology, University of Lausanne, Ludwig Institute for Cancer Research, CH

Johanna Joyce is a Professor at the University of Lausanne, Switzerland, and Full Member of the international Ludwig Institute of Cancer Research. Her laboratory investigates the microenvironment in which a tumor arises and the critical influence that non-cancerous immune and stromal cells can have on tumor progression, metastasis and therapeutic response. They have uncovered regulatory signals provided by the normal tissue stroma and immune cells to the cancer cells, and determined how normal cells can be modified by the cancer cells to produce a variety of factors that enhance tumor malignancy. A major focus of the lab's recent research has been to deeply and comprehensively

interrogate the immune landscape of brain tumors, and then use this gained knowledge to develop novel strategies to therapeutically target the tumor microenvironment.

Prof. Joyce has been recognized for her contributions to cancer research through a series of awards including the Robert Bing Prize, Cloetta Prize, Swiss Bridge Award, American Cancer Society Scholar Award, Rita Allen Foundation Award, V Foundation Award, Sidney Kimmel Foundation Award, and many others. In 2017, she was elected as a Member of EMBO and a Fellow of the European Academy of Cancer Sciences.

A SINGLE-CELL MAP OF THE DYNAMIC CHANGES DURING IMMUNE-CHECKPOINT BLOCKADE IN EARLY BREAST CANCER

Introduction. To improve our understanding of the molecular mechanisms underlying response and resistance to immune checkpoint blockade (ICB), we monitored dynamic changes within the tumour microenvironment exposed to ICB by single-cell analyses.

Materials and methods. In a window-of-opportunity setting, fresh tumor tissue was collected from treatment-naïve patients (n=29) with early breast cancer before and ± 9 days after a single dose of 200 mg pembrolizumab. Serially collected pre- and on-treatment biopsies were subjected to single-cell RNA (scRNA) and T-cell receptor (TCR) sequencing by using the Single Cell V(D)J Solution kit from 10x Genomics and Illumina sequencing. Expression matrices were generated using Cell Ranger (10x Genomics) and analyzed with Seurat.

Results. Based on the presence of clonotype expansion upon treatment, patients were classified in two groups (Es and NEs). PD1-expressing T-cells characterized by functional genes clonally expanded upon treatment in Es, while in NEs T-cells seems to be stuck at less differentiated states. In cancer cells, decreased MHC class I/II expression was detected in NEs

compared to Es, indicating problems with antigen presentation could be linked with T-cell expansion. In dendritic cells and macrophages, PD-L1/2 expression was associated with clonotype expansion, suggesting PD1 PD-L1/2 signaling as a mechanism of action for immune evasion in Es. Also, dendritic cells could enable T-cell expansion after anti-PD1 as they increase in relative frequency upon treatment and express (co-)stimulatory molecules (CD80/86, CXCL9/10) and functional molecules (LY75, TAP1). Lastly, we found an inhibitory macrophage subtype (CX3CR1+, C3+) to be increased in NEs.

Conclusion. In this study, we collected serial biopsies before and during anti-PD1 from early breast cancer patients and subjected these to single-cell profiling, enabling the characterization of the tumor microenvironment pre- and on-treatment as well as dynamic changes induced by a single-dose of ICB. By identifying various phenotypes and associated gene sets that positively or negatively correlate with T-cell expansion following anti-PD1, we present a detail



DIETHER LAMBRECHTS

VIB-KU Leuven Center for Cancer Biology, BE

Diether Lambrechts is group leader in the VIB-KU Leuven Center for Cancer Biology and Professor at the University of Leuven, Belgium. Lambrechts was trained as an engineer at the University of Leuven and worked under Peter Carmeliet on the role of vascular endothelial growth factor in amyotrophic lateral sclerosis for his PhD and postdoc until 2007. He then worked at the Wellcome Trust Centre for Human Genetics in the Nuffield Department of Clinical Medicine at the University of Oxford, England, before joining VIB as an independent VIB group leader.

Diether Lambrechts has won 3 Belgian national awards: the Karel-Lodewijk Award for Human Medicine, the Galenus Prize for Pharmacology and the AstraZeneca Award for Translational Research.

The expertise of the Laboratory for translational Genetics is focused on tackling important questions in oncology by translating genome-scale data sets into clinically applicable knowledge. Investigations are based on the application of cutting-edge sequencing technologies and bioinformatics, and on the seamless integration of genomic data sets with clinical and fundamental biological information

to generate novel insights and potent biomarkers for the field of oncology.

Since March 2015, Diether Lambrechts also acts as director of the VIB Cancer Program in Leuven.

DISSECTING THE METASTATIC MICROENVIRONMENT

The tumour microenvironment is the vital non-cancerous compartment of the tumour structure. To date, a direct investigation of the early cellular changes induced by metastatic cells in the surrounding tissue *in vivo* is difficult to achieve, especially at early micro-metastatic stages.

We developed a strategy whereby tissue infiltrating cancer cells label neighbouring cells *in vivo* and allows direct identification of metastatic niche cells within the whole tissue, to define changes in the local micro-metastatic niche. We uncovered a remarkable local lung parenchymal regenerative response, where lung alveolar epithelial cells in the niche show stem-cell features with multi-lineage differentiation potential.

We here show that those cancer associated parenchymal cells (CAPs) fuel cancer cell growth, which is further enhanced by changes in niche myeloid cells and highlight the radical tissue local remodelling accompanying cancer cell growth.

A portrait of Ilaria Malanchi, a woman with dark curly hair, wearing a dark top, standing in front of a window. The image is overlaid with a teal color filter.

ILARIA MALANCHI

Francis Crick Institute, UK

Ilaria Malanchi obtained her Master degree at the University of Siena after two years spent at the research centre of Chiron Vaccine, (later Novartis Vaccine and now GSK, Siena, Italy). She obtained her PhD from Heidelberg University (Germany) working in the laboratory of Dr Tommasino at the DKFZ. As Dr Tommasino moved his laboratory to France in 2003, she spent one year at the IARC (WHO, Lyon, France).

Her long-standing interest in the complex cancer cellular interaction *in vivo* began when she moved to the newly opened Joerg Huelsken lab at the ISREC, Lausanne, Switzerland, (now part of the Federal Swiss University (EPFL)),

to start her postdoc in 2004. During these Postdoc years she began to investigate the importance of tumour microenvironment during metastatic progression.

Building on this expertise in mouse tumour models, Ilaria set up her laboratory at the Cancer Research UK London Research Institute in 2011 (now part of the Francis Crick Institute) and has since focused the scope of investigation on the interaction between cancer cells and the surrounding tissue during tumorigenesis and metastatic progression. Ilaria was awarded tenure and promoted to Senior Group Leader at the Francis Crick Institute in 2018.

NEURONAL ACTIVITY PROMOTES THE PROGRESSION OF GLIAL MALIGNANCIES

The nervous system regulates stem and precursor cell behavior across a range of tissues. In the central nervous system, neuronal activity is a critical regulator of development and plasticity. Activity-dependent proliferation of healthy glial progenitors, oligodendrocyte precursor cells (OPCs), and the consequent generation of new oligodendrocytes contributes to adaptive myelination. This plasticity of myelin tunes neural circuit function and contributes to healthy cognition.

The robust mitogenic effect of neuronal activity on normal oligodendroglial precursor cells, a putative cellular origin for many forms of glioma, suggests that dysregulated or “hijacked” mechanisms of myelin plasticity might similarly promote malignant cell proliferation in this devastating group of brain cancers. Indeed, neuronal activity promotes proliferation and growth of both high-grade and low-grade glioma subtypes in preclinical models. Crucial mechanisms mediating activity-regulated glioma growth include secretion of BDNF and the synaptic protein neuroligin-3 (NLGN3). NLGN3 induces multiple oncogenic signaling pathways in the cancer cell, and also promotes glutamatergic synapse formation between

neurons and glioma cells. This synaptic and electrical integration of glioma into neural circuits is central to tumor progression in preclinical models.

NLGN3 is necessary for the growth of gliomas in a range of preclinical models, and therapeutic targeting of NLGN3 is presently under clinical investigation. Thus, neuron-glia interactions not only modulate neural circuit structure and function in the healthy brain, but paracrine and synaptic neuron-glioma interactions also play important roles in the pathogenesis of glial cancers.

The mechanistic parallels between normal and malignant neuron-glia interactions underscores the extent to which mechanisms of neurodevelopment and plasticity are subverted by malignant gliomas, and the importance of understanding the neuroscience of cancer.

MICHELLE MONJE

Stanford University, US

Michelle Monje, MD, PhD, is an Associate Professor of Neurology and, by courtesy, of Neurosurgery, Psychiatry, Pathology and Pediatrics. Dr. Monje received her M.D. and Ph.D. in Neuroscience from Stanford University. As a Ph.D. student under the mentorship of Dr. Theo Palmer, Dr. Monje studied microenvironmental determinants of neural stem cell fate choice. Dr. Monje completed her residency training in neurology at the Massachusetts General Hospital/Brigham and Women's Hospital/Harvard Medical School Partners program, and then returned to Stanford for a clinical fellowship in pediatric neuro-oncology and a post-doctoral fellowship with Dr. Philip Beachy. During her postdoctoral work, Dr. Monje focused on the neurodevelopmental origins of a devastating pediatric brain tumor called diffuse intrinsic

pontine glioma (DIPG).

Dr. Monje's research program studies the molecular and cellular mechanisms of postnatal neurodevelopment and pediatric gliomagenesis. This includes microenvironmental influences on neural precursor cell fate choice in normal neurodevelopment and in disease states. Areas of emphasis include neuron-glia interactions, developmental and adaptive myelination, and microenvironmental contributions to glioma pathogenesis. As a practicing neurologist and neuro-oncologist, Dr. Monje is particularly interested in the identifying novel therapeutic strategies for pediatric gliomas, the neural regulation of cancer progression and the neurological consequences of cancer treatment.

UNDERSTANDING THE MOLECULAR EVOLUTION OF TUMORS DURING THERAPY

Targeted therapies and immunotherapies represent main pillars of cancer treatment, yet how they shape tumours during treatment response and resistance and thereby influence subsequent therapeutic responses is poorly understood.

Here, we show in melanoma patients and mouse models that when tumours relapse on targeted MAPK pathway inhibitors, they are cross-resistant to immunotherapies, despite their entirely different mode of action. We find that cross-resistance is mediated via a cancer cell-instructed, immune-suppressive tumour microenvironment that lacks functional CD103+ dendritic cells, precluding an effective T cell response. Restoration of CD103+ dendritic cell numbers and functionality can re-sensitize cross-resistant tumours to immunotherapy.

Using our lineage tracing method CaTCH, which allows the retrospective isolation of founding clones prior to evolutionary selection, we demonstrate that cross-resistance is acquired during MAPKi treatment. Cross-resistance does not arise from the selective pressure of an immune response during the evolution of resistance, paradoxically it results

from the MAPK pathway, which is not only reactivated during the formation of targeted therapy resistance but has gained increased transcriptional output driving immune evasion.

Our work provides mechanistic evidence for cross-resistance between unrelated therapies and a scientific rationale for treating patients with immunotherapy before they acquire resistance to targeted therapy.

A portrait of Anna C Obenauf, a woman with long dark hair, smiling. The image is overlaid with a teal color filter.

ANNA C OBENAUF

Research Institute of Molecular Pathology (IMP), AT

Anna C Obenauf studied molecular biology at the Karl-Franzens-University in Graz and obtained her PhD with distinction in 2010 from the Department of Human Genetics at the Medical University of Graz. For her dissertation, she received the Award of Excellence, the state prize for the best dissertations. She then worked from 2010 to 2015 as a postdoctoral research associate with Joan Massagué at the Memorial Sloan Kettering Cancer Center (MSKCC) in New York, USA - the first three years of which were funded by the FWF's Erwin Schrödinger Programme. For her work "Therapy induced tumour secretomes promote resistance and tumour progression" published in Nature, she was awarded the ASCINA Prize of the Federal Ministry of Education, Science and Research in 2015.

In 2016 Anna established her lab at the Research Institute of Molecular Pathology (IMP) in Vienna. Her laboratory focuses on understanding the molecular programs that drive the progression and immune evasion of metastatic cancer, to develop rational therapeutic strategies that result in durable responses. The lab also actively explores resistance mechanisms to targeted and immunotherapies, which pose a significant challenge. Work in her lab is funded by the WWTF and in 2018, she received an ERC Starting Grant for her research project "Rational combination therapies for metastatic cancer/CombaTCancer". In 2019, she was elected as a member of the Young Academy of the Austrian Academy of Sciences.

PHOSPHATIDYLINOSITOL SATURATION DRIVES CD8+ EFFECTOR T CELL SIGNALING AND ANTI-TUMOR FUNCTION

Lipids are essential mediators of T cell signaling and function. We investigated lipid alterations during mouse and human CD8+ T cell activation *in vitro* and found that phosphatidylinositol (PI) species with a distinct acyl chain composition, marked by increased saturation, accumulated in effector T cells (TE). This unique PI signature was also evident in mouse and human CD8+ TE *in vivo*, and PI synthesis inhibition impaired TE fitness. PI synthesis depended on TE glycolysis, and co-culture of TE with B16-tumor spheroids caused depletion of TE-associated PIs.

Analysis of B16-melanoma tumor-infiltrating lymphocytes revealed that increased PI saturation predicted improved anti-tumor function following immunotherapy treatment, results also observed in circulating CD8+ T cells from patients with malignant melanoma who successfully responded to immunotherapy. Mechanistically, PI saturation was essential to maintain increased PIP2 signaling in TE.

Our findings suggest that approaches to boost PI saturation in TE could be important for cancer immunotherapy.

ERIKA PEARCE

Johns Hopkins University & Bloomberg-Kimmel Institute for Cancer Immunotherapy, US

Dr. Pearce obtained her Ph.D. in Cell and Molecular Biology in 2005 at the University of Pennsylvania in Philadelphia, where she studied the regulation of T cell responses during infection. During her postdoctoral studies, also at the University of Pennsylvania, she began her research into how cellular metabolic processes govern immune responses to infection and cancer.

She launched her independent career in 2009, holding faculty positions at the Trudeau Institute in NY and then Washington University School of Medicine in St. Louis. She moved her research group to Europe in

2015 to become a Director at the Max Planck Institute for Immunobiology and Epigenetics in Freiburg, Germany. In 2018 she was awarded the Gottfried Wilhelm Leibniz Prize from the DFG for her work on immunometabolism. In 2021 she became a Bloomberg Distinguished Professor at the Johns Hopkins University and Scientific Director of the Bloomberg-Kimmel Institute for Cancer Immunotherapy in Baltimore.

Her work continues to investigate the connection between metabolism and cell function.

CANCER EVOLUTION, IMMUNE EVASION AND METASTASIS

Evidence supports complex subclonal relationships in solid tumours, manifested as intratumour heterogeneity. Parallel evolution of subclones, with distinct somatic events occurring in the same gene, signal transduction pathway or protein complex, suggests constraints to tumour evolution that might be therapeutically exploitable. Data from TRACERx, a longitudinal lung cancer evolution study will be presented. Drivers of tumour heterogeneity change during the disease course and contribute to the temporally distinct origins of lung cancer driver events. APOBEC driven mutagenesis appears to be enriched in subclones in multiple tumour types. Oncogene, tumour suppressor gene and drug induced DNA replication stress are found to drive APOBEC mutagenesis. Phylogenetic tracking detects minimal residual disease and clonal evolution of disease from primary to metastatic sites, presenting opportunities for drug development.

On-going chromosomal instability, manifested as Mirrored Subclonal Allelic Imbalance (MSAI) is found to be a major driver of intratumour heterogeneity across cancer types, contributing to parallel evolution and selection. Subclonal

driver events, evidence of ongoing selection within subclones, combined with genome instability driving cell-to-cell variation is likely to limit the efficacy of targeted monotherapies, suggesting a need for new approaches to drug development and integration of cancer immunotherapeutic approaches. Multiple adaptive mechanisms to neo-antigen evolution have been found in TRACERx highlighting cancer chromosomal instability driving immune evasion and HLA loss and loss of clonal neo-antigens as well as epigenetic repression of neo-antigens. The clonal neo-antigenic architecture may act as a tumour vulnerability to mitigate resistance and treatment failure.

A portrait of Charles Swanton, a man with glasses and a slight smile, wearing a dark suit jacket over a light-colored shirt. The background is a solid teal color.

CHARLES SWANTON

The Francis Crick Institute, UK

Charles completed his MBPhD training in 1999 at the Imperial Cancer Research Fund Laboratories and Cancer Research UK clinician scientist/medical oncology training in 2008. Charles combines his laboratory research at the Francis Crick Institute with clinical duties at UCLH and as director of the CRUK UCL Lung Cancer Centre, focussed on how tumours evolve over space and time. Charles has helped to define the branched evolutionary histories of solid tumours, processes that drive cancer cell to-cell variation in the form of new cancer mutations or chromosomal instabilities, and the impact of such cancer diversity on effective immune surveillance and clinical outcome.

Charles was made Fellow of the Royal College of Physicians in April 2011, appointed Fellow

of the Academy of Medical Sciences in 2015, Napier Professor in Cancer by the Royal Society in 2016, appointed Cancer Research UK's Chief Clinician in 2017, and elected Fellow of the Royal Society in 2018. Charles was awarded the Stand up to Cancer Translational Cancer Research Prize (2015), Glaxo Smithkline Biochemical Society Prize (2016), San Salvatore prize for Cancer Research (2017), the Ellison-Cliffe Medal, Royal Society of Medicine (2017), recipient of the Gordon Hamilton Fairley Medal (2018), the Massachusetts General Hospital Cancer Centre Kraft Prize for Excellence in Cancer Research (2018), and the International Association for the Study of Lung Cancer (IASLC) Paul A. Bunn Scientific Award for achievements in lung cancer (2018).

HETEROGENEITY AND PLASTICITY IN HEAD AND NECK CANCER

Each tumor is composed of diverse malignant, immune and stromal cells that interact with one another and collectively determine tumor biology and clinical phenotypes. We are applying single cell RNA-seq (scRNA-seq) to clinical tumor samples to comprehensively characterize the cellular diversity within tumors and explore the function of distinct tumor subpopulations.

In this talk, I will focus on our work in head and neck squamous cell carcinoma (HNSCC) and on three subpopulations of cells with clinical significance that we identified in patient samples and are further studying using model systems. First, we found cells that have undergone a partial epithelial-mesenchymal transition (EMT). These cells are located at the invasive edge of tumors and are associated with increased chance of metastasis. We leverage this observation to define EMT patterns across many cancer types and assess their functional significance. Second, we uncovered a senescence-associated subpopulation that is predictive of clinical drug responses.

Finally, in HPV-driven tumors, we find a distinct subpopulation that is associated with

decreased survival. These subpopulations demonstrate the patterns and significance of intra-tumor heterogeneity and may help to define novel combination treatments.

A portrait of Itay Tirosh, a man with dark hair and glasses, wearing a dark polo shirt. The background is a blurred outdoor setting with green foliage. The entire image has a teal/cyan color overlay.

ITAY TIROSH

Weizmann Institute of Science, IL

Itay Tirosh obtained his BSc from Ben-Gurion University and his PhD from the Weizmann Institute of Science, both in the area of computational biology. From 2012 to 2017 he was a postdoctoral fellow at the Regev and Golub labs at the Broad Institute of MIT and Harvard, and since August 2017 he has been a Senior Scientist at the Weizmann Institute of Science, Dept. of Molecular Cell Biology.

The Tirosh lab is combining computational

and experimental methods to study human tumors as a complex ecosystem in which diverse cancer and non-cancer cells interact and collectively determine tumor biology and response to therapies. They leverage single cell technologies, computational approaches and clinical collaborations to understand the diversity of cells within human tumors, the mechanisms that maintain this diversity and the implications for cancer therapy.

DISTINCT PROPERTIES OF TUMOR-INFILTRATING IMMUNE CELLS ACROSS MULTIPLE CANCER TYPES

Understanding tumor immune microenvironments is critical for identifying immune modifiers of cancer progression and for developing cancer immunotherapies, but it is increasingly evident that different cancer types may harbor distinct characteristics of subtypes, functional states and cell-cell interactions for tumor-infiltrating immune cells.

We first performed deep single-cell RNA sequencing on CD45+ cells isolated from peripheral blood, tumor and adjacent normal tissues from cancer patients, and identified several cell subtypes specific to various tumor types. Combining published and newly generated single-cell RNA sequencing (scRNA-seq) data, we performed comprehensive analysis of tumor-infiltrating immune cell subpopulations and delineated their abundance across 21 human cancer types. Such pan-cancer analysis revealed a congruence of major myeloid lineages, dendritic cell (DC) subsets and monocyte subsets, while T cells and macrophage subsets exhibited unique transcriptomic patterns across tumor types.

The broadly present LAMP3+ cDCs could be further characterized according to their different developmental origins, highlighting

their differences in transcription factors and external stimulus. We also uncovered the complexity of cDC2s in tumors and identified a pro-inflammatory subset. Tumor-associated macrophages (TAMs) linked with tumor angiogenesis are characterized with diverse markers among different tumor types. We further examined how the tumor microenvironment would change upon treatment of immune-modulating agents, and observed the dynamic patterns associated with therapeutic responses.

Our comprehensive analyses provide deep understanding of the commonalities and differences of the tumor microenvironment across multiple cancers, and some of the features might shape how different tumors respond to immunotherapies.

A portrait of Zemin Zhang, a man with short dark hair, wearing a dark suit jacket over a dark shirt. He is smiling slightly and looking towards the camera. The background is a solid teal color.

ZEMIN ZHANG

BIOPIC, College of Life Sciences, and Beijing Advanced Innovation Center for Genomics, Peking University, CN

Dr. Zemin Zhang is a Professor of Peking University and a principal investigator at BIOPIC, and Beijing Advanced Innovation Center for Genomics. Dr. Zhang obtained his BS from Nankai University, PhD from Penn State University and postdoctoral trainings at UCSF. Dr. Zhang spent >16 years at Genentech/Roche, leading the cancer genomics and bioinformatics group to discover anticancer targets and biomarkers using genomic and computational technologies.

He has pioneered multiple research directions in computational cancer biology and cancer

genomics including the first ever whole genome tumor sequencing. He is also an inventor for >60 issued patents, and has directly contributed to the initial finding of the molecular targets of multiple cancer therapeutic agents in clinical trials.

His lab currently focuses on understanding the interplay between immune and cancer cells using both large-scale cancer genomics data and the cutting-edge single cell sequencing technologies. He is a CUSBEA Scholar as well as Cheung Kong Scholar.

DEVELOPMENT OF A MOLECULAR SIGNATURE FOR METASTATIC CANCER STEM CELLS IN ORAL SQUAMOUS CELL CARCINOMA

Leah Ambler¹, Gehad Youseff¹, Alice Scemama¹, Muy-Teck Teh¹, Ian C. Mackenzie¹, Micheal P. Philpott¹, Adrian Biddle¹

¹Blizard Institute, Queen Mary University of London, UK

Oral squamous cell carcinoma (OSCC) is one of the most prevalent cancers worldwide, resulting in 200,000 deaths annually. Metastatic status is a key predictor of outcome for patients with OSCC and is a major factor in treatment decisions. However, there is currently no effective method for predicting metastatic status at presentation, leading to ineffective treatment tailoring.

Here, we use a novel 3D model of OSCC invasion to develop a molecular signature for metastatic cancer stem cells (CSCs), with the aim that this can be used within the current treatment pathway to more accurately diagnose metastatic OSCC.

Using a 3D *in vitro* model we are able to effectively separate invasive from non-invasive cells from within OSCC cell lines, and further show that the invasive cells are enriched for the metastatic CSCs that we have previously shown to be associated with metastasis in human specimens (Youssef et al., 2020). We apply RNA sequencing and a machine learning approach

to generate a gene set that is highly predictive of patient survival and disease progression in patient cohorts with 5-year follow up data.

These results represent the first steps towards development of a molecular biomarker test that is able to stratify patients by risk of metastasis based on a metastatic CSC signature, resulting in better treatment tailoring, and ultimately improving OSCC outcomes.

Youssef et al., 2020: Disseminating cells in human tumours acquire an EMT stem cell state that is predictive of metastasis. bioRxiv. 2020:2020.04.07.029009.

CD73 + EXTRACELLULAR VESICLES INHIBIT ANGIOGENESIS THROUGH ADENOSINE A2B RECEPTOR SIGNALLING

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Pathological angiogenesis is a hallmark of several conditions including eye diseases, inflammatory diseases, and cancer. Stromal cells play a crucial role in regulating angiogenesis through the release of soluble factors or direct contact with endothelial cells.

Here, we analysed the properties of the extracellular vesicles (EVs) released by bone marrow mesenchymal stromal cells (MSCs) and explored the possibility of using them to therapeutically target angiogenesis. We demonstrated that in response to pro-inflammatory cytokines, MSCs produce EVs that are enriched in TIMP-1, CD39 and CD73 and inhibit angiogenesis targeting both extracellular matrix remodelling and endothelial cell migration. We identified a novel anti-angiogenic mechanism based on adenosine production, triggering of A2B adenosine receptors, and induction of NOX2-dependent oxidative stress within endothelial cells. Finally, in pilot experiments, we exploited the anti-angiogenic EVs to inhibit tumour progression *in vivo*.

Our results identify novel pathways involved in the crosstalk between endothelial and stromal cell and suggest new therapeutic strategies to target pathological angiogenesis.

INTRADUCTAL XENOGRAFTS OF ESTROGEN RECEPTOR-POSITIVE BREAST CANCER MODEL DORMANCY

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Estrogen receptor α -positive (ER+) breast cancer (BC) often manifests with a latent disease that recurs decades after initial diagnosis. The mechanisms governing dormancy and distant recurrence of ER+ tumors remain elusive due to the lack of preclinical models.

Here, we compare the tumor progression of ER+ and triple negative (TN) BCs by grafting cell lines and patient-derived tumor cells into the milk ducts of immunocompromised mice. In the intraductal model, both ER+ and TN BC cells disseminate already during the *in situ* stage. TN disseminated tumor cells (DTCs) proliferate and form macro-metastases, while ER+ DTCs have low proliferative indices, are arrested in the G0/G1 phase of the cell cycle, and express the dormancy marker p27. The ER+ DTCs display mesenchymal and niche-specific characteristics, as revealed by 3D single cell resolution imaging, and show molecular features of Epithelial-Mesenchymal Transition (EMT). However, EMT is neither detected in tumor cells, nor required for their invasion or dissemination, but is acquired upon their

establishment in distant sites. Dormant lung and brain DTCs revert back to a proliferative state within 3 weeks through a Mesenchymal-Epithelial Transition in *ex vivo* cultures.

We conclude that the intraductal model provides exciting new experimental opportunities to study ER+ BC dormancy, and reveals that EMT could be exploited therapeutically to prevent distant recurrence.

CONTRIBUTION OF SNAIL1 IN THE TUMOUR MICROENVIRONMENT TO THE REGULATION OF MELANOMA PROGRESSION: A TARGET FOR THERAPEUTIC INTERVENTION

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Melanoma is a very malignant form of skin cancer due to its high metastatic abilities and resistance to therapies. MAPK pathway is hyperactivated in 90% of melanomas with mutations in BRAF responsible for approximately 50% of the cases. Tumour cells reside in a highly heterogeneous tumour microenvironment (TME), composed by local and recruited cells including immune infiltrate cells, fibroblasts and blood vessels composed by endothelial cells and pericytes.

It has become clear that the crosstalk between tumour and stromal cells plays a key role in cancer progression. Activated fibroblasts, known as Cancer-associated fibroblasts (CAFs), are the main stromal cells within the reactive stroma and possess many pro-tumorigenic properties via secretion of growth factors, cytokines, chemokines and the degradation of extracellular matrix. Snail1 is an epithelial-to-mesenchymal transition transcription factor expressed during development and reactivated in pathological situations including cancer and fibrosis. Snail1 is expressed in

activated fibroblasts associated to damaged epithelial cells in renal fibrosis and increasing evidence supports its pro-tumorigenic role when expressed in CAFs from breast or colorectal cancer. We have established BRAF-driven murine melanoma cell lines and conditional mouse models to deplete Snail1 expression in TME cells and assessed its impact on melanoma progression.

Our results show that Snail1 is highly expressed in cells from TME, predominantly in CAFs. Snail1-silencing in TME decreases tumour sizes and metastatic tumour burden in lungs. The investigation of a combination therapy co-targeting tumour cells and CAFs may provide a promising new strategy to improve patient's prognosis.

TARGETING A TUMOUR-SPECIFIC SURVIVAL SWITCH IN REGULATORY T CELLS TO INCREASE ANTI-TUMOUR IMMUNITY IN LUNG CANCER

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Tumours subvert the host immune system to enable their growth and thwart immunotherapy. A key mechanism employed by many advanced tumours involves the recruitment of immunosuppressive FOXP3+ regulatory T (Treg) cells that restrain anti-cancer responses. Specifically targeting tumour Treg cells is a major goal in immunotherapy.

Using deep profiling of lung adenocarcinoma for immune and cell survival characteristics by CyTOF, we found “re-wiring” of cell death mechanisms specifically in Treg cells infiltrating tumours. This Treg cell survival switch was also observed in other tumour classes and mouse models of lung adenocarcinoma. In tumour-bearing mice, targeting the pro-survival characteristics of tumour-Treg cells with a BH3 mimetic drug in combination with anti-PD1 checkpoint blockade prolonged survival.

These data uncover a conserved cell survival switch in tumour-infiltrating Treg cells that may be exploited to enhance response to immunotherapy.

MECOM PERMITS PANCREATIC ACINAR CELL DEDIFFERENTIATION AVOIDING CELL DEATH UNDER STRESS CONDITIONS

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Stress conditions such as pancreatitis in which acinar cells dedifferentiate and acquire duct cell-specific markers, predispose for cancer formation in the pancreas. However, it remains unclear to what extent dedifferentiated acini differ from native duct cells. Moreover, most studies have been performed in mice since the availability of human cells is scarce.

Here, we applied a non-genetic lineage tracing method of human pancreatic exocrine acinar and duct cells that allowed cell-type-specific gene expression profiling by RNA sequencing. This analysis showed that human dedifferentiated acinar cells expressed genes in Pathways of cancer with prominence of MECOM, a transcription factor that was not expressed by duct cells. During mouse embryonic development, pre-acinar cells also transiently expressed MECOM and in the adult mouse pancreas, MECOM was re-expressed

when mice were subjected to acute and chronic pancreatitis, conditions in which acinar cells dedifferentiate. In human cells and in mice, MECOM expression correlated with and was directly regulated by SOX9. Mouse acinar cells that, by genetic manipulation, lose the ability to upregulate MECOM showed impaired cell adhesion, more prominent acinar cell death and suppressed acinar cell dedifferentiation by limited ERK signaling. Its role during pancreatic embryogenesis is still under investigation.

In conclusion, we transcriptionally profiled the two major human pancreatic exocrine cell types, acinar and duct cells, during experimental stress conditions. We provide insights that in dedifferentiated acinar cells, cancer pathways are upregulated in which MECOM is a critical regulator that suppresses acinar cell death by permitting cellular dedifferentiation.

TIMING COMPLEX COPY NUMBER GAINS IN WHOLE GENOME DUPLICATED TUMORS

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The accumulation of genetic copy number aberrations is a process common to the development of many cancers. Tumors that have undergone a whole genome duplication are known to have high degrees of genomic instability and often contain genomic regions that have undergone a series of genomic gains resulting in multiple copies of both alleles. The relative timing of these copy number gains is made difficult by the fact that there are multiple plausible route histories that could give rise to them. Typically, these events are either not included or the most parsimonious event history is assumed to take place with current timing methods.

Here we detail a method that infers the route taken and the timing of such gains in whole genome duplicated samples by leveraging the simultaneous occurrence of the whole genome duplication in the sample. On a representative simulated cohort, it accurately assigns route history and timing to complex copy number gains. By applying the method to 789 whole genome duplicated tumor samples from the Pan-Cancer Analysis of Whole Genomes

dataset we find that the principle of maximum parsimony is frequently violated with large numbers of gains occurring earlier than thought under this assumption.

This method allows for a more accurate inference of evolutionary histories in different cancer types and better insights on the early genomic events that occur in tumors that undergo a whole genome duplication.

MASS SPECTROMETRY IMAGING FOR THE STUDY OF INTRA-TUMOR HETEROGENEITY

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Mass spectrometry imaging (MSI) is a powerful tool for the non-labeled and parallel imaging of hundreds to thousands of molecules in a single biological tissue section. It allows obtaining tissue type-specific molecular patterns and conversely the annotation of tissues based on their molecular profiles beyond histological information.

Here, we will show how this capability can be used to discover molecularly distinct but histologically invisible tumor cell populations and how to link their presence in patient tissues to clinical phenotypes such as metastasis. We have established a subsequent workflow where MSI-guided laser microdissection can then be used to physically extract cells from these tumor cell populations for their further in-depth molecular characterization using other omics techniques. As example, we have applied the workflow to a breast cancer sample where MSI of lipids was used to obtain a spatio-molecular segmentation of the tumor. 2000 cells of these segments were then submitted to ultra-sensitive liquid-proteomics, which was able to quantifying the expression

~1500 proteins for determining the biological processes within each segment.

We will also show how the increased spatial resolution in MSI can be used to couple metabolic profiles at the single-cell level with morphometric analyses of the corresponding histological images. We used this method in a diffuse-type gastric cancer to show that the molecular profiles of muscle cells in the vicinity of tumor cells are different than from those ones further away.

In conclusion, all of this gives evidence that MSI constitutes a unique tool for the investigation of intra-tumor heterogeneity and cancer field effects.

FERROPTOSIS RESPONSE SEGREGATES SMALL CELL LUNG CANCER (SCLC) NEUROENDOCRINE SUBTYPES

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Loss of TP53 and RB1 in treatment-naïve small cell lung cancer (SCLC) suggests selective pressure to inactivate cell death pathways prior to therapy. Yet, which of these pathways remain available in treatment-naïve SCLC is unknown.

Here, through systemic analysis of cell death pathway availability in treatment-naïve SCLC, we identify non-neuroendocrine (NE) SCLC to be vulnerable to ferroptosis through subtype-specific lipidome remodelling. While NE SCLC is ferroptosis resistant, it acquires selective addiction to the TRX anti-oxidant pathway. In experimental settings of non-NE/NE intratumoral heterogeneity, non-NE

or NE populations are selectively depleted by ferroptosis or TRX pathway inhibition, respectively. Preventing subtype plasticity observed under single pathway targeting, combined treatment kills established non-NE and NE tumors in xenografts, genetically engineered mouse models of SCLC, patient-derived cells and identifies a patient subset with drastically improved overall survival.

These findings reveal cell death pathway mining as a means to identify rational combination therapies for SCLC.

THEM6-MEDIATED LIPID REMODELLING SUSTAINS STRESS RESISTANCE IN CANCER

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Despite the clinical benefit of androgen-deprivation therapy (ADT), the majority of prostate cancer (PCa) patients ultimately develop lethal castration-resistant prostate cancer (CRPC).

From an *in vivo* proteomic screen, we identified thioesterase superfamily member 6 (THEM6) as a marker of ADT resistance in PCa. THEM6 is overexpressed in androgen-insensitive cell lines and castration-resistant orthografts in comparison to their hormone-naïve counterparts. In patients, THEM6 expression correlates with aggressive disease and is associated with poor survival. THEM6 deletion reduces *in vivo* tumour growth and restores castration sensitivity in orthograft models of CRPC. Mechanistically, THEM6 is located at the endoplasmic reticulum (ER) membrane and controls lipid homeostasis by regulating the levels of ether lipids. As a consequence, THEM6 loss in CRPC cells significantly alters ER function, preventing lipid-mediated activation of ATF4 and reducing *de novo* sterol

biosynthesis. THEM6 and MYC are frequently co-amplified in cancer. Moreover, THEM6 is required for the establishment of the MYC-induced stress response, highlighting its importance in MYC-driven tumours. As a proof of concept, we show that THEM6 loss prevents ATF4 activation and reduces tumour burden in a model of triple negative breast cancer (TNBC).

Altogether our results establish THEM6 as a novel component of the treatment-induced stress response, and highlight its potential to be used as a future therapeutic target in the context of CRPC and MYC-driven cancer.

SLC4A4, THE ROLE OF A BICARBONATE TRANSPORTER IN PANCREATIC CANCER IMMUNE RESPONSE

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Standard chemotherapy had failed to provide Pancreatic adenocarcinoma (PDAC) patients with a promising treatment option and, although new immunotherapy approaches are efficient in different cancer types, PDAC tumors remain resistant. These tumors are characterized by a dense desmoplastic stroma that impedes oxygen and nutrient diffusion from the blood stream and contributes to a strong hypoxic and acidic tumor microenvironment (TME). A lot of research has been performed on the contribution of acidity to tumor progression and immunotherapy resistance. However, the role of bicarbonate transporters has been mostly neglected.

Our research focused on a sodium bicarbonate transporter, Solute Carrier Family 4 Member 4 (SLC4a4), that based on scRNA-seq data from PDAC patients is the most expressed acid extruder in PDAC and it is selectively expressed in pancreatic ductal cells. In mice, genetical and pharmacological targeting of this transporter in cancer cells increases the pH of the TME leading to an improved T cell-mediated immune response and to an increased efficacy of immunotherapy. The study of this transporter could pave the venues towards novel therapeutic strategies to render effective immunotherapeutic regimens in PDAC.

SINGLE-CELL PROFILING REVEALS A CONSERVED MYOGENIC HIERARCHY IN PEDIATRIC RHABDOMYOSARCOMAS ASSOCIATED WITH DRUG RESISTANCE

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in pediatric patients. Despite improvements in the cure rates over the last 30 years, patients with the alveolar subtype are still largely considered incurable, with 5-year overall survival rates below 40%. These patients often develop resistance towards therapy, limiting therapeutic success and making the clinical course of relapsed RMS very poor.

One of the emerging determinants of treatment failure in cancer is intratumoral heterogeneity (ITH), a yet unexplored area in RMS. In this project, we propose to characterize ITH in RMS and to decipher its involvement in drug-resistance. Using a combined approach of single-cell RNA sequencing and mass cytometry, we profiled several thousand individual cells from RMS patient-derived xenografts and cell lines and discovered that RMS tumors are composed of heterogeneous cell populations. These populations recapitulate a branched myogenic

trajectory, whereby progenitor stem-like cells (SC-like) either commit to differentiation or to actively cycling myoblasts. We show that SC-like cells are characterized by yet undescribed markers, which get upregulated upon exposure to chemotherapeutics. Similarly, markers of committed populations decrease after drug treatment, suggesting that chemotherapeutics shift cellular composition of RMS towards undifferentiated clusters. Current studies are ongoing to determine pathways underlying drug resistance in SC-like cells and ways to pharmacologically interfere with it.

Taken together, this study suggests a possible origin for RMS and has the potential to improve the clinical outcome of RMS patients by co-targeting drug-sensitive and drug-resistant subpopulations.

IDENTIFICATION OF CHITINASE 3-LIKE 1 AS A SOLUBLE IMMUNE CHECKPOINT PARALYZING THE CYTOTOXIC GRANULE MACHINERY

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Cytotoxic lymphocytes require a functional lytic granule machinery to mediate effective anti-tumor responses. We hypothesized that tumor cells can survive the lytic immune synapse (IS) as a critical step for immune evasion and cancer progression through yet unknown mechanisms.

Here, we found that Chitinase 3-like 1 (CHI3L1), an inflammatory protein, highly expressed in cancer, inhibits natural and antibody-dependent NK cell cytotoxicity. Mechanistically, CHI3L1 inhibits the correct polarization of the microtubule-organizing center (MTOC) along with the lytic granules to the IS by modulating the receptor of advanced glycation end-products (RAGE) and its downstream JNK signaling. *In vivo*, CHI3L1 administration drastically increases the growth of NK cell-sensitive tumors, while CHI3L1 blockade synergizes with anti-HER2 antibody, Trastuzumab, to cure mice with HER2+ xenografts. Accordingly, sera of Trastuzumab-

resistant HER2 breast cancer patients contain high levels of CHI3L1 and inhibit NK cell ADCC. CHI3L1 affects also CD8 T cell cytotoxicity and antitumor activity in the 4T1 triple-negative breast cancer model.

Our work highlights a new paradigm of tumor immune escape mediated by CHI3L1 which acts on the cytotoxic effector machinery by interfering with granule polarization. Therefore, targeting CHI3L1 could prevent immune escape and potentiate antibody and cell-based immunotherapies.

CANCER-ASSOCIATED FIBROBLASTS PROMOTE DRUG RESISTANCE IN ALK-REARRANGED LUNG ADENOCARCINOMA CELLS THROUGH UPREGULATION OF CHOLESTEROL BIOSYNTHESIS

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Targeted therapy interventions provide compelling response rates in ALK-rearranged lung adenocarcinoma patients. The occurrence of resistances, however, still poses a major clinical challenge. Several lines of evidence support a role of cancer-associated fibroblasts (CAFs) to such treatment insensitivities, while underlying mechanisms remain poorly understood.

We aimed to uncover the molecular networks shaping the susceptibility of lung cancer cells towards ALK inhibition in 3D co-culture settings. CAFs were capable to provoke resistance to ALK inhibition in lung tumor spheroids as observed by enhanced spheroid growth, reduced cell death and increased cell proliferation. Transcriptional differences between homo- and heterotypic tumor spheroids as interrogated by scRNA-sequencing revealed CAF-dependent changes in the metabolic profiles of lung tumor cells. *In vitro* experiments confirmed an enhanced

mitochondrial activity and increased expression level of cholesterol homeostasis-controlling genes in resistant ALK+ lung cancer cells. The establishment of this metabolic-supportive niche could be overcome by co-targeting of ALK and HMGCR, the rate-limiting enzyme of cholesterol biosynthesis. Heterocellular communication between tumor cells and the stromal cell compartment were examined through ligand-receptor-interaction analyses. CAF-derived factors were able to confer therapy resistance and induced an increased expression of cholesterol biosynthesis enzymes in lung tumor cells.

Our findings provide a rationale on how CAFs could confer therapy resistance in ALK-driven lung cancer cells, with the cholesterol biosynthesis pathway as a potential therapy target for patients with acquired resistance to ALK inhibitors.

THE RNASET2 ONCOSUPPRESSOR PROTEIN REGULATES BOTH DIFFERENTIATION AND POLARIZATION PATTERNS IN HUMAN MACROPHAGES

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T2 ribonucleases represent an evolutionarily conserved family of extracellular ribonucleases mainly involved in host defense and stress response. The human RNASET2 protein has been consistently associated with a marked tumor suppressive activity, which is likely carried out by establishing a cross-talk between cancer cells-derived RNASET2 and the tumor microenvironment (TME). Indeed, independent xenograft-based or syngeneic murine experimental models clearly showed that increased RNASET2 expression levels are consistently associated with a marked suppression of both tumorigenic and metastatic potential *in vivo*. Noteworthy, histological characterization of RNASET2-suppressed tumors revealed a marked inflammatory infiltration within the tumor mass, represented by murine stromal cells belonging to the M1 anti-tumorigenic subclass of macrophages.

In this work, we established an *in vitro* experimental model to recapitulate the activity of human RNASET2 protein on cells belonging

to the monocyte/macrophage lineage that we previously observed *in vivo*. We present here preliminary results from human promyelocytic THP-1 cells exposed to elevated RNASET2 protein levels in two independent experimental settings. Treatment of human THP-1 cells with either recombinant human RNASET2 protein or with conditioned media from RNASET2-overexpressing 22Rv1 prostate cancer cells confirmed the role of this protein in regulating the biology of human macrophages, although we observed a slightly different response in the two experimental settings.

Taken together, these data provide a further confirmation of the ability of human RNASET2 to affect human macrophage's differentiation and polarization pattern *in vitro*.

MITOCHONDRIAL TRANSLATION DRIVES METABOLIC REPROGRAMMING REQUIRED FOR METASTASIS

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Metabolic flexibility is a common hallmark of aggressive and metastatic cancers. Here, we use tRNA 5-methylcytosine (m5C) to reveal that dynamic changes in mitochondrial translation rates drive the switch between glycolytic and mitochondrial energy production required for metastasis.

We find that loss of m5C in mitochondrial tRNAMet is sufficient to repress mitochondrial translation and trigger the shift from oxidative phosphorylation (OXPHOS) to glycolysis. Glycolytic tumour cells form primary tumours but fail to metastasize in an orthotopic mouse model of human oral cancer. Instead, a small subpopulation of non-dividing tumour cells requires high OXPHOS levels for invasion and metastasis. Although this metastasis-initiating metabolic switch is highly dynamic and

reversible, we identify a mitochondria-driven gene signature predictive for metastasis and disease progression.

Finally, we demonstrate that pharmacological inhibition of mitochondrial translation blocks metastasis in orthotopic transplants. Together, our results indicate that metastasis-initiating cells can be eradicated by blocking mitochondrial translation.

INFINITE SITES VIOLATIONS DURING TUMOUR EVOLUTION REVEAL LOCAL MUTATIONAL DETERMINANTS

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The infinite sites model of molecular evolution requires that every base in the genome is mutated at most once. It is a cornerstone of (tumour) phylogenetic analysis, and is often implied when calling, phasing and interpreting variants or studying the mutational landscape as a whole. It is unclear however, whether this assumption holds in practice for bulk tumour samples.

Here we develop frameworks to model and directly detect infinite sites violations, where the same base is mutated independently on both alleles. In total, we identify 20,555 of these biallelic mutations, including 8 candidate driver events, in 722 of 2,658 (27.2%) bulk tumour samples from the ICGC/TCGA Pan-Cancer Analysis of Whole Genomes project. Violations generally occur at mutational hotspots and their frequency and type can accurately be predicted from the overall mutation spectrum. In melanoma, their local sequence context evidences how ETS and NFAT-family transcription factors create hotspots for UV damage. In colorectal and oesophageal

adenocarcinoma, respectively, violations reveal hypermutable special cases of the canonical trinucleotide contexts of COSMIC mutation signatures SBS10 (POLE proofreading) and 17 (unknown aetiology).

Taken together, the infinite sites model breaks down at the bulk level for a considerable fraction of tumours. By extension, it is untenable in most, if not all, tumours at the multi-sample or single cell level. At-scale detection of infinite sites model violations affords an intimate look at previously hidden features of mutational processes. Our findings highlight the need for accurate models of mutation rates and tumour evolution as well as their inference from sequencing data.

ACTIVATION OF DIGESTIVE-TRACT AND STRATIFIED-EPITHELIUM TRANSCRIPTIONAL PROGRAMS BY PROMOTER DNA DEMETHYLATION IN HUMAN TUMOR CELLS

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Human tumors exhibit aberrant activation of genes that are normally expressed specifically in germ cells. Their activation in tumors is due to the demethylation of their promoter region, a consequence of genome-wide DNA methylation loss in these cells. It is now recognized that several of these 'cancer-germline' (CG) genes contribute to tumor development. To date, DNA demethylation in tumors has essentially been associated with the activation of CG genes. Whether this epigenetic alteration induces other tissue-specific expression programs in tumors remains unknown.

By combining expression and methylation data of normal lung, lung adenocarcinoma (LUAD) cell lines and tumors we now show that two additional tissue-specific expression programs are aberrantly activated in tumors due to promoter DNA demethylation. The first group of genes is physiologically expressed in digestive-tract tissues and were termed 'digestive tract' genes (DT). The second group of genes was restricted to stratified epithelium-

tissues and therefore named 'stratified epithelium' genes (SE). To the contrary of CG genes, we found that DT and SE genes are activated in tumors irrespective of the overall DNA methylation content. Finally, we show that the activation of some genes belonging to the SE program is associated with poor patient survival. Overall, our study identified two tissue-specific expression programs dependent on promoter DNA demethylation for their activation in tumors.

Our data point to an activation mechanism involving targeted DNA demethylation events in these cells. Finally, the activation of these genes could be potentially exploited in the clinic as prognostic markers of LUAD.

THE MIR-143/145 CLUSTER PROMOTES MESENCHYMAL PHENOTYPIC PLASTICITY ASSOCIATED WITH RESISTANCE TO TARGETED THERAPIES IN MELANOMA

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Treatment of BRAF-mutant metastatic melanoma with MAPK pathway inhibitors (MAPKi) has revolutionized management of patients with advanced stage disease but therapeutic resistance inevitably occurs. Due to high plasticity, melanoma cells are capable of developing non-genetic resistance towards MAPK-targeted therapy that is frequently associated with dedifferentiation into a mesenchymal invasive phenotype displaying fibrotic markers and extracellular matrix (ECM) remodeling activities. However, the molecular mechanisms that regulate this resistant phenotype remain poorly defined.

In this study, an expression screening of a pool of miRNAs defined as "FibromiRs" revealed high expression of the miR-143/145 cluster in dedifferentiated invasive MAPKi-resistant melanoma cells. In addition, inhibition of the BRAFV600 pathway *in vitro* and in xenograft model triggered cluster expression along with ECM reprogramming. Remarkably, administration of the anti-fibrotic

drug Nintedanib in combination with MAPKi reverted ECM remodeling, prevented miR-143/145 cluster upregulation and delayed tumor relapse. Ectopic expression of the two miRNAs in melanoma cells caused ECM reprogramming, the switch to an invasive dedifferentiated slow-cycling melanoma cell state and resistance to MAPKi. Conversely, miR-143/145 inhibition impaired MAPKi-driven ECM remodeling and increased drug sensitivity.

These findings identify the profibrotic miR-143/145 cluster as a key regulator of phenotypic plasticity and non-genetic resistance to MAPKi in melanoma. They also provide pre-clinical evidence that normalizing the fibrotic stromal reaction driven by MAPK-targeted therapy can be exploited therapeutically to delay relapse and disease progression.

ROLE OF HYPOXIA AND HIF-1 α IN THE IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT

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Hypoxia-driven immunosuppression contributes to tumor evasion from CD8 T cell surveillance. However, the impact of the hypoxia inducible factor (HIF)-1 α and prolyl hydroxylase domain-containing enzymes (PHD), the main factors mediating cellular response to hypoxia, on CD8 T cell function remains unclear. Our aim is to dissect the direct and indirect effects of HIF-1 α in CD8 T cells and assess how they affect tumor responses to adoptive cell therapy (ACT).

Using activated P1A-specific (TCRP1A) or OVA-specific (OT-1) murine CD8 T cells in which we genetically silenced or stabilized HIF-1 α , we show that hypoxia-driven induction of activation markers (CD69, CD25, CD137, GITR), inhibitory receptors (PD-1, LAG3, TIM3, CD244) and effector molecules (perforin, granzyme-B) is HIF-1 α dependent. Furthermore, HIF-1 α signaling in CD8 T cells is crucial for hypoxia-enhanced cytotoxicity of target cells. On the other hand, HIF-1 α doesn't seem to contribute to the reduced CD8 T cell growth in hypoxia. *In*

vivo, HIF-1 α silencing in CD8 T cells abolished tumor response to ACT in transplanted tumor models (LLC-OVA, T429.1-P1A). Surprisingly, HIF-1 α silenced CD8 T cells were more efficient at rejecting autochthonous melanoma tumors expressing the antigen P1A (TiRP) compared to WT CD8 T cells upon mice pre-treatment with the lymphodepleting drug cyclophosphamide.

In summary, HIF-1 α plays an important role in cytotoxic activity and expression of functional markers in activated murine CD8 T cells *in vitro*. *In vivo*, HIF-1 α function in CD8 T cells seems to be dependent on the tumor microenvironment. Therefore, we are currently analyzing how hypoxia and HIF-1 α affects CD8 T cells infiltration and function in the different tumor models.

VALINE tRNA AMINOACYL-TRANSFERASE PROMOTES DRUG RESISTANCE IN MELANOMA THROUGH SELECTIVE mRNA TRANSLATION

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Emerging evidences indicate that tRNA expression is modulated during tumorigenesis and correlates with changes in mRNA translation of genes with specific codon usage. Our previous work uncovered the importance of wobble uridine tRNA modification in melanoma resistance to therapies. As such, tRNA dynamics contribute to key aspects of cancer biology and potentially underlie cellular plasticity within the tumor.

In this study, we combined tRNA sequencing, quantitative proteomics and systematic codon usage analysis of translated mRNA to uncover new mechanisms underlying translation reprogramming and therapy resistance in melanoma. Integrative analyses of these approaches uncovered the importance of the GTG codon (Val) and the corresponding tRNA-Val-(CAC) in resistant cells. Moreover, the expression of the valine tRNA aminoacyl-

transferase (VARS) is upregulated in resistant melanoma cultures and in patient biopsies and is correlated with poor prognosis in melanoma patients. Strikingly, VARS depletion resensitized a variety of resistant melanoma cultures to MAPK-based therapy, while it had no significant effect on untreated cells. *In vivo*, depletion of VARS synergized with MAPK-therapy to induce tumor shrinkage of resistant melanoma. At the molecular level, the combination of global proteomics and Ribo-IP experiments uncovered potential VARS translational targets. Current experiments will pinpoint the role of these candidates in melanoma resistance.

Taken together, these data uncover the role of the tRNA aminoacyl-transferase VARS and its translational targets in promoting drug resistance in melanoma.

ONCOGENIC GLI1 AND STAT1/3 ACTIVATION DRIVES IMMUNOSUPPRESSIVE TRYPTOPHAN/KYNURENINE METABOLISM VIA SYNERGISTIC INDUCTION OF IDO1 IN SKIN CANCER

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Pharmacologic targeting of Hedgehog (HH)/GLI is effective for certain blood, brain and skin cancers including basal cell carcinoma (BCC). However, limited response rates and the development of drug resistance call for improved anti-HH therapies that take synergistic crosstalk mechanisms and immune evasion strategies into account.

We demonstrated that crosstalk of HH/GLI with pro-inflammatory IL-6 signaling drives the growth of BCC (Sternberg et al., 2018). Here, we identified the immunosuppressive enzyme Indoleamine 2,3-Dioxygenase 1 (IDO1) as a novel HH-IL-6 response gene. ChIP analysis revealed co-occupancy of GLI1 and STAT3 along with active chromatin marks at the cis-regulatory region of IDO1. Elevated expression of IDO1 in human BCC with high-level HH and IL-6 signatures supports the clinical relevance of our mechanistic data. We also questioned this mechanism in human

melanoma cells where genetic inhibition of GLI1 expression prevented the induction of IDO1 in response to IL-6/STAT3 and IFN- γ /STAT1 signaling. Pharmacologic targeting of HH signaling interfered with IDO1 expression and consequently prevented the production of the immunosuppressive metabolite kynurenine (Kyn) generated by IDO1 from tryptophan. Further, inhibition of GLI1 enhanced the efficacy of the selective IDO1 inhibitor epacadostat. Of note, targeting GLI1 in melanoma cells not only reduced IDO1 expression but also attenuated Kyn-mediated T cell suppression.

We conclude that the immunosuppressive IDO1-Kyn pathway is under oncogenic HH-IL-6 and GLI-STAT cooperation in both BCC and malignant melanoma. Thus, simultaneous pharmacologic targeting of the HH/GLI, JAK/STAT and IDO1-Kyn axis displays a rational combination therapy against skin cancers.

TGFBI MODULATES TUMOR HYPOXIA AND PROMOTES BREAST CANCER METASTASIS

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Breast cancer metastasis is a complex process that depends on intrinsic characteristics of metastatic stem cells, but also on the particular microenvironment that supports their growth and modulates the plasticity of the system.

In search for microenvironmental factors supporting cancer stem cell (CSC) growth and tumor progression to metastasis, we here investigated the role of the matricellular protein Transforming Growth Factor Beta-Induced (TGFBI) in breast cancer. We crossed the MMTV- PyMT model of mammary gland tumorigenesis with a Tgfb1 ko mouse, and studied the CSC content of the tumors. We performed RNAseq on wt and ko tumors, and analysed the tumor vasculature and the immune compartment by IHC and FACS. The source of TGFBI expression was determined by qPCR and by bone marrow transplantation experiments. Finally, we performed in silico analyses using the METABRIC cohort to assess the potential prognostic value of TGFBI. Our results show that deletion of Tgfb1 led to a dramatic decrease in CSC content and lung

metastasis. Moreover, TGFBI loss resulted in tumor vessel normalisation, with improved vessel perfusion and decreased hypoxia, a major factor controlling CSCs and metastasis. Furthermore, human data mining in a cohort of breast cancer patients showed that higher expression of TGFBI correlates with poor prognosis and is associated with the more aggressive subtypes of breast cancer.

Overall, these data reveal a novel biological mechanism controlling metastasis that could potentially be exploited to improve the efficacy and delivery of chemotherapeutic agents in breast cancer.

INNATE IMMUNE EVASION REVEALED IN A COLORECTAL ZEBRAFISH XENOGRAFT MODEL

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Cancer immunoediting is a dynamic process of crosstalk between tumor cells and the immune system. Herein, we explore the fast zebrafish xenograft model to investigate the innate immune contribution to this process. Using multiple breast and colorectal cancer cell lines and zAvatars, we find that some are cleared (regressors) while others engraft (progressors) in zebrafish xenografts.

We focus on two human colorectal cancer cells derived from the same patient that show contrasting engraftment/clearance profiles. Using polyclonal xenografts to mimic intra-tumor heterogeneity, we demonstrate that SW620_progressors can block clearance of SW480_regressors. SW480_regressors recruit macrophages and neutrophils more efficiently than SW620_progressors; SW620_progressors however, modulate macrophages towards a pro-tumoral phenotype. Genetic and chemical suppression of myeloid cells indicates that macrophages and neutrophils play a crucial role in clearance. Single-cell-transcriptome analysis shows a fast subclonal selection, with

clearance of regressor subclones associated with IFN/Notch signaling and escaper-expanded subclones with enrichment of IL10 pathway.

Overall, our work opens the possibility of using zebrafish xenografts as living biomarkers of the tumor microenvironment.

LNCMELO01 INTERACTION WITH THE GTPASE RAP2C PROMOTES OXIDATIVE PHOSPHORYLATION, MELANOMA CELL SURVIVAL AND RESISTANCE TO BRAF INHIBITION

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The lineage-defining transcription factors MITF (Microphthalmia-associated transcription factor) and SOX10 promote melanoma cell proliferation and survival by regulating a repertoire of protein coding genes and the long non-coding (Lnc)RNA SAMMSON. Here we show that MITF and SOX10 regulate LncMEL01 and LncMEL02 novel lncRNAs essential for melanoma cell viability. Anti-sense oligonucleotide (ASO)-mediated co-targeting shows that together with SAMMSON, they define a network of SOX10/MITF regulated lncRNAs that act cooperatively to promote melanoma cell survival. We show that LncMEL01 interacts with the small GTPase RAP2C and together they promote optimal oxidative phosphorylation metabolism required for melanoma cell proliferation and survival. Mechanistically, LncMEL01 promotes RAP2C association with DRP1 and phosphorylation of DRP1 serine 637 increasing mitochondrial fusion and balancing ERK1/2 dependent fission. LncMEL01 and RAP2C are also essential for the metabolic switch from glycolysis to oxidative phosphorylation upon

treatment with BRAF inhibitors. Consequently, their silencing cooperates with MAP kinase pathway inhibitors to induce melanoma cell apoptosis.

These results highlight a novel lncRNA-protein complex regulating melanoma cell metabolism. Like SAMMSON, LncMEL01 is also expressed in undifferentiated and neural crest type melanoma cells involved in drug resistance and hence their co-targeting provides an attractive strategy to target the minimal residual disease state for more efficient therapeutic outcome.

INTRINSIC TUMOR PLASTICITY IN GLIOBLASTOMA ALLOWS FOR EFFICIENT ADAPTATION TO DYNAMIC MICROENVIRONMENTAL NICHES AND TREATMENT PRESSURE

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Phenotypic plasticity has recently emerged as a major contributor to the development of intra-tumoral heterogeneity and resistance to treatment. Glioblastoma (GBM) cells, -the most aggressive of brain tumors, display strong intra-tumoral heterogeneity. GBM cells exist in continuous gradients of transcriptomic states based on various cellular properties including proneural-mesenchymal axis, proliferation, stemness, and metabolism. The adaptive properties of GBM cells towards new microenvironmental niches and treatment pressure are still not well understood.

Here we examined inter- and intra-tumoral GBM heterogeneity at the single cell transcriptomic and proteomic level in treatment naive and treated GBMs. Tumor cell subpopulations were further classified based on cell membrane markers. The resulting 16 subpopulations were FACS isolated and functionally analyzed. Mathematical Markov modelling was applied to calculate state transitions in different microenvironmental conditions.

Single cell transcriptomic data showed extensive intra-tumoral heterogeneity. Treatment led to adaptation of transcriptomic states towards resistant phenotypes without underlying genetic evolution. Functional assessment of GBM subpopulations revealed that cellular states are non-hierarchical, reversible and occurred via stochastic state transitions of existing populations, striving towards an equilibrium instructed by the microenvironment.

Our data suggests that phenotypic heterogeneity in GBM results from intrinsic plasticity allowing tumor cells to effectively adapt to new microenvironments and escape treatment. Novel treatment strategies should tackle the inherent plasticity allowing GBM cells to transit towards treatment resistant states.

SENOTHERAPY AS AN EFFICIENT CONCOMITANT TREATMENT TO PREVENT PROGRESSION AND RESISTANCE IN NSCLC

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Lung cancer is the most common cause of cancer-related death worldwide. The majority of patients with non-small cell lung cancer (NSCLC) present with incurable, advanced (stage IV) disease. Chemotherapy regimens remain standard treatments for NSCLC patients, either alone or in combination with immunotherapy. Despite recent advances, the long-term outcomes of advanced NSCLC patients remain poor, with treatment resistance and progression being essentially inevitable.

Senescence is a cell autonomous response to damage/oncogenic stress characterised by stable proliferative arrest and intense inflammatory secretion, termed as SASP. Senescence is abundant in premalignant lesions and also occurs in response to therapy-induced genotoxic stress [1]. When senescent cells accumulate in tissues can drive a variety of cell autonomous and non-cell autonomous tumour-promoting effects and resistance

[2,3]. Importantly, our recent unpublished data show that chemotherapy-induced senescence severely contributes to NSCLC in xenografts and orthotopic mouse cancer models. Therefore, approaches targeting this process may improve patient outcomes. We have developed senolytic nanotechnologies [4] and prodrugs [5] in combination with senescence-inducing therapy resulting in a significantly reduction of tumour burden and reduced toxicities when compared to standard chemotherapy in a number of NSCLC *in vivo* models. This strategy may open new avenues for lung cancer management.

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EXPLORING THE LANDSCAPE OF TRANSCRIPTIONAL INTRA-TUMOURAL HETEROGENEITY IN NEUROENDOCRINE PROSTATE CANCER

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Neuroendocrine prostate cancer (NEPC) is one of the most aggressive forms of prostate cancer, with limited treatment options and poor prognosis. The design of more effective treatments for these tumours will require deeper understanding of intra-tumoural heterogeneity (ITH) in NEPC at the molecular level.

We investigated transcriptional ITH within NEPC tumours by performing single-cell RNA-seq of 7 patient-derived xenograft (PDX) models of NEPC covering multiple histological subtypes. Preliminary results show substantial heterogeneity at the transcriptional level both within and between PDXs, including considerable cell-level heterogeneity in the expression of common neuroendocrine (NE) markers. Proliferative, epithelial-mesenchymal transition (EMT) and stress response pathways were among the most frequently enriched gene expression signatures in NE cell clusters. Data from two PDXs with mixed adenocarcinoma

and neuroendocrine pathologies revealed divergent roles for transdifferentiation and selection in the emergence of NE subpopulations in each case.

This work provides new insights into the landscape of transcriptional ITH in NEPC.

COMPARATIVE STUDY OF DIMENSIONALITY REDUCTION METHODS FOR MULTI-OMICS DATA

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Background: High-throughput techniques become standard for molecular diagnostics of cancer. Their results, however, are highly dimensional, which limits their comprehension and visualization. Dimensionality reduction methods resolve this limitation by projecting data to few dimensions preserving important properties. Here we compared performance of the most popular methods in application to clustering mRNA, miRNA and epigenomics data, collected for breast tumors.

Method: The most popular dimensionality reduction methods include principal component analysis (PCA), non-negative matrix factorization (NMF), independent component analysis (ICA), multidimensional scaling (MDS), t-distributed stochastic neighbor embedding (tSNE), uniform approximation and projection (UMAP). We applied the methods to TCGA breast cancer data (BRCA) and considered three modalities: DNA methylation, microRNA and messenger RNA expression. BRCA patients fall into five cancer subtypes, which were used

as a ground truth for clustering. The quality of the projection was assessed according to: 1) running time, 2) average intra-cluster and inter-cluster distances, 3) ratio of these distances.

Results: UMAP showed the best performance in transforming data of different complexity and dimensionality, keeping samples from the same subtype clustered together. Its distinctive feature was the construction of a weighted undirected graph of a lower dimension. The running time of this algorithm changed insignificantly with an increase in the size and complexity of the data, which gave it a huge advantage over other methods. We concluded that the UMAP is the optimal algorithm for reducing the size of data for studying the high-throughput data in breast cancer data.

INTEGRATION OF PHENOTYPIC SCREEN WITH OMICS DATA REVEALED COMBINATORY TREATMENT FOR GLIOBLASTOMA

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Glioblastoma (GBM) represents an important clinical challenge. Median patient survival under the current standard-of-care, comprising surgical resection, radio-and chemotherapy, is around 15 months. This poor prognosis can partly be attributed to high tumor heterogeneity, acquisition of tumor resistance and lack of tumor-specific targets. This high level of heterogeneity is reflected by the presence of a rare subpopulation of cells which are capable of tumor initiation and self-renewal, known as glioblastoma stem-like cells (GSCs). In the past decade, genomic, transcriptomic and methylation analyses have led to a subtype classification of glioblastoma tumors, including proneural (PN), classical (CL), and mesenchymal (MES) subtypes. While their classification signatures are based on the predominant gene expression patterns in a tumor, there are clear physiological differences between the subtypes. In a kinome-wide RNAi screen aiming at identifying genetic vulnerabilities that differentiate the MES from the PN subtype, we found that silencing the

proto-oncogene tyrosine-protein kinase Src (SRC) reduced proliferation in MES but not in PN GSCs.

Using dasatinib, a SRC inhibitor, we confirmed MES GSCs are more sensitive to SRC-kinase inhibition than PN and CL GSCs. In order to find new combinatory treatment modalities to target different subtype populations within GBM, we have integrated phenotypic data, gathered through an shRNA loss-of-function screen on dasatinib-treated GSCs, with (phospho)-proteomics data. Thus we have identified the kinase Wee1, a key regulator of the G2/M cell cycle checkpoint, as a target to sensitize PN GSCs to dasatinib, highlighting a new potential combinatory treatment for glioblastoma patient.

YOUNG CUTANEOUS ADIPOCYTES TRANSFER MORE FUEL AND DRIVE MORE AGGRESSIVE MELANOMA THAN AGED ADIPOCYTES

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The skin changes with age and with exposure to external factors such as UV light. As we age, it wrinkles and sags and the layer of subcutaneous fat thins. Previous work has shown that the aged cutaneous microenvironment contributes to early-stage and primary melanoma progression. Additionally, adipocytes can be a source of fatty acids to fuel cancer and melanoma. Here we present how aged and young cutaneous adipocytes have distinct secretory profiles and contribute to melanoma progression differently.

We studied human preadipocytes obtained from human skin from donors of different ages. We differentiated preadipocytes to adipocytes and compared the phenotypic, lipid and molecular adipocyte changes by age. We then exposed melanoma cells to the secretome of young or aged adipocytes and found exposure to young adipocyte secretome increased the rate of proliferation, invasion and migration of melanoma cells more than exposure to aged adipocyte secretomes. We describe

how lipids transferred from adipocytes to melanoma cells lead to a profound shift in melanoma cell metabolism and affect oxidative stress adaptability. Moreover, we describe lipids drive a persistent activation of the pAkt signalling pathway long after adipocyte secretome exposure. The lipidomics study of the secretome reveals overall fewer lipids are transferred to melanoma cells from aged cutaneous adipocytes; and the ratio of lipids varies by age. Furthermore, melanoma invasion and migration are specifically due to the transfer of phospholipid components in the secretome.

These data may explain why aged patients with purely subcutaneous, localised in-transit metastases have a prolonged course of the disease and longer survival.

ACQUIRED RESISTANCE TO TARGETED THERAPY CONFERS CROSS-RESISTANCE TO IMMUNOTHERAPY

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Targeted therapies and immunotherapies represent main pillars of cancer treatment, yet how they shape tumors and thereby influence subsequent therapeutic responses is poorly understood.

Here, we show in melanoma patients and mouse models that when tumors relapse on targeted MAPK pathway inhibitors, they are cross-resistant to immunotherapies. We find that cross-resistance is mediated via a cancer cell-instructed, immune-suppressive tumor microenvironment that lacks functional CD103+ dendritic cells, precluding a T cell response. Restoration of CD103+ dendritic cell numbers and functionality can re-sensitize cross-resistant tumors to immunotherapy. Cross-resistance arises not from the selective pressure of an immune response but from the MAPK pathway, which is not only re-activated during resistance formation but has an enhanced transcriptional output driving

immune evasion.

Our work provides mechanistic evidence for cross-resistance between unrelated therapies and a scientific rationale for treating patients with immunotherapy before they acquire resistance to targeted therapy.

ASSOCIATION OF MELANOMA DIFFERENTIATION TRAJECTORIES UPON MAPKi WITH ACTIVATION OF CD8+ TUMOR-INFILTRATING LYMPHOCYTES

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Melanoma cells are highly plastic and can undergo dynamic differentiation transitions in response to BRAFV600-targeted inhibitors. Aiming to gain insights into the impact of fluctuating melanoma cell-state on anti-tumor T cell response, we investigated the kinetic of melanoma differentiation and its associated recognition by autologous CD8+ tumor-infiltrating lymphocytes (TILs) in the course of drug treatment.

Our current study provides the evidence that single BRAF inhibitor (BRAFi) and dual BRAFi plus MEKi (BRAFi+MEKi) treatment can drive human melanoma cells into distinct differentiation trajectories and result in two different resistant phenotypes: the BRAFi-induced differentiated and BRAFi+MEKi-induced dedifferentiated phenotypes. Using autologous human TIL/melanoma models, we observed recurrently that differentiated BRAFi-resistant tumor sublines could be more efficiently recognized by autologous CD8+ TILs, while the dedifferentiated double drug-resistant tumor cells were protected from T

cell recognition. Kinetic analysis of melanoma differentiation status in the course of BRAFi or BRAFi+MEKi treatment further revealed that activation of CD8+ TILs by drug-treated tumor cells dynamically followed the alteration in tumor differentiation states. Furthermore, re-expression of the differentiation antigen into the dedifferentiated drug-resistant melanoma cells could rescue the impaired T cell activation.

Taken together, BRAFi alone or in combination with MEKi may trigger diverse differentiation trajectory in melanoma cells and, consequently, define activity of local pre-existing tumor-reactive CD8+ T cells.

CURCUMIN INDUCES APOPTOSIS, COLLAPSE OF MITOCHONDRIAL MEMBRANE POTENTIAL, PRODUCTION OF REACTIVE OXYGEN REACTIVE SPECIES AND CELL CYCLE ARREST IN EPIDERMAL CARCINOMA CELLS

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Secondary plant metabolites are produced in response to physical, chemical or biological stress factors and synthesis of these phytochemicals allow plants to survive in unfavorable environmental conditions. So far, it was shown that several dietary polyphenols exhibit anticancer properties and have the ability to overcome multidrug resistance in malignant diseases.

The aim of the study was to investigate the effects of two dietary polyphenols, curcumin and epigallocatechin-gallate (EGCG) in A-431 human epidermal carcinoma cells. Flow cytometric measurements demonstrated that curcumin induced cell death by apoptosis, triggered the collapse of mitochondrial membrane potential ($\Delta\psi_m$), increased production of reactive oxygen species (ROS) and arrested the carcinoma cells in S phase. Furthermore, clonogenic survival and cell proliferation have been inhibited by curcumin at low concentrations. Addition of EGCG demonstrated synergic activity in regard with cell viability and cell

cycle arrest. Nevertheless, EGCG due to its anti-oxidant activity was able to scavenge ROS produced by curcumin and caution should be taken when both compounds are applied. Moreover, combined administration of both polyphenols demonstrated a biphasic effect, lower concentrations being correlated with increased proliferation while higher concentrations reduced the viability of carcinoma cells. Concentrations used in these experiments did not affect the viability of CCD-1070Sk normal skin fibroblasts, excepting the highest curcumin concentration that should not be used for future studies.

Further experiments are required to investigate the ability of dietary polyphenols in overcoming multidrug resistance in cancer cells.

SINGLE-CELL SEQUENCING REVEALS CELL ONTOGENY AND TUMOR-SPECIFIC HETEROGENEITY OF A PERIVASCULAR EPITHELIOID CELL NEOPLASM (PEComa)

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Perivascular Epithelioid Cell Neoplasms (PEComas) are a rare family of mesenchymal tumours arising at diverse locations and characterized by *TSC1-2* mutations, *TFE3* translocations or *FLCN* truncating mutations that are mutually exclusive and linked to the mTOR pathway. Although PEComas are characterised by both melanocyte and smooth muscle markers, their ontogeny remains debated to date.

Herein, we used single-cell (sc)RNAseq to determine the cell of origin and dissect tumour heterogeneity of a PEComa sample of the kidney. Genomic profiling identified a somatic *TSC2* mutation. ScRNAseq analysis captured 624 cells that could be assigned to 6 cell populations including natural killer cells, endothelium, pericytes and tumour components. Tumour cells separated in 2 distinct populations. The first showed proximity to pericytes and was characterized by high

MITF activity fitting the “myomelanocytic” phenotype and sharing expression of pericyte marker genes. This population showed high activity of MITF and TFE3 but also PPARG, CEBPA and CEBPB further suggesting their partial differentiation toward adipocytes. The second tumour population showed reduced activity of the melanocytic program replaced by expression of smooth-muscle markers.

Our data provide novel insights into the transcriptional pathways driving pericyte transformation and underlying intra-tumoral heterogeneity of PEComas. Further collaboration with the French Sarcoma Group Network (NETSARC) will allow us to extend these observations to a large cohort of patients using bulk-RNAseq. These data will allow us to better understand the heterogeneity of the disease, the roles of the MITF and TFE3 transcription factors and identify potential therapeutic targets.

IMMUNE INFILTRATING PHENOTYPES IN DIFFUSE GLIOMAS

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One of the biggest caveats in diffuse glioma therapy is suppression of anti-tumor immune response. Neoplastic cell-to-cell interactions manage to modulate tumor microenvironment, promoting favorable immediate milieus within adjacent brain tissue.

Due to the sparse knowledge regarding cerebral-native immune cell populations, the focus of this study was to analyse deeper immune infiltrating populations under tumor-predominant milieus, far from being fully understood. By combining immunophenotyping with unsupervised clustering (PhenoGraph), we aimed to follow a non-biased approach to gain insight on immune phenotypes dwelling brain malignant milieus. As a result, we detected well established immune populations alongside previously unreported T cells, exhibiting CD14 epitope. Moreover, our RNA-seq data analysis complements observed patient-unique

immune proportions, linking diagnosis and immune phenotypes together with immune microenvironments in diffuse gliomas.

IN VIVO EFFECTS OF A NOVEL YAP-TEAD INHIBITOR IN CANCER

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The Hippo pathway and its downstream effectors, the YAP and TAZ transcriptional co-activators, are deregulated with high frequency in many different types of human cancer and are required for cancer phenotypes in human cancer cells and in mouse cancer models, while largely dispensable for normal homeostasis of mice. YAP/TAZ and their partner transcription factors, the TEAD1-4 factors, are therefore heralded as promising anti-cancer targets for many cancer types.

However, mutational aberrations in known Hippo pathway components are rare and it is not known to what degree different tumors and tumors with different driver mutations (Hippo mutant and Hippo non-mutant tumors) are sensitive to YAP/TAZ-TEAD inhibition. Therefore, I'm using a battery of mouse cancer models to test the efficacy and specificity of a new class of YAP/TAZ-TEAD inhibitors that bind into the palmitoylation pocket of TEAD proteins.

UNRAVELLING THE ROLE OF EARLY DISSEMINATION IN COLORECTAL CANCER

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Metastasis is the leading cause of cancer death but little is known about this phenomenon. In the classical model of metastasis, cell dissemination occurs at late stages of tumor development. However, some studies performed in different types of cancer suggest that rare tumor cells spread to distant sites much earlier than previously believed. Despite colorectal cancer's (CRC) high incidence worldwide, early tumour dissemination has not yet been studied.

Our aims in this project were first to demonstrate that early tumour dissemination process occurs in CRC, to assess the role of early disseminated cells (eDTC) in distant organs and finally to validate data on patient blood samples.

Using an inducible mouse model that enable us to lineage trace tumoral dissemination at very early stages of tumoral development, we first demonstrate the presence of eDTCs using tissue clearing, intravital live imaging and

immunolabelling in the liver, the main organ that is prone to get colonized by metastatic CRC cell. The impact of eDTCs in the liver was assessed using CyTOF/hyperion where we can detect a strong enrichment of macrophages and cancer associated fibroblast (CAFs) suggesting a microenvironmental remodelling. This liver remodelling has a strong impact on metastatic colonisation where we have demonstrated that it enhances the welcoming of future waves of highly mutated cells. Finally, we have detected, isolated and cultured early disseminated cells from blood of patients with intestinal polyps.

In conclusion, this project functionally validates for the first time the existence of an early dissemination process in CRC and propose a causal role of these cells on early pre-metastatic niche preparation.

THE ROLE OF LYMPHATIC ENDOTHELIAL AUTOPHAGY IN LYMPH NODE T CELL TRAFFICKING

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Metastatic melanoma is associated with high therapy resistance and very poor prognosis. Emerging evidence indicates that the melanoma metastatic cascade is not only driven by cancer cell-autonomous processes, but is dictated by the dynamic crosstalk established between melanoma cells and the surrounding stroma. Melanoma cells can use the lymphatic system, important for tissue drainage, lipid trafficking and immune regulation, as a channel for cancer cell dissemination and thus tumour progression. Studies highlight an emerging role for autophagy, the major lysosomal pathway for degradation and recycling of cytoplasmic components, in regulating cancer cell-stroma interactions.

Indeed, our recent data show that lymphatic endothelial cell (LEC) autophagy has a key role in their lipid metabolism and secretion of sphingosine-1-phosphate (S1P). To clarify the specific contribution of LEC-associated autophagy, we generated a transgenic mouse model, whereby ATG5 is deleted only in LECs (ATG5LEC-KO). Our preliminary data

show that immunocompetent ATG5LEC-KO mice show reduced melanoma-induced lymphangiogenesis compared to wild type mice. Further, ATG5LEC-KO mice show an accumulation of naïve T cells in the sentinel lymph node, indicating a possible role of LEC-associated autophagy in immune response towards cancer cells. This accumulation is most likely due to the change in sphingolipid metabolism in LECs, which can interfere with normal T cell circulation.

Experiments are ongoing to elucidate the mechanistic and signalling aspects of autophagy inhibition in the lymphatic vasculature and the in vivo impact on T cell circulation, melanoma-induced lymphangiogenesis and lymphatic spread.

INHIBITING THE MNK1/2-eIF4E AXIS IMPAIRS MELANOMA PHENOTYPE SWITCHING AND POTENTIATES ANTI-TUMOR IMMUNE RESPONSES

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Melanomas commonly undergo a phenotype switch, from a proliferative to an invasive state. Such tumor cell plasticity contributes to immunotherapy resistance, however, the mechanisms are not completely understood and thus are therapeutically unexploited. Using melanoma mouse models, we demonstrated that blocking the MNK1/2-eIF4E axis inhibited melanoma phenotype switching and sensitized melanoma to anti-PD-1 immunotherapy. We showed that phospho-eIF4E-deficient murine melanomas expressed high levels of melanocytic antigens, with similar results verified in patient melanomas.

Mechanistically, we identified phospho-eIF4E-mediated translational control of NGFR, a critical effector of phenotype switching. Genetic ablation of phospho-eIF4E reprogrammed the immunosuppressive microenvironment, exemplified by lowered production of inflammatory factors, decreased PD-L1 expression on dendritic cells and MDSCs, and increased CD8⁺ T-cell infiltrates. Finally, dual blockade of the MNK1/2-eIF4E axis and the

PD-1/PD-L1 immune checkpoint demonstrated efficacy in multiple melanoma models regardless of their genomic classification. An increase in the presence of intratumoral stem-like TCF1+PD-1+CD8⁺ T cells, a characteristic essential for durable anti-tumor immunity, was detected in mice administered a MNK1/2 inhibitor and anti-PD-1 therapy.

Using MNK1/2 inhibitors to repress phospho-eIF4E thus offers a new strategy to inhibit melanoma plasticity and improve response to anti-PD-1 immunotherapy.

TEMOZOLOMIDE AND RADIATION RESISTANCE OF GLIOBLASTOMA CELLS IS ASSOCIATED WITH DNA DAMAGE SIGNALLING NETWORK

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Glioblastoma is the most common and aggressive central nervous system tumor with poor prognosis. The standard treatment consists of surgical resection of tumor, followed by radiotherapy and chemotherapy.

To decipher the mechanism under therapy resistance and develop more effective therapeutic approaches, Temozolomide (TMZ) and ionizing radiation (IR) exposed (TMZ+IR) U373 cell lines are generated. The enhancement of DNA damage response (DDR) machinery in the TMZ+IR exposed glioblastoma model was discovered by RNA sequencing, qRT-PCR, and western blots. MGMT and RAD51 were found among the top upregulated genes by RNAseq data analysis. DDR signalling contribution to therapy resistance was also shown by γH2AX and 53BP1 foci formation in TMZ+IR resistant cells. The pharmacological inhibition of essential kinases in the DDR pathway restored the TMZ+IR sensitivity.

Moreover, translation of the results from this study to glioblastoma patients could alter

the therapeutic effect of TMZ+IR and patient survival.

GENOMIC CORRELATES OF IMMUNE EXCLUSION ACROSS CANCERS

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Immune checkpoint inhibitors (ICIs) have transformed cancer care, however their effectiveness depends greatly on the immune cell presence in the tumor microenvironment (TME): tumors that are abundantly infiltrated with immune cells (*hot*) have better survival and improved response to ICIs compared to immune cell excluded (*cold*) tumors. A growing body of evidence suggests that genetic aberrations in cancer cells dictate immune contexture in the TME. In order to test this hypothesis, we performed an integrative analysis on the solid tumors in the TCGA cohort.

To systematically address the relationship between genomic aberrations and TME composition in solid tumors, we analyzed 9676 tumors across 28 different histologies. Using the transcriptome data, we have classified tumors as *cold* or *hot* by deconvolving the tumor TME. Next, we used matched genomic data of these samples to identify somatic mutations and copy number alterations associated with distinct TME phenotypes. Using evolutionary

methods and linear models, we have identified genes that are under selective pressure in *hot* and *cold* samples separately.

For instance, we found that antigen-presenting machinery (HLA-A, HLA-B, B2M, CASP8) genes are under higher selection pressure in *hot* samples, while a number of oncogenes and tumor suppressors are found to be under higher selective pressure in *cold* samples including NRAS, KRAS, SOX9 and STK11.

Finally, we extended our analysis to gene-sets to identify signalling pathways or gene regulatory networks that are altered differentially in distinct TME phenotypes.

A DEVELOPMENTAL CELLULAR HIERARCHY IN MELANOMA UNCOUPLES GROWTH AND METASTATIC PHENOTYPES

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Although melanoma is notorious for its high degree of heterogeneity and plasticity, the origin and magnitude of cell state diversity remains poorly understood. Equally, it is not known whether melanoma growth is supported by a subfraction of Melanoma Stem-like Cells (MSCs) and if so, whether MSCs and Metastasis-Initiating Cells (MICs) represent overlapping, interchangeable, or distinct cell populations.

By combining single-cell gene expression profiling, multicolour lineage tracing and quantitative modelling, we developed a spatially and temporally resolved map of the diversity and trajectories of melanoma cell states during primary tumour growth and metastatic dissemination in a clinically-relevant mouse model of melanoma. We show that melanoma growth and metastatic dissemination are fuelled, respectively, by two transcriptionally and spatially distinct

melanoma subpopulations. Our findings implicate a hierarchical model of tumour growth that is supported by a population of cancer stem-like cells, which reside in a perivascular niche and exhibit a transcriptomic signature of pre-migratory neural crest cells established transiently during embryonic development. Metastatic dissemination is, instead, driven by a “mesenchymal-like” subpopulation, which preferentially accumulates at the invading front of primary lesions. We identified the transcription factor Prrx1 as a driver of the mesenchymal-like melanoma phenotype, and demonstrate that this population fuels metastatic dissemination to lymph nodes and distant organs through an EMT-MET-like continuum.

These results will pave the way for the development of strategies that detect and, ultimately, intercept melanoma before its dissemination to vital organs.

IL1 β PROMOTES IMMUNE SUPPRESSION IN THE TUMOR MICROENVIRONMENT INDEPENDENT OF THE INFLAMMASOME AND GASDERMIN D

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Interleukin-1 β (IL1 β) is a central mediator of inflammation. Secretion of IL1 β typically requires proteolytic maturation by the inflammasome and formation of membrane pores by gasdermin D (GSDMD). Emerging evidence suggests an important role for IL1 β in promoting cancer progression in patients, but the underlying mechanisms are ill-defined.

Here, we have shown a key role for IL1 β in driving tumor progression in two distinct mouse tumor models. Notably, activation of the inflammasome, caspase-8 as well as the pore-forming proteins GSDMD and mixed lineage kinase domain-like protein (MLKL) in the host were dispensable for the release of intratumoral bioactive IL1 β . Inflammasome-independent IL1 β release promoted systemic neutrophil expansion and fostered accumulation of

T-cell suppressive neutrophils in the tumor. Moreover, IL1 β was essential for neutrophil infiltration triggered by antiangiogenic therapy, thereby contributing to treatment-induced immunosuppression. Deletion of IL1 β allowed intratumoral accumulation of CD8⁺ effector T cells that subsequently activated tumor-associated macrophages. Depletion of either CD8⁺ T cells or macrophages abolished tumor growth inhibition in IL1 β -deficient mice, demonstrating a crucial role for CD8⁺ T cell-macrophage crosstalk in the antitumor immune response.

Overall, these results support a tumor-promoting role for IL1 β through establishing an immunosuppressive microenvironment and show that inflammasome activation is not essential for release of this cytokine in tumors.

DISRUPTION OF THE CRYPT NICHE PROMOTES OUTGROWTH OF MUTATED COLORECTAL TUMOR STEM CELLS

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Recent data establish a logarithmic expansion of Lgr5+ colonic epithelial stem cells (CESCs) in human colorectal cancer (CRC). Complementary studies using the murine 2-stage azoxymethane (AOM)-dextran sulfate sodium (DSS) colitis-associated tumor model indicate early acquisition of Wnt pathway mutations drives CESC expansion during adenoma progression.

Here, sub-division of the AOM-DSS model into *in vivo* and *in vitro* stages reveals DSS induces physical separation of CESCs from stem cell niche cells and basal lamina, a source of Wnt signals, within hours, disabling the stem cell program. While AOM delivery *in vivo* under non-adenoma-forming conditions yields phenotypically-normal mucosa and organoids derived thereof, niche injury *ex vivo* by progressive DSS dose escalation facilitates outgrowth of Wnt-independent dysplastic organoids. These organoids contain 10-fold increased Lgr5+ CESCs with gain-of-function Wnt mutations orthologous to human CRC-driver mutations.

We posit CRC originates by niche injury-induced outgrowth of normally-suppressed mutated stem cells, consistent with models of adaptive oncogenesis.

DISCORDANCE OF LUMINAL A AND LUMINAL B SUBTYPES OF BREAST CANCER IN LOCOREGIONAL METASTASIS COMPARED WITH PRIMARY TUMOR

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Objective of the study: to evaluate changes in luminal A and luminal B subtypes of breast cancer in regional metastasis.

Samples of primary tumor and local metastasis obtained from 65 patients were stained immunohistochemically with antibodies to ER, PR, Her2/neu and Ki67. Primary tumor had the luminal A subtype (according to St. Gallen 2015 consensus) in 36 cases, the luminal B subtype – in 29 cases. The frequency of changes in BC subtype in metastasis was evaluated; the detected frequencies were compared using a Fischer exact probability test.

Subtype of metastasis was discordant in 28 cases (43,1%, 95% CI 31,1-55,9%). In group with luminal A subtype of primary tumor metastasis had discordant subtype in 11 cases (30,6%, 95% CI 16,9-48,3%), among which in 10 cases metastasis had luminal B subtype (27,8%, 95% CI 14,8-45,4%), in 1 – triple negative subtype (2,8%, 95% CI 0,1-16,2%) (p=0,006). In group with luminal B subtype of primary tumor metastasis had discordant subtype in 17 cases

(58,6%, 95% CI 39,1-75,9%) among which in 16 cases metastasis had luminal A subtype (55,2%, 95% CI 36,0-73,0%), in 1 – triple negative subtype (3,4%, 95% CI 0,2-19,6%) (p<0,001). Frequencies of subtype change in groups with luminal A and luminal B primary tumor subtypes differed significantly (p=0,03).

Changes in the luminal A or luminal B subtype of breast cancer in regional metastasis were observed in 43,1% of cases, while the luminal B subtype changed more frequently than luminal A. Among cases with discordance of primary tumor and regional metastases subtypes, another luminal subtype (A or B) of the metastasis was more common than the triple negative subtype.

NRF2 ENABLES EGFR SIGNALING IN MELANOMA CELLS

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Receptor tyrosine kinases (RTK) are rarely mutated in cutaneous melanoma, but the expression and activation of several RTK family members is associated with a pro-invasive phenotype and therapy resistance. Epidermal growth factor receptor (EGFR), a member of the RTK family, is only expressed in a subgroup of melanomas with poor prognosis. The insight into regulation of EGFR is important for the understanding of the development of this melanoma phenotype.

Here we describe that the transcription factor NRF2, the master regulator of the oxidative stress response, mediates the activation of EGFR in melanoma by elevating the levels of EGFR as well as its ligands EGF and TGF- α . ChIP sequencing data show that NRF2 directly binds to the promoter of EGF, which contains a canonical antioxidant response element and is induced by oxidative stress. In contrast, regulation of EGFR occurs by an indirect mechanism, which is enabled by the ability of NRF2 to block the activity of the melanocytic lineage factor MITF. MITF effectively suppresses

EGFR expression and therefore serves as link between NRF2 and EGFR. As EGFR was previously described to stimulate NRF2 activity, the mutual activation of NRF2 and EGFR pathways was investigated using indicator genes of both pathways. Interestingly, we found that the presence of NRF2 was necessary for full EGFR pathway activation. Conversely, EGF led to a profound induction of NRF2 and its target NQO1, thereby demonstrating that NRF2 and EGFR are connected in a positive feedback loop in melanoma.

In summary, our data show that the EGFR-positive melanoma phenotype is strongly supported by NRF2, thus revealing a novel maintenance mechanism for this clinically challenging melanoma subpopulation.

IDENTIFYING METABOLIC VULNERABILITIES THROUGH MASS SPECTROMETRIC IMAGING

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Heterogeneity is an omnipresent feature of tumours, which impinges on their evolution, spread and therapeutic response. Technological advances have allowed us to define this heterogeneity at the level of the genome, the transcriptome and the proteome at an ever-increasing depth, and have sparked the development of targeted therapies. In stark contrast, analysis of tumour metabolism still largely relies on bulk tissue samples. This shortcoming hampers the development of metabolic drugs targeted at certain oncotypes.

To overcome this hurdle, we have developed a number of mass spectrometric imaging (MSI) workflows that allow the correlation of different acquisition modalities. Combining isotopic tracing, fluorescent microscopy and DESI (desorption electrospray ionisation) in a model of Myc heterogeneity, we show that the more proliferative Myc-high clones have a higher flux through the central carbon metabolism, and that this coincides with an increased concentration of pantothenic acid, the backbone of Coenzyme A. To corroborate

these findings, we trace selected compounds using a further method we developed, which allows the overlay of fluorescent signals, electron microscopy and ultra-high-resolution MSI (NanoSIMS). This enabled us to reveal the clonal distribution of a number of isotopically labelled compounds down to their subcellular location.

Lastly, we show the therapeutic potential of this finding, by placing mice growing murine polyclonal tumour or PDXs on a pantothenic acid free diet, which delayed tumour growth significantly and lead to a robust reduction of the metabolic flux. In conclusion, we have established a workflow that facilitates the detection of novel metabolic vulnerabilities through correlative MSI.

IN VITRO EXHAUSTED T CELL ASSAYS FOR IMMUNO-ONCOLOGY CANDIDATES

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The need to better understand the tumour microenvironment (TME) dictates the characterization of the cell types involved, the roles they play and how they respond to treatment. To develop better cancer immunotherapies, *in vitro* primary immune cell bioassays offer an early assessment of their effects on the various players of the TME.

The chronic stimulation of the T cells in the tumour microenvironment leads to an exhausted phenotype of the cells. Those exhausted T cells are defined by an increased expression of a specific set of cell surface markers such as PD-1, TIM-3 and LAG-3 as well as an increased expression of diverse transcription factors like BLIMP-1, BATF and TOX. As exhaustion builds, the expression of these markers increases while T cell functionality decreases. The loss of functionality is defined by a reduction in cytokine secretion, proliferation, and cytotoxic activity.

Reversing their loss of functionality as a therapeutic strategy has made the

characterization of these exhausted T cells a priority.

Using primary immune cells, *in vitro* bioassays were developed to better screen the potential effect of new therapeutics on exhausted T cells. Their ability to reverse the exhausted phenotype and facilitate the anti-tumour immune response can as a result be assessed early in the drug development process.

LncRNA SWITCHES THE CHEMOTHERAPY-POLARIZED MACROPHAGES FROM TUMOR-SUPPRESSING TO TUMOR-PROMOTING PHENOTYPES IN BREAST CANCER

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Although chemotherapy can stimulate anti-tumor immunity by inducing interferon response, the functional role of tumor-associated macrophages (TAMs) in this scenario remains unclear.

Here, we found that interferon-activated proinflammatory macrophages after neoadjuvant chemotherapy enhanced anti-tumor immunity but promoted cancer chemoresistance. Mechanistically, interferon induced the expression of a cytoplasmic lncRNA, Interferon REsponsive NF- κ B Activator (IRENA) in the macrophages, which triggered NF- κ B signaling via dimerizing protein kinase R (PKR), and subsequently increased the production of pro-tumor inflammatory cytokines. By constructing macrophage-conditional IRENA knockout mice, we found that targeting IRENA in the interferon-activated macrophages abrogated their pro-tumor effects, while retained their capacity to enhance anti-tumor immunity. Clinically, IRENA expression in the post-chemotherapy macrophages was associated with poor patient

survival.

These findings present for the first time that a lncRNA can determine the dichotomy of inflammatory cells on cancer progression and anti-tumor immunity and suggests that targeting IRENA is an effective therapeutic strategy to reverse tumor promoting inflammation.

THE NEURAL CREST STEM CELL MARKER CD271 CONFERS IMMUNE EVASION TO MELANOMA CELLS

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Cutaneous melanoma, the deadliest type of skin cancer, is a very heterogeneous and plastic tumor with a high potential to metastasize. Metastasis formation has been linked to stem cell-like subpopulations of melanoma cells, which (amongst others) express the neural crest stem cell marker CD271/NGFR/P75NTR. However, the molecular mechanisms by which CD271 drives metastasis formation remain largely unknown.

Interestingly, transcriptome analysis of CD271 overexpressing human melanoma cells revealed a putative immune evasion signature, suggesting immunosuppression as a potential mechanism for CD271-mediated metastasis formation. In line with this hypothesis, we found substantially less tumor infiltrating innate immune cells in xenograft tumors of CD271 overexpressing human melanoma cells, - with a significant decrease in NK cells. Using *in vitro* cytotoxic assays, we could demonstrate that melanoma cells with endogenously or induced high CD271 levels got less killed by allogeneic and KIR-matched NK cells than

control cells with low CD271 levels. The same held true *in vivo* in that CD271 overexpressing melanoma cells got specifically less killed when injected together with human NK cells into the peritoneum of NSG mice. Finally, in an *in vivo* metastasis assay, where human NK cells were adoptively transferred into NSG mice, preliminary data showed an increased metastatic potential of CD271 overexpressing melanoma cells compared to control cells.

Together, these findings suggest an immune evasive role of CD271 that protects melanoma cells from immune eradication and thus facilitates metastases formation.

LncRNAs AS DYNAMIC COMPONENTS OF SPECIALIZED RIBOSOMES REGULATING STRESS RESPONSES TO ANTICANCER THERAPY

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Translation rewiring was recently shown to be a major evolutionary-conserved driver of invasiveness and therapy resistance in melanoma, where environmental stress conditions (including treatment itself) prime the Integrated Stress Response (ISR) with consequent block of CAP-dependent translation and induction of phenotype switching. Although lncRNAs are not or rarely translated into (small) peptides recent evidence indicates that a large fraction of them is associated with ribosomes. We therefore hypothesized that lncRNAs might be druggable regulators of protein synthesis thus contributing to melanoma-specific translation rewiring.

Here we systematically identified lncRNAs associated with the ribosome during translational rewiring and drug tolerance induction in melanoma.

Our results confirm that occasional, regulated, translation of these transcripts occurs, but it is not the rule. Among the validated hits we

identified a candidate giving rise to a small peptide with potential immune regulatory functions, and a candidate upstream of the ISR activation regulating sensitivity to target therapy, thus suggesting novel opportunities to drug this pathway.

COMBINING IMMUNOHISTOCHEMISTRY WITH GENE EXPRESSION ANALYSIS TO GAIN INSIGHT IN TUMOR-INFILTRATING LYMPHOCYTES IN BREAST CANCER

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Activating the immune system is a promising treatment strategy in breast cancer, as shown for HER2 positive and triple negative breast cancer. To date, the correlation between various subpopulations of tumor-infiltrating lymphocytes (TILs) and response to therapy is still under debate. To understand therapy response, it is critical to study the composition of TILs before and after therapy.

In this study, we evaluated TIL scoring based on immunohistochemistry and nanoString nCounter® gene expression analysis. Formalin-fixed paraffin-embedded and fresh frozen core needle biopsies of 12 breast cancer patients were included in this study. TILs were scored on tissue sections stained with standard hematoxylin-eosin or antibodies specific for CD45, CD3, CD4 or CD8. Scoring was performed twice by three pathologists with a wash-out period of three days. Increasing intra- and

inter-rater variability was observed with higher TIL numbers for all methods with the highest reproducibility observed on tissue sections stained for CD3 and CD8 positive TILs. These TIL scores correlated well with the TIL scores obtained through nanoString nCounter® gene expression analysis. Additionally, nanoString nCounter® gene expression analysis revealed 62 genes that are inversely related to both TIL scoring methods.

This study argues for the use of gene expression analysis to corroborate immunohistochemistry data as well as to provide additional information that could provide insight into the TILs' contribution to controlling breast cancer progression.

CO-OPTION OF PROGRAMS OF NEURONAL DEVELOPMENT AND FUNCTION SUPPORTS CANCER PROGRESSION: AN EMERGING ROLE FOR P73 ISOFORMS IN CANCER-NEURONAL CROSSTALK

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The effect of the neuronal system in tumor plasticity has emerged as an enigmatic aspect of cancer biology. Cancer and neuronal cells develop reciprocal interactions via mutual production and secretion of neuronal growth factors, neurotrophins and/or axon guidance molecules in the tumor microenvironment. Understanding the mechanisms underlying this process holds great potential for blocking tumor progression and improving patient survival.

Here, by applying computational and systemic approaches, we show that neural networks recapitulation by tumor cells has a universal character and a tumor type-independent nature. Genes indispensable for neuronal development, differentiation and function are ectopically activated in tumors and predict poor patient outcomes across various cancers. Tumors tend to co-opt genes essential for the development of distinct anatomical parts of the nervous system, with a frequent preference for cerebral cortex and the neural crest-derived enteric nerves. To gain further experimental

insights, we use melanoma as a representative case and show that TP73, a member of the p53 transcription factor family, is involved in the cancer-neuronal crosstalk. In particular, several TP73 isoforms simultaneously activate neurodifferentiation and stemness pathways in melanoma cells. Neurotrophic factors and genes with neuron-restricted expression are induced by p73 isoforms, along with stemness factors, during melanoma progression.

Our data yield the basis for elucidating how pathways of neural plasticity are recapitulated in the context of metastasis and highlight p73 as a promising regulator of cancer neurobiology.

TARGETING THE ATR/CHK1 AXIS POTENTIATES THE EFFECT OF GLI1 INHIBITORS IN SOLID TUMORS

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Oncogene amplification and overexpression is one of the main drivers of tumorigenesis. The amplification of chromosome regions depends on genomic rearrangements and frequently results in the formation of gene fusions. We recently identified GLI Family Zinc Finger 1 (GLI1) fusions associated with strong GLI1 overexpression in glioblastoma and sarcoma with focal amplifications on chromosome arm 12q. These data indicate GLI1 as a potential target for precision-oncology therapy.

GLI1 is a transcription factor that is activated by sonic hedgehog (SHH) signaling and its inhibition suppresses DNA repair activity in human cancers. Here, we performed an inhibitor-synergism screen targeting Hedgehog-GLI1 signaling in combination with DNA repair and cell cycle checkpoint pathways in glioblastoma and sarcoma cell lines. In the primary screen, we evaluated the sensitivity of the cell lines towards the individual compounds

and selected the inhibitors with the highest efficacy for the subsequent secondary screen in a dose-response matrix design. Both tumor entities showed high sensitivities to the SHH inhibitor JK184 and the GLI1 inhibitor GlaB. Synergistic effects were observed when either JK184 or GlaB were combined with inhibitors of the ATR/CHK1 axis, i.e. CHK1 inhibitor LY2606368 or ATR inhibitor VX-970. Independent validation of the screening results in cellular assays showed dose-dependent increases of cell apoptosis and decreases of cell proliferation in response to the individual drugs and corresponding synergistic effects in combination treatments.

In conclusion, our data suggest that concurrent targeting of the SHH/GLI1 and ATR/CHK1 axes provides a possible precision-therapy approach for tumors with high GLI1 expression.

H3K27me3 IS A DETERMINANT OF CHEMOTOLERANCE IN TRIPLE-NEGATIVE BREAST CANCER

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Residual disease, formed by cancer cells persistent to therapy, remains one of the major clinical challenges towards full cure. In triple-negative breast cancers, persistence to chemotherapy results in the highest risk of recurrence among all breast cancer subtypes. If there is now consensus that the drug-tolerant state is defined by non-genetic features, understanding the mechanisms driving tumor cells to drug-tolerance stands as a pre-requisite to efficiently fight recurrence.

Monitoring epigenomes, transcriptomes and lineages with single-cell resolution, we show that the repressive histone mark H3K27me3 is a determinant of cell fate at the onset of chemotherapy exposure. Indeed, we demonstrate that the persister expression program is primed with both H3K4me3 and H3K27me3 in unchallenged cells, H3K27me3 being the lock to its transcriptional activation. We further establish that H3K27me3 controls cell fate upon chemotherapy exposure: alleviating the genome from H3K27me3 enhances the potential of each cancer cell to tolerate chemotherapy insult. Conversely, we demonstrate that preventing H3K27me3

methylation simultaneously to chemotherapy inhibits the transition to drug-tolerant state, and delays tumor recurrence in vivo.

Our results highlight how chromatin landscapes shape the potential of cancer cells to respond to initial therapeutic insult, and pave the way for combinatorial therapy with histone demethylase inhibitor to delay recurrence.

DISCOVERY AND 3D IMAGING OF DELTANP63-EXPRESSING CELLS IN THE HUMAN PANCREATIC DUCT THAT SHARE CHARACTERISTICS WITH AN AGGRESSIVE MOLECULAR SUBTYPE OF PANCREATIC CANCER

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The aggressive basal-like molecular subtype of pancreatic ductal adenocarcinoma (PDAC) is characterized and driven by deltaNp63 expression, with hints of a duct cell origin. In many epithelia, deltaNp63+ basal cells have stem cell capacity and can generate tumors. In pancreas these cells were said not to exist.

We assessed human normal pancreas, chronic pancreatitis (a risk factor for development of PDAC) and PDAC. We developed a 3D imaging protocol to study sizeable archival patient samples at single cell resolution. We generated organoids from pancreatic ducts obtained from Sox9-eGFP reporter mice.

In normal human pancreas, very rare deltaNp63+ cells exist among the KRT19+ duct cells. Their prevalence increases in chronic pancreatitis. They also express canonical basal markers (KRT5, KRT14 and S100A2), pertain to a niche of cells expressing gastrointestinal stem cell markers but do not express the typical duct cell transcription factor SOX9. 3D views of the ductal tree show that the cells are

localized on the basal membrane of medium to large ducts and are more prevalent as multilayer dome-like structures in chronic pancreatitis. In mice, we detect induction of ΔNp63 in organoid cultures from medium to large size ducts with characteristic low Sox9 expression.

This plasticity of pancreatic duct cells may inform us on the ontogeny of the poor prognostic PDAC subtype.

SMALL CELL LUNG CARCINOMA (SCLC) METASTASIS TO A PANCREAS WELL DIFFERENTIATED NEUROENDOCRINE TUMOUR (PanWD-NET): A CASE REPORT AND ITS PITFALLS

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The well-differentiated neuroendocrine tumours (WD-NET) and poorly-differentiated neuroendocrine carcinomas (PD-NEC) are showing different pathogenesis, distinct clinico-pathological expression and outcome. According to that, the distinction between NET and NEC is based on morphology and specific cellular/architectural parameters. But this case reveals pitfalls by having two components of neuroendocrine neoplasms in a one tumour located in pancreas.

A 68-year-old man was hospitalized to the Hospital of Lithuanian University of Health Sciences for a surgical excision of pancreas tumor found during echoscopy. A tumor in size of 2,6x1,8x1,6cm microscopically consisted of two components: mostly WD-NET G1 and 7mm area of PD-NEC that revealed architectural alterations, pronounced cytological atypia, extent necrosis and high Ki67 proliferative index (~80%). Both components were positive with neuroendocrine immunomarkers, so the diagnose of WD-NET G1 with a component of PD-NEC was made. Few months later a

transbronchial biopsy revealed TTF-1 and neuroendocrine immunomarkers positive small atypical cells infiltration with expressed poorly differentiated morphology. Revised diagnosis of PanWD-NET G1 with SCLC metastasis was made after immunomarker TTF-1 positivity in PD-NEC component was approved.

WD-NET can contain regions with increased proliferation that place them in the WHO G3 category, so the first diagnose of WD-NET with PD-NEC component would be quite unusual. Although, NEC treatment of different localizations does not differ, this case showed that to solve these diagnostic issues, a coordinated and comprehensive clinical and pathological investigation is required.

ALDH-HIGH CELL SUBPOPULATIONS IN OVARIAN CARCINOMA AND THEIR SENSITIVITY TO NIFUROXAZIDE

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High grade serous ovarian carcinoma (HGSOC) is a severe form of ovarian cancer with poor prognosis. HGSOC is of unmet clinical need, as malignant or relapsed tumours typically respond poorly to existing therapies. Tumour cell subpopulations with high expression and activity of aldehyde dehydrogenase (ALDH) family proteins are a potential novel therapeutic target. High ALDH1 expression is a marker of cancer stem-like cells (CSCs) in HGSOC, and these ALDH1-High cells are associated with cell migration, tumourigenicity, chemo-resistance, and poor prognosis. Previous work by Sarvi et al (2018) showed that the 5-nitrofuranyl drug Nifuroxazide (NAZ) can selectively target and induce apoptosis in ALDH1-High cells within melanoma tumours, both *in vitro* and *in vivo*. We aim to investigate whether NAZ shows similar specific cytotoxic effects in other tumour types; particularly HGSOC, in which ALDH1-High CSCs have previously been identified.

We have shown the presence of high ALDH activity in several HGSOC cell lines, and determined in the OVCAR-8 cell line that this is likely due to elevated ALDH1A3 expression in ALDH-High cell subpopulations. High ALDH1A3 expression was also observed in HGSOC patient tumour samples by immunohistochemistry and cDNA expression analysis. We now aim to further elucidate the functional role of ALDH1A3 using knockdown experiments. We also show that ALDH-High OVCAR-8 and PEO1 cells are sensitive to treatment with NAZ *in vitro*.

We are now developing *in vivo* mouse models to determine the sensitivity of HGSOC tumours to NAZ. Additionally, we will analyse patient clinical outcome data in relation to ALDH1 expression, to elucidate potential routes for clinical patient stratification for NAZ treatment.

ROLE OF FIBROBLAST HETEROGENEITY IN IMMUNOSUPPRESSION AND RESISTANCE TO IMMUNOTHERAPY

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Cancer Associated Fibroblasts (CAF) constitute one of the most abundant stromal components in solid tumors. By combining various approaches and CAF markers, we identified 4 CAF subsets (CAF-S1 to CAF-S4) in breast and ovarian cancers (Costa, *Cancer Cell*, 2018; Givel, *Nat. Commun*, 2018). Two myofibroblastic CAF subsets CAF-S1 (FAP+ SMA+ MCAM-) and CAF-S4 (FAP- SMA+ MCAM+) accumulate strictly in cancer, while the two others are also detected in normal healthy tissues. CAF-S1 and CAF-S4 display pro-metastatic functions through complementary mechanisms (Pelon, *Nat. Commun*, 2020; Bonneau, *Breast Cancer Res.*, 2020). In addition, CAF-S1 promote immunosuppression through a multi-step mechanism. Indeed, CAF-S1 attract CD4+ CD25+ T lymphocytes, enhance their survival and stimulate their differentiation into CD25^{High} FOXP3^{High} regulatory T cell (Tregs) (Costa, *Cancer Cell*, 2018; Givel, *Nat. Commun*, 2018).

By using single cell technology on more than 19,000 single CAF-S1 fibroblasts from breast cancer and demonstrated that the CAF-S1 subset is heterogeneous and composed of

8 cellular clusters. We validated the five most abundant clusters by flow cytometry and *in silico* analyses in other cancer types, highlighting their relevance. Myofibroblasts from clusters 0 and 3, characterized by extracellular matrix proteins and TGFβ signaling, respectively, are indicative of primary resistance to immunotherapies. Cluster 0/ECM-myCAF upregulates PD-1 and CTLA-4 protein levels in Tregs, which, in turn, increases CAF-S1 cluster 3/TGFβ-myCAF cellular content.

Thus, our study highlights a positive feedback loop between specific CAF-S1 clusters and Tregs and uncovers their role in immunotherapy resistance (Kieffer, *Cancer Discovery*, 2020).

GM-CSF NITRATION IS A NEW DRIVER OF MYELOID SUPPRESSOR CELL ACTIVITY IN TUMORS

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Immunosuppression represents a major hurdle for the accomplishment of effective immunotherapy for cancer treatment. In this context, myeloid suppressor cells enroll prominent suppressive activity, directly inhibiting T cell functions and promoting tumor progression by multiple mechanisms.

The immunomodulatory cytokine GM-CSF that is secreted by tumor and immune cells is primarily responsible for myeloid cell expansion and differentiation in tumors. However, its role in cancer biology still remains controversial.

We show that GM-CSF is post-translationally modified by reactive nitrogen species (RNS) within the tumor microenvironment. The nitration of a single amino acid in the sequence of GM-CSF boosts the generation and activity of highly immunosuppressive myeloid cells in tumor-bearing hosts by over-activating distinctive molecular pathways. Collectively, our data identify the nitrated-GM-CSF as a

novel target for immune-based approaches that can be exploited to counteract tumor-induced immunosuppression.

EARLY cDC1 FUNCTION DETERMINES RESPONSE TO ANTI-CD40 AGONIST THERAPY WHILE SUPPRESSIVE IMMUNE MICROENVIRONMENTS AND LACK OF ANTIGENS SUBVERT EFFICACY

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Tumours dysregulate proper immune function during both early tumourigenesis and late-stage disease by subverting T-cell priming and inducing strong systemic immune suppression respectively. Understanding how these processes evolve during tumour progression could inform both the timing and selection of therapeutic interventions.

By treating B16 melanoma tumours with anti-CD40 (aCD40) agonist that matures antigen-presenting cells, we significantly slowed tumour growth. Using XCR1-DTR mice, we showed that the aCD40 response relied on the presence of cDC1s during tumour onset, but that cDC1s were dispensable for the therapeutic effect of aCD40 when tumours reached a volume of 100mm³. Eventually, treatment with aCSF1R after tumour control was lost prolonged survival.

Lewis lung carcinoma (LLC) tumours, which – in contrast to B16 tumours- are characterised by a robust myeloid infiltrate, contain less CD8+

T cells and were resistant to aCD40 therapy. When combined with aCSF1R to deplete TAMs, the aCD40 response remained limited. Despite increasing CD8+ T-cell infiltration into LLC tumours, by combining aCD40/aCSF1R with either Flt3L pre-treatment to increase cDC abundance, or aCD25 to deplete regulatory T cells, LLC tumour growth remained unaltered. To assess whether cancer cell-intrinsic mechanisms were inhibiting therapeutic efficacy, we treated LLC-OVA tumour bearing mice with aCD40/aCSF1R and saw a strong tumour growth suppression.

These results suggest that aCD40 efficacy relies on pre-existing CD8+ T cells that were primed by cDC1s, and that therapeutic efficacy can be limited by the lack of strong antigens or by the presence of a suppressive microenvironment.

DIGITAL PCR-BASED DEEP QUANTITATIVE ANALYSIS OF BULK TUMOURS UNCOVERS THE HETEROGENEITY AND EVOLUTION OF PRIMARY UVEAL MELANOMA

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Uveal melanoma (UM) is a rare intraocular tumour, characterised by a limited number of genetic alterations. The evolution of this malignancy remains incompletely understood. Based on bulk sequencing analyses, UM has been proposed to arise after an early punctuated burst of genetic alterations, leading to homogeneous tumours. In contrast, recent single-cell studies revealed substantial intratumour heterogeneity and higher genomic complexity.

By comparing the absolute abundances of genetic alterations present in a bulk tumour, the heterogeneity and evolution of a malignancy can be inferred. In this study, we performed a deep quantitative assessment of 82 primary uveal melanomas by novel digital PCR-based approaches. (Non-)hotspot mutations were quantified by targeted and drop-off mutation assays, while copy number alterations were sensitively measured by quantifying the allelic imbalance of heterozygous SNPs.

Gq signalling mutations were present in ~98% of the tumours. They were always

clonally abundant and therefore likely acquired in the earliest stage of UM development. Three main evolutionary subtypes could be identified based on having an *EIF1AX* mutation (~12%), *SF3B1* mutation (~20%) or monosomy 3p (~63%). These alterations were usually mutually-exclusive and (near-)clonally abundant, suggesting that they represented distinct secondary drivers. This contrasts with gains and amplifications of chromosome 8q, which showed subclonality in ~30-40% of the affected tumours and were frequently tertiary events.

Using high-resolution genetic analyses, we identified systematic differences in the evolutionary timing of genetic events in UM. Intratumour heterogeneity was observed in most tumours and suggests gradual evolution.

TACKLING BRAF INHIBITORS RESISTANCE IN HUMAN MELANOMA

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Malignant melanoma is one of the most aggressive cancers, characterized by high invasive and metastatic potential. It is responsible for the majority of skin cancer related deaths. Initially, drugs targeting constitutively active MAPK pathway caused by *BRAF* V600E mutation occurring in melanoma (e.g. vemurafenib) had excellent results and increased patient's survival. Unfortunately, patients receiving BRAF inhibitor (BRAFi) treatment relapsed after therapy, because of acquired resistance to this drug. It is known that Wnt signaling plays a role in development of resistance to targeted therapy. We found that activity of the canonical and non-canonical pathways is crucial for melanoma cells survival upon BRAF inhibition. We observed plasticity in the activation of Wnt pathway components in responses to the temporary and chronic vemurafenib treatments.

Interestingly, we found out that RNF43 in human melanoma cells is a negative regulator of the Wnt signaling pathway. Moreover, RNF43 expression is significantly lower in the metastatic growth phase of melanoma

and it is a negative survival factor for melanoma patients. Importantly, cancer cells overexpressing RNF43 lose their plasticity in the response to the vemurafenib treatment.

Furthermore, during our experiments we identified that for resistance development can be also important casein kinase 1ε (Ck1ε), which can influence BRAF V600E inhibitor resistance in melanoma metastasis. Our results indicated its increased expression in the vemurafenib resistant cells. Strikingly, kinases are druggable targets by i.e. small molecule inhibitors, what makes Ck1ε and possibly other Ck1 isoforms an attractive targets in melanoma.

THE MYOSIN II CYTOSKELETON AS A NEW VULNERABILITY IN THERAPY-RESISTANT MELANOMA

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MAPK-targeted therapies (MAPKi) and immune checkpoint blockers (ICB) improve survival of subsets of melanoma patients. However, therapy resistance is a persistent problem. Cross-resistance to MAPKi and ICB has been suggested to be driven, in part, by common transcriptomic alterations in pathways controlling invasion and metastasis. We find that adaptation to treatment and acquisition of resistance to MAPKi involve cytoskeletal remodelling and changes in expression levels in the ROCK-Myosin II pathway, which plays a key role in cancer invasion and metastasis.

Myosin II activity is decreased shortly after MAPK is blocked. However, resistant cells promptly restore Myosin II activity to increase survival, and this becomes a vulnerability, since survival of MAPKi- and ICB-resistant cells is highly dependent on ROCK-Myosin II. Efficacy of MAPKi and ICB can be improved by combination with ROCK inhibitors, which have a dual action by impairing melanoma

cell survival (through induction of lethal reactive oxygen species and unresolved DNA damage) and myeloid- and lymphoid-driven immunosuppression, overcoming cross-resistance. In human tumours, high ROCK-Myosin II activity and their associated transcriptome identify MAPKi-, ICB-resistant melanomas, and treatment-naïve melanomas with worse prognosis.

Therefore, a subset of MAPKi- and ICB-resistant melanomas is intrinsically more susceptible to ROCK-Myosin II inhibition, suggesting clinical opportunities for combination therapies.

DIETARY PALMITIC ACID PROMOTES A PROMETASTATIC EPIGENETIC MEMORY RELATED TO TUMOUR-ASSOCIATED SCHWANN CELL ACTIVATION

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Fatty acid (FA) uptake and altered tumour cell metabolism constitute hallmarks of metastasis though it is still unclear how this works, or whether all dietary FAs are prometastatic.

Here we show that dietary palmitic acid (PA), promotes metastasis in oral carcinoma and melanoma and unveil a specificity of action for distinct FAs. Strikingly, tumours exposed for a short period to a PA-rich remain highly metastatic even when serially transplanted (without further PA exposure). This PA-induced prometastatic memory requires the FA transporter CD36 as well as the epigenetically stable deposition of histone H3 lysine 4 trimethylation by the methyltransferase Set1A/COMPASS. Bulk, single-cell sequencing and spatial transcriptomics of primary tumours indicate that cancer cells that acquire the prometastatic memory alter their tumour-associated stroma inducing the activation of intratumour Schwann cells and perineural invasion, two parameters strongly correlated with metastasis but etiologically poorly understood. Mechanistically, tumour-

associated Schwann cells secrete a specialized pro-regenerative extracellular matrix reminiscent of perineuronal nets, which when ablated strongly inhibits metastatic colonization. Both the PA-induced epigenetic neural signature and its long-term boost in metastasis require the transcription factor EGR2 and the glial cell-stimulating peptide galanin.

In sum, we provide evidence for a long-term epigenetic stimulation of metastasis by a dietary metabolite related to a pro-regenerative state of tumour-activated Schwann cells. In addition to underscoring the potential danger of eating large amounts of PA, our results reveal novel epigenetic and neural-related therapeutic strategies for metastasis.

A NOVEL L-ASPARAGINASE WITH ULTRA-LOW GLUTAMINASE CO-ACTIVITY PREVENTS METASTASIS IN A BREAST CARCINOMA MOUSE MODEL

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L-Asparaginase (ASNase) is a non-human enzyme that hydrolyses the amino acid asparagine (Asn) into aspartic acid and ammonia. It is a cornerstone drug for the treatment of pediatric leukemia. Evidence is accumulating that ASNase may also have clinical potential for the treatment of certain aggressive solid cancer subtype, however continued longterm treatment is associated with severe adverse side-effects, especially in adult patients. The development of more tolerable ASNase variants could potentiate its expansion to many other aggressive cancer subtypes.

A major contributor to these adverse side-effects is the glutaminase co-activity that is present in all ASNase formulations nowadays used in the clinic. We designed a mutant *Erwinia* ASNase variant (*Era-TM*) with similar L-asparaginase activity but ultra-low glutaminase co-activity, and demonstrated that this *Era-TM* variant is as efficient in killing leukemia cells, but has significantly less

adverse side-effects compared to the wild type *Erwinia* ASNase.

A recent report demonstrated that Asn bioavailability determines the metastatic potential of a murine breast carcinoma model [1]. Using our exclusive more tolerable ASNase variant, we confirmed these findings in an independent model; 7-week continued treatment with *Era-TM* significantly decreased the metastatic nodules in the lungs of these mice without influencing primary tumor growth. Further investigation demonstrated that breast cancer cells that undergo an epithelial-mesenchymal-transition (EMT) undergo a metabolic switch that results in a dependency for exogenous Asn uptake, which drives sensitivity of these invasive and circulating tumor cells to ASNase treatment.

1: Knott SRV, et al, Nature 2018

COPY NUMBER EVOLUTION AND LOCAL IMMUNE PRESSURE IN PANCREATIC CANCER: IMPLICATIONS FOR PROGNOSIS

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Many attempts have been made to elucidate the evolution of pancreatic cancer (PC), without leading to new clinical practice. We hypothesize that the dynamic interplay between the evolutionary propensity of the tumor, along with the local pressure of the immune microenvironment, influence emergence of resistance and, hence, patient outcome.

The clonal landscape and immune profile of the regional tumor space were analyzed in nine patients diagnosed with primary resectable PC. Multiregional sampling of primary tumor and lymph node metastases was used for construction of individual tissue microarrays and DNA extraction. Targeted deep sequencing (> 590 cancer related genes) together with genome wide SNP array assay was performed prior to clonal deconvolution and phylogenetic analysis. A mathematical complexity score was developed to calculate the network extent of each evolutionary tree. Spatial variation in abundance and tumor nest proximity of immune cells was analyzed by

double immunohistochemical staining.

Copy number aberrations accounted for almost all intratumor heterogeneity and evolutionary branching, whereas known driver mutations were mainly noted in the stem. An increased tree complexity was significantly ($p < 0.001$) associated with a decreased overall survival (OS). Further, significant relationships were observed between increased tumor nest proximity of regulatory T cells and PD-L1 positive lymphocytes, respectively, with a larger-sized phylogeny, and for M2 macrophages with complex trees.

Complex evolutionary patterns imply a worse OS and certain tree characteristics are associated with the ability of various immune cells to locate closely to the tumor nest.

SLC16A1 EXPRESSION IN MELANOMA PATIENTS PRIOR TO IMMUNE CHECKPOINT BLOCKADE

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Modern therapeutic approaches like immune checkpoint blockade (ICB) have greatly improved melanoma treatment with better progression free and overall survival. Studies showed an involvement of genes contributing to tumor invasion and poor patient survival. Here we investigate the role of *SLC16A1*, coding for the MCT1 protein that shuttles lactate between hypoxic and normoxic cancer cells along with H⁺ ions.

Tasdogan et al. 2019 found that higher *SLC16A1* associated with worse overall survival in advanced melanoma patients and higher MCT1 protein levels in efficiently metastasizing xenografted melanomas. We tested patient survival using univariate and multivariate analysis in *SLC16A1* high and low groups treated with ICB in four bulk RNA sequencing cohorts. Overall and progression free survival were not associated with *SLC16A1* expression. We then analysed single cell RNA sequencing data published by Tirosh et al. 2016 and Jerby-Arnon L et al. 2018 and found *SLC16A1* was expressed in melanoma cells and macrophages, however not in other non-malignant and immune cells.

Pathway analysis for the genes correlated with *SLC16A1* showed the cell cycle pathway was associated with the gene's expression across different databases. *CDK4* and *CDK1* mRNA levels were positively correlated with *SLC16A1* in melanoma single cells. Examining immune infiltration in bulk RNA ICB cohorts, myeloid dendritic cells from XCELL assays appeared reduced with increased *SLC16A1* expression in 3 of 4 analysed cohorts.

In conclusion, as *SLC16A1* expression did not correlate with patient survival in ICB cohorts, the worse prognosis observed without ICB may also relate to an altered cell cycle and possibly a reduced antigen-presentation by myeloid dendritic cells.

SINGLE-CELL PROFILING OF MYELOID CELLS IN GLIOBLASTOMA ACROSS SPECIES AND DISEASE STAGE REVEALS MACROPHAGE COMPETITION AND SPECIALIZATION

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Glioblastomas are aggressive primary brain cancers that recur as therapy-resistant tumors. Myeloid cells control glioblastoma malignancy, but their dynamics during disease progression remain poorly understood.

Here, we employed single-cell RNA sequencing and CITE-seq to map the glioblastoma immune landscape in mouse tumors and in patients with newly diagnosed disease or recurrence. This revealed a large and diverse myeloid compartment, with dendritic cell and macrophage populations that were conserved across species and dynamic across disease stages. Tumor-associated macrophages (TAMs) consisted of microglia- or monocyte-derived populations, with both exhibiting additional heterogeneity, including subsets with conserved lipid and hypoxic signatures. Microglia- and monocyte-derived TAMs were self-renewing populations that competed for space and could be depleted via CSF1R

blockade. Microglia- derived TAMs were predominant in newly diagnosed tumors, but were outnumbered by monocyte-derived TAMs following recurrence, especially in hypoxic tumor environments.

Our results unravel the glioblastoma myeloid landscape and provide a framework for future therapeutic interventions.

FEATURES OF THE GROWTH AND PHENOTYPE OF TUMOR CELLS DURING THE REPOPULATION OF MATRICES OF VARIOUS MURINE ORGANS

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The use of decellularized matrices, which can be repopulated with cells of the required type for solving a particular research problem, is currently gaining special popularity.

The aim of our work was to study the influence of the matrix of various organs on the features of changes in the phenotype of metastatic tumor cells. The SKOV-3 line was chosen for repopulation of matrices of different murine organs.

To obtain scaffolds, organs were sequentially kept in 0.5% Triton X-100, 0.5% SDS, 1% SDC, and 0.075% SDS. The obtained samples were placed in PBS for a day, after which they were incubated for 72 hours in McCoys medium with 30% serum.

For recellularization (RCL), each matrix was placed in a well of a plate with reduced adhesion, after which 2x10⁶ SKOV-3 cells were injected inside the matrix. After repopulation, the matrices were incubated for 7 days on orbital shaker. On days 3 and 5, the medium

was removed, centrifuged, and the resulting cell pellet was re-injected.

To determine the degree of repopulation, histological, cytomorphological and DNA concentration analyzes were performed.

According to the results of the study, an uneven growth of the cell population on different types of matrix was shown. Change in the cell phenotype from pronounced mesenchymal to pseudo epithelial was found in some types of matrix. It was noted that cells exhibit different behavior depending on the density of the matrix and co-localization. We assume that in the future RCL matrices can be used for a more detailed study of the mesenchymal-epithelial transition of cancer cells and metastasis processes.

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THE INCREASING COMPLEXITY OF THE MELANOMA TRANSCRIPTOMIC LANDSCAPE

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Melanoma is notorious for its high degree of intratumoral heterogeneity (ITH) and plasticity, the key features known to promote disease progression and therapy resistance. The advent of single cell genomics allows to study this ITH to an unprecedented level of resolution.

In this study, single cell RNA-seq data were generated using the 10x Chromium technology from 5 primary murine tumors (*Tyr::NRasQ61K^o;Ink4a-I*-model), and 17 human metastatic lesions from treatment naïve melanoma patients. Malignant cells were identified based on inferred copy number variations and specific melanoma gene expression signatures and analyzed using Seurat, Harmony, SCENIC, CytoTRACE, and CellPhoneDB packages. In total we obtained ~27K and ~8K malignant cells from mouse and human lesions, respectively. This cross-species comparison allowed the identification, in addition to the previously described melanocytic (MITF^{high}) and mesenchymal-like (MITF^{low}) states, novel discrete and

shared states, thereby demonstrating that the melanoma transcriptional landscape is more complex than previously thought.

We observed that each melanoma state varied in levels of immune related genes with one being characterised by particularly high levels of genes involved in antigen processing and presentation. Importantly, our analyses showed that ITH cannot be solely explained by genetic makeup, as shown by the discrepancy of transcriptional and genetic (CNV) cluster identity but rather by specific cell-to-cell interactions with the TME. Finally, we confirmed a non-random geographical distribution of our melanoma cell states by mapping them spatially (Visium, 10x genomics). These results contribute to deeper understanding of melanoma tumor microenvironment.

MATHEMATICAL MODELING OF RESISTANCE TO TREATMENT AND HETEROGENEITY IN MELANOMA

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Cutaneous melanoma is a highly invasive tumor and most patients with advanced melanoma have a poor clinical outcome. The most frequent mutations in melanoma affect the BRAF oncogene, a protein kinase of the MAPK pathway. Therapies targeting BRAF/MEK are effective for only 50% of the patients and almost systematically generate resistance. Genetic and non-genetic mechanisms of drug resistance associated with the strong heterogeneity of melanoma cells are still poorly understood.

Recently we have introduced a novel mathematical formalism allowing to cope with all these aspects and proposed three types of models for the mechanisms of heterogeneity and resistance. A first model considers mutational mechanisms. Using this model and extant data we showed that the hypothesis of punctuated equilibrium corresponding to a mutation rate that is strongly inhomogeneous in time should be favoured. It also allowed us to investigate the effect of combining dabrafenib and ipilimab therapies and showed

that treating with ipilimumab first and with dabrafenib later is the optimal protocol schedule. A second model considering metabolic remodelling between high glycolysis and high oxidative phosphorylation states predicts the possibility of resensitisation after a drug holiday. A third, expression based model, driven by single cell RNAseq data recapitulates single cell expression dynamics and predicts zoning, with sensitive cell distributed in regions inside the tumor, less accessible to the drug and resistant cells distributed in drug accessible regions.

Currently we are refining these models using annotated features of H&E-stained and spatially resolved multiplexed Hyperion images of tissue sections.

SIALIC ACIDS IN PANCREATIC CANCER CELLS DRIVE TUMOUR-ASSOCIATED MACROPHAGE DIFFERENTIATION VIA THE SIGLEC RECEPTORS SIGLEC-7 AND SIGLEC-9

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Changes in glycosylation during tumour progression are a key hallmark of cancer. One of the glycan moieties generally overexpressed in cancer are sialic acids, which can induce immunomodulatory properties via binding to Siglec receptors. We here show that Pancreatic Ductal Adenocarcinoma (PDAC) tumour cells present an increased sialylation that can be recognized by Siglec-7 and Siglec-9 on myeloid cells.

We identified the expression of the α 2,3 sialyltransferases ST3GAL1 and ST3GAL4 as main contributor to the synthesis of ligands for Siglec-7 and Siglec-9 in tumour cells. Analysing the myeloid composition in PDAC, using single cell and bulk transcriptomics data, we identified monocyte-derived macrophages as contributors to the poor clinical outcome. Tumour-derived sialic acids dictate monocyte to macrophage differentiation via signalling through Siglec-7 and Siglec-9. Moreover, triggering of Siglec-9 in macrophages reduce inflammatory programmes, while increasing PD-L1 and IL-10 expression, illustrating that

sialic acids modulate different myeloid cells.

This work highlights a critical role for sialylated glycans in controlling immune suppression and provides new potential targets for cancer immunotherapy in PDAC.

IMPACT OF NOVEL SMALL-MOLECULES COMPOUNDS ON E-CADHERIN RECOVERY AT CELL-CELL CONTACTS THROUGH HAKAI ACTION IN HUMAN COLORECTAL ADENOCARCINOMA CELLS

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Hakai is an E3 ubiquitin-ligase protein that mediates E-cadherin ubiquitination, endocytosis and finally degradation, leading the alterations of cell-cell contacts. E-cadherin, a tumour suppressor responsible for cell-cell adhesion in the epithelium and a well-recognised hallmark of the epithelial-to-mesenchymal transition (EMT). Hakai is important for tumour progression *in vitro* and *in vivo* in different carcinomas.

By virtual screening, we have identified novel small-molecule inhibitors directed against the domain responsible for Hakai activity and show the antitumor effect *in vitro*. Novel small-molecules compounds were tested to analyse expression and localization of E-cadherin in colon cancer cell line by immunocytochemistry using Structured Illumination Microscopy platform. Image acquisition and Algorithm CyteSeer® was used to quantify the E-cadherin location. Cell cycle analysis was performed measuring relative DNA content by quantifying Total Integrated Intensity (TII) of the Nuclear Image (Ni) on the Nuclear Mask (Nm). We have validated the effect of at least two new compounds (A and B) from the nineteen tested

that show a significant increase in E-cadherin recovery at the cell junctions and an improved potency. These two compounds strongly decrease cell number compared to treated with DMSO vehicle control. Compound A where arrested the cell cycle in G0/G1 phase.

Our results show that least two compounds show improve efficacy from previously reported in terms E-cadherin recovery at cell-cell contacts and decrease cell number. Hakai inhibitors may be effective strategy to inhibit EMT through Hakai action. Further studies are needed to elucidate their antitumor action in human colon cancer progression

IMMUNOLOGICAL RESPONSE AND RESISTANCE OF HUMAN CANCERS TO PD-1 BLOCKADE

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Immune checkpoint treatment targeting the PD-1/PD-L1 pathway has revolutionized cancer treatment for many tumor types. While numerous studies have demonstrated clinical activity upon anti-PD-1 treatment, the immunological alterations that are induced in human cancer tissue by this treatment are not yet very well understood. In this study, we developed and employed a patient-derived tumor fragment (PDTF) platform to assess early immunological changes upon *ex vivo* anti-PD-1 treatment. In addition, we characterized baseline tumor properties to identify causes of upfront resistance to PD-1 blockade.

Using the PDTF platform, we assessed early immunological responses upon *ex vivo* PD-1 blockade in 37 tumors from five different cancer types. Importantly, we found that the capacity for immune reactivation by PD-1 blockade strongly correlated with clinical

response and was predicted by the baseline presence of tertiary lymphoid structures and their components. Moreover, by assessing immune composition, spatial distribution and immune reactivity of the tumors, we identified distinct microenvironment subtypes that were associated with upfront resistance to anti-PD-1. Potential causes for non-response included lack of immune infiltration, T cell exclusion, and absent/low T cell recognition of the tumor.

Altogether, we here show that human *ex vivo* technologies such as the PDTF platform allow to dissect therapy-induced immune reactivity in human cancers. By linking immunological response to baseline tumor properties, such technologies should furthermore support the identification of biomarkers as well as the development of novel personalized treatment strategies.

HETEROGENEITY IN PHGDH PROTEIN EXPRESSION POTENTIATES CANCER CELL DISSEMINATION AND METASTASIS

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Cancer metastasis requires the transient activation of cellular programs enabling dissemination and seeding in distant organs. Genetic, transcriptional and translational intra-tumor heterogeneity contributes to this dynamic process. Beyond this, metabolic intra-tumor heterogeneity has also been observed, yet its role for cancer progression remains largely elusive.

Here, we discovered that intra-tumor heterogeneity in phosphoglycerate dehydrogenase (PHGDH) protein expression drives breast cancer cell dissemination and metastasis formation. Specifically, we observed intra-tumor heterogeneous PHGDH expression in primary breast tumors, with low PHGDH expression being indicative of metastasis in patients. In mice, Phgdh protein,

but not mRNA, expression is low in circulating tumor cells and early metastatic lesions, leading to increased dissemination and metastasis formation. Mechanistically, low PHGDH protein expression induces an imbalance in glycolysis that can activate sialic acid synthesis. Consequently, cancer cells undergo a partial EMT and show increased p38 as well as SRC phosphorylation, which activate cellular programs of dissemination. In turn, inhibition of sialic acid synthesis through knock-out of cytidine monophosphate N-acetylneuraminic acid synthetase (CMAS) counteracts the increased cancer cell dissemination and metastasis induced by low PHGDH expression.

In conclusion, we find that heterogeneity in PHGDH protein expression promotes cancer cell dissemination and metastasis formation.

FIELD CANCERIZATION IN MAMMARY TISSUE IS DRIVEN BY PROTECTION MECHANISMS THAT CLEAR MUTATIONS

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Cells with mutations in oncogenic driver genes are abundant in tissues of healthy individuals, but they rarely develop into tumors. Yet, the underlying protection mechanisms that prevent tumor formation are largely unknown.

To resolve these mechanisms in breast tissues, and understand how they may be overcome by mutant cells to drive field cancerization, we compare the clonal fate behavior of cells that acquire oncogenic (Brca1^{-/-};Trp53^{-/-}) mutations with those bearing neutral mutations in the mouse mammary gland epithelium. From the quantitative analysis of the clonal dynamics in normal tissue, we find that the ductal epithelial network is supported by a short, lineage-restricted, stem-progenitor cell hierarchy. This hierarchy confers the first protection mechanism against field cancerization as only clones initiated in the stem cell compartment survive over the short-term. At longer times, local tissue remodeling during the estrous cycle leads to stochastic collective stem cell amplification and loss. This process provides a second mechanism of protection, leading to the elimination of the majority of mutant clones, while massively

accelerating the expansion of a minority of clones that, by chance, survive. Eventually, this "inflationary" process of geometric clone expansion becomes restrained by the one-dimensional geometry of the ducts, providing a third mechanism to protect the epithelium against uncontrolled colonization by mutant clones.

Together, these findings reveal layers of protection that serve to eliminate the majority of cells that acquire chance somatic mutations at the expense of driving the accelerated expansion of a minority of cells, which can colonize large areas leading to field cancerization.

CHRONIC INFLAMMATION AND ENHANCED TUMOR PLASTICITY INFERRED BY CLONAL EVOLUTION OF A TUMOR SUBPOPULATION WITH PERSISTENT ACTIVATION OF NUCLEAR DNA-DAMAGE REPAIR SIGNALS POST RADIOTHERAPY

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Molecular mechanisms governing late radiation induced inflammatory and immune effects remain elusive. We sought to identify the sensing event and downstream executive processes underlying tissue radiation memory and tumor plasticity.

We present proliferative propagation of unresolved, residual radiation-induced DNA damage foci (rRIF) as a source of delayed inflammation following radiotherapy. Image guided (IF+DEPararray) single-cell transcriptomics (10X) revealed a characteristic gene expression signature for rRIF positive subpopulation enriched for innate inflammatory signaling. Mechanistically, expression of these genes correlated with formation of micronuclei and activation of cGas-STING-NFKB-signaling. rRIF-positive cells preserved proliferative potential, lacked characteristics of senescent cells like distension of constitutive heterochromatin and

expression of mobile elements. Moreover, they did not present a target for the senolytic drug Navitoclax. In contrast, time-lapse microscopy demonstrated clonal inheritance of persistent RIF (pRIF) over multiple generations. Formation of pRIF positive colonies underscored high proliferative capacity of these cells and was associated with increased presence of micronuclei and NFKB activation. Clonally expanded pRIF-positive cells maintained pRIF-rates for >1 month maintaining characteristic pro-inflammatory expression signature of rRIF cells, reminiscent of tissue radiation memory. *In vivo*, rRIF/pRIF was detected during recurrence of irradiated A549 xenografts, what was accompanied by a persistent innate immune response on the transcript-level.

Our findings provide an explanatory mechanistic model for chronic inflammation after radiation.

CELLULAR PLASTICITY OF THE ALDEHYDE DEHYDROGENASE POPULATION AS ESCAPE MECHANISM FROM IONIZING RADIATION IN PROSTATE CANCER

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Tumors are heterogeneous containing a cancer stem cell (CSC) subpopulation defined by increased self-renewal, tumorigenic capacity and therapy resistance. However, therapeutic pressure itself is one of the key factors driving tumor cell plasticity and clonal selection. Previously we demonstrated that irradiation (IR) enriches for aldehyde dehydrogenase-positive (ALDH+) CSCs in prostate cancer (PC) cell lines and xenografts.

We hypothesize that the IR-induced increase of ALDH+ CSCs is both stochastic via a reduction of sensitive tumor cells as well as deterministic through cell conversion of ALDH- into ALDH+ cells. To investigate the cellular mechanisms of irradiation-induced plasticity we used a competitive co-culture and co-transplantation setup with ALDH+ and ALDH- cells that were isolated from either tdTomato or blue fluorescent protein labelled PC cell lines. Within the xenograft

tumors we confirmed dynamic changes of the ALDH+ population and conversion of ALDH- into ALDH+ during tumor progression that was further increased upon IR. We compared the clonogenicity and spherogenicity of the IR-induced ALDH+ cell population in comparison to the original ALDH+ cells and found no functional differences. We assume additional factors influence dynamics and radiosensitivity of ALDH+ population as cellular heterogeneity and sub-clonal cooperation. Hence, we compared clonal distribution and cellular heterogeneity of ALDH+ and ALDH- in two PC cell lines after IR using multiplex gene expression analysis of 18 samples with 10 pooled cells. Using cluster analysis, we found a reduced heterogeneity after IR in both populations.

In summary, IR induces ALDH+ cells from ALDH- population in xenografts that affects tumor responsiveness.

IMPACT OF WOBBLE tRNA MODIFICATION AND CODON-SPECIFIC TRANSLATION REGULATION IN MELANOMA IMMUNE RESPONSE

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Malignant melanoma is the deadliest form of skin cancer. The breakthrough discoveries on the role of the immunity system in tumor development led to new and effective treatments for melanoma patients. Nevertheless, poor response and acquired resistance limit the benefits of immunotherapy. We recently demonstrated that wobble uridine (U34) tRNA modification promotes codon-specific translation reprogramming during melanoma resistance to targeted therapy. Moreover, impairment of U34-enzymes strongly limits melanoma resistance to BRAF inhibitors (1).

Here, we investigate the implication of wobble uridine (U34) tRNA modification in modulating the antitumoral immune response in melanoma. We found that the depletion of U34-enzymes promotes an anti-tumoral immune response in melanoma in immunocompetent mice. Surprisingly, we highlight that CD4⁺ T cells are the main driver of the anti-tumoral response, while CD8⁺ T

cells remain dispensable. Loss of U34-enzymes results in specific protein aggregation and increased autophagy that colocalize with MHC II in melanoma cells. Strikingly, genetic ablation of MHC II complex in melanoma cells abolished the anti-tumoral immune response induced by the depletion of the U34 enzymes in immunocompetent mice. These data suggest that perturbation of codon-specific translation by modulation of U34-tRNA modification leads to generation and presentation of neo-antigens through a non-canonical mechanism. We are currently investigating this hypothesis.

Taken together this project will indubitably shed light on the role of tRNA modification and codons specific translation regulation in cancer immune response and will pinpoint new therapeutic targets for immunotherapy.

LEUKAEMIA INHIBITORY FACTOR SIGNALLING FOR TARGETING CANCER STEM CELLS IN GASTRIC ADENOCARCINOMA

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Cancer stem cells (CSCs) chemo-resistance mechanisms contribute to tumour maintenance and dissemination. Though rare, not all CSCs are involved in tumour metastasis. Some CSCs, Metastasis initiating cells (MICs), possess exacerbated epithelial-to-mesenchymal transition (EMT) and invasive characteristics facilitating tumour primary site evasion and initiate metastasis. Hippo pathway involvement in gastric tumorigenic and invasive properties has recently been established and Leukaemia Inhibitory Factor Receptor (LIFR) and its ligand LIF were found to inhibit gastric CSC tumorigenic properties through activation of Hippo kinases LATS1/2.

Since LIF impact on gastric MIC invasive properties has never been investigated, this study evaluated LIF effect on MIC invasive properties in GC cell lines and patient-derived xenograft (PDX) cells. RTqPCR and immunofluorescence were used to decipher LIF treatment effect on EMT markers expression as well as on invasive phenotype of

GC cells. LIF effect on invasion capacity of GC cells was evaluated by using collagen-coated transwell® inserts. XMU-MP-1 was used as Hippo kinase inhibitor to evaluate Hippo pathway participation to observed effects. LIF treatment decreased GC cell lines and PDX cells invasion capacity *in vitro*. In addition, decrease in EMT state, linked to the decrease in invasion was observed. LIF anti-invasive effect was reversed by Hippo kinase inhibition, highlighting the role of the Hippo pathway in LIF-dependent effects in GC cells.

In conclusion, this study first displays Hippo kinases- dependent LIF anti-invasive properties in GC cells. LIF treatment could in fine constitute a new CSCs-targeting strategy to help decrease relapse cases and bad prognosis in GC.

PHOTOCONVERSION-BASED ISOLATION UNRAVELS PHENOTYPIC TRANSITIONS IN ASYNCHRONOUSLY GROWING METASTATIC COLONIES

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Cancer patients often harbor occult metastases, a potential source of relapse that is targetable only through systemic therapy. Studies of this occult fraction have been limited by a lack of tools with which to isolate discrete cells on spatial grounds.

We developed a photoconversion-based isolation technique allowing efficient recovery of cell clusters of any size – including single-metastatic cells – which are largely inaccessible otherwise. In a spontaneous liver metastasis model of pancreatic cancer, we compared occult microcolonies with overt macrometastases coinciding in the same organ. Transcriptional profiling of spontaneously arising microcolonies revealed a surprising degree of phenotypic heterogeneity and enrichment of specific molecular programs in microcolonies and macrometastases. Microcolonies manifested significantly low biosynthetic pathway activity accompanied by decreased pRB phosphorylation levels and functionally reduced proliferation propensity. In contrast, microcolonies were strongly

enriched for a pro-inflammatory phenotype associated with NF- κ B/AP-1 signaling trading-off with hypoxic response in macrometastases. Pharmacological inhibition of NF- κ B depleted microcolonies but had no effect on macrometastases, suggesting microcolonies are particularly dependent on this pathway.

Taken together, our study presents a powerful experimental approach for studying the earliest stages of metastatic formation and suggests that phenotypic transitions in asynchronously growing metastatic colonies generate an underappreciated layer of tumor heterogeneity that leads to variations in response to therapy.

ADAPTIVE TRANSLATIONAL REPROGRAMMING OF METABOLISM LIMITS RESPONSE TO TARGETED THERAPY IN BRAFV600 MELANOMA

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Despite the success of therapies targeting oncogenes in cancer, clinical outcomes are often limited by a residual disease that results in relapse. This residual disease is characterized by drug-induced adaptation, that in melanoma involves altered glycolytic and mitochondrial metabolism.

To examine how targeted therapy reprograms metabolism in BRAFV600 melanoma cells we employed a genome-wide functional genomics screen and gene expression profiling. This systematic approach revealed post-transcriptional regulation of metabolism following BRAF inhibition (BRAFi), involving selective mRNA transport and translation. Poly-ribosome profiling confirmed extensive reprogramming of mRNA translation following BRAFi, including translational up-regulation of mitochondrial oxidative phosphorylation (OXPHOS) and “buffering” of glycolysis. As proof of concept we demonstrate the RNA binding kinase UHMK1 associates with mRNAs that encode OXPHOS and glycolysis proteins and is required for their efficient transport

and translation during adaptation to BRAFi. Importantly, inactivation of UHMK1 induces cell death in BRAFi treated cells by further attenuating glycolysis and preventing adaptive mitochondrial reprogramming, and delays resistance to BRAF and MEK combination therapy *in vivo*.

Our data suggests UHMK1 regulates therapy-induced metabolic adaptation by controlling the abundance of metabolic enzymes through the export and translation of the mRNA that encode them. We propose a model wherein adaptive mRNA trafficking and translation pathways contribute to metabolic plasticity underpinning targeted therapy response, and we suggest inactivation of these pathways may delay or prevent development of resistance.

DISTRIBUTION AND PROGNOSTIC SIGNIFICANCE OF GLUCONEOGENESIS AND GLYCOLYSIS IN HUMAN LUNG CANCER

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Gluconeogenesis, mediated by phosphoenolpyruvate carboxykinase (PEPCK), is an alternative source of glycolytic intermediates in cancer cells lacking glucose. The interplay of glycolysis and gluconeogenesis in cancer is still poorly understood.

We analyzed the expression of GLUT1, the prime glucose transporter, and of PCK1 and PCK2, the cytoplasmic and mitochondrial isoforms of PEPCK, in 450 samples of non-small cell lung cancer (NSCLC) and in 54 NSCLC metastases using tissue microarrays and whole tumor sections. Spatial distribution was assessed by automated image analysis. We found that PCK2 was preferentially expressed in the lung adenocarcinoma subtype, while GLUT1 expression was higher in squamous cell carcinoma. GLUT1 and PCK2 were inversely correlated, GLUT1 showing elevated expression in larger tumors while PCK2 was highest in smaller tumors. A mixed

phenotype showing the presence of both, glycolytic and gluconeogenic cancer cells was frequent. In lung adenocarcinoma, PCK2 expression was associated with significantly improved overall survival, while the opposite was found for GLUT1. The metabolic tumor microenvironment and the 3-dimensional context appear to modulate both pathways, since PCK2 expression preferentially occurred at the tumor margin. Moreover, hypoxia regulated both, glycolysis and gluconeogenesis, in NSCLC cells *in vitro*, albeit in opposite directions. PCK1/2 expression was enhanced in metastases compared to primary tumors, possibly related to the different microenvironment.

The frequent co-activation of glycolysis and gluconeogenesis in lung cancer should be considered in potential antimetabolic therapeutic strategies.

SINGLE-CELL CHARACTERIZATION OF MINIMAL RESIDUAL DISEASE IN HER2-POSITIVE BREAST CANCER

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Minimal residual disease (MRD) consists of dormant cancer cells that have evaded treatment and remain undetectable by methods currently used in clinics. Small populations of these cells, present either at the primary tumor site or in distant locations as micrometastases, could eventually lead to disease relapse. Identifying intrinsic or therapy-induced features of these cells can help us understand how MRD is formed. However, the studies on the progression of MRD are challenging due to barriers in obtaining the samples from patients over the course of treatment, as well as a lack of methods to characterize the heterogeneity of rare populations.

To overcome this and mimic MRD in breast cancer, we used 3D organoid cultures derived from mammary glands of an inducible TetO-MYC/TetO-Neu/MMTV-rtTA mouse model that allows the recapitulation of carcinogenesis, from initiation to relapse. We further investigated, at the single-cell level, genomic heterogeneity of populations at different stages of tumorigenesis, as well as after treatment

with doxorubicin, a chemotherapeutic agent commonly used in both early-stage and metastatic breast cancer. We applied the template strand sequencing method, Strand-seq, in combination with a computational framework, single-cell tri-channel processing (scTRIP), to detect structural variations in individual cells that represent the different disease stages.

DISTINCT CONTRIBUTIONS OF PARTIAL AND FULL EMT TO BREAST CANCER MALIGNANCY

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Epithelial-mesenchymal transition (EMT) is a multistep process of cell de-differentiation which confers carcinoma cells with a variety of malignant characteristics. Due to the transient and reversible nature of the process, cancer cells may transit between various stages of an EMT continuum, including epithelial, partial EMT or full EMT, and the actual status of cancer cells and the kinetics of cellular state transition *in vivo* as well as the consequences thereof have remained elusive.

We have established tamoxifen inducible dual recombinase lineage tracing systems combined with live cell imaging and 5-cell RNA-sequencing analyses to trace cancer cells undergoing a partial or a full EMT in the MMTV-PyMT mouse model of metastatic breast cancer. In primary tumours, cancer cells infrequently undergo an EMT, and most of these cells transition between epithelial and partial EMT states, but rarely undergo a full EMT. Consequently, cells that have ever undergone a partial EMT

dynamically transit between various EMT states, and contribute to lung metastasis and chemoresistance. In contrast, full EMT cells mostly retain a mesenchymal phenotype and stably reside in perivascular regions, but fail to colonize the lungs. However, full EMT cancer cells are enriched in recurrent tumors upon chemotherapy.

Our findings suggest that cancer cells in various stages of the EMT continuum contribute differentially to hallmarks of breast cancer malignancy, such as cell migration, invasion, metastasis and chemoresistance.

A MOLECULAR MAP OF HISTOLOGIC INTRA-TUMOR HETEROGENEITY IN LUNG ADENOCARCINOMA

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Lung adenocarcinoma (LUAD) is characterized by diverse histologic patterns of tumor and immune cells that lead to spatial intra-tumor heterogeneity. The relative prevalence of these patterns is used in the clinic for LUAD classification and is associated with patient prognosis. However, the molecular features driving LUAD histologic heterogeneity are still largely unknown.

Here, we performed histologically-guided multiregion sampling of 10 early-stage human LUADs, and we assayed the genetic, epigenetic, and transcriptomic features of each histologic pattern. We found that patterns were not driven by specific genetic alterations, but they rather exhibited diverse epigenetic and transcriptional profiles. The progression from the least (lepidic) to the most (solid) aggressive pattern was characterized by de-differentiation and increased proliferation of the tumor cells. A transcriptional signature that reflected this progression was predictive

of overall survival across independent LUAD cohorts, and revealed a continuous transition from lepidic to solid phenotypes when applied to single cell datasets. Finally, patterns showed heterogeneous interactions with the tumor microenvironment, even within the same tumor. Immune cell infiltration was low in lepidic and it significantly increased in acinar and solid patterns. In addition, spatial proteomic profiling revealed that the solid regions exhibited features of immune exclusion.

In summary, we showed that LUAD histologic patterns are driven by non-genetic changes reshaping cancer cell identity, and they are associated with diverse microenvironments. Our results provide a detailed molecular map of LUAD histologic intra-tumor heterogeneity.

LOSS OF SNAI1 INDUCES CELLULAR PLASTICITY IN INVASIVE TRIPLE-NEGATIVE BREAST CANCER CELLS

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The transcription factor SNAI1 mediates epithelial-mesenchymal transition (EMT), regulates fibroblast biology and controls plasticity in cell differentiation and invasive potential during embryogenesis and in the context of cancer, including breast carcinoma. As previously established, we first demonstrate that high SNAI1 expression characterizes triple-negative breast carcinomas (TNBC) that usually lack expression of E-cadherin (CDH1).

Using CRISPR/Cas9-mediated knockout of SNAI1 in a TNBC cell model, we observed a partial reversion of CDH1 expression and maintenance of the functionally related transcription factors SNAI2 and ZEB1/2 that like SNAI1 promote the development of a mesenchymal phenotype. Downregulation of cell adhesion molecules in SNAI1 knockout cells resulted in their poor adhesion to extracellular matrices and decreased invasive potential in a 3D matrix-embedded microenvironment. Loss of SNAI1 further resulted in higher expression of the transcription factor FOXA1, a known regulator of luminal progenitor specification

during normal mammary gland differentiation. FOXA1 upregulation correlated with a specific gene program that reappeared in the TNBC cells, including nuclear hormone receptors, and an ability of the mutant cells to assemble 3D mammospheres more effectively than parental carcinoma cells. The data establish that loss of SNAI1 in the context of TNBC promotes an intermediary phenotype of luminal progenitor-like cells, that presents higher degree of differentiation plasticity but lower invasive potential.

This study offers a new view on the functions of SNAI1 in breast cancer providing evidence for separate roles in tumor cell invasiveness and progenitor cell plasticity.

ELUCIDATING THE LINK BETWEEN PSORIASIS AND SKIN CANCER

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Psoriasis is a chronic inflammatory skin disease that affects 3% of the general population. Although there is growing evidence linking chronic inflammatory conditions with increased cancer risk, a possible correlation between psoriasis and skin cancer is still elusive.

Genetically engineered mouse models (GEMMs) with inducible epidermal deletion of c-Jun and JunB in basal keratinocytes (DKO*) [1] or hair follicle stem cells (DKO*K15) [2] were used to model psoriasis and investigate how local and/or systemic inflammation could affect skin cancer development. Here, we show that papilloma development induced by the DMBA/TPA chemical carcinogenesis protocol is significantly decreased in both GEMMs with psoriasis-like disease compared to non-psoriatic littermate controls. Molecular analyses revealed increased circulating TSLP, IL-17, IL-6, and S100A9 in psoriasis-like mice, suggesting that these mediators may modulate papilloma formation. Our data supports the hypothesis that psoriasis-like inflammation shapes a tumour suppressive

microenvironment, thus preventing papilloma development. By combining lineage tracing and spatially resolved transcriptomics, we aim to uncover the molecular profile of chemically-induced skin tumours in mice with and without psoriasis-like disease, as well as the cellular dynamics that orchestrates the tumour microenvironment.

Ultimately, our goal is to identify novel targets to develop cancer prevention strategies, as well as biomarkers to identify psoriatic patients that might be at risk of developing skin cancer.

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DECOUPLING EPITHELIAL-MESENCHYMAL TRANSITIONS FROM STROMAL PROFILES BY INTEGRATIVE EXPRESSION ANALYSIS

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Epithelial-to-mesenchymal transition (EMT) is the most commonly cited mechanism for cancer metastasis, but it is difficult to distinguish from profiles of normal stromal cells in the tumour microenvironment.

In this study we used published single cell RNA-seq data to directly compare expression of mesenchymal signature genes in cancer and stromal cells. Informed by these comparisons, we developed a method to deconvolve the mesenchymal signature in bulk RNA-seq data into 'true' EMT and stromal components. This offers an opportunity for a pan-cancer analysis of EMT using the wealth of readily available bulk tumour transcriptome data, enabling a characterisation of EMT across hundreds of tumours and an examination of its association with metastasis and other clinical features. We show that classical EMT marker genes often primarily reflect stromal content, while our inferred cancer-cell-specific EMT signatures usually do not correlate with metastasis.

This study demonstrates the importance

of distinguishing 'true' EMT from stromal contributions in order to elucidate the therapeutic relevance of EMT in cancer.

MAPK ACTIVITY GRADIENTS ARE KEY DRIVERS OF COLORECTAL CANCER CELL DEVELOPMENT AND TARGETED THERAPY RESISTANCE

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In colorectal cancer, oncogenic mutations transform a hierarchically organized and homeostatic epithelium into invasive cancer tissue lacking visible organization.

We sought to define colorectal cancer cell types and signals controlling their development. More than 30,000 epithelial single cell transcriptomes of tumor and matched non-cancerous tissue of twelve colorectal cancer patients were clustered into six patient-overarching groups defined by differential activities of oncogenic signaling pathways such as mitogen-activated protein kinase and oncogenic traits such as replication stress. RNA metabolic labeling and assessment of RNA velocity in patient-derived organoids revealed developmental trajectories of colorectal cancer cells organized along a gradient of mitogen-activated protein kinase activity. This was in contrast to trajectories of normal colon organoid cells showing graded Wnt activity.

Experimental therapies targeting the EGFR-BRAF-MEK axis in colorectal cancer organoids

induced cell plasticity overriding default developmental trajectories, providing a basis for non-genetic resistance to targeted therapies.

THERAPEUTIC DEPLETION OF CCR8+ TUMOR-INFILTRATING REGULATORY T CELLS ELICITS ANTITUMOR IMMUNITY AND SYNERGIZES WITH ANTI-PD-1 THERAPY

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Modulation and depletion strategies of regulatory T cells (Tregs) constitute valid approaches in antitumor immunotherapy but suffer from severe adverse effects due to their lack of selectivity for the tumor-infiltrating (ti-) Treg population, indicating the need for a ti-Treg specific biomarker.

We employed single-cell RNA-sequencing to discern two ti-Treg populations, one of which is characterized by the unique expression of Ccr8 in conjunction with Treg activation markers. Ccr8 is also expressed by dysfunctional CD4+ and CD8+ T cells, but the CCR8 protein was only prominent on the highly activated and strongly T-cell suppressive ti-Treg subpopulation of mouse and human tumors, with no major CCR8-positivity found on peripheral Tregs. CCR8 expression resulted from TCR-mediated Treg triggering in an NF- κ B-dependent fashion, but was not essential for the recruitment, activation nor suppressive capacity of these cells. To target ti-Treg, we generated two CCR8-specific and -blocking nanobodies (Nbs) that recognize distinct

epitopes on the CCR8 extracellular domain. These Nbs were formulated as tetravalent Nb-Fc fusion proteins for optimal CCR8 binding and blocking, containing either an ADCC-deficient or an ADCC-prone Fc region. While treatment of tumor-bearing mice with a blocking ADCC-deficient Nb-Fc did not influence tumor growth, ADCC-prone Nb-Fc elicited antitumor immunity and reduced tumor growth in synergy with anti-PD-1 therapy. Importantly, ADCC-prone Nb-Fc specifically depleted ti-Tregs in a NK cell-dependent fashion without affecting peripheral Tregs.

In conclusion, we propose anti-CCR8 biologicals as novel, preclinically validated cancer immunotherapeutics.

TUMOR SIALYLATION: A MASTER REGULATOR OF ANTI-TUMOR IMMUNITY IN COLORECTAL CANCER

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Abnormal levels of sialylation are observed in many types of cancer, including colorectal cancer (CRC), where they are associated with increased invasion and metastasis. Tumor-exposed glycans, especially sialic acids, are thought to play a key role in immunosuppression, but how they impact immune responses in CRC remains unclear. Previously, we generated a murine MC38 CRC glycovariant without sialic acids using CRISPR/Cas9 technology. Unexpectedly, abrogation of sialic acid expression on MC38 cells resulted in significantly increased *in vivo* tumor growth and increased CD8+ T cell apoptosis in the tumor microenvironment (TME)¹. Thus, we hypothesized that the level of sialic acids governs the effectiveness of the anti-tumor immune response, whereby high sialylation results in immune evasion due to an increase in regulatory T cells in the TME², and complete removal of sialic acids induces immune cell death.

Our aim is to uncover how sialylation affects anti-tumor immunity in CRC by generating

syngeneic CRC glycovariants (MC38 and CT26 cells) with different levels of sialylation. Once the cell lines are validated, we will characterize the *in vivo* immune landscape of the different sialo-variants and evaluate their responsiveness to immunotherapy. Our findings will be also confirmed using metastatic tumor samples of CRC patients that have different sialo-immunoscores.

A MULTI-OMICS ANALYSIS OF METASTATIC MELANOMA IDENTIFIES A GERMINAL CENTER-LIKE TUMOUR MICROENVIRONMENT IN HLA-DR- POSITIVE TUMOUR AREAS

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The emergence of immune checkpoint inhibitors has dramatically changed the therapeutic landscape for patients with advanced melanoma. However, relatively low response rates have prompted the search for predictive biomarkers. Aberrant expression of Human Leukocyte Antigen-DR (HLA-DR) by melanoma cells has been given a positive predictive value, but it is still unknown why this is the case.

Using a multi-omics approach, we investigated the microenvironment of HLA-DR positive metastatic melanoma samples. First, using spatial single-cell multiplexed immunohistochemistry, we detected an enrichment of professional antigen presenting cells, enhanced cytotoxic T cell exhaustion and higher PD-1 expression in immune cells in HLA-DR positive areas. In turn, HLA-DR positive melanoma cells expressed significantly higher PD-L1. In parallel, transcriptomic analysis on

micro-dissected tissue revealed an increase of IFN γ signalling, an enhancement of leukocyte adhesion and mononuclear cell proliferation in HLA-DR positive areas compared to HLA-DR negative areas. Finally, multiplexed ELISA highlighted the presence of germinal-centre associated cytokines CXCL12, CXCL13 and CCL19 in HLA-DR positive lesions which, together with IFN γ and IL4, could serve as biomarkers to identify samples with melanoma-specific HLA-DR expression.

To conclude, our results suggest HLA-DR positive areas in melanoma attract the antitumour immune cell infiltration by creating a dystrophic germinal centre-like microenvironment where enhanced antigen presentation leads to exhaustion and correlates with higher efficacy of anti-PD-1 treatment due to simultaneous higher levels of PD-L1 and PD-1 expressions.

A NOVEL MOUSE MODEL RECAPITULATES ALL THE PHASES OF HUMAN MELANOMA

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Melanoma, the most aggressive skin disease, is caused by malignant transformation of epidermal melanocytes due to oncogenic mutations. Initially, melanoma cells proliferate in the epidermis and then enter the dermis where they acquire a dormant state. After several years, they gain an aggressive phenotype and eventually metastasize. However, the molecular and cellular mechanisms that enable a melanoma cell to invade the dermis need to be elucidated.

In the past years, different laboratories have been trying to develop melanoma mouse models to deeply characterize early events of melanoma progression. Nevertheless, no breakthrough has been made at this stage. Currently, melanoma mouse models do not recapitulate the human anatomico-physiological features of the disease. In mice, opposed to humans, hair follicle melanocytes that are malignantly transformed contribute to melanoma initiation and progression.

Therefore, our lab has pioneered a unique genetically engineered mouse model (GEMM) on a Tabby background, which lacks hair follicles in the tail. Hence, we observed that

this melanoma GEMM, that has a melanocyte-specific BrafV600E mutation and Pten deletion, mimics all the different human melanoma stages.

By single cell RNAseq, we characterized the melanoma cells during the invasive phase, where the majority of melanoma cells are in the dermis, and identified a novel plastic cell state between the classical and deeply studied proliferative and invasive melanoma cell states. Further profound characterization of the mechanisms behind early dermal invasion at a transcriptional and histopathological level can lead to the discovery of novel biomarkers and therapeutic approaches, increasing survival of patients with melanoma.

MODELLING FUNCTIONAL AND PHENOTYPIC INTRATUMORAL HETEROGENEITY USING PATIENT-DERIVED BREAST CANCER ORGANOIDS

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Intratumoral heterogeneity is implicated as a leading contributor to anti-cancer therapy resistance. To overcome this, a deeper understanding of the impact of clonal diversity within a tumor on the response to treatment is needed.

Here, we used the human breast cancer organoid technology to model intratumoral heterogeneity and identify mechanisms that underlie treatment susceptibility. Organoids have proven to be reliable *in vitro* models that mimic the original tumor phenotype and drug response. We have recently established a robust method to establish, manipulate, and transplant patient-derived breast cancer organoids. By implementing subcloning methods we have generated a biobank of over 100 clonal organoid lines, derived from 3 parental organoid cultures of different breast cancer subtypes (triple negative, HER2+). By exposure of individual clones to a variety of therapies, including chemotherapy, PARP inhibition, and an emerging T-cell based cellular immunotherapy called TEGs, we revealed remarkable variation in treatment response

in between individual clones and compared to the parental culture. Bulk RNA sequencing of top differentially responding clones to TEGs revealed significant transcriptomic variability between treatment-sensitive and treatment-resistant clones. We thereby identified several candidate genes overrepresented in susceptible or resistant clones, which have to date not been implicated in T cell recognition and are currently being followed-up on to validate if their modulation can reverse TEG resistance.

Together, these results demonstrate that patient-derived organoids display both functional and transcriptomic heterogeneity and can be applied to identify key determinants of therapy response.

DISTINCT ALTERATIONS IN THE PI3K SIGNALING PATHWAY DIFFERENTIALLY AFFECT THE LOCAL AND SYSTEMIC IMMUNE LANDSCAPE IN MOUSE MODELS OF INVASIVE LOBULAR CARCINOMA

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Despite significant benefits of the use of conventional therapies, approximately 30% of breast cancer patients relapse and develop metastases. The use of immunotherapy has shown great success in treating multiple cancers. However, treatment of breast tumors remains challenging. A key obstacle in breast immune-oncology is the unexplained interpatient heterogeneity in the composition and function of the tumor microenvironment (TME). The large diversity and plasticity of immune cells in the breast TME can exert both anti-tumor and pro-tumor activities.

Recently it has become evident that oncogenic pathways in cancer cells can influence the immune landscape. The phosphoinositide-3 kinase (PI3K) signaling pathway is the most commonly altered pathway in breast cancer and an important player in tumor development. Using transgenic mouse models of invasive lobular carcinoma, we show that distinct PI3K alterations in breast cancer cells drive tumorigenesis, but differentially shape their immune landscape. Extensive immune

profiling of blood and mammary tumors by high dimensional flow cytometry showed significant differences between the models. The immune cell composition of mammary tumors with a mutation in phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) is dominated by myeloid cells, whereas loss of phosphatase and tensin homolog (PTEN) increased the numbers of infiltrating CD8+ T and B cells. We are currently dissecting the mechanisms by which distinct PI3K alterations shape their unique immune landscape.

Ultimately these results will provide a greater understanding of interpatient heterogeneity and open new avenues for the design of personalized immunomodulatory strategies.

THERAPY-INDUCED EPIGENETIC INACTIVATION OF MCT1 RENDERS TUMOR CELLS VULNERABLE FOR MCT4 INHIBITION

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Metabolic plasticity in cancer cells makes very challenging the use of metabolism-interfering agents as a monotherapy due to occurrence of drug resistance. Still, therapeutic strategies could exploit such drug-induced metabolic rewiring in tumor cells. Our study aims to identify the optimal combination of metabolism-targeting drugs, in order to prevent a possible escape for cancer cells. To do so, we used 3-bromopyruvate (3-BrPA) as a first drug to induce metabolic addictions to given pathways that could be targeted in a second time (synthetic lethality).

We established three different 3-BrPA-resistant (3-BrPA-R) cell lines (from bladder, cervix and colorectal cancers) after a chronic exposure of parental cells with increasing drug concentration. We report that exposure to 3-BrPA leads to the epigenetic inactivation of the monocarboxylate transporter MCT1 in cancer cells, thereby driving resistance but also preventing the use of extracellular lactate as a fuel. We further observe that unexpectedly 3-BrPA-R cancer cells mostly rely on glycolysis to grow, with MCT4 becoming essential to support lactate flux. This shift makes cancer cells particularly suited to adapt to hypoxic

conditions within 3D tumor spheroids. By contrast, genetic and pharmacological blockade of MCT4 activity significantly reduces the growth of 3-BrPA-exposed cancer cells and prevents 3-BrPA-resistance establishment.

In conclusion, our study allows to envision a new evolution-based therapeutic modality according to which epigenetic adaptation of tumor cell metabolism to a first-line therapy makes them more vulnerable to a second-line treatment.

ACTIVATION OF THE INTEGRATED STRESS RESPONSE CONFERS VULNERABILITY TO MITORIBOSOME-TARGETING ANTIBIOTICS IN MELANOMA

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The ability of tumour cells to adapt to environmental stress, including therapeutic insult, contributes to tumour evolution and drug resistance. Drug-tolerant persister (DTP) cells from multiple cancer types often exhibit elevated activation of the integrated stress response (ISR), which promotes survival by dampening CAP-dependent mRNA translation, one of the most energy-demanding cellular processes.

Here we show that activation of ISR in DTP cells promotes selective translation of a subset of mRNAs encoding for mitochondrial proteins. Moreover, DTP survival upon therapeutic pressure relies on the ISR-dependent concomitant upregulation of mitochondrial protein synthesis, a vulnerability that can be exploited using FDA-approved mitoribosome-targeting antibiotics. Accordingly, such agents sensitise to MAPK inhibition, thereby delaying

–and even preventing– the development of resistance in BRAFV600E PDX models. Additionally, this treatment hampers the growth of therapy-resistant and NRAS-mutant cutaneous melanomas as well as uveal melanomas and prevents resistance to both immunotherapy and targeted therapy. Consistently, a melanoma patient exposed to doxycycline, a mitoribosome-targeting antibiotic commonly used to treat infections, experienced a complete and long-lasting response of a treatment-resistant lesion.

Our study indicates that the repurposing of mitoribosome-targeting antibiotics offers a rational salvage strategy for targeted therapy in BRAF-mutant melanoma, and a therapeutic option for the treatment of NRAS-driven and immunotherapy-resistant cutaneous and uveal melanomas that can be easily implemented in the clinic.

SINGLE-CELL TRANSCRIPTOMIC ANALYSIS REVEALS SHIFTS IN GLIOBLASTOMA CELL COMPOSITION IN DIFFERENT BMP4-TREATED PRIMARY TUMOR CULTURES

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Glioblastoma (GBM) is the most aggressive primary brain tumor. Its cellular heterogeneity, which includes glioma tumor-initiating cells with stem cell moieties (GSCs), co-determines therapy resistance and tumor recurrence. Bone Morphogenetic Protein-4 (BMP4) promotes astroglial differentiation and suppresses oligodendrocyte production in GSCs, causing increased survival in mice. BMPs exert effects in cell-type and context dependent fashion, but also generate subtle heterogeneity in transcriptional response. We studied first the cell viability of BMP4-treated primary GBM cultures and then used single-cell RNA-sequencing (scRNA-seq) on two differently responding cultures, and found correlation between these responses and therapy sensitivity.

Cell viability was assessed in 17 primary BMP4-treated GBM. We selected one culture in which this treatment had high *in vitro* therapeutic efficacy, and one in which the treatment was ineffective, for analysis by scRNA-seq, and then validated the results on the initial panel of 17 cultures.

In the culture with high *in vitro* therapeutic efficacy, BMP4 induces the formation of a large new cell population displaying decreased cell proliferation, increased migration, and apoptosis. Astroglial differentiation was induced in all BMP4-treated cultures, while neuronal differentiation was reduced most in the cultures with low or without *in vitro* therapeutic efficacy. In addition, OLIG1/2 mRNA and protein levels seemed predictive for BMP4-therapy efficacy, while activation of EIF2 was a suitable, immediate marker for this.

scRNA-seq of *in vitro* GBM cultures provides advanced insights into the mechanism underlying therapy efficacy of BMP4.

OVERCOMING RESISTANCE OF MESENCHYMAL COLON CARCINOMA TO TNF INDUCED NECROPTOTIC CELL DEATH WITH ANTIPSYCHOTIC DRUGS

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Epithelial to mesenchymal transition (EMT) leads to a subset of cancer cells that obtain a higher invasive potential and form metastasis. Cancer cells can show resistance towards apoptotic cell death inducers. Therefore necroptosis, another form of regulated cell death which is highly immunogenic, is an interesting cell death modality for cancer treatment.

An in-house doxycycline-inducible system was developed in HT29 colon carcinoma, driving ZEB1 expression and the EMT process *in vitro* coupled to fluorescent reporters. This system allows a side-by-side comparison of epithelial (E) and mesenchymal (M) cells in the same cellular context. The inflammatory cytokine TNF is known to activate pro-survival pathways as well as to induce the formation of pro-death complexes, resulting in apoptotic or necroptotic cell death. Results show that M cells in caspase-deficient conditions are resistant towards TNF induced necroptotic cell death, while E cells die completely by the same treatment. The observed resistance is cell autonomous and unique for the M state, as the reverse EMT process (MET) renders E cells again sensitive to necroptosis. Screening

of an FDA approved drug library led to the identification of antipsychotic drugs as cell death sensitizers in resistant M cells. Further data showed that EMT transitioned HT29 cells produce higher levels of pro-inflammatory cytokines compared to E cells and these levels increase further during necroptotic cell death induction.

By setting up a combination therapy of currently used chemotherapeutics with antipsychotic drugs tumor treatment might be improved by targeting cells that escape apoptotic cell death and at the same time increase immunogenicity of cancer cell death.

ZEB2 EXPRESSION DRIVES CELLULAR PLASTICITY RESULTING IN HIGH INTRA-TUMOR HETEROGENEITY IN SQUAMOUS CELL CARCINOMA OF THE SKIN

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Tumor heterogeneity and cellular plasticity contribute to cancer progression and therapy resistance, and significantly worsens patient prognosis.

We found that conditional expression of the EMT-associated transcription factor ZEB2 in combination with p53 loss in basal epidermal cells results in skin cancer formation. These cancers represent remarkably high intra-tumor heterogeneity with tumours showing a mixed epithelial-mesenchymal or fully mesenchymal appearance strongly resembling carcinosarcomas. Intra-tumor heterogeneity identified *in vivo* was further retained in early passage tumor-derived cell cultures. Interestingly, based on single cell RNA expression analysis, different cellular differentiation states along the epithelial to mesenchymal axis were identified. Intermediate EMT states were found to give rise to both the epithelial and mesenchymal cancer cell subpopulations whereas fully epithelial and fully mesenchymal-like cells

remained stable over time.

Together, these data provide evidence that ZEB2 controlled EMT in skin epidermal cells results in high cancer cell plasticity, which can result in carcinosarcoma formation.

HETEROGENEITY OF PROSTATE CANCER AT THE GENOMIC AND TRANSCRIPTOMIC LEVEL

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Prostate cancer represents the most frequently diagnosed cancer and the second leading cancer-related cause of death in men in most regions of the world. Although 90% of prostate cancers are localized at diagnosis, 30-50% of patients show biochemical relapse after prostatectomy or radiotherapy (1). Despite of progress in diagnosis, prognosis, and prediction, the treatment options of the disease in the metastatic or resistant stage are limited.

Furthermore, therapy resistance, e.g. against androgen deprivation therapy, occurs, resulting in castration-resistant prostate cancer or metastatic castration-resistant prostate cancer (2). The heterogeneity of prostate cancer, with more than 80% of tumors showing multiple foci, complicates diagnosis and therapy of the disease. Thus, to investigate tumor heterogeneity, we have analyzed multiple regions from mono- and multifocal prostate tumors regarding genomic variants and gene expression levels. We used these samples to distinguish inter-patient and intratumor heterogeneity. Furthermore, we

revealed differences between primary and lymph node lesions.

We believe, that the analysis of intra-patient prostate cancer heterogeneity will provide additional information for diagnostic and therapeutic decisions.

1. Lalonde, E. et al. Tumour genomic and microenvironmental heterogeneity for integrated prediction of 5-year biochemical recurrence of prostate cancer: a retrospective cohort study. *Lancet Oncol.* 15, 1521–1532 (2014).
2. Watson, P. A., Arora, V. K. & Sawyers, C. L. Emerging mechanisms of resistance to androgen receptor inhibitors in prostate cancer. *Nat. Rev. Cancer* 15, 701–711 (2015).

CHROMATIN-REMODELING SUBUNIT SMARCB1 REGULATES CELL IDENTITY AND SENSITIVITY TO FERROPTOSIS IN RENAL MEDULLARY CARCINOMA

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Renal medullary carcinoma (RMC) is a malignant neoplasm arising from the kidney medulla afflicting young patients bearing sickle cell trait. RMC tumors are characterized by bi-allelic loss of the SWI/SNF subunit *SMARCB1* and are highly aggressive with a median overall survival of 8 months and resistance to drugs commonly used against other renal cell carcinomas highlighting the need for novel therapies.

Using single-cell (sc)RNA sequencing of naïve and treated RMC tumors and bulk transcriptomic data from RMC cohorts we identified the putative RMC cell-of-origin as cells from the thick ascending limb (TAL) of the renal nephron. We characterize multiple tumour cell states in a gradient from epithelioid cells that partly retained their TAL identity to a distinct group of sarcomatoid cells that underwent EMT. Using these gene signatures, we show that primary tumour sections from a cohort

of 11 patients comprised varying proportions of each cell state resulting in varying degrees of intra-tumour heterogeneity. Remarkably, only cells with a sarcomatoid signature were found in regional lymph node metastasis from the same patients. Our data point to a mechanism for malignant RMC transformation where TAL identity is lost through repression of their master transcription factor TFCEP2L1 concomitantly with a transcriptional switch that promotes GPX4 expression and resistance to ferroptotic cell death. Using cellular models, we demonstrate that RMC resistance to ferroptosis is repressed upon *SMARCB1* re-expression that additionally leads to reduced expression of MYC targets and restoration of TFCEP2L1 expression.

We propose that sensitivity to ferroptosis is a key event that links RMC transformation with sickle cell trait.

DELAYED IMMUNE PRESSURE IN NEVER-SMOKER LUNG CANCER SCULPTS TUMOUR EVOLUTION

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Lung cancers arising in never-smokers (NS) and ever-smokers (ES) can be viewed as distinct diseases with major clinical differences, including poorer responses to immunotherapies in NS patients. Yet it is currently unknown how the 'soil' in which these cancers arise might influence therapy responses. We hypothesised the immune environment within the lung, particularly tissue-resident memory T cells (TRM), constitutes a selective pressure upon tumour evolution in a discrete manner between ES and NS patients.

We used CyTOF to profile the immune landscape in lung tissue, early-stage tumours and late-stage tumours. We discovered reduced immunosurveillance by TRM in the lung tissue of NS and diminished activity of CD8⁺ T cells within NS tumours compared to ES tumours. We used sequencing of lung cancers from multiple sites to assess the impact of these distinct immune selective pressures on tumour immunogenicity during evolution. We detected early tumour immune

escape processes only in ES patients, resulting in lower frequencies of shared neoantigens. In contrast, less immune escape events resulted in a greater proportion of NS patients with high frequencies of shared neoantigens. We activated the T cell co-stimulatory molecule, ICOS, with an agonist antibody and enhanced T cell proliferation in NS lung T cells as well as in a murine lung adenocarcinoma model.

Overall, we have demonstrated a lack of TRM immunosurveillance in NS tumours correlates with low selective pressure to evade the host immune system. The resulting T cell quiescence and clonal neoantigens within NS tumours creates an opportunity for alternative immunotherapies targeting T cell co-stimulatory molecules to promote anti-tumour immunity in these patients.

EXPLOITING TUMOR HETEROGENEITY IDENTIFIES LCK AS A DRIVER OF INVASION AND MIGRATION IN ORAL CANCER

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Cancer metastasis is the main cause of lethality and continues to be challenging to treat clinically and investigate experimentally. The 5-year survival rate for patients diagnosed with advanced stage oral cancer is 30%. Hence, the identification of therapeutic targets is an urgent need. However, tumors are comprised of a heterogeneous mix of cells with distinct genetic and molecular profiles that can differentially promote metastasis making therapy development a challenging task.

Here, we leveraged intratumoral heterogeneity to identify druggable drivers of cancer cell motility. We applied 2D migration and 3D matrigel-based invasion assays to characterize the invasive heterogeneity among and within 4 human oral cancer cell lines. We identified SAS cells as a highly invasive cell line and subclonal analysis yielded a panel of 20 clones with different invasive capacities. Of note, the expression of *CDH1*, *CDH2* and *VIM* did not

correlate with the invasiveness of individual clones suggesting that EMT is not a major driver of invasion in this cell system.

Subsequently, we applied RNA-Seq to map the transcriptomes of weak and strongly invasive subclones as well as primary tumors and matched metastasis. This integrative expression analysis identified the tyrosine kinase LCK as a druggable target associated with invasion and cancer metastasis. In fact, inhibition of LCK using siRNAs or chemical inhibitors like A-770041 or dasatinib blocked invasion of highly aggressive SAS cells. Intriguingly, reduction of LCK activity increased the stability of focal adhesion sites, formation of adherens junctions and induced cell differentiation.

Our study suggests that targeting LCK might be a promising anti-metastasis therapy in oral cancer.

MELANOMA REGULATORY HETEROGENEITY IN STEADY-STATE AND DURING PHENOTYPE SWITCHING

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Melanoma is notorious for its cellular heterogeneity, which is at least partly due to its ability to transition between alternate cell states. Similarly to EMT, melanoma cells with a melanocytic phenotype can switch to a mesenchymal-like phenotype. However, scattered emerging evidence indicates that additional, intermediate state(s) may exist.

In order to search for such new melanoma states and decipher their underlying gene regulatory network (GRN), we extensively studied ten patient-derived melanoma cultures by single-cell RNA-seq of >39,000 cells. Although each culture exhibited a unique transcriptome, we identified shared gene regulatory networks that underlie the extreme melanocytic and mesenchymal cell states, as well as one stable and distinct intermediate state. The intermediate state was corroborated by a distinct open chromatin landscape and governed by the transcription factors SOX6, NFATC2, and EGR3. Single-cell migration assays established that this intermediate state also produces an intermediate migratory phenotype. Through a dense time-series sampling of single cells and dynamic GRN inference, we unraveled the sequential and

recurrent arrangement of transcriptional programs at play during phenotype switching that ultimately lead to the mesenchymal cell state. This exhaustive analysis of melanoma cell state diversity indicates that additional states exist between the two extreme melanocytic and mesenchymal-like states.

The GRN we identified may serve as a new putative target to prevent the switch to the mesenchymal cell state and thereby, acquisition of metastatic and drug resistant potential.

INTERROGATING INTRATUMORAL TRANSCRIPTOMIC HETEROGENEITY AND PLASTICITY AS RESISTANCE MECHANISMS IN GLIOBLASTOMA

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Intratumoral heterogeneity is one of the major barriers in the treatment of many tumor types. In glioblastoma (GBM) – the most aggressive brain tumor – survival of patients following standard therapy has stagnated to an average of 14 months, largely due to the inherent heterogeneity and phenotypic plasticity of GBM cells. Patient-derived orthotopic xenografts (PDOX) are important tools for preclinical cancer studies. We have shown that they recapitulate histological and molecular features of patient tumors, thus producing more clinically relevant study outcomes when compared to other preclinical models.

To understand the impact of treatment on intratumoral heterogeneity and interactions of tumor cells with the microenvironment, we applied single-cell RNA-seq in GBM PDOX models generated from naive and treatment-exposed patient tumors. Here we show that PDOX models recapitulate all the major cell types and transcriptional programs reported in GBM patient samples. We demonstrate that GBM tumor cells occur in distinct phenotypic

states dictated by microenvironmental cues and treatment-induced pressure. Non-neoplastic cells present within xenografts undergo transcriptomic adaptation similar to the GBM microenvironment in patients. Analysis of treated PDOXs and longitudinal PDOX models derived from patients prior and after treatment allows us to investigate short-term and stable long-term transcriptomic adaptations. We aim to reveal key molecular regulators enhancing tumor cell plasticity and resistance, that could be targeted in combinatory treatments.

Our GBM PDOX models provide a platform for high-quality pre-clinical research and an improved clinical relevance in the development and testing of novel therapeutics.

DISSEMINATING CELLS IN HUMAN TUMOURS ACQUIRE A HYBRID EMT STEM CELL STATE THAT IS PREDICTIVE OF METASTASIS

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Cancer stem cells undergo epithelial-mesenchymal transition (EMT) to drive metastatic dissemination in experimental cancer models. However, tumour cells undergoing EMT have not been observed disseminating into the tissue surrounding human tumour specimens, leaving the relevance to human cancer uncertain.

Here, we identify a stem cell state that retains the epithelial marker EpCAM after undergoing EMT and exhibits enhanced plasticity. This afforded the opportunity to investigate whether retention of EpCAM alongside upregulation of the EMT marker Vimentin and the plasticity marker CD24 can identify disseminating stem cells in human oral cancer specimens. Examining disseminating tumour cells in the stromal region of 84 human oral cancer specimens, evenly divided into metastatic and non-metastatic specimens, we see a significant enrichment of EpCAM, Vimentin and CD24 co-stained cells in metastatic specimens. Through training an artificial neural network on the EpCAM, Vimentin and CD24 co-staining,

we predict metastasis with high prognostic accuracy in the out-of-sample validation set. RNAseq analysis reveals a distinct gene expression signature for the hybrid stem cell state, and this signature is predictive of tumour recurrence and overall survival in an analysis of 202 head and neck tumour samples from The Cancer Genome Atlas (TCGA) cohort.

We have observed, for the first time, disseminating hybrid EMT stem cells in patient histological specimens and demonstrated their utility for predicting metastatic disease.

Youssef et al., 2020: Disseminating cells in human tumours acquire an EMT stem cell state that is predictive of metastasis. bioRxiv. 2020:2020.04

LOSS OF PRC2 OR KMT2D-COMPASS UNLOCKS DISTINCT EMT TRAJECTORIES THAT CONTRIBUTE DIFFERENTIALLY TO METASTASIS

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The epithelial-mesenchymal transition (EMT) is a key cell-biological program enabling carcinoma cell phenotypic plasticity. Accumulating evidence suggests EMT programs do not operate as a stereotypical program that functions as a binary switch, shifting cells from an epithelial (E) to a mesenchymal (M) state. Instead, EMT programs generate cells that enter into a series of intermediate states arrayed along the E-M phenotypic spectrum. At present, we still lack a coherent understanding of how carcinoma cells control their entrance into and residence in these various intermediate states, and which EMT intermediate state(s) favor the metastatic process.

Here we characterize a new layer of EMT-regulating machinery, consisting of two chromatin-modifying complexes, PRC2 and KMT2D-COMPASS, that function as critical regulators to maintain stable residence of both normal and neoplastic cells in an epithelial state via regulating the downstream response of EMT-inducing signals. Loss of

either of these two complexes destabilizes the epithelial phenotype and unlocks two distinct EMT trajectories. Dysfunction of PRC2, but not KMT2D-COMPASS, yields a quasi-mesenchymal state that is associated with highly metastatic capabilities and poor survival of breast cancer patients.

These observations uncover a novel mechanism that regulates E-M plasticity, specifies which intermediate EMT state a cell will reside in, and thereby determines the metastatic ability of carcinoma cells. Our data also suggest that in developing PRC2 inhibitors as anti-neoplastic drugs, great caution should be applied to patient cohort selection and careful monitoring of unanticipated side-effects.



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Custom Cloning

• Viral Vectors • CRISPR Vectors • shRNA Vectors



CRISPR & shRNA Libraries

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Custom Stable Cell Lines



Research and GMP quality

The VIB Grand Challenges program (GCP) was initiated to increase the societal impact of VIB research, by financially supporting reverse translational research projects i.e. projects that start from a specific problem and that are translated into practical solutions for consumers, farmers, and patients. A major requirement to receive GCP funding is that the consortium is made up of interdisciplinary collaborations and beyond borders where VIB researchers collaborate with non-VIB researchers such as clinicians from various hospitals, agricultural institutions and other universities. Concretely, 8 projects have been selected that could provide direct added value for citizens, farmers, or patients in the short term. In addition, three projects have been co-funded since the start of the COVID-19 crisis to gain more insights into the course of the disease and investigate new treatment options with existing drugs.

The POINTILLISM project was selected during the first call of the Grand Challenges program and focusses on unraveling the single cell tumor microenvironment. In this context, a unique collection of pre- and on-treatment biopsies from patients enrolled in four immunotherapy trials which involved treatment with immune checkpoint blockers (ICB) was established. These samples were used to unravel dynamic (spatial) maps of the entire tumor ecosystem before and during ICB treatment using innovative and integrative single-cell profiling methods. On the short term, this allowed: 1) monitoring of therapeutic response at unprecedented resolution, 2) acquisition of information on resistance (patient- or treatment level) to ICB, and 3) distilling novel biomarkers predictive of response to ICB.

