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Preisverleihung

Charles Rodolphe Brupbacher Preis für Krebsforschung 2017

Award Ceremony

Charles Rodolphe Brupbacher Prize for Cancer Research 2017

Charles Rodolphe Brupbacher Preis für Krebsforschung 2017

Die Stiftung verleiht alle zwei Jahre den Charles Rodolphe Brupbacher Preis für Krebsforschung an Wissenschaftler, die auf dem Gebiet der Grundlagenforschung hervorragende Leistungen erbracht haben. Die Preisverleihung findet statt im Rahmen eines internationalen wissenschaftlichen Symposiums, an dem auch der öffentliche Charles Rodolphe Brupbacher Vortrag gehalten wird.

Der Preis für das Jahr 2017 wird verliehen an:

Laurence Zitvogel, Paris, Frankreich
Guido Kroemer, Paris, Frankreich und
Sir Adrian Peter Bird, Edinburgh, U.K.

Charles Rodolphe Brupbacher Prize for Cancer Research 2017

Biennially, the Foundation bestows the Charles Rodolphe Brupbacher Prize for Cancer Research upon a scientist who has made extraordinary contributions to basic oncological research. The Award Ceremony takes place within the framework of a Scientific Symposium, which includes the Charles Rodolphe Brupbacher Public Lecture.

The recipients of the 2017 Awards are:

Laurence Zitvogel, Paris, France
Guido Kroemer, Paris, France and
Sir Adrian Peter Bird, Edinburgh, U.K.

Begrüssung
Prof. Dr. Michael Hengartner, Rektor der Universität Zürich

Mozart: Trio KV 502, 1. Satz

Preisverleihungen

Laudatio
Laurence Zitvogel, MD/PhD und Guido Kroemer, MD/PhD
durch
Miriam Merad, MD/PhD

Referat der Preisträgerin
Laurence Zitvogel, MD/PhD

Beethoven: Rondo e cappriccio

Referat des Preisträgers
Guido Kroemer, MD/PhD

Chopin: Cello Sonata Op. 65, Largo

Laudatio
Sir Adrian Peter Bird, PhD
durch
Josef Jiricny, PhD

Referat des Preisträgers
Sir Adrian Peter Bird, PhD

Piazzolla: Invierno porteno

Schlussworte

Apéro

Introduction
Prof. Dr. Michael Hengartner, Rector of the University of Zurich

Mozart: Trio KV 502, first movement

Awards

Laudatio
Laurence Zitvogel, MD/PhD and Guido Kroemer, MD/PhD
by
Miriam Merad

Acceptance Speech
Laurence Zitvogel, MD/PhD

Beethoven: Rondo e cappriccio

Acceptance Speech
Guido Kroemer, MD/PhD

Chopin: Cello Sonata Op. 65, Largo

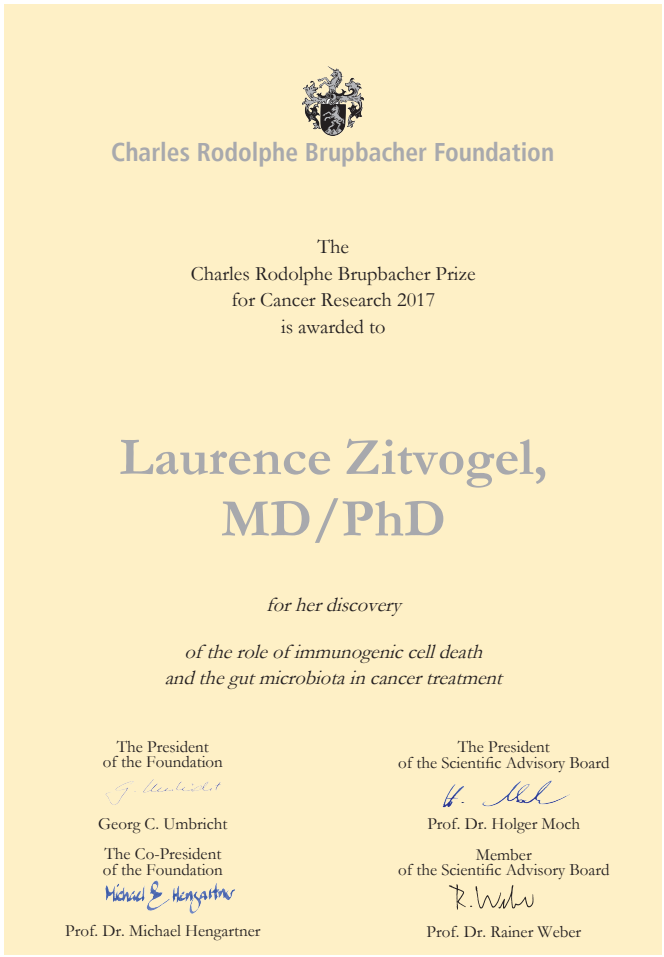
Laudatio
Sir Adrian Peter Bird, PhD
by
Josef Jiricny, PhD

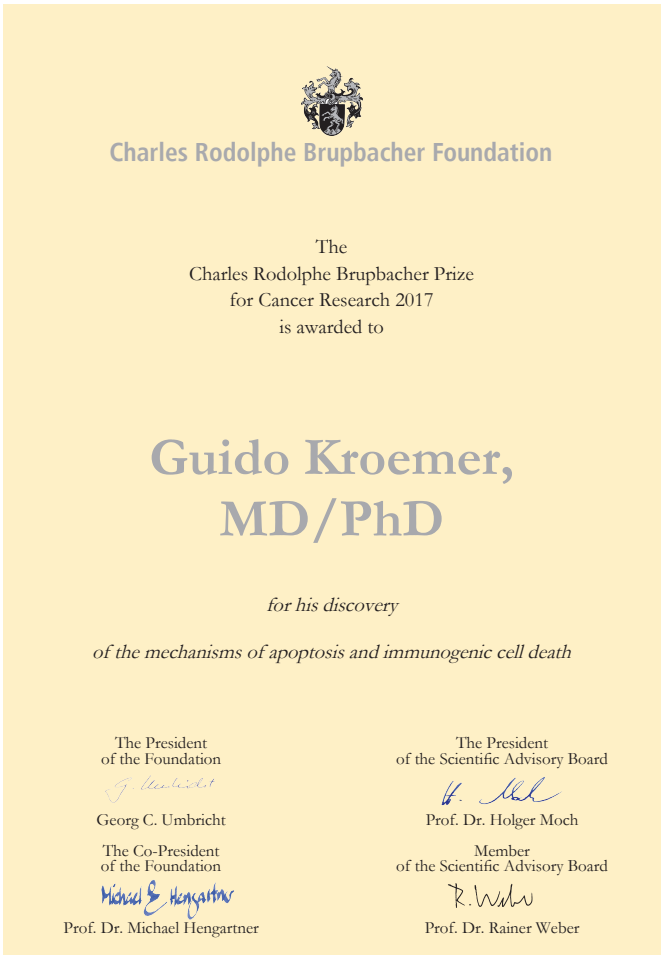
Acceptance Speech
Sir Adrian Peter Bird, PhD

Piazzolla: Invierno porteno

Final address

Apéro





Laudatio

Miriam Merad

It is my great pleasure to give the laudation for Laurence Zitvogel and Guido Kroemer, winners of the Brupbacher Prize for Cancer Research in recognition of their contribution to cancer immunology research.

I am standing here presenting this eulogy today because of a fortunate encounter I have made with Laurence Zitvogel 20 years go. In 1996, I was a 3rd resident in hematology/oncology at the Gustave Roussy Cancer Institute in Paris, when I was introduced to Laurence, a new attending clinician who had just returned from the United States after 3 years spent in a cancer immunology research lab. Laurence's passionate teaching of cancer immunotherapy, which contrasted so much with the discussions we had on the clinical floors, inspired me to learn immunology and, twenty years later, there is still so much to learn and discover.

Laurence was born in France in a suburb of Paris from a family of Artisans. When she was six-years old, she announced to her family that she will become a doctor, and the long suffering and early death of a father she adored strengthened her intention. To this day, Laurence's vocation to seek novel cures and alleviate suffering has remained intact.

Laurence attended La pitie Salpetriere's Medical School in Paris and very early decided to specialize in Oncology. Frustrated by her training in clinical oncology and saddened by the bleak outcome of her patients, Laurence decided to take some time off from the clinical training to learn about immunology, as she had become fascinated by the process of vaccination and immune protection. Intrigued by the work of Steven Rosenberg, a surgical oncologist at the NIH and a pioneer of tumor immunotherapy, she decided to join the laboratory of Michael Lotze, a former Rosenberg's trainee, who was leading a Cancer Immunology laboratory in Pittsburgh University. After a successful time there, she returned to Paris to start her own laboratory at Gustave Roussy Cancer Research Institute , where she has been since. Soon after starting her laboratory , she became interested in the role of targeted and conventional therapy on the immune system. She discovered that imatinib (Glivec), a tyrosine kinase inhibitor targeting the oncogenic kinases Brc-Abl, c-Abl and c-Kit, promotes tumor response partly though its ability to stimulate natural killer (NK) cells to attack tumor cells, a findig that was to dramtically influence her subsequent research with Guido Kroemer. It is at Gustave Roussy that Laurence met Guido for the first time, a meeting that was to transform their personal and professional lives.

Guido is a trained pathologist, immunologist and molecular biologist. In the mid 90's he discovered that mitochondrial membrane permeabilization constitutes the central checkpoint of apoptosis, thereby laying the grounds for the comprehension of regulated cell death. Kroemer's team discovered that, in mammalian cell death, mitochondrial membrane permeabilization constitutes the point-of-no-return of the lethal process and thus defines the lethal checkpoint. Instead of considering apoptosis as a process dominated by proteases and nucleases, cell death is now viewed as a process that is largely controlled by mitochondria. This discovery has had impotant implications for the understanding of cell death. A prolific author and one of the most cited biologist in the world, Guido obtained many scientific awards including the International Cell Death Society Life Achievement Award and the European Cell Death Society Career Award.

In contrast to most immunologists at that time, who focused mainly on tumor vaccines or T cell adaptive therapies, Laurence and Guido inspired by Laurence's finding on the immunogenic role of Glivec and Guido's work on the mechanisms of cell death started to question whether all types of cell death were equal and searched for compounds that could induce tumor cell death in a way that could be recognized by the immune system. They tested hundreds of compounds for more than 10 years and together they finally discovered that anthracyclines and oxaliplatin, two common chemotherapy agents, can induce a type of cancer cell death that elicits an anticancer immune response, hence allowing the immune system to control residual tumor cells. They identified the distinct molecular alterations that lead a dying cell to induce an immune response, distinguish this type of cell death from conventional apoptosis and showed that the immune response against dying tumor cells controls the clinical outcome of many chemotherapy regimen, both in mouse models and in cancer patients. These results led to a novel paradigm they named immunogenic cell death, a paradigm that revolutionizes our understanding of anti-cancer chemotherapies and should influence the development of combination chemo-immunotherapy regimen for the treatment of cancer patients.

During the course of these studies, Laurence became fascinated by the differential effects of chemotherapy on the immune system and she started systematically to monitor immune responses induced upon administration of chemotherapy. Soon she discovered that cyclophosphamide induced the release of interleukin-17, which was essential for the induction of antitumor immunity and that IL-17 release was dependent on the presence of gut comensal microbes. This led her to hypothesize for the first time the key role of the gut microbiome in the control of chemotherapy-mediated antitumor immunity. This work, published in "Science" in 2013, was soon followed by another breakthrough. Prompted by the finding that the checkppoint blockade of CTLA4 leads to gut tissue damage, Laurence then searched whether the gut microbiota was also instrumental in inducing tumor response to CTLA4 blockade. In a subsequent study, also published in "Science", she showed that, in mouse experimental tumor models, CTLA4-mediated antitumor immunity was also dependent on the presence of gut commensals. The discovery of the role of the gut microbiome in cancer treatment had enormous implications for the field and ignited a worldwide effort in academia and industry to develop novel microbe-based therapies to potentiate antitumor therapies.

I have met many physicians and scientists throughout my professional life, but to date I have not met scientists as passionate and as engaged as the Zitvogel & Kroemer team. They have dedicated their intellectual and personal lives to the finding of a cure for cancer and their passion has transformed our understanding of antitumor immune response and dramatically influenced and inspired generations of scientitists throughout the world.

It is my distinct pleasure today, Laurence and Guido, to present you this well-deserved Charles Rodolphe Brupbacher Prize for Cancer Research in recognition of your contribution to cancer immunology research.

Laurence Zitvogel

Summary Curriculum vitae

Appointment Scientific Director of OncoImmunology, Gustave Roussy Cancer Center (GRCC)

Address 114 Rue Edouard Vaillant
94800 Villejuif, France

Date of Birth December 25, 1963

Current Positions

2016– Director, Torino-Lumiere, Program Project, Paris, France
2011– Member of Co-Directorate, Gustave Roussy Cancer Campus, Villejuif, France
2003– Full Professor, Immunology & Biology, Kremlin Bicêtre School of Medicine, University Paris XI
2002– Co-Director, Center of Clinical Investigations in Biotherapies of Cancer, GRCC-Curie, Paris
2000– Director, Laboratory "Tumor immunology and immunotherapy" INSERM U1015, GRCC
1998– Hospital Practioner, Breast Cancer Department, Clinical attending, GRCC

Previous Positions

1995-2000 Associate Professor, Clinical attending, Medical University of Paris XI, Villejuif
1995-1998 Post-doctoral fellowship, Adenovirus Gene Therapy, Pr Pericaudet's lab, Villejuif, France

1994-1995 Assistant Professor, University of Pittsburgh, Pittsburgh Cancer Institute, USA
1992-1994 Instructor, University of Pittsburgh, Pittsburgh Cancer Institute, USA
1990 Master in Tumor Immunology, Prof. Fridman's lab. Institut Curie, Paris

Education

1987 MD, School of Medicine, Pitié Salpétrière, University of Paris VI, France
1992 Board Certificate, Medical Oncology, University Paris VII, France
1995 PhD, Immunology, University Paris VII, France - Pittsburgh Cancer Institute, Pittsburg, PA, USA
1998 Habilitation, University Paris XI, France

Fellowships and Awards

2014 Swiss Bridge Award for Cancer Research, Switzerland
2013 Ligue Française contre le Cancer, Research Prize, Conseil Général des Yvelines, Versailles
2012 Member of the National Academy of Medicine, Biology Division, Paris, France
2012 Permanent member of the European Academy of Cancer Sciences, ECCO
2011 Medical Research Prize, Price Raymond Rosen Fondation pour la recherché médicale, France
2007 INSERM Prize for Translational Research, French Medical Research Council (INSERM)
2007 Gallet & Breton Prize, National Academy of Medicine, Paris, France
2005 Charles Oberling Prize, Senate of the French Republic, Paris, France
2000 Gustave Roussy Prize, National Academy of Sciences, Paris, France
1999 Prize of the Chancellery, University of Paris City Hall Paris
1996 Ligue Française contre le Cancer, Research Prize, Conseil Général Haute Loire, France
1995 Merit Award, Society for Biological Therapy, Nappa Valley, CA, USA
1994 Presidential Award, American Society of Clinical Oncology, LA, CA, USA
1992 Vocation Prize, Bleustein Blanchet Foundation, Paris, France
1992 Gold Medal, Internal Medicine, first Prize, Assistance Publique-Hôpitaux de Paris

Principal Commissions of Trust

2016– Scientific Advisory Board (SAB), Transgene, Paris-Illkirch, France
2015– SAB, Lytix Ltd., Oslo, Norway
2015– SAB, NeoVacs, Paris, France
2015– SAB, GSK, Philadelphia, PA, USA
2014– Board of Directors/Executive Board, Transgene, Paris, France
2014– Board of Directors/Executive Board, National Institute of Cancer (INCA), Paris
2011-2014 SAB, DKFZ, Heidelberg, Germany
2011 Helmoltz Foundation, research committee
2001-2003 French Medical Research Council (INSERM)
1997 EORTC, Immunology Scientific Commitee

Organization of Scientific Meetings

2017 Organizing comittee, AACR Conference, Washington DC, DC, USA
2016 Organizing comittee, Cold Spring Harbor, Shuzhu, China
2015-2016 Organizing comittee, AACR/CIMT/CRI/EATI Conf. Tumor Immunology NYC, USA
2013 Co-Organizer, Keystone Symposium, Vancouver, British Columbia, Canada
2013 Coordinator, Cancéropôle Ile-de-France, Microbiota Conf, Paris, France
2011 Co-Organizer, Keystone Symposium, Santa Fe, New Mexico, USA
2006-2016 President and co-organizer, Miltenyi Immunology Annual Conferences, Paris, France
2015 Organizing comittee, AACR Conference on Tumor Immunology, San Diego, CA, USA

2013 Organizing comittee, ECDO Conference, Paris, France

Principal Editorial Activities

2012– Editor-in-Chief, OncoImmunology (Landes Bioscience), Austin, Texas, USA
2010-2016 Editor (Immunology), Cell Death & Disease (Nature Publishing Group), London, UK
2005-2012 Associate Editor, Cancer Research (AACR), Philadelphia, PA, USA

Guest Editor

2016 J. Clinical Investigations (series on exosomes biology)
2008 Current Opinion in Immunology (Springer)
2008 Cell Death Differentiation (Nature Publishing Group)
2007 Immunological Reviews (Munksgaard-Springer)

Major Collaborations

Together with some hundreds of collaborators/co-authors, our team has published close to 360 PubMed-indexed papers (search Zitvogel_L). The most important collaborative efforts include G. Kroemer from Les Cordeliers, University Paris Descartes (230 common publications) on the immunogenic cell death concept (together with Dr D. Green, St. Judes Hospital in Memphis, Dr M. Pittet, Harvard Medical School), Mark J. Smyth from QIMR Berghofer Medical Research Institute in Australia (>20), the Dendritic cell team (Dr Miriam Merad, Mont Sinai, NYC, USA, Dr Florent Ginhoux, A*Star, Singapoor, Dr Federica Sallusto, Bellinzona, Switzerland, Dr Dhodapkar, University of Yale, Connecticut, USA) the Microbiota team (Dr Ivo Gomperts Boneca, G. Eberl, and Dr Mathias Chamaillard, Institut Pasteur, Lille and Paris, France, Dr Joel Doré and Dr P. Lepage, Metagenopolis, INRA, Jouy-en-Josas, France), the Clinical team for breast cancer and melanoma (Dr F. André, Dr S. Delaloge, Gustave Roussy Cancer Center, Dr D. Jaeger, DKFZ, Heidelberg, Germany, Dr J. Wolchock, MSKCC, NYC, USA, Dr M. Maio, University of Siena, Italy, Dr B. Weide University of Tübingen, Germany), and the exosome team (Dr S. Amigorena, INSERM, Institut Curie, Dr Clotilde Théry, Institut Curie, Dr O. Lantz, Institut Curie).

Acceptance Speech

Laurence Zitvogel

Preamble

Prof. L. Zitvogel, MD. PhD. is 52 and graduated in Medical Oncology, School of Medicine, University of Paris in 1992 before starting her scientific career at the University of Pittsburgh, Pennsylvania, US. She established her own lab at the Institut Gustave Roussy in Villejuif. She developed her career in the field of cancer immunology and immunotherapy and reconciled basic and translational research to design novel cancer vaccines and conduct Phase I and II trials. Her scientific discoveries over the last 20 years rely on 4 pillars.

1: The role of dendritic cells and their exosomes in cancer immunology and immunotherapy

Having triggered the therapeutic potential of DC for cancer therapy, Pr Zitvogel investigated the cellular mechanisms whereby DC mediated tumor regression in vivo. She unraveled four novel and critical biological pathways: DC not only elicit T cell responses but also trigger the activation of innate effectors such as NK and NKT cells (Ikarashi et al. J. Exp. Med. 2001, Zitvogel L J Exp Med 2002, Fernandez et al. Nat. Med. 1999, Fernandez et al. Eur. Cyt. Netw. 2002, Terme et al J. Immunol. 2004, Borg et al. JCI, 2004, Walzer et al. Blood 2005). DC/NK cell cross-talks appeared critical to dictate cognate immune responses (in the context of viruses or tumors) and control certain types of human malignancies (such as leukemia, gastrointestinal sarcoma and neuroblastoma), DC directly interact with T cells but also secrete membrane vesicles called "exosomes" that bear major complex histocompatibility molecules and heat shock proteins inducing, on their own, antitumor effects (Zitvogel et al Nat Med 1998, Wolfers et al Nat Med 2001, Thery, Zitvogel, and Amigorena, Nat Rev Immunol 2002, André et al The Lancet, 2002, André et al J Immunol 2004, Chaput et al, J Immunol 2004, Taieb J, J. Immunol 2006). Having demonstrated the immunogenicity of DC-derived exosomes in vitro and mouse models, Pr Zitvogel conducted two clinical trials based on patents and support from a Biotech Cie at first. Indeed, in collaboration with Institut Curie, she launched a Phase I trial using autologous DC derived-exosomes in stage IV melanoma patients in an academic cell therapy unit (Escudier et al J Transl Medicine 2004, results confirmed in parallel by an American team (Morse et al J Transl Med 2004). Exosomes were able to restore NKG2D-expression levels in both CD8+ T cells and NK cells due to their high contents in IL-15R and MICA/B (Viaud et al PloS One, 2009). A Phase II in non small cell lung cancer using second generation exosomes (from DC propagated in GM-CSF/IL-4/IFNγ) has been completed and submitted to JCI. It demonstrates the bioactivity of DC IFNγ exosomes on NK cell functions in NSCLC, specifically on NKp30 effector functions due to B7-H6 expression on exosome membranes. This illustrates that therapeutic intervention on the host immune system using exosomes may be of therapeutic value.

A novel DC subset (called "IKDC" for IFN producing killer DC), with a unique morphology and unique potentials (IFNγ secretion and TRAIL-dependent lysis in contact with a variety of transformed cells) involved in tumor immunosurveillance (J. Taieb, Nature Med, Feb. 2006).

Moreover, facing the reality of tumor-induced tolerance, she undertook the investigation of DC pathophysiology during tumor progression in mouse and human specimen. She discovered two novel concepts of immunosuppression: i) tumor cells pervert DC and convert them into TGF- secreting cells promoting the expansion and accumulation of naturally occurring regulatory T cells (suppressor T cells, Treg) (Ghiringhelli et al J Exp Med 2005a), ii) such Treg interfering with not only conventional T cells but also blunting all NK cell functions in tumor bearing hosts (Ghiringhelli et al J Exp Med 2005b).

2: The role of NK cells in human malignancies and discovery of NKp30-associated biomarkers

Her team valued the study of a potential impact of NK cells in tumor immunosurveillance. She conducted mouse models of transplantable tumors and studied human malignancies. In addition to the well recognized prognostic value of NK cells in leukemias, her team contributed to highlight for the first time the critical prognostic role (and predictive value of NKp30 isoforms) of NK cells in gastrointestinal sarcoma (GIST) and high grade neuroblastoma (HGNB).

She first characterized various subsets of NK cells in mouse models of expanding tumors such as IKDC (a subset of CD11b+ class II+ NK capable of APC functions, Terme et al. Cancer Res. 2008, a subset of regulatory NK cells CD27+ Kit+, dictated by IL-18, Terme et al. Cancer Res. 2010, Cancer Res. 2011).

Next, she reported that NK cells are major components of human gastrointestinal sarcoma and high grade neuroblastoma and are endowed with prognostic value in large cohorts of metastatic patients (Borg C, J.Clin. Invest 2004, Ménard C et al Cancer Res. 2006).

Based on the finding that the NK specific NKp30 receptor was selectively downregulated in tumors, her team was the first to describe a post-transcriptional regulation of three distinct NKp30 isoforms and their functional consequences on NK cell effector properties and patients prognosis (Delahaye, Nat Med 2011, Rusakiewicz, Cancer Res 2013, Semeraro, Sci Transl Med in press, Rusakiewicz, JCI submitted).
Importantly, her medical background and constant preoccupation for clinical care in oncology led her to highlight a novel mode of action of the c-Kit tyrosine kinase inhibitors (the paradigmatic STI571/imatinib mesylate/Gleevec° used for chronic myeloid leukemia and gastrointestinal sarcoma (GIST)). Her team showed that STI571, in addition to cell autonomous effects on tumor cells, exerts potent NK cell-mediated tumor regression in vivo in tumor models resistant to the antiproliferative effects of STI571 in vitro. This statement also applies to humans since GIST -bearing patients treated with STI571 exhibit enhanced NK cell effector functions after 2 months of therapy (Borg et al J. Clin Invest 2004, Ménard C, Cancer Res 2009). Moreover, STI571-induced NK cell triggering is an independent surrogate marker of efficacy of Gleevec° associated with prolonged disease free survival (Borg et al J Clin Invest 2004, Ménard et al. Cancer Res 2009). This discovery prompted her to find a biomarker of response to imatinib, by describing isoforms of NKp30 activating receptors dictating the prognosis of GIST (Delahaye N et al. Nat. Med. 2011, Rusakiewicz S, Cancer Res 2013). Together with Novartis Pharma, she launched a Phase I/II study combining STI571 to drugs enhancing NK cell activation (Cyclophosphamide and IL-2, Locher C, OncoImmunology, 2013, Chaput N, OncoImmunology 2013).

3: The concept of immunogenic cell death: how chemotherapy can be viewed as a cancer vaccine?
Our groups (Zitvogel L in collaboration with G. Kroemer) invalidated the dogma that apoptosis is a non-immunogenic cell death modality. We demonstrated that, depending on the upstream triggers, apoptosis can be immunogenic and hence alert the innate immune system and instruct it to stimulate a cognate response against dead-cell antigens. This has opened a new field of research at the frontier between immunology and cell biology, allowing us to define the molecular properties of immunogenic cell death (ICD). We found that ICD is characterized by autocrine stimulation of type 1 interferon (IFN) receptors (and the TLR3/TRIF pathway), the pre-apoptotic exposure of calreticulin (CRT) on the cell surface, release of ATP during the blebbing phase of apoptosis, and post-apoptotic exodus of the chromatin-binding protein high mobility group B1 (HMGB1). Type 1 interferon secretion depends on the stimulation of TLR3, CRT exposure on an endoplasmic reticulum stress response, ATP release on pre-mortem autophagy, and HMGB1 exodus on secondary necrosis. CRT, ATP and HMGB1 interact with three receptors (CD91 receptor, purinergic P2Y2 or P2X7 receptors, and toll-like receptor 4, respectively) that are present on the surface of dendritic cells or their precursors. CD91, P2Y2, P2RX7 and TLR4 promote engulfment of dying cells, attraction of dendritic cells, production of interleukin-1β and presentation of tumor antigens, respectively. We have launched and then proven the hypothesis that the immune response against dying tumor cells dictates the therapeutic success of anticancer chemotherapy, both in mouse models and in cancer patients (Obeid et al. Nat Med. 2006, Apetoh et al Nat. Med. 2007, Ghiringhelli et al. Nat. Med. 2009, Ma Y, JEM 2011, Michaud M et al. Science 2011, Menger L, Sci. Transl. Med 2012, Senovilla L, Science 2012, Sistigu et al. Nat Med 2015).
Obviously, this discovery has had major consequences for the comprehension, conception and implementation of anticancer chemotherapies. Indeed, we postulate that, at least in certain cases, both classical and targeted anticancer therapies require an active contribution of the immune system to be optimally efficient. We obtained clinical evidence that this hypothesis holds true for anthracycline-treated breast cancer, oxaliplatin-treated colorectal cancer, and imatinib-treated gastrointestinal stromal tumors.

4: The unsuspected role of gut microbiota in cancer therapies
Her team has recently highlighted the crucial role of gut microbiota in eliciting innate and adaptive immune responses beneficial for the host in the context of effective therapies against cancer (chemotherapies, immunotherapy based on immune checkpoint blockers).
1/ Context of cyclophosphamide (CTX):
Chemotherapeutic agents, by compromising, to some extent, the intestinal integrity, facilitate the gut permeability and selective translocation of Gram positive bacteria in secondary lymphoid organs. There, anti-commensal pathogenic TH17 T cell responses are primed, facilitating the accumulation of TH1 helper T cells in tumor beds post-chemotherapy as well as tumor regression. Importantly, the redox equilibrium of myeloid cells contained in the tumor microenvironment is also influenced by the intestinal microflora, contributing to tumor responses. Hence, the anticancer efficacy of alkylating agents is compromised in germ-free mice or animals treated with antibiotics. These findings represent a paradigm shift in our understanding of

the mode of action of many compounds having an impact on the host-microbe mutualism (Viaud S, Science 2013).These findings have been extended to platinum salts (oxaliplatine, cis-platine) as well as to a combination of anti-IL-10R mAb+CpG for Iida et al. Science Nov 2013 (Trinchieri's group at the NIH, USA).

2/ Context of CTLA4 blockade :

The immune checkpoint blocker (ICB) anti-CTLA4 Ab is a first-in class compound approved for reinstating cancer immunosurveillance and prolonging survival in metastatic patients. However, this clinical benefit is often associated with immune –related side effects at sites exposed to commensal flora such as the large intestine. Uncoupling efficacy from toxicity is a challenging issue for the future development of ICB. Her team showed (and submitted to Science) that the antitumor effects of CTLA4 blockade, largely dependent upon Toll like receptor (TLR)2/ TLR4 receptors, markedly rely on the regulatory commensal Bacteroides fragilis (Bf) (in coordination with Burkholderia cenocepacia). Innate signaling induced by specific TLR2/TLR4 agonists failed to compensate the lack of tumoricidal activity mediated by CTLA4 blockade in germ free (GF) or antibiotics-treated mice while the IL-12-dependent cognate immunity directed against Bf could do so. Hence, anti-CTLA4 Ab elicited protective Bf-specific Th1 immune responses in specific pathogen free (SPF) mice that could be substituted, in GF animals, by oral Bf, purified Bf-associated polysaccharides or a Bf-specific adoptive T cell transfer, without triggering overt colitis. Ipilimumab could also restore Bf-specific Th1 immune responses in a fraction of advanced melanoma patients. This study unravels the key role of B.fragilis in the immunostimulatory effects of anti-CTLA4 Ab, opening up novel strategies to safely broaden its clinical efficacy (Vétizou et al. Science Nov. 2015). At the same time, Gajewski's group in Chicago showed that Bifidobacteria from the gut influence the tumor microenvironment in such a way that anti-PDL-1 Ab can induce a prominent anticancer immune responses (Sivan et al. Science Nov. 2015).

Most significant publications

• Zitvogel L, Ayyoub M, Routy B, Kroemer G. Microbiome and Anticancer Immunosurveillance. Cell. 2016 Apr 7;165(2):276-87.

• Vétizou M, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C, Rusakiewicz S, Routy B, Roberti MP, Duong CP, Poirier-Colame V, Roux A, Becharef S, Formenti S, Golden E, Cording S, Eberl G, Schlitzer A, Ginhoux F, Mani S, Yamazaki T, Jacquelot N, Enot DP, Bérard M, Nigou J, Opolon P, Eggermont A, Woerther PL, Chachaty E, Chaput N, Robert C, Mateus C, Kroemer G, Raoult D, Boneca IG, Carbonnel F, Chamaillard M, Zitvogel L. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science. 2015 Nov 27;350(6264):1079-84.

• Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillère R, Hannani D, Enot DP, Pfirschke C, Engblom C, Pittet MJ, Schlitzer A, Ginhoux F, Apetoh L, Chachaty E, Woerther PL, Eberl G, Bérard M, Ecobichon C, Clermont D, Bizet C, Gaboriau-Routhiau V, Cerf-Bensussan N, Opolon P, Yessaad N, Vivier E, Ryffel B, Elson CO, Doré J, Kroemer G, Lepage P, Boneca IG, Ghiringhelli F, Zitvogel L. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science. 2013 Nov 22;342(6161):971-6.

• Zitvogel L, Galluzzi L, Viaud S, Vétizou M, Daillère R, Merad M, Kroemer G. Cancer and the gut microbiota: an unexpected link. Sci Transl Med. 2015 Jan 21;7(271):271ps1.

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Guido Kroemer

Summary Curriculum vitae

Appointment Professor, University Paris Descartes

Address Centre de Recherche des Cordeliers
15 rue de l'Ecole de Médecine
F-75006 Paris, France

Date of Birth June 11, 1961.

Education

1985 MD-PhD (Immunology), University of Innsbruck, Austria
1990 Habilitation (Pathology), Medical University of Innsbruck, Austria
1992 PhD (Molecular Biology), Autonomous University of Madrid, Spain

Academic Appointments/Affiliations

1982-1985 Student Professor, University of Innsbruck, Austria
1984 Instructor, Wayne State University, Detroit, MI, USA
1985-1988 Assistant Professor, University of Innsbruck, Austria
1988-1990 Postdoctoral Fellow, Collège de France, Nogent-sur-Marne, France
1990-1992 Group Leader, Center of Molecular Biology, Madrid, Spain
1992 Visiting Research Scientist, University of California (UCSF); San Francisco, CA, USA
1992-1993 Group Leader, National Center for Biotechnology, Madrid, Spain
1993-1996 Senior Scientist (INSERM), CNRS, Cancer Research Center; Villejuif, France
1996-1999 Research Director (INSERM), CNRS, Cancer Research Center, Villejuif, France
1999-2001 Burnham Fellow, Burnham Institute La Jolla, CA, USA
2000-2010 Research Director, Institut Gustave Roussy/ INSERM, Villejuif, France
2010-present Full Professor, University of Paris Descartes, Paris, France
2013-2014 Visiting Professor, University of Rome, Tor Vergata, Rome, Italy
2015-present Foreign Adjunct Professor, Karolinska Institute & Hospital, Stockholm, Sweden

Medical Appointments

1990-2000 Associate Professor, Medical University of Innsbruck, Innsbruck, Austria
2000-2010 Consultant, Institut Gustave Roussy Villejuif, France
2003-2010 Interface contract, INSERM/Institut Gustave Roussy, Paris/Villejuif, France
2010-present Hospital Practitioner, Hôpital Européen Georges Pompidou, Paris, France

Administrative Appointments

1993-present Scientific Director, Laboratory "Apoptosis, Cancer & Immunity", Villejuif + Paris, France
2002-present Board of Directors, European Cell Death Organization, Ghent, Belgium
2007-2014 Director, INSERM Unit 848, Villejuif, France

2010-present Director, Metabolomics & Cell-Biology platforms, Gustave Roussy, Villejuif, France
2011-present Director, LabEx Immuno-Oncology, Paris, France
2011-present Founding President, European Academy of Tumor Immunology (EATI), Paris, France
2012-2014 President, European Cell Death Organization (ECDO), Ghent, Belgium
2013-present Director, Paris Alliance of Cancer Research Institutes (PACRI), Paris, France
2014-present Deputy Director, Cordeliers Research Center (CRC), Paris, France
2016-present Board of Directors, European Network for Cancer Immunotherapy, Mainz, Germany

Appointments in Pharmaceutical Industry and Biotechnology

1994-1997 Permanent consultant, Kabi Pharmacia, Lund, Sweden
2000-2002 Permanent consultant, Institut Scientifique Aventis, Ivry-sur-Seine, France
2003-2006 Scientific Advisory Boardn Apogenix Biotechnology Co., Heidelberg, Germany
2004-2010 Scientific Advisory Board, IOM Ricerca Catania, Italy
2005-present Permanent consultant, Bayer-Schering/Bayer-Healthcare, Berlin, Germany
2008-2010 Permanent consultant, Enzo Lifesciences Inc., Lausen, Switzerland
2009-present Advisor, Discovery Oncology Board, Bayer Healthcare, Berlin, Germany
2014-present Oncology Scientific Advisory Board, Hoffmann-La Roche Ltd., Basel, Switzerland
2015-present Scientific Advisory Board, Lytix Ltd., Oslo, Norway
2015-present Chairman of Scientific Advisory Board, Medicenna Therapeutics, Vanvouver, Canada
2015-present Board of Directors/Executive Board, Bristol Meyers Squibb Foundation, Paris, France
2016-present Permanent consultant, Genmab SA, Kopenhagen, Denmark
2016-present Founder, EverImmune (Biotech company), Paris, France

Major editorial appointments

2010-2016 Editor-in-Chief, Cell Death & Disease (Springer/Nature), London, UK
2012- Editor-in-Chief, OncoImmunolog (Francis & Taylor), Philadelphia, PA, USA
2013-2017 Editor-in-Chie, Microbial Cell (Shared Science Publishers), Graz, Austria
2014- Editor-in-Chief, Molecular & Cellular Oncology (Francis & Taylor), Philadelphia, PA, USA
2016- Deputy Editor, Cell Death & Differentiation (Springer/Nature), London, UK
2017- Founding Editor-in-Chief, Cell Stress (Shared Science Publishers), Graz, Austria

Other editorial appointments (selection)

1996-2002 Executive Editor, Immunology Letters (European Federation of European Societies)
1998-2016 Receiving Editor, Cell Death and Differentiation (Nature Publishing Group)
2000-2007 Associate Editor, Mitochondrion (Elsevier)
2000-2009 Associate Editor, Cancer Research (American Association for Cancer Research)
2002- Editorial Board Member, EMBO Journal (Nature Publishing Group)
2002-2011 Editorial Board Member, EMBO Reports (Nature Publishing Group)
2005- Editorial Board Member, Autophagy (Landes Bioscience Publisher)
2005-2010 Regional Editor, American Journal of Immunology (Science Publications, NY)
2007- Editorial Board Member, Cell Cycle (Landes Bioscience, Taylor & Francis)
2008- Editorial Board Member, Aging-US (Impact Journals)
2008- Editor, Biochemical and Biophysical Research Communications (Elsevier)

2009- Editorial Board Member, Cancer Research (American Association for Cancer Research)
2009- Associate Editor, Autophagy (Landes Bioscience, Taylor & Francis)
2009- Reviews Editor, Oncogene (Nature Publishing Group)
2009- Associate Editor , Journal of Molecular Cell Biology (Oxford University Press)
2010- Founding Editor, American Journal of Cancer Research (e-Century Publishing)
2010- Founding Editor, Oncotarget (Impact Journals)
2011- Editorial Board Member, Molecular Oncology (Elsevier)
2011-2013 Specialty Chief Editor, Frontiers in Oncology (Frontiers, Lausanne, Switzerland)
2012-2013 Advisory Board Member, Seminars in Immunopathology (Springer)
2013- Editorial Board Member, EMBO Molecular Medicine (Wiley)
2013- Editorial Board Member, Molecular & Cellular Biology (American Society for Microbiology)
2013- Reviewing Editor Science Signaling (American Assoc. for the Advancement of Science)
2015- Editorial Board Member, Oncogenesis (Nature Publishing Group)
2015- Editorial Board Member, Cell Research (Nature Publishing Group)
2015- Editorial Board Member, Cell Discovery (Nature Publishing Group)
2016- Editorial Board Member, Signal Transduction and Targeted Therapy (Nature Publishing Group)

Honors/Awards (selection)

1984 Kamillo Eisner Award, Kamillo Eisner Foundation, Hergiswil, Switzerland
1985 Prize for the best MD/PhD thesis, Austrian Society for Allergology & Immunology, Vienna, Austria
1988 Erwin Schrödinger Fellowship, Austrian National Research Foundation, Vienna, Austria
1988 Hoechst Prize, University of Innsbruck, Austria
1996 Annual Research Prize, Ligue Nationale contre le Cancer, Paris, France
1997 Jean Valade Prize, Fondation de France, Paris, France
1997 Charles Oberling Prize, Senate of the French Republic, Paris, France
1998 Research Prize, Conseil Général des Yvelines, Versailles, France
1998 Monika Kutzner Cancer Research Prize, Academy of Sciences of Berlin-Brandenburg, Berlin, Germany
1999 Gallet & Breton Prize, National Academy of Medicine, Paris, France
1999 Medical Research Prize, Fondation pour la Recherche Médicale, France
2000 Jacques Sylvain Bourdin Prize, Ligue Nationale contre le Cancer, Paris, France
2000 Elected EMBO member, European Molecular Biology Organization, Heidelberg, Germany
2000 INSERM Prize for Pathophysiology, French Medical Research Council (INSERM), France
2003 Novum Lecture, Karolinska Institute & Nobel Assembly, Stockholm, Sweden
2006 Severo Ochoa Centennial Lecture, Spanish Society for Biochemistry, Alicante, Spain
2006 Descartes Prize, European Commission, Bruxelles, European Union
2007 Elected foreign member, Austrian Academy of Sciences, Vienna, Austria
2007 Elected member (Academician), German Academy of Sciences (Leopoldina), Halle, Germany
2007 Carus Medal, German National Academy of Sciences (Leopoldina)
2007 Elected member (Academician), Academia Europaea, London, UK
2007 Grand Prix Mergier-Bourdeix, French Academy of Sciences, Institut de France, Paris, France
2008 Carus Prize, City of Schweinfurth, Germany
2008 John Humphrey Lecture, Imperial College, London, UK
2008 Elected member (Academician), European Academy of Sciences and Arts, Salzburg, Austria

2009 Charles Darwin Lecture, Leopoldina Symposium on Cell Death, Wurzburg, Germany
2009 Dautrebande Prize, Belgian Royal Academy of Medicine, Bruxelles, Belgium
2009 Elected member (Academician), European Academy of Cancer Sciences, Amsterdam, Netherlands
2010 AXA Chair for Longevity Research, AXA Research Foundation, Paris, France
2010 Elected member (Academician), European Academy of Sciences, Liège, Belgium
2010 Jesus Montoliu Honorary Lecture, Institute of Biomedical Research of Lleida, Spain
2010 Elected member (Academician), European Academy of Tumor Immunology, Paris, France
2010 Duquesne Prize, National League against Cancer, Paris, France
2010 ECDO Career Award, European Cell Death Organization (ECDO), Ghent, Belgium
2011 Doctor honoris causa, University of Buenos Aires, Argentina
2011 ICDS Lifetime Achievement Award, International Cell Death Society (ICDS), New York, NY, USA
2011 Coup d'élan Prize, Bettencourt-Schueller Foundation, Neuilly, France
2012 Lambertsen Honorary Lecture, University of Pennsylvania, Philadelphia, PA, USA
2012 Ramalingaswami Memorial Lecture, National Institute of Immunology, New Delhi, India
2012 Léopold Griffuel Prize, Association for Cancer Research, Paris, France
2013 ERC Advanced Investigator Award, European Research Council (ERC), Bruxelles, European Union
2014 Karolinska Research Lecture, Medical Nobel Institute and Nobel Assembly, Stockholm, Sweden
2014 Mitjavile Prize, National Academy of Medicine, Paris, France
2015 Senior member, Institut Universitaire de France, Paris France
2015 Ohdang Distinguished Award, Pharmaceutical Society of Korea, Seoul, South Korea
2015 Galien Award for Pharmacological Research, Prix Galien de la Recherche Pharmaceutique, Paris, France
2016 International Prize for Oncology 'Ramiro Carregal', Rosaleda Foundation, Santiago de Compostela, Spain
2016 Leopoldina Lecture, International Conference on Innate Immunity, Berlin, Germany
2016 Grand Prix Claude Bernard, Science and Medicine Prize of the City of Paris, France
2016 Honorary professor, Center of Systems Medicine, Chinese Academy of Science, Suzhou

Cancer cell stress and death: cell-autonomous and immunological considerations

Guido Kroemer

Cancer can be viewed in two rather distinct ways, namely (i) as a cell-autonomous disease in which malignant cells have escaped control from cell-intrinsic barriers against proliferation and dissemination or (ii) as a systemic disease that involves failing immune control of aberrant cells. The first vision has been prevailing in the area of cancer research over the last 50 years, while the latter has gained vast support only during the past few years.

In the cell-autonomous vision of cancer, the supreme goal of cancer research is to identify drugs that selectively kill cancer cells, much like antibiotics kill microbial pathogens yet spare host cells. This can be achieved by cytotoxic chemotherapies that kill rapidly proliferating cells (and hence induce the death of cancer cells more efficiently than normal host cells) or more elegantly – at least in theory – by identifying tumor cell specific signal transduction pathways that are responsible for the malignant phenotype and that can be regarded as cancer cell-specific therapeutic targets. Such tumor-specific targets would allow for "personalized" therapeutic interventions guided by the rules of "precision" medicine. Although some "personalized" therapies have undoubtedly been successful (such as the utilization of imatinib and its follow-up drugs for the treatment of Philadelphia-positive chronic lymphoid leukemia, CML, and gastrointestinal stromal tumors, GIST), thus far most "personalized" anticancer drugs have failed in clinical trials.

Adopting the view of cell biologists, my team has long been investigating one peculiar characteristic of cancer cells, namely their intrinsic resistance against lethal stimuli, meaning that malignant cells survive in conditions in which normal cells would succumb. This particular characteristic renders cancer cells resistant against otherwise lethal endogenous stress (such as lack of trophic transport, hypoxia, shortage of nutrients etc.) as well as against therapeutic interventions with cytotoxic chemotherapeutics and radiotherapy. We discovered in 1994 (and published for the first time in 1995) that, in programmed cell death, mitochondrial membrane permeabilization constitutes the point of no return of the lethal process and hence defines the cell death checkpoint. This discovery has initiated a scientific revolution in that it led to an operational redefinition of apoptosis (the prevalent form of regulated cell death), changed the method of apoptosis detection and conditioned the theoretical framework allowing for the ordering of pro-apoptotic signaling molecules. Instead of considering apoptosis as a process dominated by proteases and nucleases, cell death is now viewed as a process that is largely controlled by mitochondria. This has had far-reaching implications for the therapeutic manipulation of cell death, including the chemotherapeutic induction of cell death in cancer cells, which can be achieved by directly triggering mitochondrial permeabilization, as well as for the prevention of unwarranted cell death in stroke and infarction, which can only be achieved when targeting pre-mitochondrial or mitochondrial (but not post-mitochondrial) events. We explored the fine mechanisms of mitochondrial cell death control, as well as the molecular pathways that explain the inhibition of cell death in cancer cells, upstream of or at the level of mitochondria. We found that pro- and anti-apoptotic proteins of the BCL2 family regulate mitochondrial membrane permeability through interactions with proteins from the ATP synthasome and lipids. Beyond these mechanistic details, our work contributed to the comprehension of the mode of action of a series of oncogene products, in particular the proteins from the Bcl-2 family.

At a subsequent step of our work on the cell biology of cancer, we turned to autophagy, which is a bulk degradation pathway in which portions of the cytoplasm are enwrapped by membranes to form vesicles, so-called autophagosomes, which subsequently deliver their content to lysosomes. We noted that cell death is often preceded by cytoplasmic vacuolization with formation of autophagosomes, yet that suppression of autophagy by appropriate pharmacological and genetic interventions did not prevent cell death but rather accelerated the apoptotic or necrotic demise of stressed cells. We deciphered part of the molecular crosstalk between apoptosis and autophagy, showing that proteins with BH3 domains (BCL2 family members carrying BCL2 homology domains) can control both catabolic events and that activation of prominent elements of the classical NFκB activation pathway (in particular TAK1 and the proteins of the IKK complex) are required for the optimal induction of autophagy. We discovered that the pro-apoptotic tumor suppressor protein p53 plays a dual role in the control of autophagy, namely as an autophagy-inducing transcription factor and as an autophagy-repressing cytoplasmic factor. We also found that STAT3 can inhibit autophagy via the inhibition of PKR. We identified spermidine as a novel, non-toxic inducer of autophagy and

determined its mode of action as a life span-extending drug in yeast, nematodes, flies and mice. We accumulated extensive evidence that acetyl-coenzyme A and protein acetylation repress autophagy and that "caloric restriction mimetics" including spermidine induce autophagy via deacetylation reactions. We launched the (still valid) hypothesis that all longevity extending manipulations, be they metabolic, pharmacological or genetic, must induce autophagy to be efficient.

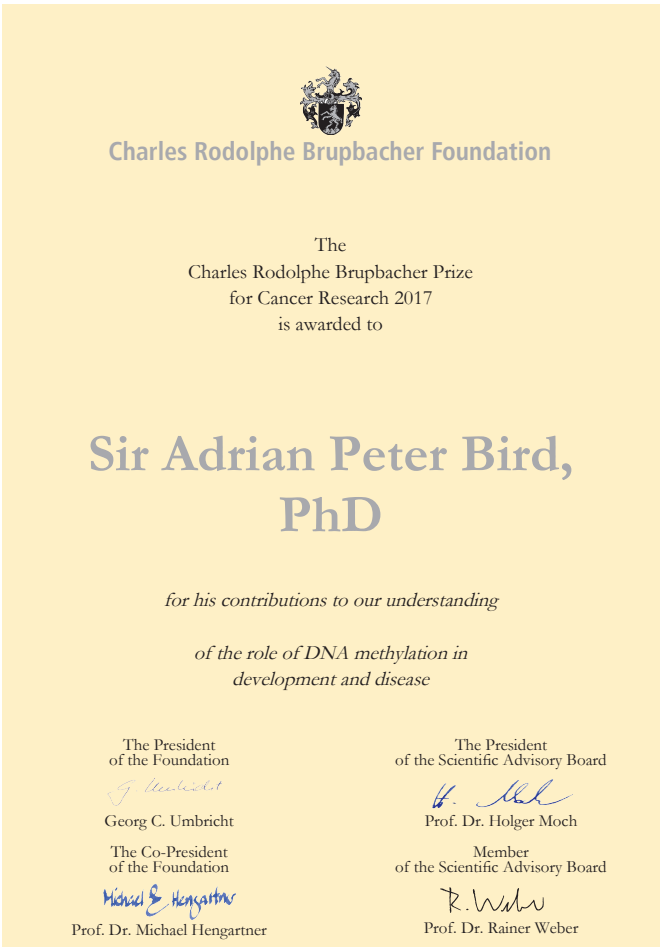
As we were working on the cell biology or apoptosis, autophagy and necrosis, we were wondering whether these cell stress and death mechanisms might influence the perception of cancer cells by the immune system. Driven by a discussion with my spouse, Laurence Zitvogel, as well as by our prior discovery that mitochondrial permeabilization constituted a lethal event that causes apoptosis accompanied by caspase activation as a default pathway, yet necrosis when caspases are inhibited, I launched a working hypothesis that a posteriori (and luckily) turned out to be wrong. I thought that, as the most prevalent pathway of physiological cell death execution, apoptosis would be immunologically silent (or even tolerogenic), while necrosis would be immunogenic. Hence, we injected CT26 colorectal cancer cells into immunocompetent BALB/c mice (which bear the same major histocompatibility locus as CT26 cells) and treated them with anthracycline-based chemotherapy alone, knowing that anthracyclines induce apoptosis, or anthracyclines plus a broad-spectrum caspase inhibitor (Z-VAD-fmk), knowing that this combination induces necrosis. We expected that the latter pro-necrotic regimen would be much more efficient than the pro-apoptotic treatment in stimulating an anticancer immune response and hence reducing tumor growth. To my dismay, the experiment yielded exactly the opposite result. Anthracyclines alone efficiently reduced tumor growth, while anthracyclines combined with caspase inhibitors failed to do so. Moreover, the capacity of anthracyclines to mediate tumor growth control were lost when CD8-positive cytotoxic T lymphocytes were depleted from the mice, indicating that their therapeutic action was entirely dependent on a cellular immune response. Driven by these unexpected results and a long-standing collaboration with Laurence Zitvogel, my group radically changed the working hypothesis to postulate that some chemotherapeutic agents including anthracyclines can stimulate a modality of cell death that we baptized "immunogenic cell death" (ICD) and that stimulates anticancer immunosurveillance through a partially caspase-dependent pathway.

In subsequent rounds of experiments, we demonstrated that, depending on the upstream triggers, apoptosis can be immunogenic and hence alert the innate immune system and instruct it to stimulate a cognate response against dead-cell antigens. This has opened a new field of research at the frontier between immunology and cell biology, allowing us to define the molecular properties of ICD. We found that ICD is characterized by autocrine stimulation of type 1 interferon (IFN) receptors, the pre-apoptotic exposure of calreticulin (CRT) on the cell surface, release of ATP during the blebbing phase of apoptosis, and post-apoptotic exodus of annexin A1 (ANXA1) and the chromatin-binding protein high mobility group B1 (HMGB1). Type 1 interferon secretion depends on the stimulation of TLR3, CRT exposure on an endoplasmic reticulum stress response, ATP release on pre-mortem autophagy, and annexin A1/HMGB1 exodus on secondary necrosis. CRT, ATP, ANXA1 and HMGB1 interact with four receptors (CD91 receptor, purinergic P2Y2 or P2X7 receptors, formyl peptide receptor-1 [FPR1], and toll-like receptor 4 [TLR4], respectively) that are present on the surface of dendritic cells or their precursors. CD91, P2Y2, FPR1, P2RX7 and TLR4 promote engulfment of dying cells, attraction of dendritic cells, juxtaposition of dendritic and dying cells, production of interleukin-1β and presentation of tumor antigens, respectively. Local induction of endoplasmic reticulum stress in the tumor bed and systemic induction of autophagy increase anticancer immune responses. We have launched and then proven the hypothesis that the immune response against dying tumor cells dictates the therapeutic success of anticancer chemotherapy, both in mouse models and in cancer patients. Obviously, this discovery has had major consequences for the comprehension, conception and implementation of anticancer chemotherapies. Indeed, we postulate that, at least in certain cases, both classical and targeted anticancer therapies require an active contribution of the immune system to be optimally efficient. We obtained clinical evidence that this hypothesis holds true for anthracycline-treated breast cancer, oxaliplatin-treated colorectal cancer, and imatinib-treated gastrointestinal stromal tumors, among others.

Since macroautophagy/autophagy generally increases the fitness of cells (and entire organisms) as well as their resistance against endogenous or iatrogenic stress, it has been widely proposed that inhibition of autophagy would constitute a valid strategy for sensitizing cancer cells to chemotherapy or radiotherapy. Colliding with this cell-autonomous vision, however, we found that immunosurveillance against transplantable, carcinogen-induced or genetically engineered

cancers can be improved by pharmacologically inducing autophagy with caloric restriction mimetics, which are non-immunosuppressive but rather immunostimulatory. This positive effect depends on autophagy induction in cancer cells and is mediated by alterations in extracellular ATP metabolism, namely increased release of immunostimulatory ATP and reduced adenosine-dependent recruitment of immunosuppressive regulatory T cells into the tumor bed. The combination of autophagy inducers and chemotherapeutic agents is particularly efficient in reducing cancer growth through the stimulation of CD8+ T lymphocyte-dependent anticancer immune responses.

Altogether, our past and present research indicates that the efficacy of conventional and targeted anticancer agents does not only involve direct cytostatic/cytotoxic effects, but also relies on the (re)activation of tumor-targeting immune responses. Chemotherapy can promote such responses by increasing the immunogenicity of malignant cells, or by inhibiting immunosuppressive circuitries that are established by developing neoplasms. These immunological "side" effects of chemotherapy are desirable, and their in-depth comprehension will facilitate the design of novel combinatorial regimens with improved clinical efficacy.



Laudatio

Josef Jiricny

The Watson-Crick model of DNA consists of two anti-parallel sugar-phosphate chains that are held together in the double helix by pairs of complementary bases: adenine/thymine and guanine/cytosine. But DNA of most organisms contains also a fifth base, 5-methylcytosine, first described in 1925 by Johnson and Coghill as a constituent of nucleic acid isolated from Mycobacterium tuberculosis. The biological roles of 5-methylcytosine have remained enigmatic for many years until the demonstration that it is a key component of the bacterial restriction/modification system, which brought Werner Arber, Daniel Nathans and Hamilton Smith the 1978 Nobel Prize in Physiology or Medicine, and made restriction enzymes the primary tools of molecular biology. Four decades have passed since then, but the role of 5-methylcytosine in eukaryotic DNA metabolism is still shrouded in mystery. We know that the sperm methylation pattern is largely erased after fertilization and that methylation is gradually reintroduced during embryogenesis and differentiation, but the processes that regulate the cell type- and tissue-specific methylation patterns remain to be elucidated. We have also learned that DNA can be not only methylated, but also demethylated, and that aberrant methylation can lead to disease - including cancer. Again, how these processes are regulated remains to be discovered. However, we have learnt a great deal about 5-methylcytosine metabolism during the past three decades and much of our knowledge came from the laboratory of Adrian Bird.

Adrian spent his doctoral and postdoctoral time in Max Birnstiel's laboratory, first in Edinburgh and then in Zurich, studying the amplification of ribosomal DNA in Xenopus laevis. In this organism, genomic rDNA in somatic tissues is highly methylated, while the extrachromosomal amplicons are unmethylated. When he returned to Edinburgh to establish his own group, Adrian set out to study the methylation pattern of these loci using the newly-available methylation-sensitive restriction enzymes. Moving from frog to sea urchin to mice, he noted that while genomic DNA was largely resistant to cleavage with HpaII, a restriction enzyme inhibited by methylation, a small fraction was cut into very small fragments, which he called HpaII tiny fragments, or HTF islands. These genomic features, later renamed CpG islands, were subsequently shown to be frequently associated with active housekeeping genes and to be generally unmethylated. Their aberrant methylation, for example in cancer cells, resulted in transcriptional silencing and Adrian was able to show that the latter phenomenon was linked to the binding of factors displaying high affinity for densely-methylated DNA. These polypeptides share a conserved "methylated-CpG binding domain", and have many interesting biological roles. Thus, MBD2 and 4 have been shown to suppress intestinal tumourigenesis. However, one member of this protein family, MeCP2, stands out from the rest. Adrian's laboratory established a knock-out mouse model and noted that its pathology was reminiscent of that seen in individuals affected with a severe neurological disorder, Rett Syndrome (RTT), which affects around one

person (predominantly female) in 10'000. Indeed, RTT patients could be shown to carry mutations in the MeCP2 gene. Importantly, the Bird laboratory showed that the resulting severe neurological phenotype is reversible when the protein is re-expressed, which promises that the Rett Syndrome might be curable in the future.

Adrian has unquestionably made a major contribution not only to our understanding of DNA methylation, but also to the entire field of epigenetics, which plays a key role in development and in disease. But his influence is much broader that that. He is a role model for young scientists as a teacher, as a tutor and as a member of numerous advisory boards. He is also a member of key strategic bodies that decide on the future of scientific research. With people like Adrian at the helm, the ship of science need not fear even the stormiest seas.

Sir Adrian Peter Bird

Summary Curriculum vitae

Appointment Buchanan Professor of Genetics

Address
University of Edinburgh
The Wellcome Trust Centre of Cell Biology
The King's Buildings, Mayfield Road
Edinburgh EH9 3JR
United Kingdom

Date of Birth July 3, 1947

Education

1968-71 PhD, University of Edinburgh
Supervisor: Dr. Max L. Birnstiel
Thesis title: "The Cytology and Biochemistry of Ribosomal DNA amplification in Xenopus laevis oocytes."

1965-68 BSc.(Hons.) Biochemistry 2(1)
University of Sussex

Post-Doctoral Positions

1974-75 Swiss National Fund Fellowship
University of Zürich, Switzerland
Laboratory of Prof Max L Birnstiel
1971-73 Damon Runyan Memorial Fellowship
Yale University, USA, Laboratory of Dr. J.G. Gall

Positions held

2011– Associate Faculty at The Sanger Institute, Cambridge
1999–11 Director, Wellcome Trust Centre for Cell Biology, University of Edinburgh
1990– Buchanan Chair of Genetics, University of Edinburgh
1987–90 Senior Scientist, Research Institute for Molecular Pathology, Vienna, Austria
1987 Head of Structural Studies Section, MRC Clinical and Population Cytogenetics Unit Western General Hospital, Edinburgh, U.K.
1975–86 Research Group Leader, MRC Mammalian Genome Unit, Edinburgh, U.K.

Awards & Honors

2016 Harold Ackroyd Lecture, Caius College, Cambridge
2016 Francis Crick Lecture, MRC Laboratory of Molecular Biology, Cambridge
2016 Shaw Prize in Life Science and Medicine, Hong Kong
2015 Jordan Translational Medicine Lecture, University of Oxford
2014 BBVA Frontiers of Knowledge Award, Madrid
2014 Jacob's Ladder Norman Saunders International Research Prize, Toronto
2014 Knighthood New Year's Honours list
2014 Rosalind Franklin Lecture, King's College London
2013 GlaxoSmithKline Prize Lecture, Royal Society, London
2012 Royal Society GlaxoSmithKline Prize Lecture and Medal
2012 Jean Shanks Lecture, Academy of Medical Sciences, London
2011 Gairdner International Award, Toronto
2010 Honorary Doctorate, Sussex University
2008 Charles-Léopold Mayer Prize, French Academy of Sciences, Paris
2007 Novartis Medal of The Biochemical Society
2005 Commander of the Order of the British Empire
2002 Almroth Wright Lecturer, Imperial College London
2002 34th Bruce-Preller Prize Lectureship, Royal Society of Edinburgh
1999 Louis-Jeantet Prize for Medicine, Geneva
1999 Gabor Medal of the Royal Society, London
1986 Federation of European Biochemical Societies Anniversary Prize of the Gesellschaft für Biologische Chemie

Learned Societies

- Elected Foreign Associate, US National Academy of Sciences (2016)
- Elected Member of EMBO Council (2016)
- Honorary membership of the Biochemical Society (2012)
- Elected Fellow of the Academy of Medical Sciences (2001)
- Elected Fellow of the Royal Society, Edinburgh (1994)
- Howard Hughes International Fellow (1993-98)
- Elected Fellow of the Royal Society, London (1989)
- Elected to European Molecular Biology Organisation (1987)

External Committees

- International jury for the Start and Wittgenstein Prize of the Austrian Science Fund (2016-2021)
- Governing Body, Lister Institute for Preventive Medicine (2015)
- Adviser to the National Autism Project, The Shirley Foundation, UK (2015)
- Trustee and Chair of Research Strategy Committee at Cancer Research UK (2010-present)
- Scientific Advisory Boards: Gurdon Institute, Cambridge; Friedrich Miescher Institute, Basel; Stratified Medicine Scotland, Glasgow; Crick Institute, London
- Trustee of the Kirkhouse Trust, Scotland (2001-present)
- Trustee of the Rett Syndrome Research Trust, USA (2008-present)
- Editorial Boards: PeerJ
- Governor of the Wellcome Trust (2000-2010)

Public Engagement

- Rett Syndrome Anniversary family weekend, Northampton (2015)
- Abcam Scientist of the month (July 2015)
- HowTheLightGetsIn Philosophy Festival, Hay on the Wye, Wales (2013)
- Café Scientifique, Cockermouth, UK (2012)

Keynote Lectures

- London Chromatin Club Meeting, University College London (2016)
- SyBoSS Conference, Oberstdorf (2015)
- Edinburgh University Alumni weekend (2015)
- Keystone Symposia on DNA methylation/epigenetics, Keystone, Colorado (2015)
- Newlife Foundation Brighter Minds Symposium, London, UK (2014)
- Chromatin & Epigenetics Conference Cold Spring Harbor, New York (2012)
- EMBO Dynamic Genome Meeting, Nice (2013)
- 12th International Workshop of Developmental Nephrology, Edinburgh (2013)
- Plenary speaker Killam lecture series, Dalhousie University, Halifax, Nova Scotia (2013)
- Glasgow University Wellcome Trust 4yr PhD Scientific Retreat (2013 Guest Lecturer)
- Chromatin, Replication and Chromosomal Stability Conference, University of Copenhagen (2013)
- Epigenesys Meeting, University of Cambridge (2013)
- Epigenetics & Chromatin Meeting, Cold Spring Harbor (2012)

Teaching

PhD training

- Supervised 30 post-graduate students; 24 as 1st supervisor and 6 as 2nd supervisor (all competed successfully)

Undergraduate training

- Lectures to 4th year students in "Gene Expression", "Molecular Biology of Disease" and "Medical Biology

Patents/commercialisation

- CXXC Patent: Identification of CpG islands licenced to New England Biolabs, Oncomethylome Sciences, Sigma Aldridge, Qiagen and Active Motif.
- Monoclonal antibody against hydroxymethylcytosine (collaboration with Babraham Institute). Licensed to Diagenode.
- MBD reagents: antibodies licensed to Millipore (Upstate)
- pET Expression vector expressing MBD2b. Licensed to Applied Biosystems

Current Research Funding

Rett Syndrome Research Trust
"A research consortium to define the function of MeCP2: A step towards the development of therapeutics for treating Rett Syndrome"
Amount awarded: £673,484
Dates: 14 Jan 2014 - 15 Jan 2017

Sylvia Aitken Charitable Trust
Understanding Rett Syndrome by generating animal models with altered versions of the MeCP2 gene
Amount awarded: £181,924
Dates: 1 Sept 2014 - 30 Aug 2017

Wellcome Trust Studentship
Grant ref: 100469
"Genetic manipulation of MeCP2 in the human neuronal LUHMES cell line with a view to gene therapy of Rett Syndrome
Amount awarded: £151,134
Dates: 1 Oct 2012 - 30 Sept 2016

Wellcome Trust Investigator Award
Grant ref: 107930
"Towards understanding and treatment of MeCP2-related disorders"
Amount awarded: £2,814,000
Dates: 1 Jan 2016 – 31 Dec 2020

European Research Council Advanced Grant
Grant ref: EC 694295 Gen-Epix (ERC-AdG)
"Genetic determinants of the epigenome"
Amount awarded: €2,499,717
Dates: 1 Jun 2016 – 31 May 2021

DNA methylation patterns and cancer

Sir Adrian Peter Bird

The instructions for constructing the human body are stored by the genome as a sequence of DNA bases. This chemical code directs the synthesis of thousands of different proteins needed for life. To facilitate the controlled release of the information, the genome acquires chemical marks in the form of methyl groups that are added to genomic DNA as an extra layer of "epigenetic" regulation. The human genome has high DNA methylation levels throughout, but this "sea" of methylation is interrupted by short "islands" that are methylation-free. These so-called CpG islands coincide with many gene control regions and they serve as platforms for binding of enzymes that modify chromosome structure, thereby facilitating gene regulation. In cancer cells, CpG island control regions often acquire DNA methylation, leading to aberrant shutdown of the associated gene. In fact altered methylation patterns in tumours usually affect large domains that include many genes and also inter-genic DNA, but the underlying cause for this epigenomic de-regulation is unknown. Our recent research implicates proteins that recognize short DNA sequence motifs in modulating DNA methylation and other features of the "epigenome". The results suggest that the epigenome, which is often thought to respond to the external environment, is greatly influenced by the "hard-wired" sequence of the genome. Proteins that appear to link the genome with the epigenome are frequently mis-expressed in cancer and are likely to be important contributors to oncogenesis.

Bisherige Preisträger
Previous Laureates

1993

Arnold J. LEVINE
Department of Molecular Biology, Lewis Thomas Laboratory, Princeton University, Princeton, NJ, USA
«Functions of the p53 Gene and Protein»

David P. LANE
Cancer Research Campaign Laboratories, Department of Biochemistry, University of Dundee, Dundee, Scotland
«The p53 Pathway, Past and Future»

1995

Alfred G. KNUDSON
Fox Chase Cancer Center, Philadelphia, PA, USA
«Hereditary Cancer»

Robert A. WEINBERG
Whitehead Institute for Biomedical Research, Department of Biology, MIT, Cambridge, MA, USA
«Genes and Cancer»

1997

Laurent DEGOS
Institut Universitaire d'Hématologie, Hôpital Saint Louis, Paris, France
«Differentiation Therapy of Cancer»

Zhen-yi WANG
Shanghai Institute of Hematology, Rui-Jin Hospital Shanghai, Second Medical University, Shanghai, China
«Treatment of Acute Promyelocytic Leukemia with All-Trans Retinoic Acid. A Model of Differentiation Therapy in Cancer»

1999

George KLEIN
Microbiology and Tumor Biology Center (MTC)
Karolinska Institute, Stockholm, Sweden
«Cancer and the New Biology»

Harald ZUR HAUSEN
Deutsches Krebsforschungzentrum, Heidelberg, Germany
«Cancer Causation by Viruses»

2001

Brian DRUKER
Oregon Health Sciences University, Portland, OR, USA
«STI571: A Tyrosine Kinase Inhibitor for the Treatment of CML – Validating the Promise of Molecularly Targeted Therapy»

2003

Rudolf JAENISCH
Whitehead Institute for Biomedical Research, Department of Biology, MIT, Cambridge, MA, USA
«Nuclear Cloning and the Reversibility of Cancer»

Erwin F. WAGNER
Institute of Molecular Pathology, Vienna, Austria
«Unravelling the Functions of AP-1 (Fos/Jun) in Mouse Development and Disease»

2005

Mariano BARBACID
Centro Nacional de Investigaciones Oncológicas, Madrid, Spain
«The Molecular Bases of Human Cancer: a 25 Year Journey»

Klaus RAJEWSKY
The CBR Institute for Biomedical Research,
Harvard Medical School, Boston, MA, USA
«The Janus Face of Antibody Formation: Protective Function and Tumor Risk»

2007

Lloyd J. OLD
Ludwig Institute for Cancer Research, New York, NY, USA
«Contributions to the Field of Cancer Immunology»

Robert D. SCHREIBER
Department of Pathology and Immunology, Washington University School of Medicine, St.Louis, MO, USA
«Cancer Immunoediting: Deciphering the Complex Interaction Between Immunity and Developing Tumors»

Mark J. SMYTH
Cancer Immunology Program, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia
«Extrinsic Tumor Suppression by Innate and Adaptive Immunity»

2009

Nubia MUÑOZ
National Cancer Institute, Bogota, Colombia
«From Causality to Prevention: The Case of Cervical Cancer»

Sir Richard PETO
Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom
«The Absolute Benefits of Anti-cancer Drugs and of Tobacco Control»

2011

Jan HOEIJMAKERS
Department of Genetics, Erasmus Medical Center, Rotterdam, The Netherlands
«DNA Damage and its Impact on Cancer, Aging and Longevity»

Bert VOGELSTEIN
The Sidney Kimmel Comprehensive Cancer Center
Johns Hopkins University, Baltimore, MD, USA
«The Genetic Basis of Human Cancer and its Implications for Patient Management»

2013

Michael KARIN
Department of Pharmacology
University of San Diego, California, USA
«Inflammation and Cancer: Effects, Mechanisms and Therapeutic Implications»

2015

Joan MASSAGUÉ
Sloan Kettering Institute
Memorial Sloan Kettering Cancer Center
«Molecular Basis of Metastasis»

Irving L. WEISSMAN
Institute for Stem Cell Biology and Regnerative Medicine
Stanford University
«The view from stem cell land: Stem cell biology in regeneration and cancer»

Programm des Wissenschaftlichen Symposiums 2017

Program of the Scientific Symposium 2017

Charles Rodolphe Brupbacher Symposium 2017
Breakthroughs in Cancer Research and Therapy

Tuesday, January 31, 2017

19:00 – 20:00 Charles Rodolphe Brupbacher Public Lecture
Aula of the University of Zurich, Main Building, Rämistrasse 71, 8006 Zurich

Reinhard Dummer, Zurich
Ist Hautkrebs heilbar?

Wednesday, February 1, 2017

12:00 – 13:30 Registration / Sandwich Lunch

13:30 – 15:00 Cancer Genomics
Chair: Holger Moch, Zurich

Richard Houlston, London
Polygenic susceptibility to colorectal cancer - mechanisms and impact

Jussi Taipale, Stockholm
Systems biology of cancer

Charles Swanton, London
Cancer chromosomal instability, diversity and evolution: TRACERx

15:00 – 15:30 Coffee break

15:30 – 17:30 Cancer Signalling and Targeted Therapy
Chair: Nancy Hynes, Basel

Myles Brown, Boston
Hacking the hormone code in cancer

Yibin Kang, Princeton
Stromal niches in bone that mediate the seeding, progression and treatment resistance of metastatic breast cancer

Lew Cantley, New York
PI 3-kinase and cancer metabolism

Gerard Evan, Cambridge
How and Why Ras and Myc cooperate

18:30 – 21:30 Posters, Beer and Pretzels
Evening for all participants

Thursday, February 2, 2017

08:00 – 08:30 *Registration and Coffee*

08:30 – 10:00 **Genomic Instability**
Chair: Lauri Aaltonen, Helsinki

Simon Boulton, London
Mechanism and regulation of DNA-protein crosslink repair by the DNA-dependent metalloprotease SPRTN

David Pellman, Boston
Rapid evolution of the cancer karyotype

Angelika Amon, Cambridge (MA)
Aneuploidy and cancer - a complicated relationship

10:00 – 10:30 *Coffee break*

10:30 – 12:00 **Cancer Epigenetics**
Chair: Josef Jiricny, Zurich

Adrian Bird, Edinburgh
Genetic determinants of the epignome in development and cancer

Maarten van Lohuizen, Amsterdam
Context-dependent roles of polycomb repressors in cancer and development

Margaret Goodell, Houston
DNMT3A in hematopoietic stem cells, cancer and aging

12:00 – 13:30 *Lunch and Coffee*

13:30 – 15:00 **Cancer Stem Cells**
Chair: Markus G. Manz, Zurich

Kornelia Polyak, Boston
Heterogeneity in breast cancer

Benjamin Ebert, Cambridge (MA)
Clinical and biological consequences of clonal hematopoiesis

Yusuf Hannun, Stony Brook (NY)
Contributions of extrinsic factors to cancer

15:00 – 15:30 *Coffee Break*

15:30 - 17:00 **Tumour Microenvironment, Tumour Immunity**
Chair: Alexander Markham, Leeds

Miriam Merad, New York
Contribution of myeloid cells to tumour outcome

Guido Kroemer, Villejuif, Paris
Cell death in pathophysiology: inexorable, avoidable or desirable

Laurence Zitvogel, Villejuif, Paris
The unsuspected impact of the gut microbiota on cancer immunosurveillance

17:15 – 18:45 **Award ceremony**
Charles Rodolphe Brupbacher Prize for Cancer Research 2017
All participants are kindly invited to attend

19:00 – 19:30 *Apéro*

Friday, February 3, 2017

08:00 – 08:30 Coffee

08:30 – 10:00 Breakthroughs in Cancer Therapy
Chair: Miriam Merad, New York

Michael Hallek, Cologne
Towards control of chronic lymphocytic leukemia

Jedd Wolchok, New York
Combination checkpoint blockade:
combinations and mechanisms

Mark O'Connor, Cambridge (UK)
Development of olaparib: the first cancer therapy
based on a targeted DNA damage response inhibitor

10:00 – 10:30 Coffee break

10:30 – 11:30 Breakthroughs in Cancer Biology
Chair: Markus G. Manz, Zurichl

Alberto Bardelli, Turin
Cancer evolution as a therapeutic target

David Lane, Singapore
Drug discovery in the p53 pathway

11:30 – 12:00 Young Investigator Awards
Referees: Josef Jiricny, Nancy Hynes

Abstracts
Eingeladene Redner

Abstracts
Invited Speakers

Öffentlicher Vortrag:
Ist Hautkrebs heilbar?

Reinhard Dummer

Die Haut ist das Organ, das am häufigsten von einer bösartigen Erkrankung betroffen ist. Das Lebenszeitrisiko für einen Schweizer, eine bösartige Hautkrebserkrankung zu entwickeln, liegt bei etwa 30%.
Klinische Untersuchungen haben gezeigt, dass jeder zweite über 70-jährige Schweizer bereits von Hautkrebs betroffen war. Die häufigsten Hautkrebserkrankungen gehen von den verhornenden Keratinozyten (Oberhautzellen) aus. Dazu gehören die sogenannten Basalzellkarzinome und die spinozellulären Karzinome der Haut.
In der Oberhaut finden sich auch zahlreiche pigmentbildende Zellen, die den Ursprung für das Melanom der Haut (häufig schwarzer Hautkrebs genannt) darstellen.
Hautkrebserkrankungen können in der Regel durch operative Massnahmen oder auch durch Radiotherapie sicher geheilt werden. Deswegen kann man klar sagen: Die meisten Hautkrebserkrankungen sind heilbar.
Liegen jedoch Ableger in inneren Organen vor, waren die Überlebensaussichten insbesondere beim schwarzen Hautkrebs (Melanom) sehr schlecht. Bis zum Jahr 2010 sind wir davon ausgegangen, dass innerhalb eines Jahres 75% aller betroffenen Patienten an der Krankheit versterben müssen. Neue Entwicklungen haben nun diese schlechten Aussichten deutlich verbessert.
Molekularbiologische Untersuchungen konnten zeigen, dass sich das Melanom aus verschiedenen Untergruppen zusammensetzt. Je nach den im Erbgut vorliegenden genetischen Veränderungen können heute Behandlungen ausgewählt werden. Diese Behandlungen führen bei bis zu 80% der Patienten zu einem schnellen Rückgang der Ableger (Metastasen). Ein Teil der Patienten kann auch durch diese Behandlung über lange Zeit profitieren.
Die andere Revolution im Bereich der Hautkrebserkrankungen ist durch Erkenntnisse der Immunologie entstanden. Ausführliche Untersuchungen der Aktivierung von T-Lymphozyten (weisse Blutkörperchen) und die Blockierung ihrer Funktionen ergaben neue Zielstrukturen für die Immunbehandlung von Hautkrebserkrankungen. Der Erfolg dieser Behandlungen ist häufig nicht kurzfristig (innerhalb von Monaten) zu sehen, sondern langfristig. Er äussert sich über eine Gruppe von Patienten die ein Langzeitüberleben (> als 5 Jahre) erzielen. Der Anteil dieser Patienten hat in den letzten Jahren kontinuierlich zugenommen. Lag er im 2010 bei etwa 5% gehen wir heute davon aus, dass 40 – 45% aller Patienten ein Langzeitüberleben erreichen können.
Können wir diese Hautkrebserkrankungen heilen? Die Antwort lautet aus meiner Sicht zum Teil ja; aber es gibt noch viel zu tun.
Universitäre Einrichtungen sind hier sehr gefordert. Wichtig ist eine intensive Zusammenarbeit mit Patientengruppen (Selbsthilfegruppen) um den politischen Boden zu bereiten, dass diese Patienten in den Zentren behandelt werden, die in der Lage sind, Gewebeproben und Blutproben kontinuierlich zu analysieren und herauszufinden warum immer noch die Hälfte der Patienten nicht auf die oben genannten Behandlungen ansprechen. Solche Untersuchungen benötigen die Unterstützungen von medizinischen Bioinformatikern, die gewohnt sind mit grossen Datenmengen umzugehen und am allerwichtigsten ist und bleibt, dass Patienten so viel wie möglich im Rahmen von prospektiven kontrollierten Studien behandelt werden.

Polygenic susceptibility to colorectal cancer - mechanisms and impact

Richard Houlston

The Institute of Cancer Research, Sutton, UK

Inherited susceptibility contributes significantly to colorectal cancer (CRC) risk. High-risk mutations in APC and the mismatch repair genes account for <5% of all CRC and most of the heritable risk appears to be polygenic. This model of CRC has been supported by genome-wide association studies (GWAS). Individually, these variants have only a modest impact on CRC risk, but the combined effect of multiple single-nucleotide polymorphisms (SNPs) has the potential to achieve a degree of risk discrimination that is useful for population-based prevention and screening programmes. Mechanistically, the identification of this class of susceptibility has provided new insights into CRC biology. Many of the SNPs associated with CRC often reside within regulatory elements and exert effects through long-range regulation of gene expression. The application of Hi-C based strategies to the CRC risk loci is allowing us to identify key long-range chromatin interactions in cis and trans involving the gene regulatory elements that play a role in oncogenesis.

Systems biology of cancer

Jussi Taipale

Karolinska Institutet, Sweden

Cancer is the most complex genetic disease known – mutations in more than 380 genes have been associated with the formation of different types

of malignant tumors in humans. Yet, the malignant phenotype is simple, characterized by unrestricted growth of cells that invade neighboring healthy tissue and in many cases metastasize to distant organs. One possible hypothesis explaining the complexity of cancer genotypes is that oncogenic mutations would commonly activate cell type specific upstream mechanisms, which would then drive the expression of a common set of downstream genes responsible for the cancer phenotype. We are taking a systems-biology approach to identify such mechanisms, and to understand how lineage-specific factors collaborate with oncogenic signals to drive cell proliferation. For this purpose, we have developed computational and experimental methods to identify direct target genes of oncogenic transcription factors that are commonly activated in major forms of human cancer. In addition, we have used high-throughput RNAi screening to identify genes required for cell cycle progression. Combining these two sets of data allows the identification of specific transcription factors and gene regulatory elements that drive growth in particular tissues and tumor types. This analysis has identified MYC and CDK4/6/7/CCRK families as common targets of lineage-specific oncogenic pathways. The same mechanisms were also identified by analysis of common genome-wide association signals from multiple cancer types. Our results indicate that lineage-specific oncogenic transcription factors commonly regulate the same set of target genes important for growth control, and pave the way for the development of broadly-active antineoplastic and chemopreventive agents against cancer.

1. Sur, I., and Taipale, J. The role of enhancers in cancer. Nature Reviews Cancer, in press, 2016.

2. Jolma, A., Yin, Y., Nitta, K.R., Dave, K., Popov, A., Taipale, M., Enge, M., Kivioja, T., Morgunova, E. and Taipale. J. DNA-dependent formation of transcription factor pairs alters binding specificity. Nature 527: 384-388, 2015.

3. Yan, J., Enge, M., Whitington, T., Dave, K., Liu, J., Sur, I., Schmierer, B., Jolma, A., Kivioja, T., Taipale, M., and Taipale, J. Transcription factor binding in human cells occurs in dense clusters formed around cohesin anchor sites. Cell, 154: 801-816, 2013.

4. Jolma, A., Yan, J., Whitington, T., Toivonen, J., Nitta, K. R., Rastas, P., Morgunova, E., Enge, M., Taipale, M., Wei, G.-H., Palin, K., Vaquerizas, J. M., Vincentelli, R., Luscombe, N. M., Hughes, T. R., Lemaire, P., Ukkonen, E., Kivioja, T. and Taipale J. DNA-binding specificities of human transcription factors, Cell, 152: 327-39, 2013.

5. Sur, I., Hallikas, O., Vähärautio, A., Yan, J., Turunen, M., Enge, M., Taipale, M., Karhu, A., Aaltonen, L. A., and Taipale, J. Mice lacking a Myc enhancer element that includes human SNP rs6983267 are resistant to intestinal tumors. Science 338: 1360-3, 2012.

Cancer chromosomal instability, diversity and evolution: TRACERx

Charles Swanton

The Francis Crick Institute, London, UK

CIncreasing 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. Drivers of tumour heterogeneity will be presented that 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. Genome doubling, occurring early or late in tumour evolution, exacerbates chromosomal instability contributing to intercellular heterogeneity and poor outcome. Ongoing chromosomal instability is found to be a major driver of ongoing intratumour heterogeneity in non-small cell lung cancer, contributing to parallel evolution and selection. The finding of subclonal driver events combined with genome instability driving cell to cell variation is likely to limit the efficacy of targeted monotherapies, suggesting the need for new approaches to drug development and clinical trial design and integration of cancer immunotherapeutic approaches. Emerging data from TRACERx, a longitudinal lung cancer evolution study will be presented and the use of the clonal neo-antigenic architecture of tumours to develop precision immune-oncology will be discussed.

Hacking the hormone code in cancer

Myles Brown

Dana-Farber Cancer Institute and Harvard Medical School, Boston, USA

Endocrine therapies targeting the estrogen receptor (ER) are the mainstay of treatment for the majority of breast cancers. While ER antagonists and aromatase inhibitors are effective adjuvant therapy for patients with early stage ER+ breast cancers, patients with advanced disease invariably develop resistance. Using genome-wide CRISPR screens we have identified genes essential for the growth of ER+ breast cancers including key components of the ER signaling pathway such as ER itself, ER coregulators and transcription factors we previously identified as playing important roles in ER function. Importantly, we also identified genes whose loss confers endocrine resistance. Most strikingly, loss of CSK, a negative regulator of SRC family kinases (SFK), is sufficient to drive estrogen independent growth in culture and in xenografts and to confer resistance to ER antagonists. Examination of the ER cistrome revealed an ER bound enhancer upstream of the CSK transcription start site and deletion of this enhancer was also sufficient to block the estrogen induction of CSK and to promote estrogen independent growth. A synthetic lethality screen for genes essential in the absence of CSK identified the PAK2 kinase. Inhibitors of PAK2 or SFK block estrogen-independent breast tumor growth and synergize with the complete ER antagonist fulvestrant. These findings reveal an estrogen-induced negative feedback loop that constrains the growth of ER+ tumors thereby limiting the efficacy of therapies that inhibit ER and suggest a previously unappreciated therapeutic route to overcoming endocrine resistance in breast cancer.

Stromal niches in bone that mediate the seeding, progression and treatment resistance of metastatic breast cancer

Yibin Kang

Department of Molecular Biology, Princeton University, Princeton, USA

During cancer metastasis, disseminated tumor cells often hijack existing physiological cellular interactions to facilitate their seeding, survival and outgrowth in distant organs. Bone metastasis is a frequent occurrence in breast cancer, affecting more than 70 % of late stage cancer patients with severe complications such as fracture, bone pain, and hypercalcemia. The pathogenesis of osteolytic bone metastasis depends on cross-communications between tumor cells and various stromal cells residing in the bone microenvironment.

E-selectin is an adhesion molecule normally functioning to recruit leukocytes during infection or vascular damage. E-selectin is also thought to be a major component of the hematopoetic progenitor cell (HPC) niche in the bone. We show that E-selectin functions as an essential component of the endothelial niche for bone metastasis, wherein glycosylated E-selectin ligands expressed by metastatic breast cancer interact with endothelial E-selectin to promote the survival and proliferation of metastatic tumor cells.

We further identified Jagged1 as a TGFβ target gene in tumor cells that engaged bone stromal cells through the activation of Notch signaling to provide a positive feedback to promote tumor growth and to activate osteoclast differentiation. Using genetically modified mouse models, we revealed a surprising role of Jagged1 in promoting chemoresistance of bone metastasis. Chemotherapy of bone metastasis induced elevated expression of Jagged1 in osteoblasts, which provide a pro-survival niche for tumor cells in the bone.

These findings support the notion that development of organ-specific metastasis depends on the interactions between tumor cells and various stromal niche components in a given organ. Importantly, therapeutic targeting of Jagged1 and E-selectin significantly reduce bone metastasis and sensitize them to chemotherapy, suggesting possible avenues to dramatically improve the treatment of metastatic bone disease.

PI 3-kinase and cancer metabolism

Lewis C. Cantley

Meyer Cancer Center, Weill Cornell Medicine and New York Presbyterian Hospital, New York, NY, USA

In general cancer cells produce higher levels of reactive oxygen species (ROS) than normal cells due to increased rates of metabolism and defective mitochondria. In order to survive under conditions of high ROS, cancer cells typically turn on pathways for generating NADPH and glutathione to bring ROS levels back to homeostasis. Activating mutations in PIK3CA or loss of PTEN or activating mutations in KRAS can stimulate glucose uptake and metabolism and pathways for generating NADPH and glutathione to suppress ROS. A detailed understanding of the biochemical mechanisms by which cancer cells suppress excess ROS may suggest new therapies for inducing synthetic lethality in tumors that evolve in specific mutational backgrounds. Our research using human cancer cell lines and genetically engineered mouse models suggests new approaches for killing cancer cells by targeting metabolic pathways for ROS suppression that allow cancer cells to survive.

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2. DeNicola GM, Chen PH, Mullarky E, Sudderth JA, Hu Z, Wu D, Tang H, Xie Y, Asara JM, Huffman KE, Wistuba II, Minna JD, DeBerardinis RJ, Cantley LC. NRF2 regulates serine biosynthesis in non-small cell lung cancer. Nat Genet. 2015 Dec;47(12):1475-81. doi: 10.1038/ng.3421. Epub 2015 Oct 19. PMID: 26482881

3. Hu H, Juvekar A, Lyssiotis CA, Lien EC, Albeck JG, Oh D, Varma G, Hung YP, Ullas S, Lauring J, Seth P, Lundquist MR, Tolan DR, Grant AK, Needleman DJ, Asara JM, Cantley LC, Wulf GM. Phosphoinositide 3-Kinase Regulates Glycolysis through Mobilization of Aldolase from the Actin Cytoskeleton. Cell. 2016 Jan 28;164(3):433-46. doi: 10.1016/j.cell.2015.12.042. PMID: 26824656

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How and why RAS & MYC cooperate

Gerard Evan, Trevor Littlewood, Nicole Sodir, Roderik Kortlever, Luca Pellegrinet, Tania Campos

Department of Biochemistry and Cambridge Cancer Centre, University of Cambridge, UK

Cancers arise through somatic clonal accumulation of mutations that disrupt the intra- and extracellular networks that normally restrain untoward growth, proliferation, survival and invasion. As expected of a pathology arising through random mutation, adult cancers exhibit astonishingly genetic diversity, occasioning huge efforts to catalog and categorize them and, on the basis of their idiosyncrasies, develop tailored precision therapies. However, this preoccupation with the differences between, and within, individual cancers overlooks an equally remarkable feature: namely, that cancers of a particular tissue type look and behave remarkably similarly and share similar signature oncogenic mutations. Such tissue-specific uniformity indicates an underlying commonality in evolution and oncogenic mechanism that may, in turn, indicate common therapeutic vulnerabilities. To explore the basis of this remarkable, tissue-specific commonality, we have developed switchable genetic mouse models that allow for the real time toggling of Ras and Myc function in the epithelial compartments of differing target tissues in vivo. Ras and Myc are each, alone, relatively weak oncogenes but when combined they potently synergize to drive tumorigenesis. However, over 30 years on from the discovery of Ras/Myc cooperation, there is still no consensus as to the mechanisms that underlie that cooperation. The oncogenic capacity of Ras+Myc is tissue-agnostic, driving tumorigenesis in all tested tissues and implying they must interact with a common, tissue-independent interface. Nonetheless, the tumors they induce display the distinct tissue-specific phenotypes of spontaneous cancers arising in those same tissues; for example, generating highly angiogenic and inflammatory cancers in lung but highly avascular and desmoplastic cancers in pancreas. Hence, the signature phenotypes of cancers are inherent properties of each cell/tissue type. In normal cells, Ras and Myc act physiologically as common

downstream effectors for the diverse mitogenic signaling pathways that drive proliferation of cells and regeneration of tissues. We will demonstrate how oncogenic Ras and Myc hack the local regenerative programs of each tissue, leading to the distinct phenotypes of different cancers. Our data also imply that the distinctive features of advanced cancers, including pleiotropic stromal changes such as angiogenesis, activation of mesenchymal cells, inflammation and immune suppression, are not cancer neomorphisms or selected mechanisms of escape but, instead, attributes of normal tissue regeneration.

Mechanism and regulation of DNA-protein crosslink repair by the DNA-dependent metalloprotease SPRTN

Simon J. Boulton

The Francis Crick Institute, London, UK

Covalent DNA-protein crosslinks (DPCs) are caused by certain exogenous agents (e.g. ionizing radiation, UV light) as well as by endogenously produced reactive molecules (e.g. formaldehyde, acetaldehydes). Notably, formaldehyde is directly produced within chromatin as a by-product of the histone demethylation reaction. DPCs are highly toxic DNA lesions that interfere with essential chromatin transactions such as replication and transcription [1]. However, very little was known about DPC-specific repair mechanisms until the recent identification of a DPC processing protease in yeast, Wss1, which permits replication in the presence of DPCs and provides resistance towards DPC-inducing agents [2]. Intriguingly, Wss1 is a DNA-dependent protease, which degrades DNA-bound substrates in vitro irrespective of identity. Importantly, in Xenopus egg extracts a DPC containing plasmid is repaired by a similar mechanism indicating that protease-based DPC repair is conserved [3]. However, the identity of the DPC protease operating in frog extracts and in mammals has remained elusive.

Here we identify the metalloprotease SPRTN as the DPC protease acting in higher eukaryotes [4]. SPRTN-deficient cells accumulate DPCs and are hypersensitive to DPC-inducing agents. Using cellular, biochemical and crystallographic studies we establish the mechanism of SPRTN's catalytic activity, which is triggered upon DNA binding. We demonstrate that SPRTN undergoes autocatalytic cleavage upon binding to double or single stranded DNA. Strikingly, however, substrates are only degraded when bound to single stranded DNA revealing that autocleavage and substrate cleavage occur by distinct mechanisms. Given that SPRTN is associated with the replisome these data raise the possibility that DPCs may be specifically processed on ssDNA when exposed upon replication fork stalling. Structural analysis of the protease domain coupled with SAXS and hydrogen/deuterium exchange mass-spec data have revealed that DNA-dependent catalysis is associated with a conformational change in the catalytic site, which allows the enzyme to engage with itself or its substrates dependent on the mode of DNA binding. We assume that this regulatory mechanism is important to prevent promiscuous proteolysis of non-DNA bound proteins. Our studies also reveal that SPRTN's potentially toxic proteolytic activity is constrained by an ubiquitin-switch that controls lesion accessibility. SPRTN is constitutively mono-ubiquitylated in cells, a form that is excluded from chromatin. Upon detection of DPCs, SPRTN is rapidly de-ubiquitylated allowing it to engage with its substrates. Finally, SPRTN deficiency in humans results in Ruijs-Aalfs syndrome, which is characterized by genomic instability, premature aging and early-onset hepatocellular carcinoma [5]. Our detailed analysis of the molecular defects of SPRTN patient variants provides the first molecular explanation for the pathology of this disease. We propose that faulty DPC repair is the molecular defect underlying Ruijs-Aalfs syndrome [4].

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Rapid evolution of the cancer karyotype

David Pellman

Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA

Recent genome sequencing has uncovered new patterns of mutagenesis that appear to originate from localized bursts of chromosome rearrangement. One such pattern is chromothripsis, where extensive chromosome rearrangement occurs on only one or a few chromosomes, distinguished by a unique DNA copy number pattern. We developed an approach to combine live-cell imaging and single cell genome sequencing (Look-Seq) that enabled us to recreate chromothripsis in the laboratory, demonstrating that it can be generated within a single cell cycle from aberrant nuclear structures (micronuclei) that are common in cancer. We demonstrated that chromothripsis can generate small chromosomal circles, the first step in the formation of double minute chromosomes, a major source of oncogene amplification. These findings begin to develop a mechanistic basis for chromothripsis, potentially providing generalizable insights into the role of altered nuclear architecture in cancer. Here, I will discuss our progress in understanding the mechanism of chromothripsis. I will discuss the contributions of both chromosome fragmentation and localized abnormalities in DNA replication (chromoanasynthesis). I will describe new experiments suggesting that unscheduled DNA replication during mitosis may be a source of localized chromosome rearrangement.

Aneuploidy and cancer – a complicated relationship

Stefano Santaguida, Jason Sheltzer, Sarah Pfau and Angelika Amon

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Aneuploidy is a hallmark of cancer. Changes in chromosome copy number have been proposed to drive disease by modulating the dosage of cancer driver genes and by promoting cancer genome evolution. Given the potential of cells with abnormal karyotypes to become cancerous, a key question is whether pathways exist that limit the prevalence of such cells. We established a system that allows us to induce chromosome mis-segregation at will and to investigate the immediate consequences of aneuploidy on cellular physiology. This analysis led to two remarkable discoveries. First, we found that chromosome mis-segregation causes DNA replication defects and DNA damage in the subsequent cell cycle. This observation shows that whole chromosome gains and losses are sufficient to precipitate genomic instability, which could explain why most cancer genomes are unstable. Second, we found that cells with highly aneuploid karyotypes induce an inflammatory gene expression signature. This gene expression signature is biologically relevant. Highly aneuploid cells are recognized and eliminated by natural killer (NK) cells in vitro. These findings suggest that cells with highly aberrant karyotypes are recognized and eliminated by the innate immune system. We propose that cells with abnormal karyotypes generate a signal for their own elimination that may serve as a means for cancer cell immunesurveillance.

Genetic determinants of the epigenome in development and cancer

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DNA methylation patterns are often disrupted in cancer, but neither the origins nor the consequences of these epigenomic shifts are fully understood. In part this reflects our incomplete understanding of the functional significance of this epigenetic mark in maintaining gene expression programmes. A small number of genes are silenced by DNA methylation during development, but switching of most genes with CpG island promoters does not involve DNA methylation changes. In cancer, however, inappropriate de novo methylation of CpG island promoters is invariably associated with silencing and these abrupt, but discrete, DNA methylation changes are often accompanied by long-range modulation of the low-density methylation that pervades most of the genome. The latter phenomenon has received relatively little attention, although long-standing evidence suggests that low-density methylation inhibits weak promoters and may therefore function to reduce baseline transcription. Methyl-CpG binding proteins, which recruit various histone deacetylase complexes may help to mediate these effects. We are studying the origin of changes in the epigenome, including DNA methylation, in development and cancer. Our hypothesis is that proteins recognizing short, frequent DNA sequence motifs play a major role and we have found several candidates that were previously implicated as regulators of pluripotency and cancer. Biochemical and genetic evidence

supports the view that gene expression within multi-gene domains is tuned by modulating the epigenome in this way.

Context-dependent roles of polycomb repressors in cancer and development

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Repressive Polycomb-group (Pc-G) protein complexes and the counteracting Trithorax-group (Trx-G) of nucleosome remodeling factors are involved in the dynamic maintenance of proper gene expression patterns during development, acting at the level of chromatin structure. As such, they are important controllers of cell fate and differentiation, by acting to stabilize cell fate. When deregulated, these master switches of gene expression are strongly implicated in formation of a diverse set of cancers. Examples are the Pc-G gene Bmi1 and Ezh2 which are overexpressed in many cancers including Glioblastoma. This opened up large interest from Pharma companies to develop specific Ezh2 inhibitors for therapeutic purposes. However, recently Ezh2 has also been found to be mutated/inactivated in several forms of cancer, suggesting a highly context-dependent role as either an oncogene or tumor suppressor. This underscores the importance of how to unravel and understand these context dependencies in relevant preclinical models. We will discuss a recent prime example, studying the driver-mutation depend effects of gain or loss of Ezh2/PRC2 in non-small cell lung cancer driven by mutant Kras. This highlights important consequences for tumor progression and epigenetic treatment options, which will be discussed.

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DNMT3A in hematopoietic stem cells, cancer and aging

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DNA methyltransferase 3a (DNMT3A) has recently emerged as an important tumor suppressor in hematologic malignancies, and its ablation in mouse hematopoietic stem cells inhibits differentiation. We will describe the use of DNMT3A knockout mice to study its role in myeloid and lymphoid malignancy development and its function in maintaining global DNA methylation in normal and aging hematopoietic stem cells.

Heterogeneity in Breast Cancer

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With rare exceptions tumors are thought to originate from a single cell. Yet, at the time of diagnosis the majority of human tumors display startling heterogeneity in many structural and physiological features, such as cell

size, shape, metastatic proclivity, and sensitivity to therapy. This intratumor heterogeneity for heritable traits is a fundamental challenge in breast cancer, underlying disease progression and treatment resistance. Yet our understanding of its mechanisms, and as a consequence, our ability to control it remains limited. This is largely due to the cancer-gene and cancer cell-focus of mainstream cancer research and the reliance on experimental models that poorly reproduce this key aspect of the human disease.

We have been developing methods that allow for the assessment of cellular genetic and phenotypic heterogeneity within tumors at the single cell level in situ. Using these methods, we have analyzed breast tumor samples before and after pre-operative chemotherapy, or at different stages of disease progression. We found that tumors with the lowest pretreatment genetic diversity responded the best to treatment and that distant metastatic lesions had higher genetic diversity compared to primary tumors and lymph node metastases. More recently we have also been analyzing changes in immune cell composition during breast tumor progression and how this may impact selection for cancer cells with features that facilitate immune escape.

Lastly, we have been working on developing experimental models of intratumor clonal heterogeneity in breast cancer and utilize these to assess the functional relevance of clonal interactions in metastatic progression and therapeutic responses. We found that polyclonal tumors are commonly metastatic, even though none of the individual clones present in them showed this behavior in monoclonal tumors.

Our results emphasize the need to study tumors as a whole and to understand the properties of cancer cell populations that compose tumors and interactions among them to be able to predict tumor evolution during disease progression and cancer therapies.

Clinical and biological consequences of clonal hematopoiesis

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Clonal hematopoiesis of indeterminate potential (CHIP) is a common, age-associated condition. CHIP is defined by the presence of clonal, somatic mutations in the blood. We have characterized the presence of clonal, somatic mutations in exome sequencing data from peripheral blood DNA from over 60,000 individuals and examined the phenotypic consequences of these mutations. The mutations identified in CHIP are the same as those found in hematologic malignancies such as myelodysplastic syndrome and myeloproliferative neoplasms. Indeed, the mutations identified in CHIP, including mutations in DNMT3A, TET2, and ASXL1, are lesions that are commonly acquired early in the genetic ontogeny of hematologic malignancies, prior to the development of overt disease. Consistent with the concept that CHIP is a pre-malignant state, CHIP is associated with an increased risk of hematologic malignancy. Individuals with CHIP have a 10-fold increase in the risk of hematologic malignancies, and approximately 0.5 to 1% of individuals with CHIP progress to hematologic malignancy per year. Individuals with CHIP, generally bearing just a single mutation in a driver gene, do not have altered blood counts, but do have an elevated red blood cell distribution width (RDW). Clonal mutations are present in terminally differentiated blood cells and have the potential to alter the phenotype and functional properties of blood cells. Most strikingly, individuals with CHIP have a 40% increase in overall mortality which is due, at least in part, to an elevated risk of cardiovascular disease. Some aspects of the aging phenotype of hematopoiesis may be related to the acquisition of clonal, somatic mutations.

Contributions of extrinsic factors to cancer

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The determination of the contributions of extrinsic vs intrinsic factors to the development of cancer is of high significance as it relates to our understanding of cancer pathogenesis. Moreover, such determinations have consequences to defining public policies and the allocation of resources towards cancer prevention. These issues have been the subject of a recent 'debate'. We have advanced several lines of investigation that suggest that extrinsic factors contribute at least 70% of cancer risk. These involve a reanalysis of results that were recently compiled on the correlation of cancer risk to stem cell division. We conducted a thought experiment that demonstrates that this correlation does not imply causation, and more importantly, it does not distinguish the contribution of extrinsic factors from intrinsic ones. We analyzed a multitude of epidemiologic data. We also analyzed recent results on mutational signatures in cancer. Finally, we developed a novel biologically-based model to evaluate cancer risk. The concordance between these (mostly orthogonal) approaches was very high. These results will be presented and discussed.

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Contribution of myeloid cells to tumour outcome

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My laboratory studies the mechanisms that control the development, homeostasis and function of tissue resident myeloid cells, which mostly consist of macrophages and dendritic cells.
Macrophages are hematopoietic cells that perform tissue-specific functions critical for regulating and maintaining organ homeostasis. In contrast to most other hematopoietic cells, macrophages that reside in quiescent tissues originate from early hematopoietic precursors that take residence in tissues prior to birth. In this presentation, I will discuss how tissue cues control macrophage functional diversity to ensure tissue integrity. I will also discuss recent data from our laboratory showing that developmental diversity contributes to shaping macrophage functional specialization and that ontogenically distinct macrophages differently regulate tumour response to radiation therapy.
In contrast to macrophages, DCs are constantly repopulated by DC restricted precursors that are recruited from the blood circulation. Our laboratory has contributed to the mapping of the regulatory network of DCs, and the identification of a lineage of DC, the CD103+ DC, which are specialized in the induction of CD8+ T-cell immunity. Here I will discuss recent data from the laboratory that revealed the critical role for tumour-associated CD103+ DC in tumour response to checkpoint blockade and argue for the need to study human CD103+ DC equivalent in clinical Cancer Immunotherapy

Cell death in pathophysiology: inexorable, avoidable or desirable

Guido Kroemer (1-6)

(1) Equipe 11 labellisée par la Ligue contre le Cancer, Centre de Recherche des Cordeliers; Paris, France; (2) Cell Biology and Metabolomics platforms, Gustave Roussy Cancer Campus; Villejuif, France; (3) INSERM, U1138, Paris, France; (3) Université Paris Descartes, Sorbonne Paris Cité; Paris, France; (4) Université Pierre et Marie Curie, Paris, France; (5) Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP; Paris, France; (6) Karolinska Institute, Department of Women's and Children's Health, Karolinska University Hospital, Stockholm, Sweden

Beyond the few potentially immortal gametes that our body produces, mortal (somatic) cells fall into two classes, those that are constantly renewed from proliferating stem cells, such as epithelial cells and leukocytes, and those that are not (or scarcely) renewed after birth, such as neurons or cardiomyocytes. While the former are programmed to undergo cell death and are replaced throughout life, the latter must endure until we expire. Excessive cell death, in particular in post-mitotic tissues, precipitates degenerative states, while the failure to timely execute death in renovating tissues contributes to hyperplasia and cancer. Our initial contribution to cell (death) biology consisted of the discovery that mitochondrial membrane permeabilization marks the inexorable point of no return of lethal pathways, explaining why the inhibition of proteases and nucleases that contribute to cellular dismantling downstream of mitochondria cannot provide durable cytoprotection. We also demonstrated that autophagy is not a self-destructive pathway, as had initially been thought, but rather a potent cytoprotective mechanism that, if induced at the whole-organism level, can increase the longevity of several animal species. Thus, autophagy contributes to the avoidance of the death of cells and organisms within the context of hormesis, the phenomenon whereby exposure to low, sublethal doses of an otherwise toxic agent subsequently increases resistance against damage and death. More recently, we invalidated the dogma that apoptosis, which is one particular modality of programmed or regulated cell death, is by definition a non-immunogenic cell death modality. We found that, depending on the upstream triggers and the premortem stress responses, apoptosis can be immunogenic and hence alert the innate immune system, instructing it to stimulate specific responses against dead-cell antigens. Several successful anticancer drugs that have saved millions of life-years induce this particularly 'desirable', immunogenic cell death type and hence convert cancers into a therapeutic vaccine that (re)activates tumor-specific immune responses. The induction of autophagy

is one of the premortem events that favors the immunogenicity of apoptotic corpses.

The unsuspected impact of the gut microbiota on cancer immunosurveillance

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Cancer susceptibility and progression result from a complex interplay between gene regulation and the environment[1]. Many epithelial and hematopoietic neoplasias are under strong immuno-surveillance, as indicated by numerous studies revealing that the density, composition and functional state of immune infiltrates dictates patient prognosis and therapeutic response to adjuvant or neoadjuvant chemotherapy[2-4] and immune checkpoint blockers[5-7]. The recognition of cancer cells by immune effectors requires two parameters, namely, antigenicity (the presence of tumor-associated antigens that usually either result from mutations yielding mutated proteins or the ectopic expression of genes/proteins that are usually only transcribed/translated in embryonic development or in the testis) and adjuvanticity (the presence of co-stimulatory signals that activate innate immune effectors).

Communities of commensal microbes inhabiting our intestine influence the surrounding environment, and appear to play a role not only in intestinal carcinogenesis, but potentially throughout the body[8]. Some observations suggest that distinct bacteria have a beneficial role in combatting cancer[9-11]. First, total body irradiation promoted a LPS/TLR4-dependent activation of antigen presenting cells, thereby facilitating the efficacy of adoptive T cell transfer[12]. Second, ROS and TNFα-mediated antitumor effects of tumor infiltrating myeloid cells were mediated by bacterial TLR4 agonists during platinum-based anticancer therapies and an immuno-modulatory regimen[13]. We reported that the antitumor efficacy of metronomic dosing of the alkylating agent cyclophosphamide (CTX) was compromised in germ-free mice, or in animals treated with broad-spectrum antibiotics. Indeed, CTX altered the integrity of the intestinal barrier, promoting the translocation of Gram+ bacteria that mounted effector pathogenic $CXCR3^+CCR6^+$ ($IL\text{-}17^+IFN\gamma^+$) Th17 (pTh17) and memory Th1 immune responses associated with tumor control[14]. Our group identified E. hirae and Barnesiella intestinihominis, which act in concert, in altering the tumor microenvironment post-CTX[17]. Importantly, we showed that CD4 positive Th1 immune responses towards E. hirae (strain 13144) or B. intestinihominis that are memory MHC class II-restricted were associated with prolonged progression-free survival in end stage lung and ovarian cancer patients who were previously treated with chemotherapy[35]. Finally, we and others extended these findings to immune checkpoint blockers, demonstrating that Bacteroidales and Burkholderiales or Bifidobacteriales orders influenced the tumor microenvironment (TME) contributing to the efficacy of anti-CTLA4 or anti-PDL-1 Ab respectively[15, 16]. Hence, we postulate that the intestinal ecosystem controls not only the gut, but also provokes systemic effects that shape the tumor microenvironment.

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Towards control of chronic lymphocytic leukemia

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BIOLOGY: The use of whole exome sequencing and large, annotated clinical CLL databases has allowed describing the genomic landscape in CLL. Recurrent mutational patterns suggest that inflammatory pathways, B-cell receptor signaling and differentiation, Notch signaling, Wnt signaling, DNA damage control, chromatin modification and RNA and ribosomal processing are frequently altered in CLL (Landau et al., 2015). In addition, the interaction of CLL cells with their microenvironment is essential for CLL pathogenesis. In knock out models, leukemia associated macrophages (LAM) were shown to be important components of the microenvironment of CLL cells (Reinart et al., 2013). Even conventional therapeutics such as chemotherapy with alkylators and monoclonal antibodies mediate their effects through compartment restricted interactions with macrophages (Pallasch et al., 2014). Finally, agents supposed to exclusively target B-cell receptor associated kinases such as Lyn or BTK seem to exert essential effects through the modulation of the leukemic microenvironment, since targeted deletions of these kinases reduce the capacity of macrophages to "feed" CLL growth (Nguyen et al., 2016).

PROGNOSIS: The clinical course of CLL is highly variable but can be predicted today by a plethora of clinical, biological, genetic and molecular factors (Cramer and Hallek, 2011). The key challenge is to define a limited number of meaningful tests to predict the prognosis and/or response to treatment with reasonably high reliability. Based on a recent effort to construct a comprehensive prognostic score (Pflug et al., 2014), we have teamed up with an international consortium of study groups to create the CLL International Prognostic Index (CLL-IPI). Data of 3472 treatment-naïve patients from France, Germany, UK, USA and Poland were used, and multivariate analyses were performed using 27 baseline factors and overall survival (OS) as endpoint. Two separate datasets of 838 patients from the Mayo Clinic and 416 from a Scandinavian population-based cohort were added for external validation. Five independent prognostic factors were identified: TP53 deletion and/or mutation, IGHV mutational status, serum β2-microglobulin, clinical stage, and age. Using a weighted grading of the independent factors, a prognostic index was derived separating four risk groups with significantly different OS at five years: low (93·2%), intermediate (79·3%), high (63·3%), and very high risk (23·3%) [$P < 0{\cdot}001$; C-statistic, $c=0{\cdot}723$]. These risk groups were confirmed by all validation datasets. The CLL-IPI will allow creating sufficiently homogenous populations of patients at high risk to be included in trials on innovative drugs, while sparing others from unnecessary therapies.

THERAPY: Inspired by some of the above progress in our understanding of the biology of CLL, new therapeutic options have become available. As a consequence, the management of patients with CLL is currently undergoing significant changes. During the last decade the outcome of first-line therapies was markedly improved. It could be demonstrated for the first time that the choice of an initial treatment (by adding CD20 antibodies to chemotherapy) created a survival benefit for young, fit and elderly, unfit CLL patients (Goede et al., 2014; Hallek et al., 2010). More recently, with the advent of targeted agents such as ibrutinib (Byrd et al., 2013), idelalisib (Furman et al., 2014), or venetoclax (Roberts et al., 2016; Stilgenbauer et al., 2016), our therapeutic

armamentarium has increased even further. It is now our task to optimize therapeutic combinations to create long-lasting remissions, if not cure for CLL patients. One of these concepts uses sequential, targeted therapies to eradicate residual disease. The treatment intensity is tailored by assessing minimal residual disease (Hallek, 2013). First results obtained by this type of therapy have been promising.

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Combination checkpoint blockade: combinations and mechanisms

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Given the activity noted with both CTLA-4 or PD-1 blockade, clinical trials are now investigating combination checkpoint blockade. The most mature data with a combination of ipilimumab + nivolumab in melanoma showed a response rate of 60% in the context of increased, yet manageable toxicity. Such responses are generally durable, even when treatment was stopped early for toxicity. Unlike in studies of PD-1 blockade monotherapy, there was no significant difference in clinical activity based on tumor expression of PD-L1. This approach has gained regulatory approval for metastatic melanoma and is in late stage clinical trials for other malignancies. Attention is being paid to the reasons underlying the efficacy of checkpoint blockade in certain malignancies. One hypothesis has been that cancers having a high mutational load may be more amenable to immune modulation by virtue of the larger number of potential neo-epitopes present, fostering baseline immune recognition that can then be potentiated by checkpoint blockade. We have found that melanoma patients having long term clinical activity with ipilimumab have a significantly greater median number of non-synonymous passenger mutations, compared with patients who do not respond or those who have only short-term regression. Strategies to enhance baseline immune reactivity are therefore necessary to investigate as means to improve the impact of checkpoint blockade on a broad spectrum of cancers. The presence of suppressive myeloid cells in the tumor microenvironment also is emerging as a mechanism of resistance to the anti-tumor activity for checkpoint blockade. Strategies to overcome this include inhibition of CSF-1R signaling, IDO activity and selective suppression of PI3K-γ.

Development of olaparib: the first cancer therapy based on a targeted DNA damage response inhibitor

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An underlying hallmark of cancers is their genomic instability (1), which is associated with a greater propensity to accumulate DNA damage. Historical treatment of cancer by radiotherapy and DNA-damaging chemotherapy is based on this principle, yet it is accompanied by significant collateral damage to normal tissue and unwanted side effects. Targeted therapy based on inhibiting the DNA damage response (DDR) in cancers, offers the potential for a greater therapeutic index by tailoring treatment to patients with tumours lacking specific DDR capabilities.

There are at least three key aspects of DDR that are different in cancers compared with normal cells that provide an opportunity to target DDR and generate new cancer therapies (2). These are represented by a greater level of endogenous DNA damage in cancers, increased levels of replication stress and the loss of one or more DDR pathway or capability that occurred during oncogenesis. Exploiting a DDR dependency using a small molecule inhibitor to generate cancer-specific cell killing has also been described as synthetic lethality and was exemplified preclinically by a thousand-fold increase in the sensitivity of BRCA deficient cells to poly (ADP-ribose) polymerase (PARP) inhibitors than BRCA proficient cells (3). The clinical validation of this synthetic lethality using the oral PARP inhibitor olaparib (4, 5) has led to the approval of the first DDR-based cancer medicine in ovarian cancer.

This presentation will cover both the development history and challenges leading to the regulatory approval of olaparib, as well as the potential for further expanding the clinical utility of PARP inhibitors beyond the treatment of BRCA mutant ovarian cancer.

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Cancer evolution as a therapeutic target

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When metastatic cancers are challenged with targeted agents, almost invariably a subset of cells insensitive to the drug emerges. As a result, in most instances, targeted therapies are only transiently effective in patients. Strategies to prevent or overcome resistance are therefore essential to design the next generation of clinical trials. How can we overcome the near-certainty of disease recurrence following treatment with targeted agents? To address this question, a deeper understanding of the evolutive nature of cancer cells is necessary. We used colorectal cancer (CRC) as a model system to test the hypothesis that the emergence of drug resistance can be controlled through understanding tumor evolution. We find that clonal dynamics can be monitored in real time in the blood of patients, and liquid biopsies can be used to intercept the emergence of resistant clones before relapses are clinically manifest. We discovered that a multistep clonal evolution process driven by progressive increases in drug fitness underlies the development of resistance in cells and patient avatars. To have long-term efficacy, the use of targeted therapies must take into account the continuous evolution of cancer cells, that is to say, therapies must adapt to tumor evolution. One possibility is to anticipate the changes the tumors will make. For example, by knowing in advance how CRC cells overcome resistance to EGFR blockade, we devised further rounds of therapy. Another approach is to unleash the ability of cancer cells to be recognized by the immune system. We tested this possibility in syngeneic mouse models of colorectal tumors. Our findings indicate that inactivation of DNA repair triggers dynamic neoantigen evolution, impairs cancer growth and leads to prolonged therapeutic responses.

Drug discovery in the p53 pathway

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Most current small molecule drugs that have found clinical utility follow Lipinski's "rule of five" which describe the tolerated size, chemical and biophysical properties of our current "block buster" orally active drugs. These constraints are however broken by a number of natural products such as cyclosporin, which despite violating Lipinski's rules are still very effective medicines. The advantage of such natural products is their ability to act on targets that lack the small hydrophobic binding pockets normally targeted by more conventional drugs. This enhanced target space is especially valuable when devising drugs that target the intracellular protein protein interactions that control many signaling pathways. In an attempt to mimic the action of these natural products we have been examining the use of stapled peptides and mini-proteins as intracellular therapeutics. These approaches allow the development of exceptional molecules in terms of ligand interaction but the challenge of understanding intracellular delivery remains. The p53 pathway has proved to be an ideal test system in which to explore routes of delivery and our latest results will be presented that suggests the existence of novel pathways from the cell surface to the cell nucleus. The existence of such novel potential cell signaling pathways opens up many targets for drug discovery that were previously elusive. Already the stapled peptides that we have produced that target Mdm2 and Mdm4 have shown a number of properties that distinguish them from the currently available small molecules that target this interaction. Firstly they show exceptionally high affinities for their targets, secondly it has been possible to design molecules that bind to both Mdm2 and Mdm4 of both human and mouse origin, something that has eluded the designers of current small molecules and finally using a powerful system of protein evolution we were readily able to develop variants of Mdm2 that were resistant to small molecule inhibitors but not to the stapled peptides. X-ray structure analysis confirmed modeling studies that this was due to the much larger region of Mdm2 involved in binding to

the stapled peptide that exactly mimicked the physiological interaction with p53. A deeper understanding of the requirements for effective uptake and target engagement by this new class of molecules promises to open up an exceptional prospect for a new class of therapeutic entities.

Poster Abstracts

1

MiR-29b Mediates NF-kB signaling in KRAS-induced non–small cell lung cancers

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microRNAs (miRNAs), short regulatory sequences at the posttranscriptional level, are important mediators of signaling pathways that act as backups of transcriptional control. To identify miRNAs implicated in epidermal growth factor receptor (EGFR) signaling, transformed bronchial epithelial BEAS-2B cells were retrovirally transduced with KRASG12V and alterations in miRNA expression were assessed by microarray analysis. Here we show that miR-29b is significantly induced by mutant KRAS in bronchial epithelial and non-small cell lung cancer (NSCLC) cell lines as well as in primary NSCLC tissue. In agreement with these results, inhibitors of EGFR and MEK resulted in reduced levels of miR-29b while inhibitors of PI3K had no effect. KRASG12V-transduced BEAS-2B cells were significantly more protected from extrinsic apoptosis than control transduced cells, but co-transduction of cells with KRASG12V and anti-miR-29b constructs sensitized cells to apoptosis indicating that miR-29b is a mediator of KRAS-induced resistance to apoptosis. Protection from extrinsic apoptosis was due to enhanced nuclear factor κB (NF-κB) activity. The ubiquitin-editing enzyme tumor necrosis factor alpha-induced protein 3 (TNFAIP3/A20) is a negative regulator of NF-κB signaling. Enhanced NF-κB activity elicited by miR-29b was due to targeting TNFAIP3/A20. Overexpression of miR-29b-refractory TNFAIP3 restored NF-κB activity as well as extrinsic apoptosis, demonstrating that TNFAIP3 is a relevant target of miR-29b. Interestingly, miR-29b conferred sensitivity to cisplatin-induced intrinsic apoptosis by targeting Mcl-1. Thus, miR-29b tips the balance from extrinsic apoptosis towards intrinsic apoptosis. Our results indicate that miR-29b can act either as an oncogene or tumor suppressor gene depending on the external stimulus.

2

Lymphotoxin-beta receptor signalling regulates self-renewal during stress induced hematopoiesis

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Aim: TNF receptors (TNFRs) are involved in multiple pathways mediating apoptosis, proliferation and cell survival. TRAF-binding TNFRs are predominantly expressed on Hematopoietic stem cells (HSCs) and immune cells. The HSCs are responsible to replenish all blood cell lineages. The balance between self-renewal, proliferation and quiescence is tightly regulated to remain the stem cell pool and to guarantee the differentiation into all hematopoietic cell lineages during homeostasis and stress-induced hematopoiesis. Whether TNFRs play a role in regulation of self-renewal in HSCs remain elusive. Here, we examine the impact of TNFR lymphotoxin-beta receptor (LTβR) on HSCs during steady-state and stress-induced hematopoiesis.

Material and Methods: HSC numbers and function in the bone marrow during steady-state hematopoiesis were studied in LTβR-/- and C57/BL6 mice. To study the role of LTβR-signalling during hematopoietic reconstitution, we performed serial competitive repopulation assays by generating mixed chimeras with LTβR-/- and C57/BL6 LSK cells. To address whether LTβR-signalling regulates HSC dormancy, we evaluated the effects of genotoxic stress mediated by 5-fluorouracil (5-FU) exposure on HSCs.

Results: We show for the first time that LTβR is expressed by the majority of HSCs and progenitor cells. LTβR-signalling did not affect steady-state hematopoiesis; however, during serial transplantation experiments, LTβR-signalling led to increased asymmetric division and proliferation, resulting in HSC exhaustion. Furthermore, genotoxic stress promoted increased cell cycling in LTβR-deficient HSCs.

Conclusion: Our results identify a novel negative regulatory role for the TNFR LTβR in balancing self-renewal and dormancy of HSCs during-stress-induced hematopoiesis.

3

Using phosphoproteomics for investigating MET oncogene addiction and the MET-DDR crosstalk

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The MET receptor tyrosine kinase has acquired widespread attention in the field of cancer research since its discovery, which occurred thirty years ago. Indeed, its role in promoting oncogenic processes, invasiveness and metastatic events in numerous cancers has been well established. Moreover, MET inhibition (METi) was found to confer radiosensitivity to MET-addicted cell lines, potentially through the complex re-wiring of DNA damage response (DDR) signalling pathways following acute disruption of oncogene activity. The concept of oncogene addiction was coined more than a decade ago to describe that, despite the diverse array of genetic lesions that characterize cancer cells, some tumors rely on individual dominant oncogenes for growth and survival. Implicit in this dependency is the extremely sensitivity to the targeted inhibition of the addicting oncogene, thus revealing a promising Achilles' heel of cancer cells. While accumulating data have proven the importance of this concept both in basic research as well as in therapeutic settings, the molecular pathways underlying this phenomenon and the link with DDR remain poorly understood. Therefore, the major aim of this project is to identify a signature of protein phosphorylation that is descriptive for METi-mediated radiosensitivity observed in MET-addicted cellular models and that could predict the response of MET-positive cellular models to MET inhibition (METi), alone or in combination with ionizing radiation (IR). To this purpose, we performed a targeted proteomics approach based on Selected Reaction Monitoring (SRM) in order to monitor phosphorylation changes of 127 candidate proteins in nine MET-positive cellular models upon METi, IR and the combination of the two perturbations. Remarkably, METi and IR appear to regulate the phosphorylation status of a subset of analyzed proteins in a synergistic manner in MET-addicted cellular models. Moreover, METi alone seems to control the phosphorylation status of key players of DDR signaling pathways in MET-addicted cell lines. Interestingly, not only crucial regulators of DDR-related processes, but also key nodes of a plethora of other cellular processes seem to be regulated in MET-addicted cellular models upon METi. Finally, subsequent analysis of cellular models addicted to other oncogenes upon exposure to their respective inhibitors, alone or in combination with IR, seems to suggest that the composite phosphosignature identified has the potential to describe addiction to other oncogenes beyond MET, suggesting that oncogene addiction is governed by shared dominant pathways that, once disrupted, lead to radiosensitivity. In this respect, the identification of a composite phosphosignature for oncogene addiction would allow more accurate patient stratification and personalized clinical settings, both for single agents as well as for combination therapies.

4

TGF-β pathway-mediated escape from VEGF blockade is linked with angiogenesis and immune-suppression in murine glioma models

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The vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β are key target molecules in glioblastoma that are interdependently regulated. Both pathways drive key malignant features in glioma cells such as angiogenesis, invasiveness and immunosuppression. Here we explore whether TGF-β acts as an escape pathway from VEGF inhibition using gene expression profiling and in vitro and in vivo studies in a panel of syngeneic mouse glioma models. In vivo, single agent activity was observed for the VEGF antibody B20-4.1.1 in three (SMA-540, SMA-560 and GL-261), and for the TGF-β receptor I antagonist LY2157299 in two (SMA-540 and SMA-560) of four mouse glioma models. Transcriptomic profiling of the four mouse glioma models revealed that SMA-497 and GL-261, while unresponsive to LY2157299, were the most immunogenic tumors as defined by Gene Ontology; specifically, up-regulation of chemokine/chemoreceptors genes involved in immune cell recruitment and interferon-related genes were found. Co-targeting of VEGF and TGF-β was ineffective in one refractory model

(SMA-497), in which no major changes in tumor immune-infiltrating cells were detected upon mono- or co-treatment settings. Significant prolongation of survival in the GL-261 model was associated with early stage increased infiltration of CD8+ cells, lower numbers of CD11b+ macrophages/microglia and sustained suppression of angiogenesis compared to anti-VEGF treatment alone. Phosphorylated SMAD2 was increased in both tumor cells and CD45+ leukocytes during anti-angiogenic treatment pointing to the possibility of a TGF-β-induced immunosuppressive micro-milieu that is efficiently counteracted by concomitant administration of LY2157299.
In conclusion, our study highlights the biological heterogeneity of glioblastoma even among simple mouse models and illustrates that co-targeting of the VEGF and TGF-β pathways might lead to improved tumor control in subsets of glioblastoma linked with angiogenesis impairment and general reduction of the immune-suppressive phenotype

5

BRCA1-regulated RRM2 expression protects glioblastoma cells from endogenous replication stress and promotes tumorigenicity

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Error-free and timely regulation of cell cycle progression is crucial for genome integrity maintenance. Cells are particularly sensitive during S phase when DNA damage causes replication fork stalling or collapse, collectively referred to as replication stress (RS), one of the emerging hallmarks of cancer. Our recent study showed that BRCA1, traditionally regarded a tumor suppressor, plays an unexpected tumor-promoting role in glioblastoma (GBM). Higher BRCA1 positivity was associated with shorter survival of glioma patients and the abrogation of BRCA1 function in GBM led to enhanced RS, DNA damage (DD) accumulation and impaired tumor growth. Mechanistically, we identified a novel role of BRCA1 as a transcriptional co-activator of RRM2 (catalytic subunit of ribonucleotide reductase), whereby BRCA1-mediated RRM2 expression protects GBM cells from endogenous RS, DD and apoptosis. We believe that apart from the discovery of the BRCA1-RRM2 interplay as a novel example of non-oncogene addiction in GBM, our results also offer testable predictive biomarkers (BRCA1/RRM2), and therefore may inspire further work to validate clinical relevance of our present findings.

6

Coupling enhanced mitochondrial respiration to robust store-operated Ca2+ entry in amplified lipid rafts of tumorigenic cells

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Mitochondrial respiration is emerging as an attractive target for cancer therapy. Respiration is strongly promoted by Ca2+, but its targeting through alteration of Ca2+-signaling in neoplastic cells has not been explored. Here employing tumorigenic and non-tumorigenic cell variants of murine and human melanoma, we present facilitation of mitochondrial function by store-operated Ca2+-entry (SOCE) and interruption of this pathway by ablation of cholesterol-enriched lipid raft microdomains in tumorigenic cells. The pathway was indicated by intense mitochondrial O2-consumption and tubular networks, coinciding with amplification of rafts, strong raft-localized SOCE and low basal activity of the mitochondrial fission protein Drp1 in these cells. Drp1 was repressed through phosphorylation via the adenylyl cyclase (AC)/protein kinase A (PKA) axis. Inhibition of AC or PKA reactivated Drp1 and fragmented mitochondria that nearly abolished SOCE-induced mitochondrial Ca2+-uptake and O2-consumption. Reactivation of Drp1 also followed blockade of SOCE or its down-stream effector calmodulin (CaM). Upon CaM- or AC-blockade, mitochondrial structure and O2-consumption were preserved by the membrane-permeable PKA agonist 8-Br-cAMP or the dominant-negative Drp1 mutant (DN-Drp1). Upon SOCE blockade, however, 8-Br-cAMP and DN-Drp1 preserved mitochondrial networks but not O2-consumption, suggesting that raft-localized SOCE augments respiration by (i) directly fueling Ca2+ to mitochondria and (ii) sustaining mitochondrial integrity through the CaM/AC/PKA/Drp1 cascade. Indeed, CaM SOCE-dependently bound AC in tumorigenic cells rafts, the disruption of which with

the cholesterol-extracting agent methyl-β-cyclodextrin reproduced the inhibitory effect of the SOCE-, CaM-, AC- and PKA-blockade on mitochondrial function. Together, the results underscore the distinctive melanoma rafts as potential targets for tumor-suppressive modulation of mitochondrial function in melanoma.

7

Stem cell factors-based identification and functional properties of in vitro-selected subpopulations of malignant mesothelioma cells

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Malignant mesothelioma (MM) are highly aggressive, most often asbestos-related tumors. The relative 5-year survival rate is between 5% and 10%, one of the reasons being the rapid tumor recurrence following first line therapy. The first line therapy most often consists of chemotherapy (cisplatinium and/or antifolates), sometimes accompanied by radiotherapy and/or surgery. Cancer stem cells (CSC) have been studied in many tumor types and are assumed to:

a) be responsible to initiate tumorigenesis;

b) being more resistant towards chemotherapeutic treatments;

c) driving relapse after therapeutic interventions.

An OCT4/SOX2 reporter approach served to identify a subpopulation of human and murine MM cells. Their identification was based on the expression of the enhanced green fluorescent protein (eGFP), which was driven, by OCT4 and SOX2, both described as core stem cell transcription factors.

These cells revealed experimentally to be, compared to the non-CSC or the bulk mass of reporter-negative cells:

i) more resistant towards chemotherapeutics (cisplatinium) in vitro;

ii) more susceptible to the FAK (focal adhesion kinase) inhibitor Defactinib (VS-6063);

iii) cells, bearing a higher tumor-initiating capacity in vivo in xenograft and allograft mouse models;

iv) not sensitive against overexpression of the tumor suppressor NF2;

v) equally sensitive to down-regulation of calretinin, the most specific and sensitive marker for MM, which was shown to be essential for MM cell survival in vitro.

Lentivirus transduction was used to insert the reporter into human and murine mesothelioma cell lines; in the human cell line ZL55 we found 4.8% of reporter-positive cells and 7.8% were eGFP-positive in the murine cell line RN5.

These reporter selected cells were subjected to cisplatinium treatment and the IC50 value (1.75 μM) of eGFP(+) cells was nearly twice as high (0.91 μM) as in the bulk eGFP(-) cells. Interestingly, compared to the exposure to the focal adhesion kinase (FAK) inhibitor (VS-6063) the reporter-positive cells were more sensitive with an IC50 of 2.41 μM compared to 6.46 μM for the bulk-cells. We could show that the reporter-selected cells had a higher tumor-initiating capacity not only in immuno-deficient NOD/SCID gamma mice, but also after intraperitoneal injection of RN5 murine mesothelioma cells immuno-competent C57Bl/6J mice.

In addition, the subpopulations of FACS-selected eGFP(+) cells were more resistant towards asbestos-induced acute cytotoxicity. The lentiviral-based overexpression of functional NF2 (neurofibromatosis 2, merlin), which is a tumor suppressor mutated or lost in nearly half of all MM, did not affect proliferation and viability of CSC-enriched MM populations. On the other hand the proliferation/survival was strongly impaired in eGFP(-) bulk cells. Interestingly, down-regulation of the most sensitive and specific marker for MM, calretinin, via lentiviral-mediated delivery of shRNA, strongly decreased proliferation and viability of CSC-enriched populations, to the same extent as of eGFP(-) cells. This hints towards an essential function of calretinin also in CSC-enriched cell populations.

In summary, we have enriched and characterized a small MM cell subpopulation that bears all expected CSC characteristics. We anticipate that therapeutic interventions aimed at specifically targeting of MM CSC might represent a novel strategy for the treatment of this currently incurable disease.

8

The role of host IL-1α and the microbiome in acute colitis

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Interleukin-1α (IL 1a) is a pleiotropic cytokine, which acts as an alarmin during inflammation. It is expressed in intestinal epithelial cells (IECs) under homeostatic conditions and is released from damaged IECs and initiates inflammation. The effect of IL-1α on acute colon inflammation was assessed in DSS-induced colitis model, using complete IL-1α mice KO mice and mice with a specific deletion of IL-1α in IECs, we observed a very mild form of colitis with significantly better repair compared to control mice. Thus, various possible mechanisms involved in IL-1α-induced colon inflammation were studied. Changes in the microbiome could explain how deficiency of IL-1α leads to reduced DSS-induced damage of intestinal mucosal barrier, as well as high expression of tight junction proteins, preservation of goblet cells or retained epithelial barrier functions. Thus IL-1α, which induces exacerbated colon inflammation, may be critical in driving the pathologic breakdown of barrier integrity. Co-housing experiments combined with metagenomic analysis confirmed the role of the specific microbiota in IL-1α KO mice as compared to control mice. After DSS administration, IL-1α KO co-housed mice developed a disease phenotype similar to DSS-treated control mice rather than to DSS-treated control IL-1α KO mice. Goblet cells in co-housed mice were also similar to their distribution to control mice. Metagenomic 16s analysis, obtained from fecal samples, revealed a significant shift in ß-diversity towards control and co-housed mice. These results confirmed that IL-1α is a key molecule in acute colon inflammation and affects barrier functions and colon microbiota.

9

Chromatin remodelers as potential new targets for therapy of pediatric sarcoma

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Fusion-positive rhabdomyosarcoma (FP-RMS) is a pediatric malignancy driven by the fusion transcription factor PAX3-FOXO1, which generates an aberrant gene expression signature leading to cell transformation. Since FP-RMS cells are highly addicted to the fusion protein, it is in focus as target for alternative therapies. Nevertheless, PAX3-FOXO1, as a transcription factor, does not contain structural cavities and has a low druggability. We therefore hypothesize that we can affect this aggressive subtype of RMS by targeting the co-regulators that collaborate with the fusion protein in regulating transcription.

Recently, we have identified the NuRD (Nucleosome Remodeling and Deacetylase) complex as a potential partner of PAX3-FOXO1 in gene expression modulation. The NuRD complex is unique among chromatin remodeling complexes due to its dual enzymatic activity (histone deacetylation through HDAC1/2 and nucleosome positioning by CHD4 - chromodomain-DNA-binding protein 4), offering new possible therapeutic targets.

Silencing of two core members of NuRD, CHD4 and RBBP4, led to a drastic decrease in FP-RMS cell viability. Additionally, CHD4 depletion caused a complete regression of mouse tumour xenografts, but it did not affect proliferation of myoblasts, fibroblasts or fusion negative RMS cells, despite the fact that these cells also carry high CHD4 expression levels.

We further investigated the nucleosome remodeler CHD4 and learnt that it affects the expression of approximately 50% of PAX3-FOXO1 target genes with most of these genes being upregulated, suggesting an activating role for CHD4 in these cases. Consistent with a positive effect of CHD4 on gene expression, ChIP-seq experiments with FP-RMS cell lines demonstrated that NuRD occupies promoter and enhancer regions of highly expressed genes and co-localizes with the fusion protein at regulatory regions of a subset of its target genes.

Next, we studied the influence of this nucleosome remodeler on the chromatin status by DNase hypersensitivity assays and determined that the presence of a DNase signal at PAX3-FOXO1 binding sites is concordant with the pres-

ence of CHD4. Hence, we suggest a scenario where CHD4 plays an essential role on FP-RMS tumorigenesis by allowing chromatin to acquire an open architecture that enables PAX3-FOXO1 mediated gene expression.
In summary, our data propose that CHD4 has a crucial role as a co-regulator of PAX3-FOXO1 driven gene expression. To our knowledge, CHD4 is the first identified chromatin remodeler associated with PAX3-FOXO1 transcriptional activity, thus highlighting the relevance of epigenetic regulation in FP-RMS tumour development and opening chromatin remodelling as a possible new field of action against this tumor, which is driving ongoing work aimed at finding first-in-class small molecules to inhibit CHD4 function.

10

Cancer Type-Specific SPOP Mutants Alter BET Protein Levels and Inhibitor Responses

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It is generally assumed that recurrent mutations within a given cancer driver gene elicit similar drug responses. Recently, cancer genome studies have delineated recurrent but divergent missense mutations in the substrate recognition domain of the ubiquitin ligase adaptor SPOP in endometrial and prostate cancer. Their therapeutic implications remain incompletely understood. Here, we analyzed changes in the ubiquitin landscape induced by endometrial cancer-associated mutations of SPOP and identified altered ubiquitylation in a subset of proteins. Of these, bromodomain and extra-terminal (BET) proteins BRD2, BRD3, and BRD4 emerged as SPOP-CUL3 substrates that are increasingly recognized and degraded by endometrial type of SPOP mutants. The resulting reduction of BET protein levels sensitized cancer cells to BET inhibitors – an attractive class of anti-cancer therapeutics. Conversely, prostate cancer-specific mutants of SPOP impaired ubiquitylation and degradation of BET proteins promoting resistance against their pharmacologic inhibition. These results uncover a paradoxical aspect of oncogenomics, whereby mutations within the same domain of a particular gene evoke opposing drug susceptibilities. More specifically, we provide a molecular rational for the use of BET inhibitors to treat endometrial but not prostate cancer patients with SPOP mutations.

11

Regulation of the oncogenic potential of CDKN1A by the tumor suppressor SOCS1 in hepatocytes

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Suppressor of cytokine signaling 1 (SOCS1) is considered a tumor suppressor due to frequent epigenetic and micro-RNA-mediated repression of the SOCS1 gene expression in hepatocellular carcinoma and in diverse other cancers. Consistent with this notion, SOCS1-deficient mice display increased susceptibility to hepatocarcinogenesis induction by diethylnitrosamine (DEN). We have previously shown that SOCS1 promotes oncogene-induced senescence in a cellular model by promoting the activation of p53 by ATM/ATR kinases. Therefore, we examined the p53 activation in SOCS-deficient livers following exposure to DEN. Activation of p53 was not impaired in SOCS1-deficient livers. Surprisingly, SOCS1 deficiency increased the expression of the p53 target gene Cdkn1a and its protein product by transcriptional and post-translational mechanisms. Even though CDKN1A generally functions as a tumor suppressor via inhibition of the cell cycle in the nucleus, its cytoplasmic retention can promote oncogenesis by promoting cell survival. We show that SOCS1 deficiency promotes cytosolic retention of CDKN1A and inhibits cell death in the liver. In vitro, SOCS1-deficient hepatocytes display resistance to apoptosis and increased cell proliferation, both of which are reversed by knockdown of CDKN1A by siRNA. Finally, we show that loss of CDKN1A abrogated the increased susceptibility of SOCS1 deficient mice to DEN-induced HCC. These findings indicate that SOCS1 plays a critical role in controlling the oncogenic potential of CDKN1A.

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SF3B1 is a dosage-dependent oncogenic transcriptional cofactor of HIF1α in pancreatic cancer

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Dysfunction of splicing factors is a signature feature of many human cancers and originates, at least in part, from mutations in core splicing factor genes and/or changes in the cellular concentrations of their gene products. Yet, how such cancer-associated alterations contribute to malignant progression remains poorly understood.
Here we report that in the context of hypoxia, the splicing factor 3b subunit 1 (SF3B1) assumes a splicing-independent function as an interaction partner and transcriptional cofactor of HIF1α crucial for efficient binding to and activation of HIF target genes, including SF3B1 itself, and for glycolytic reprogramming. In human pancreatic ductal adenocarcinoma (PDAC), which thrives in hypoxic microenvironments, high HIF1α and hypoxic target gene expression strongly correlates with high SF3B1 levels. Moreover, in a Kras-G12D/Trp53R172H-driven PDAC mouse model, in which hypoxia manifests already at the pancreatic intraepithelial neoplasia (PanIN) stage, the levels of SF3B1 and HIF1α are both robustly induced. Importantly, limiting SF3B1 expression in this mouse tumor models by conditionally ablating one copy of Sf3b1, results in reduced Hif target gene expression, glycolysis, inflammation and stromal fibrosis and a failure of PanIN lesions to progress to invasive PDAC. Tumor organoids derived from these mice, when exposed to hypoxia, demonstrate similar impairments in hypoxia-inducible gene activation, glycolytic metabolism and growth.
Together, our findings reveal a novel function of SF3B1 in reinforcing HIF transcription programs and disclose that this function represents a dosage-dependent oncogenic activity of SF3B1 critical for PDAC initiation and progression, indicating that targeting this interaction may provide novel therapeutic opportunities to treat this disease.

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Stochastic but clustered re-appearance of normal and cancer stem cells: Experimental evidence and model predictions

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Undifferentiated stem cells have the capability to differentiate into specialized cells. A similar situation is assumed to prevail in cancer cells; the subpopulation of cancer stem cells (CSC) has a higher tumor-initiating capability than non-CSC. By using a genetically-encoded stemness reporter the "stemness" of individual cells was continuously monitored in human and murine malignant mesothelioma (MM) cells, as well as in normal mouse mesothelial cells. Both differentiation and de-differentiation processes were observed, but with different probabilities. De-differentiation i.e. re-appearance of stem cells from non-stem cells is an extremely rare event. However, if it is occurring, the probability of the neighboring cells to also become stem cells is increased resulting in a clustered re-appearance of stem cells. Cell cultures starting from isolated completely non-CSC (even derived from a single non-CSC) generated CSC with time. On the other hand, a fraction of initially pure CSC lost stemness with time, resulting in a steady-state equilibrium between CSC and non-CSC populations. Our numerical simulations are based on spatially interacting Markov-chain processes. Considering also the different apoptotic, and mitogenic rates and stem cell transition rates, our tumor growth model is well suited to accurately predict the occurrence and spreading of CSC within a tumor mass.

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The role of miR-143/5 cluster in chondrosarcoma progression

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Chondrosarcoma (CHS) is the second most frequent bone sarcoma with a poor patient prognosis when tumors metastasize (5-year survival rate <25%). Due to the insufficient understanding of the genetic drivers that

contribute to CHS, molecules that could be targeted therapeutically are still missing, leaving surgical resection of tumors as the only possible treatment. Hence, there is an urgent need for the discovery of novel relevant genes in CHS. In addition to this, deregulated miRNAs are known to play a pivotal role in cancer, including CHS, and determining the miRNAs-regulated genes is a valuable approach for target discovery.

We performed a Next-Gen sequencing study of miRNAs in CHS tumor samples (4 benign, 3 low grade, 3 high grade CHS) and compared it with other global miRNA studies in CHS. From this meta-analysis we selected those miRNAs which consistently showed to be deregulated in tumor samples. We found that miRNA levels of the cluster miR-143/miR-145 (located on chromosome 5q33.1) inversely correlate with tumor grade, suggesting that these miRNAs are relevant for CHS progression. To understand the role of miR-143 and miR-145, we established CHS cell lines stably over-expressing both miRNAs. In vitro functional assays demonstrated that miR-143/miR-145 over-expression reduces the ability of cells to form colonies in soft agar, which is in agreement with the tumor-suppressor role of this cluster previously reported in other types of cancer.

In parallel, we performed a whole transcriptome analysis by Next-Gen sequencing of the same tumor samples and looked at the expression levels of putative gene targets of miR-143 and miR-145 (determined by different miRNA target prediction databases). Interestingly, we observed that multiple potential targets of the miRNAs are upregulated in high-grade CHS tumors, suggesting that both miRNAs are a part of the complex network of post-transcriptional regulation. We validated these findings by qRT-PCR in tumor samples and in our transduced cell lines and confirmed that the levels of two novels genes in CHS (Fascin-1 and EVA1, relevant for motility and adhesion, respectively) are significantly down-regulated in samples over-expressing miR-143/miR-145. Additionally, we showed that Fascin-1 protein levels are reduced in cells over-expressing the cluster.

In summary, this work shows a robust approach for the discovery of CHS drivers based on the initial identification of aberrantly expressed miRNAs. Increasing the levels of miR-143/miR-145 -or reducing the levels of its targets- represent new promising therapeutic approaches to be tested in CHS.

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Crosstalk of the HGF/c-MET and TGF-β pathways in glioblastoma

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Multiple target inhibition has gained considerable interest in combating drug resistance in glioblastoma, however, understanding the molecular mechanisms of crosstalk between signaling pathways and predicting responses of cancer cells to targeted interventions has remained challenging. Despite the significant role attributed to transforming growth factor (TGF)-β family and hepatocyte growth factor (HGF)/c-MET signaling in glioblastoma pathogenesis, their functional interactions have not been well characterized. Using genetic and pharmacological approaches to stimulate or antagonize the TGF-β-pathway in human glioma-initiating cells (GIC), we observed that TGF-β exerts an inhibitory effect on c-MET phosphorylation through the activation of non-canonical, SMAD-independent pathways, including mitogen-activated protein kinase (MAPK)/ extracellular signal-regulated kinase (ERK) and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB / AKT) pathways. Moreover, a comparison of c-MET-driven and c-MET independent GIC models revealed that TGF-β inhibits stemness in GIC at least in part via its negative regulation of c-MET activity, suggesting that stem cell maintenance may be controlled by the balance between these two oncogenic pathways. Importantly, immunohistochemical analyses of TGF-β and p-c-MET in human glioblastoma specimens supports a concept of negative regulation between these pathways.

These novel insights into the crosstalk of two major pathogenic pathways in glioblastoma may explain some of the disappointing results when targeting either pathway alone in human glioblastoma patients and inform on potential future designs on targeted pharmacological or genetic intervention

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STAT3/LKB1-Dependent Metabolic Reprogramming Dictates Metastatic Potential and Therapeutic Response in Prostate Cancer

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Tumor recurrence in metastatic diseases and therapy resistance arise from metabolic reprogramming of cancer cell functionally driven by the activation of oncogenes or the loss of tumor suppressor genes. Metformin is currently the most widely used glucose-reducing drug prescribed to millions of people worldwide and also frequently to diabetic prostate cancer (PCa) patients. Metformin exerts antineoplastic effects, induces metabolic reprogramming and leads to decreased tumor nicotinamide adenine dinucleotide (NAD+). Here we show that the administration of metformin in an androgen receptor (AR)-sensitive PCa suppressed tumor progression and is clearly dependent on the signal transducer and activator of transcription 3 (STAT3) and alternate reading frame protein (ARF) axes. However, AR-independent PCa acquired metformin resistance, formed enlarged tumors and showed an increased proliferation accompanied by high expression of MYC and loss of liver kinase B1 (LKB1). Genetic inactivation of the phosphatase and tensin homolog (PTEN) in prostate epithelium triggers the induction of LKB1 in a STAT3-dependent manner. In contrast, deletion of STAT3 promotes loss of LKB1 resulting in oncogenic MYC overexpression and metastatic formation. These findings implicate an important role of LKB1 in metabolic reprogramming of metastatic PCa and highlight STAT3 and LKB1 as key regulators of cancer metabolism and potential prognostic markers of resistant PCa.

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Challenging pathologist's eye with computer-based intensity measurement in PTEN immunohistochemistry

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PTEN is major tumor suppressor and cells are ultra-sensitive to dosage changes as well as in cells there is no alternative protein. As it is involved in PI3K/Akt pathway it could serve as a surrogate biomarker for hyperactivation of this signaling pathway, indicating when inhibitors might be more efficient. Therefore, it is important to measure PTEN loss, one off best way to measure protein expression is using immunohistochemistry (IHC) this is considered as a valuable read out, but unfortunately there is no international consensus regarding an optimal IHC protocol as well as no definition of best cut –off for PTEN positive versus PTEN negative. Therefore, in this Lungscape project by ETOP (European Thoracic Oncology Platform) we performed external quality assessment (EQA) for IHC and as well as correlated pathologist's H-scores with computer-generated intensity measurements.

EQA consisted of 5 PTEN+, 5 PTEN- tumor surgical specimens and 1 PTEN+ and 1 PTEN- cell line. Whole sections of these samples were centrally stained with 3 different antibodies (MmAb 6H2.1 DAKO, RmAb 138G6 Cell Signaling and RmAb SP218 Spring Bioscience) and assembled in webbook. From these cases an EQA tissue microarray (TMA) were made and stained locally with SP218. All staining's were finally analyzed by ETOP pathologists' (H-scores 0-300) and computer using ImageJ for pixel-based intensity on tumor epithelia.

All 3 antibodies successfully could differentiate PTEN+ and PTEN- cases (Table 1). Computer H-scores is in alignment with pathologists' score, but

with lower averages. On TMAs, computer scores showed lower inter-center variability. For 138G6 and SP218, sensitivity and specificity are high for moderate values of threshold h (0-20), but DAKO had higher background.
SP218 yields a clean IHC and separates well PTEN+ from PTEN- cases arguing for standardization across laboratories. Pathologists tend to H-score higher than the computer, likely due integration of various signal intensities.

Table 1. Descriptive statistics for pathologists vs computer H-scores on whole sections stained with the 3 different antibodies

	Antibody	PTEN	Mean	Median	Min	Max
Pathologists	SP218	pos.	157	165	10	290
		neg.	4	0	0	80
	138G6	pos.	148	140	0	300
		neg.	2	0	0	100
	6H2.1	pos.	259	270	30	300
		neg.	24	5	0	130
Computer	SP218	pos.	75	75	23	130
		neg.	0	0	0	1
	138G6	pos.	51	44	10	123
		neg.	0	0	0	1
	6H2.1	pos.	151	136	121	200
		neg.	23	5	1	63

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Synergistic cytotoxicity of a recombinant anti-PSMA immunotoxin in combination with docetaxel against prostate cancer

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In this study we investigated a new combination therapy with a recombinant anti-PSMA immunotoxin and the cytostatic agent docetaxel. The immunotoxin consists of an anti-PSMA single chain antibody fragment (scFv) as binding and a truncated form of Pseudomonas aeruginosa Exotoxin A (PE40) as toxin domain. The immunotoxin induced apoptosis and specifically reduced the viability of androgen-dependent LNCaP and androgen-independent C4-2 prostate cancer cells. A synergistic cytotoxic activity was observed in combination with docetaxel with IC50 values in the low picomolar or even femtomolar range. Moreover, combination treatment resulted in an enhanced antitumor activity in the C4-2 SCID mouse xenograft model. This highlights the immunotoxin as a promising therapeutic agent for a future docetaxel-based combination therapy of advanced prostate cancer.
This work is supported by the Deutsche Krebshilfe (Grant No. 110993 to P. Wolf).

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The helicase and nuclease activities of DNA2 enzyme can be selectively inhibited by small molecule compounds

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Background: DNA2 is an essential enzyme and a key player in DNA replication and repair. It is conserved from yeast to humans and possesses two catalytic activities: an ATP-powered helicase and a nuclease activity. DNA2 was found to be overexpressed in human cancers and the expression correlated with the disease outcome, while depletion of DNA2 inhibited xenograft growth in mice (Peng et al., 2012; Strauss et al., 2014). DNA2 is essential for replication, thus its elevated activity is possibly important for cancer progression and allows the tumor to cope with replication stress, providing a growth advantage to cancer cells. Therefore, targeting DNA2 is a promising approach for cancer therapy.
Methods: A high throughput screening of 100'000 small molecule compounds and purified Dna2 from S. cerevisiae was conducted using a FRET-based assay. The best hits were further verified with yeast and human Dna2/DNA2 proteins, as well as in cell-based assays. The properties of the selected compounds were analyzed in in vitro assays with respect to effects on both

helicase and nuclease activities of Dna2/DNA2, as well as in cell-based homologous recombination (HR) reporter assays.
Results: We could identify multiple compounds selectively inhibiting the DNA2 nuclease and helicase activities. They reduced the HR frequency, as well as the viability of U2OS cells.
Outlook: The effectiveness of the DNA2 inhibitors have to be further tested in vivo.

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The role of L1CAM in ovarian cancer stem-like cells

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Accumulating evidence indicates that many solid tumors, including ovarian cancer (OC), contain small populations of tumor-initiating cells, the so-called cancer stem-cells (CSCs). Overlapping cell populations expressing high aldehyde dehydrogenase (ALDH) activity in combination with different cell surface markers such as CD24, CD44 and CD133 have been characterized as ovarian CSCs, showing high resistance against conventional therapies (1). Recently, it was shown that L1 cell adhesion molecule (L1CAM) is a CSC-specific marker in glioblastoma (2). L1CAM is a highly glycosylated type I transmembrane protein that plays a role in the development of the nervous system and in human cancer. In cancer, L1CAM expression induces motile and an invasive phenotype, supporting aggressive tumor growth, metastasis and chemoresistance. It is now proved that only CSCs have the ability to proliferate under non-differentiating and non-adherent conditions, forming three-dimensional multicellular tumor spheroids which are very aggressive in growth and show reduced response to chemotherapeutic drugs in vitro.
The aim of our research is to elucidate the biological role of L1CAM expression in ovarian CSCs. Specific populations of cells expressing L1CAM alone or in combination with other CSC-markers (ALDH and CD133) were isolated from established ovarian cancer cell lines. Thereafter, we assessed clonogenic and spherogenic capacity as well as radiation responsiveness of these cells. The results indicated that L1CAM+ population has higher spherogenic and clonogenic properties in comparison to L1CAM-/CD133- cell population. Interestingly, we found that the cell subsets defined by the expression of both L1CAM/CD133 and L1CAM/ALDH have the highest clonogenic and spherogenic capacity. Moreover, L1CAM+/CD133+ and L1CAM+/ALDH+ cell populations retain the highest clonogenic capacity after irradiation. These results indicate that L1CAM might influence the functional properties of OC stem-like cells.

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Targeted 'stealth' adenoviruses for the cell-specific delivery of cancer therapies

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Our group has developed an adenoviral delivery system that allows for specific targeting of adenovirus serotype 5 (Ad5) to discrete cell populations through the use of interchangeable designed bispecific designed ankyrin repeat protein (DARPin) adapters. In contrast to other strategies utilizing Ad5 for its oncolytic properties, we have engineered Ad5 into a non-oncolytic, 'shielded' delivery vehicle for therapeutic genes due to its large packaging

capacity (up to 36 Kb) and the feature that the viral DNA remains episomal rather than randomly integrating into host chromosomes, providing an additional safety margin for clinical applications. The generation of this generic delivery system has enabled its use as a therapeutic platform. The primary approach aims as at targeting adenoviral particles to selectively transduce tumor cells or tumor stromal cells with the genes encoding cocktails of secreted monoclonal antibodies (mAb)- and/or other protein-based therapeutics (e.g. cytokines). The transduced subpopulation of cells then serves as a 'biofactory,' secreting therapeutic combinations that act in a paracrine fashion within the tumor microenvironment to collectively target multiple oncogenic pathways, increase anti-tumor immunity, and/or enhance tumor perfusion with additive or synergistic effects to reduce the risk of tumor escape and the development of drug resistance. We argue that in situ therapeutic production could provide an attractive alternative to treatment with repeated high bolus injections of drug combinations, as secretion by the tumor itself could provide high local concentrations that act in a paracrine fashion over an extended duration with limited toxicity to peripheral tissues. In a secondary immunotherapy application, bispecific adaptors have been generated for mediating viral delivery to the surface of discrete T cell populations (e.g. CD4+, CD8+) as potential in vivo delivery vehicles for cell-mediated immunotherapies. We propose that this approach could provide a less cumbersome alternative to ex vivo modification of T cells with tumor-specific T cell receptors (TCRs) or chimeric antigen receptors (CARs) for autologous transfer.

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Investigation of Glucocorticoid-Induced Radioresistance in Tumor Cell Lines

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Targeted radionuclide therapy exploits the cytotoxic effects of radioactivity caused by selective tumor-targeting radioligands. To control therapy or disease-related side effects, such as nausea and inflammation, the therapy is often combined with glucocorticoids (GCs). Apart from the beneficial effects of corticosteroids, a number of studies suggest that GCs can induce apoptosis resistance towards cytotoxic therapies in various solid tumor types in vitro and in vivo [1,2].

The goal of this study was to investigate several tumor cell lines with regard to the development of radioresistance upon exposure to GCs and to evaluate a possible correlation to glucocorticoid receptor (GR) expression. Therefore, in vitro cell viability experiments and Western blot analysis were performed in a selection of human tumor cell lines. The cells were exposed to GCs and cell-specific targeting agents labeled with the therapeutic radionuclide ^{177}Lu ($T_{1/2}$ = 6.65 d, $E\beta_{-av}$ = 134.2 keV) known to reduce cell viability. After one week, the cell viability was assessed using a colorimetric assay (MTT).

The results indicated that GCs had a significant radioprotective effect in some cell lines exposed to the radioactive targeting agents. Among those were KB cells (human cervical tumor cells) or AR42J cells (rat pancreatic tumor cells) in which cell viability was recuperated by 50% if the cells were co-treated with GCs, as compared to control cells treated with the radiopharmaceutical only. However, GCs did not have any observable effect on the cell viability of other tumor cell lines, such as PC-3 cells (human prostate tumor cells) or IGROV-1 cells (human ovarian tumor cells). Western blot analysis revealed that KB and AR42J cells expressed the GR, while PC-3 or IGROV-1 cells did not express the GR protein.

Our data suggest that the application of GCs induces radioresistance in GR-expressing tumor cells but not in cells lacking the receptor. In future experiments, the observed effect will be investigated in vitro in a broader selection of cell lines and in vivo using tumor-bearing mice. Should our observation be confirmed in a preclinical setting, it may be necessary to re-consider the application of GCs in patients undergoing radionuclide therapy as they may impact the therapeutic outcome.

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Replication fork slowing and reversal upon genotoxic stress require PCNA polyubiquitination and ZRANB3 DNA translocase activity

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CDNA damage tolerance during eukaryotic replication is orchestrated by PCNA ubiquitination. While monoubiquitination activates mutagenic translesion synthesis, polyubiquitination activates an error-free pathway, elusive in mammals, enabling damage bypass by template switching. Fork remodeling is driven in vitro by multiple enzymes, including the DNA translocase ZRANB3, shown to bind polyubiquitinated PCNA. However, whether this interaction promotes fork reversal and template switch in vivo was unknown. Here we show that damage-induced fork reversal in mammalian cells requires PCNA ubiquitination, UBC13 and K63-linked polyubiquitin chains, previously involved in error-free damage tolerance. These mutations affecting fork reversal also induced unrestrained fork progression, suggesting fork remodeling as a global fork slowing and protection mechanism. Fork slowing and reversal in vivo also require DNA translocase activity and PCNA interaction of ZRANB3, which is thus a key effector of error-free DNA damage tolerance. Targeting these fork protection systems may represent a promising strategy to potentiate cancer chemotherapy.

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Improved cancer immunotherapy by a CD25-mimobody conferring selectivity to human interleukin-2

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Introduction: Interleukin (IL)-2 is an essential cytokine for the homeostasis and activation of T cells. It can stimulate cells expressing different levels of dimeric or trimeric IL-2 receptors (IL-2Rs). The administration of IL-2 at high doses proved its benefit for patients with advanced cancer through the stimulation of cells expressing high levels of dimeric IL-2Rs ($CD8^+$ T cells and NK cells). By contrast most of the IL-2-related side effects observed in patients treated with IL-2 have been associated to the binding of the cytokine to its IL-2 receptor-α (CD25) subunit, preferentially expressed by immunosuppressive $CD4^+$ T regulatory cells. Specific anti-IL-2 monoclonal antibodies (mAbs) have previously shown to lead to a vigorous activation of effector immune cells in mice. Currently, no mAb specific to human (h) IL-2 suitable for clinical development in cancer immunotherapy is available. In order to cover this clinical need, we developed a rational approach to generate and identify a high affinity anti-hIL-2 antibody specifically blocking the CD25 binding site (termed NARA1). In vivo, hIL-2 in complex with NARA1 resulted in preferential stimulation of $CD8^+$ T cells and NK cells allowing potent anti-tumor control in two orthogonal pre-clinical relevant melanoma models.
Methods: In order to screen anti-hIL-2 antibodies obtained after immunization and hybridoma generation, we developed a screening approach for the detection of specific anti-hIL-2 antibodies covering the CD25 binding site of the cytokine. The best monoclonal antibody, NARA1, was isolated and fully characterized in vitro using surface plasmon resonance (SPR) and x-ray crystallography in complex with hIL-2. We injected wild-type (Wt) mice with hIL-2/NARA1 complexes in order to check levels and kinetic proliferation of relevant immune cell subsets. Finally, we investigated the anti-tumor effect of hIL-2/NARA1 complexes in comparison to hIL-2 in two syngeneic (B16-F10) and in one spontaneous metastatic (Tyr::N-RasQ61K Ink4a$^{-/-}$) melanoma mouse models. In order to confirm which cells and mechanisms were responsible for the obtained anti-tumor response, hIL-2/NARA1 complexes were used for the treatment of Tcrbd$^{-/-}$, Rag1$^{-/-}$and Wt mice depleted of $CD4^+$ or $CD8^+$ T cells bearing B16-F10 tumor cells. Finally, we checked the adverse effects of hIL-2/NARA1 complexes in comparison to hIL-2 by determining liver toxicity and lung edema.
Results: Only 0.2% of all anti-hIL-2 mAbs found by our screening approach bound the target epitope and were further characterized. SPR analysis showed that NARA1 was efficiently blocking the CD25 binding site of hIL-2 therefore acting as a high affinity CD25-mimobody. Determination of

the crystal structure allowed us to identify the binding epitope of NARA1. Residues of hIL-2 contacting NARA1 are clearly involved in the binding to CD25. In vivo, hIL-2/NARA1 complexes preferentially expand $CD8^+$ T cells and NK cells, while the expansion of $CD4^+$ T regulatory cells remains low. The immuno-stimulatory effect of hIL-2/NARA1 complexes leads to potent anti-tumor responses correlating with higher levels of $CD8^+$ T cells in the tumor site. The anti-tumor effect observed was clearly dependent on $CD8^+$ T cells that remain less exhausted that the ones generated upon conventional hIL-2 immunotherapy. Moreover, hIL-2/NARA1 complexes lead to favorable benefit to adverse effect ratios, as shown treating Tyr::N-RasQ61K $Ink4a^{-/-}$ mice which developed less skin melanoma nodules and lung metastases while tolerating well several cycles of hIL-2/NARA1 complexes.
Conclusion: We were able to generate and fully characterize the first anti-hIL-2 mAb able to potentiate the immuno-stimulatory properties of hIL-2, thus meriting further investigation for clinical development.

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Analysis of TIP5-mediated epigenetic alterations in prostate cancer

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Besides been one of the most common types of cancer in men, prostate cancer (PCa) has an unpredictable clinical history; while most tumors are indolent, some patients display lethal phenotypes. Treatment options for metastatic disease are not curative since patients inevitably acquire resistance and relapse. A particular feature of PCa is the lack of somatic mutations and an altered epigenome. We have recently shown that TIP5 is highly expressed in metastatic PCa and establishes epigenetic silencing of genes commonly repressed in metastatic tumors. Mass-spectrometry analysis indicate that in metastatic PCa cell line (PC3), TIP5 interacts with components of the NuRD, SWI/SNF chromatin remodeling complexes and Polycomb repressive complex 2 (PRC2), which are associated with epigenetic and gene regulation. TIP5 has a role in proliferation, invasion and cancer stem cell like (CSC) features. PCa is of epithelial nature and cancer stem cells have been implicated in cancer relapse after primary treatment. To determine whether TIP5 might represent a therapeutic target for metastatic PCa, we have established a culture condition that allows the isolation of spheres from the heterogeneous PC3 cell population. RNAseq analysis revealed that spheres resemble stemness features such as downregulation of developmental and differentiation genes and upregulation of CSC markers such as LGR5 and ALDH1A2. Remarkably, TIP5 knockdown impairs the formation of spheres. Similarly, treatment with chemical probes against TIP5-bromodomain (BRD) compromise the ability of PC3 cells to originate spheres without affecting proliferation of PC3 cells. These findings evidence the role of TIP5 in the regulation of CSC features and that TIP5 is a potential therapeutic target in CSCs.

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TNF receptor 2 regulates RIP1-dependent cell death in refractory leukemia

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The identification of molecular determinants that regulate sensitivity to specific agents is essential for the development of new therapeutic approaches in cancer. We have earlier shown that a subset of refractory acute lymphoblastic leukemia (ALL) samples respond to SMAC-mimetic (SM) induced IAP depletion by concurrently inducing RIP1-dependent apoptosis and necroptosis. Comparative gene expression profiling indicated a correlation of sensitivity to SM with the expression of TNF receptor 2 (TNFR2) in primary ALL. Using an independent cohort of primary chemotherapy-resistant ALL samples, we found that high TNFR2 expression predicted in vitro sensitivity to SM. High TNFR2 levels also correlated with higher expression of TNFR1. Deletion of either TNFR1 or TNFR2 using CRISPR/Cas9 in patient-derived ALL conferred resistance to treatment with SM, indicating that TNFR1 and 2 are both functionally required for cell death. In agreement with an important role for TNFR2 in the response to SM, the overexpression of TNFR2 leads to increased sensitivity to TNF through activation of the TNFR1/RIP1 death

axis. On the mechanistic level, recruitment of RIP1 to TNFR1 is a key event in the activation of cell death, which is abolished in TNFR2-deficient leukemia. Taken together, our data reveal a novel function of TNFR2 in cell death signalling, as TNFR2 predicts sensitivity to SMAC mimetics and plays a key role in modulating a switch from RIP1-controlled cell survival to cell death.

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Diagnostic potential of micro RNAs expression profiles in breast and gynecologic cancer

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Purpose: Various microRNAs act as key regulatory factors in tumorbiological processes, with both, oncogenic as well as tumor suppressive functions. This study focused on the putative biomarker properties of 25 microRNA types, investigated in three breast cancer (BC), endometrial cancer (EC) and ovarian cancer (OC) in vitro models, respectively, to determine their diagnostic potential.
Material and methods: The expression pattern of 25 microRNA (miR) specimen were investigated in three established BC, EC and OC cell lines, respectively. Quantitative analysis was performed by RNA isolation and subsequent realtime PCR. Therefore, relative quantification of the different microRNA types resulted from ΔCt method, normalized against medium expression values of the housekeeping microRNAs RNU 48, miR 16, miR 26b and miR 103, respectively.
Results: Expression analyses could determine a subgroup of ten different miRNA types (miRs: -let 7b, -21, -30a, -30c, -30e, -27a, -222, -29a, -128.1, -9) that allow an expression level based discrimination between BC and OC cell types. Six miRNA types exhibit distinguishing characteristics in regard to BC vs. EC cell expression profiles (miRs: -30a, -30e, -29a, -15b, -200b, -222). Four miRNA types were identified with altered expression levels that may account for diagnostic purposes in the determination of EC against OC (miRs: -92a, -106b, -200b, -222).
Conclusion: We were able to demonstrate the feasibility to detect distinct miRNA expression profiles for each tumor entity. We hypothesize, that these distinct patterns offer the possibility for specific discrimination between breast, endometrium and ovarian cancer. Based on the current results, the biomarker function of microRNAs in cancer management could be a useful diagnostic feature to identify a tumor entity specific microRNA signature.

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Histone Acetyl-transferases as Putative Tumor Suppressors in Diffuse Large B-cell Lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma, contributing to approximately 30% of newly diagnosed cases per year. This type of lymphoma arises from mature B-cells at different stages of differentiation, and is characterized by a diffuse growth of neoplastic large B-lymphocytes.
Alterations in chromatin modifiers are one group of genetic aberrations that are likely to contribute to DLBCL pathogenesis. Genetic inactivation of the chromatin modifiers, the acetyl-transferases CBP (CREB-binding protein) and p300 (E1A-binding protein p300), has been reported in approximately 40% of DLBCL cases. Mutations in these chromatin modifiers have been characterized as early events in lymphomagenesis, however little is known about their molecular functions and contribution to the onset and development of DLBCL.
A better understanding of the functional role of alterations in chromatin modifiers and the molecular pathways affected could help identify new oncogenic and tumor suppressive players in DLBCL pathogenesis, and potentially have a prognostic value that defines a different therapeutic strategy depending on the mutational status of the modifiers.
My aims are designed to provide a comprehensive understanding of the role of the chromatin modifiers, CBP and p300, in epigenetic regulation of tumor suppressor genes in DLBCL. More specifically, we use a panel of DLBCL cell lines in conjunction with in vivo mouse models and high throughput sequencing to identify pathways that are epigenetically regulated by acetylation of histones in DLBCL.
We have made use of the CRISPR/Cas9 genome editing system to introduce

mutations that phenocopy the cases seen in patient material. By introducing the generated knockout clones into different in vivo models, we aim to understand the effect of these mutations on lymphomagenesis under different conditions. Our in vivo system makes use of the activation-induced cytidine deaminase (AID)-Cre/loxP system to allow for an inducible knockout of the histone acetyl-transferases specifically in B cells upon antigen stimulation. In addition, we are employing a second-hit model whereby another one of the well-known oncogenes is expressed.

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An immune biomarker signature identifies chemoresistant ovarian cancer patients

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Ovarian cancer is the most common cause of gynecological cancer associated death. Despite advances in disease knowledge, 5-year survival has inched up from 40% to only 45% during the last two decades. Surgery and adjuvant platinum-based chemotherapy remain the principal treatment of ovarian cancer patients. However, half of the patients are intrinsically resistant to cytotoxic therapies and relapse within 1 year after completing treatment. Tumor microenvironment can also affect the success of chemotherapy. Since ovarian tumors are highly immunogenic, the current study was designed to investigate the relationships between tumor microenvironment and host immune system and their capabilities in predicting differential chemotherapy response in ovarian cancer patients.

We performed the expression profiling of 111 parameters, including cytokines, drug resistance-associated genes, stemness-related proteins, and lymphocyte subtyping in 35 blood and tumor samples of ovarian patients (16 sensitive and 19 resistant) using flow cytometry, antibody arrays and quantitative PCR.

We observed differences in marker expression between patients. Correlation analysis revealed several related clusters. The strongest cluster includes both systemic and tumor-specific parameters, among them immune (intratumoral CTL, intratumoral Treg, serum CXCL10, tumoral CD86), drug resistance (tumoral ATP11B, ABCC1, ATP7B, ABCC10), and stemness (tumoral CD133, CD24, Snail, Slug, FN1, PCNA) parameters.

In addition, after dividing patients into platinum-resistant and ¬sensitive groups, we derived a following immune signature with a potential predictive value for chemotherapy resistance: CCL2, CCL3, CCL4, CXCL5, CXCL9, CXCL11, IFNγ, IL-8, ABCB1, ABCC10, Oct3/4. Combined analysis of twelve-biomarker panel demonstrated high predictive power with 84.43% and 71.43% specificity.

In conclusion, our results suggest that immune profiling of ovarian cancer patients might represent a useful tool for the foreseeing of response to platinum therapy. Elevated expression of pro-inflammatory macrophage-attracting chemokines in resistant patients could suggest the role of the innate immune compartment and the potential benefit from combining chemotherapy with immunotherapy.

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Characterization of tumor-derived exosomes in osteosarcoma established cell lines

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Osteosarcoma (OS) is the most common primary malignant bone tumor in children and adolescents. It has a high predisposition towards pulmonary metastasis, which is the major cause of death in patients. 20% of the patients' exhibit detectable metastases during initial diagnosis while 80% of the patients, who initially present a localized disease, progress to develop metastases. Hence, a comprehensive understanding of the biological mechanisms of OS metastasis must be gained in order to combat patient mortality. In the recent past it has been demonstrated in several carcinoma models that exosomes play a role in metastasis progression. However, their relevance in OS metastasis currently remains speculative.

Exosomes are small membrane extracellular vesicles (30-100nm) that are secreted by both normal as well as malignant cells. Their content cargo primarily consists of mRNA, microRNAs and proteins. The transfer of exosomal cargo facilitates both local as well as systemic communication between donor parental and recipient target cells. Several types of tumour cell lines

have been documented to produce elevated levels of exosomes. Moreover, increasing evidence demonstrates that tumour cells utilize exosomes to educate distant metastasis prone organs towards a pre-metastatic niche or a supportive metastatic microenvironment. This enables disseminated tumour cells to graft and establish metastatic colonies.

To understand more about OS derived exosomes we have isolated exosomes from a panel of 8 OS established cell lines. Isolation of exosomes from conditioned cell culture media was performed using differential ultracentrifugation. Based on the downstream analysis, the vesicles were resuspended in appropriate buffers, namely RIPA buffer for Western blot analysis and PBS for nanoparticle tracking analysis (NTA) and RNA isolation. Establishing the average size of isolated extracellular vesicles was initially performed by Nanosight (NTA) analysis and the primary peak size for all 8 cell lines derived vesicles were found to be between 80 and 120 nm. The size was subsequently confirmed using electron microscopy. The micrographs also demonstrated that the vesicles were free of contaminating proteins and have the typical cup shaped morphology of exosomes. The 143B and HU09 cell lines were the highest producers of extracellular vesicles in the investigated panel of OS cell lines. Moreover, Western blot analysis for all the samples showed that exosome associated markers such as Alix, CD9 and CD81 were highly upregulated in the vesicles compared to the respective parental cell extracts, while calreticulin, an endoplasmic reticulum localized protein, was not detectable in any of the samples indicating no intracellular vesicle contamination. Molecular RNA profiling via next generation sequencing of exosomal mRNA derived from 143B cells was done in order to identify the relevant exosomal transcripts that significantly contribute towards the modulation of the pulmonary pre-metastatic niche.

In conclusion, our study has been able to confirm that all the 8 cell lines produce extracellular vesicles that are consistent with the size, morphology and protein expression signatures of exosomes as described in the literature. We are currently evaluating exosomal mRNA derived from 143B cells which are involved in communication between the primary tumor and the distant target organs of metastasis in a pre-metastatic environment.

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Synergistic activity of NKG2D-based chimeric antigen receptor (CAR)-T cells and radiotherapy against glioma

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Introduction: Glioblastoma is the most common primary brain tumor in adults and virtually always lethal despite a multimodal treatment regimen including surgery, chemotherapy and radiotherapy. Therefore, novel treatment modalities are needed. Adoptive immunotherapy with genetically engineered T cells that express a chimeric antigen receptor (CAR) to recognize and eliminate tumors in a MHC-independent manner is an emerging strategy that has led to remarkable responses in hematologic malignancies. CARs that target the natural-killer group 2-member D (NKG2D) system elegantly use the promiscuous binding properties of the NKG2D receptor that binds to several tumor-associated ligands. NKG2D-based CAR T cells have never been investigated against glioma and it is unknown if this strategy can overcome the challenges of this tumor entity and if it could even be implemented in established conventional treatment regimens.

Methods: CAR T cells were generated by retroviral transduction of splenocytes derived from C57BL/6 or VM/Dk mice to express a synthetic receptor with the extracellular domain of NKG2D, a transmembrane domain and the intracellular CD3ζ domain. As a control, splenocytes were transduced with wildtype-NKG2D (wtNKG2D). For in vitro studies, murine glioma cells (GL-261, SMA-560, SMA-540, SMA-407) were co-cultured with chNKG2D or wtNKG2D T cells and we assessed the cytolytic activity and IFN-g production by flow cytometry. For in vivo studies, we used GL-261 cells syngeneic to C57BL/6 mice and monitored tumor growth by magnetic resonance imaging. For in vivo tracking of CAR T cells, we used fluorescence molecular tomography (FMT), flow cytometry and immunohistochemistry. Long-term survivors were re-challenged with another tumor implantation and tumor-infiltrating and peripheral lymphocytes were analyzed by flow cytometry. To study the combination of radiotherapy with CAR T cells, mice received a single subtherapeutic dose of local irradiation.

Results: In all murine glioma cell lines, chNKG2D T cells had a significantly higher specific cytolytic activity compared to wtNKG2D T cells. Furthermore, chNKG2D T cells produced more IFN-g. In vivo, intravenously injected chNKG2D T cells migrated to the orthotopic tumor site, were tolerated without toxicities, prolonged the survival and cured a fraction of tumor-bearing mice. This anti-tumor effect was even more pronounced in case of intratumoral CAR T cell administration. Survivors were long-term protected against tumor re-challenge. Mechanistically, this was not the result of a classical immune memory response, but rather due to local persistence of chNKG2D T cells. Ra-

diotherapy, as part of the standard treatment regimen against glioblastoma, augmented the effect of chNKG2D T cell therapy already after a single application of a subtherapeutic dose. We identified two underlying mechanism for this synergistic activity. First, a direct tumor-cell related effect as demonstrated by an increased cytolysis and IFN-g production after co-culture of low-dose pre-irradiated GL-261 cells with chNKG2D or wtNKG2D T cells and second an indirect migration-related mechanism as demonstrated by increased accumulation of CAR T cells within irradiated tumors in vivo.
Conclusion: We provide the first systematical preclinical assessment of NKG2D-based CAR T cells against glioma. This strategy was effective, tolerated without toxicities, conferred long-term protection and was synergistic with radiotherapy. These findings could provide a rationale to test this immunotherapeutic strategy also in human glioma patients.

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A mass cytometry-based atlas reveals novel subpopulations of macrophages and T cells associated with immune checkpoint regulation and clinical features of renal carcinoma

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Immune cells in the dynamic tumor microenvironment modulate all aspects of tumor development. Macrophages and T cells are key components of this ecosystem, yet a comprehensive characterization of their phenotypic diversity, their relationships within the ecosystem and correlation with clinical data is absent. Here we use mass cytometry in combination with novel antibody panels to perform an in-depth immunoprofiling of samples from a cohort of 73 clear cell renal cell carcinoma (ccRCC) patients, with a special emphasis on T cells and macrophages. In a total of 3.5 million measured single cells, we identified 17 tumor-associated macrophage subsets and 22 T cell subsets, many with novel and surprising marker combinations. Correlation analysis revealed rules that determine the structure of the tumor-associate microenvironment and a specific macrophage subset associated with exhausted T cells. By correlation with clinical data, subsets that accurately predict time to relapse and overall survival were identified. In summary, we present the most comprehensive atlas of the immune tumor microenvironment available to date. This study revealed potential biomarkers and targets for renal cell carcinoma therapy and validated tools that can be used for analysis of other tumor types.

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Unbiased analysis of the radiotherapy response to identify targets that support clinical efficacy

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Radiotherapy (RT) is a palliative as well as a curative treatment for cancer. RT induces irreversible DNA damage. However, it is clear that RT supports tumor-specific immunity. In fact, activation of the immune system is a central part of the clinical response. Previous work in our laboratory showed that activation of tumor-associated dendritic cells (DCs), which in turn support effector $CD8^+$ T cells in the tumor is crucial for therapeutic efficacy. Recently, RT-induced, local activation of complement and subsequent production of pro-inflammatory anaphylatoxins has been identified as an essential upstream event to RT-induced DC activation and protective $CD8^+$ T cell immunity.
RT is a major disturbance and presumably influences many, yet unidentified pathways leading to a multitude of downstream changes, which are currently unknown. My hypothesis is that some pathways affected by RT support the clinical efficacy of RT while other antagonize it. Consequently, selective promoting or suppressing particular pathways may improve the clinical efficacy

of RT. In my project I aim to investigate early RT-induced changes as well as changes occurring when the tumor starts to regrow. Therefore, I will perform unbiased analysis of tumor samples isolated at different time points after RT. The analysis includes RNA- and microRNA-sequencing as well as mass spectrometry for the investigation of metabolites. In follow-up experiments, I will interfere with selected pathways in order to promote tumor-specific immunity and increase the efficacy of radiotherapy.

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Transfer of cGAMP via gap junctions from tumor to dendritic cells - a potential mechanism of innate tumor immune sensing

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Spontaneous antitumor immune responses occur in different type of cancers. In particular, the infiltration of CD8-positive T cells into solid tumors is associated with immunosurveillance of the cancer and a beneficial prognosis for the patient.
In this context, one particular innate immune mechanism is of interest in which tumor-derived DNA is sensed in the cytosol of tumor-infiltrating dendritic cells (DCs) via the cyclic GMP-AMP-snythase (cGAS)/stimulator of IFN genes (STING) pathway. Activation of the cGAS/STING pathway in DCs leads via the second messenger cyclic GMP-AMP (cGAMP) to the induction of type I interferons, which seem to be essential for the generation of tumor-specific CD8-positive T cells. Nonetheless, little is known on the specific tumor cell characteristics and the detailed molecular mechanism by which this tumor cell-DC-interaction induces type I interferons.
Our preliminary data suggest that the transfer of cGAMP from tumor cells to DCs via gap junctions composed of connexin 43 mediates the type I interferon production in DCs. We assessed this question by in vitro co-culture experiments of various tumor cells and bone marrow derived dendritic cells (BMDCs) or the DC cell line DC1940. The magnitude of DC stimulation and type I interferon expression was analyzed by qRT-PCR, ELISA, Western blotting and flow cytometry. In our set up, tumor cells themselves did not produce a type I interferon response despite cGAS/STING expression. However, we identified a clear correlation of cGAS expression in tumor cells and the capacity to induce type I interferons in DCs. Moreover, silencing of the gap junction gene encoding connexin 43 in tumor cells reduced the type I interferon expression in co-cultured DCs. Together, these results indicate a potential mechanism of innate immune sensing of tumors involving the transfer of cGAMP to DCs via gap junctions.
Further validation of this mechanism would have major implications on cancer prognosis and immunotherapy by enhancing the innate antitumor immune response.

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Anti-glioma activity of oligonucleotide-mediated TGF-β1/2 inhibition

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Transforming growth factor (TGF)-β is a potent cytokine with multiple biological activities, which has become an attractive target in glioblastoma because of its immunosuppressive properties and role in migration and invasion. TGF-β signaling is highly active in high-grade gliomas and elevated TGF-β activity has been associated with poor clinical outcome.
Here we examine the effects of two novel TGF-β_1 or TGF-β_2 specific phosphorothioate locked nucleic acid- (LNA) modified antisense oligonucleotide gapmers, ISTH1047 and ISTH0047 respectively, on the downstream signaling and growth of human LN-308 and murine SMA-560 glioma cells in vitro. Their ability to inhibit TGF-β_1 or TGF-β_2 was also assessed in orthotropic xenogeneic and syngeneic in vivo glioma models.
Both antisense oligonucleotides silence their corresponding target and inhibit downstream SMAD-2 phosphorylation. Moreover, inhibition of TGF-β_1 or TGF-β_2 mRNA expression by ISTH1047 or ISTH0047 reduces both the invasive potential of glioma cells, with no effect on cell viability. In vivo systemic administration of ISTH1047 or ISTH0047 significantly suppresses TGF-β_1 or TGF-β_2, as well as down-stream target PAI-1 mRNA expression respectively in murine glioma-bearing hemispheres. TGF-β_1 or TGF-β_2 inhibition significantly prolonged the median survival of mice bearing SMA-560 tumors.
These data suggest that directly targeting TGF-β_1 or TGF-β_2 using these

novel antisense oligonucleotides represents a promising approach to inhibit TGF-β signaling in glioblastoma with potential therapeutic activity.

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FAN1 acts in concert with ubiquitylated PCNA to alleviate replication stress and maintain genome stability

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FAN1 nuclease is recruited to interstrand cross-links (ICLs) by monoubiquitylated (Ub) FANCD2, which interacts with a ubiquitin-binding zinc finger (UBZ) domain at the FAN1 N-terminus. Surprisingly, disruption of this domain does not affect sensitivity to ICL-inducing agents; instead, it causes chromosomal instability. We now show that FAN1 contains a previously uncharacterised PCNA interacting peptide (PIP) motif that acts in concert with its UBZ domain to recruit FAN1 to Ub-PCNA accumulated at replication forks stalled not only at ICLs, but also at secondary structures such as G-quartets. This interaction slows replication fork progression and thus prevents their collapse. Importantly, inhibition of PCNA ubiquitylation in FAN1-depleted cells alleviates genomic instability and it might therefore also slow disease progression of FAN1 mutation carriers afflicted with karyomegalic interstitial nephritis (KIN).

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A Metabolomic Profiling Approach for Identification of Metabolic Changes Associated with MET Targeting in MET-Addicted and Non-MET-Addicted Models

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Deregulated activity by the MET receptor tyrosine kinase is often associated with poor clinical prognosis. Furthermore, MET signaling confers resistance to anti-cancer treatments by protecting tumors from DNA-damaging agents. In order to gain insight into the link between MET and the DNA damage response, we carried out MS-based metabolomics analysis (high-throughput flow-injection analysis on an Agilent 6550 time-of-flight instrument) and multivariate statistics to study metabolites and metabolic pathways in various cell lines overexpressing the MET receptor.
In the current work, several MET-addicted and non-addicted cell lines have been used. The cell line panel includes the gastric carcinoma cell lines GTL-16, MKN45, SNU5, KATOII, SNU638, Hs746T; the non-small cell lung cancer cell lines EBC-1, H1993, H1648, H820 and HCC827. We inhibited MET in these cell lines using the novel specific small molecule tyrosine kinase inhibitor tepotinib, which is currently under clinical evaluation. The phosphorylation levels of MET and the activation of its downstream signalling pathways were assessed by Western blotting. Preliminary experiments showed alterations in energy metabolism, tricarboxylic acid cycle (TCA) cycle and amino acid metabolism. We identified one metabolite that is consistently altered only and across all MET-addicted cellular systems: 5'-Phosphoribosyl-N-formylglycinamide, which is involved in the de novo purine synthesis pathway. Interestingly, the complementary transcriptomics analysis of EBC-1 and GTL-16 cells showed that critical purine synthesis enzymes are downregulated upon MET inhibition (METi), a finding further validated by quantitative Real-Time PCR. Currently, we are focusing on transcription factors known to be involved in the purine synthesis pathway such as E2F1, which is downregulated in the transcriptomics dataset upon METi. Given the data obtained so far, we hypothesize that MET-independent E2F1 expression may potentially lead to a rescue of MET-addicted cancer cells after METi, thus deepening our understanding over the role of the MET oncogene in metabolism of cancer pathogenesis.

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Treatment of AML with immunotherapies

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Introduction: Immunotherapies hold promise and have made major progress in the treatment of hematological malignancies in recent years. One particularly effective approach is the use of chimeric antigen receptor (CAR) T cells, consisting of high affinity single-chain monoclonal antibodies or single-chain variable fragments (scFv), linked to the signaling machinery of the T-cell receptor and costimulatory molecules. Recently, ground-breaking clinical responses to CAR T- cell therapy in CD19+ B-cell malignancies have been reported. Acute Myeloid Leukemia is a clonal disorder of the hematopoietic stem/progenitor cells (HSPCs) and contains a subpopulation of leukemia-initiating cells (LIC) that can self-renew and give rise to the hierarchy of maturing blasts. While the proliferating mature blast pool is highly sensitive to chemotherapy, the more quiescent LICs are relatively resistant and can be a source of relapse. We postulate that the only way to lasting success in poor-risk disease is to radically eliminate LICs and accept collateral damage to HSCs that, subsequently, can be replaced by transplantation. We aim to create a platform for the generation of human CAR T-cells directed against leukemic and HSC antigens, the first of which will be c-Kit (CD117).
Methods: We will use mouse AML models as well as humanized models carrying human AML cell lines and primary human leukemia to evaluate safety and efficacy of Chimeric Antigen Receptor (CAR) T-cell, Bispecific T-cell engaging (BiTEs) antibodies and monoclonal depleting antibodies. The capacity to eliminate LICs as well as bystander effects on healthy hematopoiesis will be assessed.
Results: For the mouse AML models, we have modified a murine erythroleukemia (FBL3) cell line and mouse AML (TIB-49) cell line to express murine c-Kit (CD117) and GFP as a reporter gene. For the humanized AML model, we characterized the human AML cell line (Kasumi-1) which naturally expresses c-Kit. Additionally, we have modified the Kasumi-1 cells to express human CXCR4 to facilitate in vivo homing and engraftment in immunodeficient mice as well as to express GFP as a reporter gene. We have confirmed that the mouse cell lines engraft in wild type mice C57BL/6 and that the Kasumi-1 cells engraft in NSG immunodeficient mice. We used the previously published anti-mouse cKit depleting antibody (ACK2) and confirmed that we can deplete HSPCs in wild type mice and to a greater degree in immunodeficient mice. In parallel, we used a phage display library and recombinant soluble mouse cKit protein to identify a novel anti-mouse cKit antibody binding fragment. The new antibody fragment (D9) was expressed as a monoclonal IgG format, a bispecific T cell engager (BiTE) antibody format and it was cloned into a mouse chimeric antigen receptor (CAR) T cell lentivector construct. We have confirmed binding of the D9 IgG antibody and D9 BiTE antibody to mouse cKit+ leukemia cell lines and confirmed binding of the D9 IgG antibody to cKit+ cells in primary bone marrow of mice. Binding of the D9 BiTE to CD3 was also confirmed using primary mouse T cells. The D9 IgG antibody was further modified to have a mouse IgG2a FC domain in order to allow for FC mediated depletion of target cells. Experiments comparing the ACK2 and D9 IgG2a antibody are underway. In vitro assays are underway to determine the leukemia cell killing capacity of the D9 BiTE antibody and D9 CAR T cells.
Conclusion: Our goal is to use these immunotherapies to radically eliminate both AML-LICs and HSCs by targeting non-tumor-selective HSC antigens and to subsequently deal with the life-threatening HSC depletion by allogeneic HSC transplantation.

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Induction of Dormancy in Hypoxic Human Papillomavirus-Positive Cancer Cells

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Oncogenic human papillomaviruses (HPVs) are closely linked to major human malignancies, including cervical and head and neck cancer. It is widely assumed that HPV-positive cancer cells are under selection pressure to continuously express the viral E6/E7 oncogenes, that their intracellular p53 levels are reconstituted upon E6/E7 repression, and that E6/E7 inhibition phenotypically results in cellular senescence. Here, we show that hypoxic conditions, as they are often found in subregions of cervical and head and neck cancers, enable HPV-positive cancer cells to escape from these regulatory principles: E6/E7 is efficiently repressed, yet, p53 levels do not increase. Moreover, E6/E7 repression under hypoxia does not result in cellular senescence, due to impaired mTOR signaling via the inhibitory REDD1/TSC2 axis. Instead, a reversible growth arrest is induced that can be overcome by reoxygenation. Impairment of mTOR signaling also interfered with the senescence response of hypoxic HPV-positive cancer cells towards prosenescent chemotherapy. Collectively, these findings indicate that hypoxic HPV-positive cancer cells can induce a reversible state of dormancy, with decreased viral

antigen synthesis and increased therapeutic resistance, and may serve as reservoir for tumor recurrence upon reoxygenation.

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Overcoming Chemoresistance in KRAS-mutant lung cancer

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Introduction: Lung cancer is the most common and deadliest cancer among all malignant tumors, with a five-year survival rate of 16%. Patients with mutated KRAS lung adenocarcinoma have the poorest overall survival. The only treatment for this type of lung cancer is chemotherapy, and conventional chemotherapeutics often can neither stop tumor growth nor prevent its relapse due to tumor resistance. The molecular mechanisms underlying this phenomenon remain poorly defined, highlighting an urgent need to understand the molecular mechanisms and to develop more efficient treatments.
Results: To unveil the potential mechanism of KRAS-mutant lung cancer resistance to chemotherapy, and eventually reveal new therapeutic targets which can boost the efficacy for the treatment of KRAS-driven lung cancer. In this study, we first fraction a drug resistant subpopulation by sphere formation, then we did a pharmacological screen to identify a inhibitor which can specificly target drug resistant cells, and finally we found that mTOR activation confers chemoresistance in KRAS-mutant NSCLC. Moreover, combining mTOR inhibition with chemotherapy is a novel & effective strategy for treating KRAS-mutant lung cancer.

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Dendritic cell-derived exosomes in cancer therapy

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Exosomes are nano-sized membrane vesicles derived from the late endosomal compartment. They are capable of transferring proteins, lipids and RNA between cells. B cell and dendritic cell (DC)-derived exosomes express major histocompatibility complex (MHC) class I and II, as well as costimulatory molecules (CD80/86) and can initiate T cell responses. Several clinical trials have shown DC-derived exosome-based cancer immune therapy to be safe, but limited in inducing antigen-specific T cells. Thus, a better understanding of how to modify DC-derived exosomes is crucial to induce better immunostimulatory effects.
We have shown that exosomes loaded with the NKT cell ligand alpha-galactosylceramide (αGC) and the model antigen ovalbumin (OVA) activate NKT cells, induce strong NK and $\gamma\delta$ T cell innate immune responses, and induce OVA-specific T and B cell responses far better than only OVA-loaded exosomes. Exosomes loaded with αGC/OVA decreased tumour growth and increased median survival compared to exosomes loaded with OVA only or soluble αGC + OVA alone. Furthermore, exosomal MHC class I is dispensable for the induction of antigen- specific T cell responses if whole OVA is present. We have shown that OVA-loaded DC-derived exosomes from MHCI-/- mice induce antigen-specific T cells to the same extent as wild type exosomes. Even exosomes with MHC class I and II mismatch induced tumour-infiltrating CD8 T cells and increase survival in a B16 melanoma model. Our studies provide new opportunities for increasing the immunogenicity of DC-derived exosomes for cancer treatment and opens up new possibilities for the design of allogeneic exosome-based vaccines and therapies.

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Pre-clinical evaluation of novel Exendin-4 derivatives for non-invasive insulinoma diagnosis

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The glucagon-like peptide-1 receptor (GLP-1R) is highly expressed on the pancreatic β-cells, which produce and secrete insulin. β-cells are involved in diseases of the glucose metabolism, such as type 1 and type 2 diabetes mellitus and congenital and adult hyperinsulinemic hypoglycemia. Furthermore, GLP-1R is also overexpressed in β-cell derived tumors, like insulinomas, as well as several other cancers (reviewed in [1]). Exendin-4, a peptide ligand of GLP-1R, has already been used in clinical studies for the detection of insulinomas by SPECT/CT and PET/CT [2, 3]. However, its major drawback is the high kidney uptake, which decreases imaging quality while exposing the patients to elevated radiation burden. Here, we show how the addition of an albumin-binding moiety (ABM) to Exendin-4 strongly improves biodistribution and SPECT/CT imaging outcomes while retaining its high specificity to GLP-1R.

We used ^{111}In-labeled derivatives of Exendin-4 that carry an albumin-binding moiety. We compared the new peptides to the lead compound (Exendin-4 derivative without albumin-binding moiety) in vitro, determined their stability in human blood plasma, receptor affinity as well as cell internalization, and employed CD1 nu/nu mouse models carrying GLP-1R positive tumors for in vivo experimentation. In these studies, we investigated the biodistribution and SPECT imaging properties of the peptides.

The peptides were shown to have high affinity to the GLP-1 receptor (IC_{50} of 3.7 ± 0.6 to 15.1 ± 0.8 nM) and internalized well into cells cells (39 ± 2 to 56 ± 2% within 2 hours), thus outperforming the peptide without albumin-binding moiety (41 ± 4% within 2 hours). The stability in human blood plasma was slightly improved by the addition of the albumin-binder. In the in vivo studies, the new derivatives exhibited a significantly reduced kidney-uptake (between 48.38 ± 14.93 and 71.23 ± 16.02% i.A./g, 4 hours p.i.) compared to the lead peptide (160.98 ± 28.53% i.A./g, 4 hours p.i.) leading to favorable kidney-to-tumor ratios.

In conclusion, this study demonstrated that the addition of an albumin-binding moiety to radiolabeled Exendin-4 strongly decreased kidney accumulation while having positive effect on tumor uptake. Thus, Exendin-4 derivatives with an albumin-binding moiety could present a viable class of diagnostic tracers for the detection of insulinomas and other GLP-1R positive tissue in clinical application.

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Prognostic relevance of lung squamous cell carcinoma tumor fragmentation, a histologic marker of increased tumor invasiveness

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Accurate tumor classification is an important step for adequate cancer management. Next to tumor staging, histologic subtyping aims at further stratifying patients into additional prognostic groups. In this study, we addressed tumor fragmentation (TF) as a potential marker of tumor invasiveness for lung squamous cell carcinoma (LSCC), on two independent clinical cohorts (tissue microarray: n=208; whole sections: n=99).

TF scores were quantitatively addressed on pan-cytokeratin stained LSCC, using an image processing tool (ImageJ). Clinical data analysis revealed that increasing TF was associated with blood vessel infiltration ($p<0.01$) as well as decreased overall and relapse-free survival on both patient cohorts ($p<0.01$). The poor prognostic value of TF was validated on an external clinical cohort from The Cancer Genome Atlas (TCGA: n=326) using human-based scores.

In conclusion, tumor fragmentation is a histologic parameter associated with increased tumor aggressiveness which could be relevant for refining current tumor grading systems. Additionally, the proposed approach could be potentially applied to other solid human carcinoma.

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Epigenetic Therapy a Good BET for Glioblastoma? Systems biology approach to identify a rational combination therapy

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Our aim is to evaluate whether epigenetic drugs, like bromodomain and extra-terminal proteins (BET) inhibitors would be suitable for treating glioblastoma. In the 1st step we have evaluated the biological activity of the tool drug JQ1, a small molecule inhibitor of BET, using glioblastoma derived sphere (GS) lines. The results suggest intermediate sensitivity of GS to JQ1. Importantly, we observe that JQ1 impairs self-renewal capacity of all 4 tested GS lines. JQ1 treatment decreases the number of cells in S-phase in one of the GS lines, but it is reversible upon drug withdrawal. JQ1 induces apoptosis in two of the three tested GS. Moreover, we have observed that after 24 hours of JQ1 treatment, LN-2683-GS line seems to undergo differentiation.
In the 2nd step we aim at disturbing the system by BET inhibition in order to identify disturbed pathways that we hypothesize could be informative for potential vulnerabilities that can be targeted with a second drug. Here we report the data from differential gene expression profiles using RNA-Seq of LN-2683-GS line treated with JQ1 or DMSO over a 48 hours time course. Gene set enrichment analysis (GSEA) revealed several significantly disturbed pathways including a set of genes that is associated with response to Interferon alpha (IFN-alpha). Validation experiments in vitro confirmed that JQ1 down-regulates the expression of MX1 and OAS1 which are members of IFN-alpha response signature. The mechanism underlying this down-regulation and the relevance to the real glioblastoma microenvironment will be explored.
Moreover, the sets of genes that are disturbed in response to histone deacetylase inhibitors (HDACi) are enriched in our GSEA. Here we report promising results that show synergy between JQ1 and HDACi Trichostatin A(TSA). Further research will be focused on better understanding the mechanism of this synergy

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Transcription (co)factor network analysis of TGFb-induced EMT

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EMT (epithelial to mesenchymal transition) induces tumorigenic cells to undergo major transcriptional reprogramming that leads to the conversion of benign, adhesive cells to malignant, invasive cells with increased resistance to chemotherapy.
To study the signaling network driving this cellular de-differentiation, we induce EMT in murine epithelial cells (NMuMG/E9) with TGFb and follow the phenotypic changes over time. We have screened 1440 transcription (co) factors and 57 miRNA for their functional contribution to EMT based on phenotypic changes and the ability of the cells to migrate. 59 different transcription factors, epigenetic modulators and miRNA were followed up by RNA-sequencing after siRNA-mediated knockdown or miRNA overexpression, respectively. Comparing these perturbation experiments with transcriptional changes during a timecourse of TGFb-induced EMT, we infer a transcription (co)factor network that drives EMT progression based on hierarchical clustering and nested effects models.
With this approach, we hope to identify network hubs and associated pathways that could represent pharmacological targets of EMT.

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Systems-level comparative transcriptomic analysis of fibroblasts from pre-tumorigenic skin and wounds implicates Runx2 as a master transcriptional regulator of Activin-stimulated tumorigenesis and healing

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Tumors have been described as "wounds that do not heal" (Dvorak, 1986), yet the molecular similarities between tumors and wounds, which could in-

spire new therapies for combating carcinogenesis and improving healing, are still insufficiently characterized. Fibroblasts in the granulation tissue of wounds and in the stroma of tumors deposit and remodel the extracellular matrix (ECM) and establish the essential microenvironment for healing and tumorigenesis. Activin is a potent growth and differentiation factor, which is overproduced in a variety of tumors and early in healing wounds. We have previously shown that Activin, when overexpressed in keratinocytes of transgenic mice (K14-Act), acts in a paracrine fashion on stromal cells to enhance skin repair and to accelerate cancer formation in models of chemically- and HPV8-induced skin tumorigenesis. To characterize the effect of Activin on fibroblasts, we isolated these cells via fluorescence-activated cell sorting from normal skin, healing wounds and pre-tumorigenic skin lesions of K14-Act and control mice, and then performed RNA-sequencing followed by comprehensive comparative systems biology analyses using array data from human cancers and wounds. Ingenuity Pathway Analyses (IPA) of the genes upregulated by activin in pre-tumorigenic and wound fibroblasts revealed strong activation of genes associated with cell proliferation, migration, cytoskeletal dynamics, and ECM remodeling; the genes were furthermore significantly enriched with curated cancer-associated genes. Gene Set Enrichment Analyses (GSEA) showed that the genes upregulated by Activin in pre-tumorigenic and wound fibroblasts were highly enriched in the transcriptomes of (i) cancer-associated fibroblasts from human breast, prostate, lung, oral mucosa and skin, (ii) stroma from human breast, liver and lung cancers, (iii) human pre-tumorigenic skin lesions (Actinic Keratosis), and of (iv) human skin wounds. Further systems-level analyses of the most enriched and clinically relevant genes shared by wound and pre-tumorigenic fibroblasts identified the transcription factor Runx2 as a regulatory hub that connects multiple dysregulated pathways that may be responsible for the pro-healing and pro-tumorigenic phenotypes of Activin-stimulated fibroblasts. Indeed, bioinformatics analysis of data from the cancer genome atlas (TCGA) showed strong co-expression between RUNX2 and Activin, and high overlap between the genes co-expressed with both RUNX2 and Activin, suggesting significant co-regulation in clinical tumor samples. Many of the shared co-expressed genes were also upregulated in fibroblasts from wounds and pre-tumorigenic skin of K14-Act mice. In vitro studies confirmed that Activin induces expression of Runx2 as well as its classical and predicted clinically relevant target genes in primary dermal fibroblasts. In vivo, immunohistochemical and flow cytometry analyses showed strikingly greater percentages of fibroblasts expressing high levels of Runx2 in mouse skin wounds and established tumors compared to normal skin and thus under conditions when activin is overexpressed. These data suggest that Runx2 is an Activin-regulated transcription factor that is overexpressed in cutaneous wound and cancer fibroblasts, which integrates multiple dysregulated pathways. Further functional studies will reveal the role of the Activin-Runx2 axis in regulating fibroblast function during skin healing and tumorigenesis and as a potential novel target for the inhibition of tumor progression and/or enhancement of wound repair.

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3' UTR mediated posttranscriptional regulation of mesothelioma marker calretinin

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Calretinin (CALB2, CR) is a diagnostic and prognostic marker in malignant pleural mesothelioma (MPM). We previously reported that calretinin is regulated at mRNA level. The presence of a medium size 3' untranslated region (3'UTR-573nt) in additional two non-coding alternative transcripts arising from the CALB2 gene suggest a possible post-transcriptional mechanism regulating calretinin expression. The CALB2 3'UTR contains microRNA target sites and an A/U-rich element (ARE) putative cis-regulatory elements. Our aim was to investigate the role of the CALB2 3'-UTR in the post-transcriptional regulation of calretinin expression in MPM.

Using the pmirGLO dual-luciferase expression vector, the complete CALB2 3'-UTR fragment was inserted downstream of the firefly luciferase reporter gene. Activity of the CALB2 3'-UTR was quantified after transient transfection into mesothelioma cells. In silico analysis TargetScan and AREsite2 were employed to predict potential microRNAs binding site or ARE element, respectively. Site-directed mutagenesis was employed to mutate consensus sequence of the previously predicted functional cis-elements. Subsequently, luciferase activity and calretinin expression were evaluated after overexpression or inhibition of the predicted microRNAs. In addition, calretinin protein, assessed by immunohistochemistry, and miR-30 expression, were investigated in a cohort of MPM patients (N=60).

The addition of the CALB2 3'-UTR significantly downregulated the luciferase ac-

tivity in MPM cells. Bioinformatic analysis predicted target sites for miR-30 family members, miR-9 and ARE element within CALB2 3'- UTR. Mutational analysis of ARE site resulted in further destabilization of the reporter and a cytosolic protein binding ARE sequence could be detected. The mutation of two miR-30-binding sites abolished calretinin 3'UTR destabilization effect. Transient delivery of a miR-30e-5p mimics or anti-miR into MPM cells resulted in significant decrease/increase of the luciferase reporter expression and protein. However, overexpression of CALB2-3'UTR quenched the effect miR-30e-5p on the protein, most likely by sequestering the mimics, suggesting a possible ceRNA (competitive endogenous RNA) network. Finally, expression of miR-30e-5p was found to negatively correlate with the calretinin expression in a cohort of MPM patient samples.
Our data show for the first time the role of miR-30e-5p in the post-transcriptional negative regulation of calretinin expression via interaction with its 3'-UTR. We also demonstrated a role for ARE sequences in calretinin stabilization and possible physiological role of calretinin alternative transcripts.

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Modulation of tumor hypoxia for hypofractionated radiotherapy

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Introduction: Tumor hypoxia is associated with resistance to radiotherapy, an increased risk of metastases and poor clinical prognosis. Reactive oxygen species are generated in response to ionizing radiation (IR) and produce amongst others irreversible DNA double-strand breaks followed by chromosomal aberrations and cell death. This IR-induced cytotoxic effect is less abundant under hypoxia and thus hypoxic cells are more resistant to IR. Hence reoxygenation or a reduction of the hypoxic tumor fraction by a combined treatment modality with a pharmaceutical agent is of high interest to reduce the required dose of IR and thereby to further minimize normal tissue toxicity. Here we investigated the combined treatment modality of the novel anti-hypoxia compound myo-inositol trispyrophosphate (ITPP) in combination with IR.
Methods: ITPP was developed as an allosteric effector of hemoglobin to lower the oxygen/hemoglobin affinity thereby resulting in an enhanced release of oxygen upon tissue demand e.g. in hypoxic tumors. The capability of tumor reoxygenation by ITPP was serially probed by a non-invasive hypoxia-directed ODD-luciferase-based bioimaging approach and by immunohistochemistry (pimonidazole, CAIX) in FaDu HNSCC- and A549-lung carcinoma derived tumor xenografts. Tumor growth delay was determined on treatment with ITPP and a single high dose of IR (10 Gy).
Results: Using our in vivo bioimaging approach, we confirmed as part of pharmacodynamic-oriented in vivo studies increased pO2 starting 2 hours after ITPP application. Dose-titration studies indicated a maximal dose of 3g/kg of ITPP that was administered on two consecutive days followed by immediate irradiation 2 hours after the second application of ITPP. Interestingly ITPP alone did not affect the growth of FaDu tumor xenografts but significantly sensitized the tumor to a single dose of irradiation (10 Gy). Immunohistochemical analysis of the hypoxic marker CAIX validated this rapid decrease of tumor hypoxia in response to ITPP. Furthermore, the IR-induced tumor hypoxia observed at 4 days after IR may well be associated with a decrease in tumor vascular density, which was apparently prevented by combinatorial treatment with ITPP.
Conclusion: ITPP administration induces an immediate increase of oxygen availability that can be exploited by a combined treatment modality with IR as shown in our HNSCC tumor model. ITPP also seems to protect the tumor vasculature upon IR, which may positively impact on the hypoxia status. Overall our results support the strong rationale to combine ITPP with IR for hypoxic tumors, but also demonstrate the need for further pharmacodynamic studies to investigate the optimal combination regimen in view of hypofractionated radiotherapy.

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Identification of a MET-eIF4G1 translational regulation axis that controls HIF-1α levels under hypoxia

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Aims: Poor oxygenation is a common biologic feature involved in aggressive manifestations of solid tumors. Hypoxic areas of tumors have been reported to overexpress the MET tyrosine kinase, a high affinity receptor for hepatocyte growth factor (HGF). As both MET and hypoxia are determinants that affect cellular responses to DNA-damaging agents, we aimed to elucidate the effect of MET inhibition on MET-overexpressing tumor cell lines under hypoxic conditions.
Methods: MET-overexpressing cancer cell lines were exposed to a hypoxic environment (1.5% O_2) and MET was inhibited by a highly specific pharmacological inhibitor. Gene transcription was assessed by qRT-PCR and protein expression by western blotting or IHC. Protein-protein interactions were recorded by immunoprecipitation and specific phosphorylation changes by selective reaction monitoring (SRM). A 3D ex vivo model consisting of organotypic xenograft tissues was used to confirm the in vitro observations. Patients TMAs from the Institutes of Pathology of the Basel University Hospital and the Inselspital Bern were analyzed.
Results: MET inhibition reduces the protein levels of the key hypoxic regulator HIF-1α and its targets. Receptor targeting interferes with translation of HIF-1α through reduction in phosphorylation of the eukaryotic translation initiation factor eIF4G1 on Ser-1232. This phenomenon coincides with increased interaction between eIF4G1 and another member of the eIF4F complex. Reduced eIF4G1 phosphorylation can be observed not only in 2D cell line cultures, but also in a 3D ex vivo model. In patients' samples, MET, eIF4G1 and HIF-1α tend to be co-expressed in the same tumor regions.
Conclusion: We identified a new branch of the crosstalk between hypoxia-related signaling and MET receptor activity. Interruption of this pathway using MET inhibitors may contribute to antitumor activity of these agents in hypoxic malignancies, leading potentially to better therapeutic outcomes.

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MAP4K4 is overexpressed in Medulloblastoma and promotes tumor cell invasion

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Medulloblastoma (MB) is the most common malignant brain tumor in childhood. Metastatic dissemination of MB is preceded by the infiltration of tumor cells into the brain tissue, with craniospinal irradiation being the most important therapy to target disseminating MB cells. However, such treatment causes severe long-term morbidity, therefore, therapies that target tumor cell infiltration and metastatic dissemination specifically, are urgently needed to increase efficaciousness of therapy and decrease late-treatment complications.
We have identified the Ser/Thr MAP4K4 as a pro-migratory kinase in MB. We have found that it promotes cell motility and invasiveness downstream of Hepatocyte Growth Factor (HGF)-c-Met signaling pathway and further contributes to irradiation (IR) – induced cell dissemination in MB. Tumor tissue micro array and quantitative PCR methods revealed increased expression of MAP4K4 in a number of primary MB samples compared to normal cerebellum, suggesting that MAP4K4 could increase the invasive potential of the MB tumor cells. Indeed, pharmacological or genetic interference with MAP4K4 disrupted transmembrane c-Met receptor as well as integrin β1 (ITGB1) internalization and activation, two mechanisms involved in cell dissemination regulation.
Combined, our data reveal a novel, druggable mechanism we suspect to sustain an invasive and irradiation-resistant phenotype, through enhancing endocytic activity and the control of adhesion receptor turnover, through the proto-oncogenic kinase MAP4K4.

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Gene-based analysis of copy number aberration patterns in cancer with an integrative outlook on other mutation types

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Cancer is a genomic disease, characterized by the accumulation of different types of changes to the DNA sequence of the affected cells, such as point mutations (SNV), insertions, deletions, as well as chromosomal rearrangements resulting in losses or duplications of large genomic regions.

While the prevalence of copy number changes (CNA) differs widely between tumour types, on an average approximately 14% of a given cancer genome is in an imbalanced state due to gain/loss of regions from several kb to whole chromosomes. These copy number aberrations have the potential to affect a multitude of genes with potential cancer "driver" function, as well as in the maintenance of required basic cellular functions. Since most individual CNAs involve larger chromosomal regions and encompass many genes in a single amplification or deletion, the identification of cancer supporting genes based solely on the analysis of CNA poses challenges both with regard to the quantity of needed datasets as well as regarding the applied methodologies. The traditional focus on minimal overlapping regions in recurring CNA for inferring "driver" functionality has pointed to some well defined genes affected by recurring and frequent focal CNAs, such as e.g. MYCN, ERBB2, CDKN2A/B, ERBB2, TP53, REL, BCL2. Detecting focal CNAs (operationally defined as <= 3 Mbp) is based on pretext of considering smaller sized CNA for more gene exclusivity, and the reasoning that such focal changes may reflect a "targeted" interference of relevant genetic functionality. In our analyses, we identify focal CNAs in our collection of more than 50'000 cancer genome profiles and evaluate them for their relation to cancer classification, ontogenetic pathway involvement as well as affection by other mutation types.

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Defining DDR functions during transcription-replication interference

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The DNA replication machinery continuously encounters impediments that slow replication fork progression and threaten timely and error-free replication. Transcription has been proposed to be a major source of DNA replication stress (RS), and failure to assist fork progression across transcription units may compromise genome integrity. Despite the fact that altered gene expression programs and genome instability drive cancer development, the cellular mechanisms that deal with transcription-replication interference remain poorly understood. Using targeted high-content microscopy-based RNAi screens, we recently identified several newly emerging cancer genes with roles in RNA metabolism and transcription-related processes as modulators of RS. Based on these results, we revealed that depletion of genes involved in regulating RNA polymerase II pause-release strongly impacts RS resilience. While inhibition of transcription and origin firing both suppressed RS stress-induced DNA damage under these conditions, knockdown of the DNA damage response (DDR) kinase ATM or its targeted inhibition surprisingly accelerated the transition from stalled replication forks to chromosome breaks. These findings suggest an unanticipated role of ATM in replication fork protection during transcription-replication interference. Consistently, we provide evidence that transcription-associated replication stress leads to activation of ATM signaling prior to fork collapse. Our results therefore suggest that stalling of RNA polymerase II leads to co-operative DDR kinase signaling and pose deregulated transcription as a chief source of replication stress and cancer-associated genome instability. This in turn may have important implications regarding the use of DDR kinase inhibitors in combinatorial cancer chemotherapy regimens.

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A novel approach to investigate cellular and molecular determinants of brain tissue invasion in medulloblastoma

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Medulloblastoma (MB) is a paediatric cancer of the brain that arises in the cerebellum. While disseminating tumour cells cause cerebellar and leptomeningeal metastases, it is not well understood how MB cells from the primary tumour infiltrate the cerebellar tissue. In vitro techniques used to address the mechanisms of tumour cell dissemination fail to mimic the natural microenvironment. Moreover, the current experimental in vivo model systems focus on endpoint analysis of metastatic spread, while local brain tissue infiltration, the underlying cause for distal dissemination, remains unexplored. This calls for an appropriate model system wherein living cerebellar tissue can be used as an ideal matrix for studying primary MB tumour cell invasion. Hence, we have developed a novel approach to investigate brain tissue infiltration of MB tumour cells using organotypic cerebellar slice cultures (OCSCs). We have validated maintenance of cellular and structural components of cerebellum tissue in OCSCs for up to 30 days ex vivo and shown that

the tissue slices represent a physiologically relevant matrix to foster tumour cell growth and model brain tissue infiltration. Using cell-based models of Sonic hedgehog (SHH) and group 3 (G3) MB cells, our study revealed that SHH MB cells invade the cerebellar tissue either as single cells or in clusters, depending on the available growth factor signalling. In contrast, G3 MB cells are characterised by massive expansion of the tumour cell mass by hyper proliferation of infiltrating and non-infiltrating cells, which may explain the more aggressive nature of this subgroup in the patients.
We are now using this model as a pre-clinical platform to a) validate potential druggable therapy targets directed towards SHH and group 3 MB tumour cells b) determine alteration(s) in extracellular matrix composition in the vicinity of tumour cells and c) determine activation of glial cells in response to tumour growth.
Together, our study validates the use of OCSCs as a suitable model to investigate the cellular and molecular determinants of brain tissue invasion in medulloblastoma.

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Activin A regulates mDia2 function in wound and cancer-associated fibroblasts

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A series of studies have revealed remarkable similarities between wounds and tumors. Among the most important connections are the cellular parallels between the wound granulation tissue and the tumor stroma, including the large number of fibroblasts. This is of particular interest, since the important role of cancer-associated fibroblasts (CAFs) in tumor development and progression is being increasingly recognized. However, the functional activities of fibroblasts and their gene expression pattern at different stages of wound healing and tumor development remain poorly characterized.
Previous results from our laboratory showed that Activin A, a member of the TGF-b superfamily of growth and differentiation factors, is strongly upregulated in healing skin wounds as well as in epithelial skin tumors in mice and humans. This is functionally relevant, since Activin A overexpression promoted wound healing, but also formation of malignant skin tumors in different mouse skin cancer models. Interestingly, we observed a significant accumulation of fibroblasts in the pre-tumorigenic ear skin of activin-overexpressing mice (K14-Act mice) and enhanced mRNA levels of several CAF markers. These findings prompted us to study the effect of Activin A on fibroblasts. We showed that Activin A promotes fibroblast proliferation and migration in vitro, but inhibits the contractile ability of these cells. The pro-migratory activity of Activin A- treated fibroblasts was reflected by an increase in filopodia formation. The key target in the generation of filopodia is the formin mDia2, an effector of RhoGTPases. Functional analysis coupled with genome-wide RNA profiling of isolated fibroblasts from wounds and tumors identified mDia2 as a critical target of Activin A. Notably, intradermal injection of human squamous cell carcinoma cells overexpressing Activin A into NOD-Scid mice evoked up-regulation of mDia2 in fibroblasts and aggressive tumor formation. These results suggest that Activin A may promote skin tumor development and progression at least in part via activation of mDia2 expression by fibroblasts, thereby creating a pro-tumorigenic microenvironment. In addition, it may promote fibroblast migration into wounded skin, thereby accelerating the healing process. Future studies will reveal if targeting mDia2 expression/function in fibroblasts can be explored to inhibit tumor growth and progression, in particular in the presence of high Activin A levels, and if a transient increase in mDia2 expression levels stimulates wound healing.
Taken together, our results provide further insight into the parallels between wound healing and tumor formation and identified a novel Activin A target that is likely to mediate Activin A function in wounds and skin cancers.

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Tumor suppressive mechanisms of S1PR2 signaling in DLBCL

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Diffuse large B cell lymphoma (DLBCL) is the most frequently occurring B cell lymphoma in adults. We have recently shown that the hematopoietic oncoprotein Forkhead box Protein 1 (FOXP1) transcriptionally represses Sphingosine 1 Phosphate Receptor 2 (S1PR2) in DLBCL, thereby facilitating cancer progression. The aim of this project is to determine other transcriptional regulatory mechanisms of S1PR2 and its general contribution to lymphoma progression.
Preliminary data points to a potential regulation of S1PR2 via non-canonical SMAD5 mediated TGFβ signaling. We found that TGFβ decreases cell viability and increases apoptosis in a subset of DLBCL cell lines, while showing

an increase in S1PR1 expression on RNA level. Furthermore, SMAD5 is phosphorylated upon TGFβ treatment in susceptible cell lines and was found to bind to the promoter regions of S1PR2 using a ChIP approach. Knockdown of FOXP1 in combination with TGFβ treatment leads to a synergistic decrease in cell viability and increase in S1PR2 expression. Knockout of S1PR2 in DLBCL cell lines via CRISPR results in a survival benefit in in vitro and in subcutaneous in vivo models, further highlighting the importance of S1PR2 signaling as tumor suppressive mechanism in DLBCL.
Thus, evidence suggests a link between suppressive TGFβ signaling and S1PR2 signaling in DLBCL, which could present a potential new tumor suppressive mechanism. With ongoing research we hope to clarify the ongoing tumor suppressive signaling involving TGFβ and S1PR2 in DLBCL and to further characterize the importance of S1PR2 signaling in DLBCL to find potential new therapeutic strategies for patients.

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AQP1 influences tumor cell water permeability and mobility

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Purpose: Cell water permeability has been implicated in mechanisms of tumor cell migration and agility. Aquaporin 1 (AQP1) is a water channel that is up-regulated in several solid tumors. The aim of this study was to elucidate how AQP1 expression influences functional behaviour of neuroblastoma cells.
Methods: Cell permeability of neuroblastoma cells was visualized using confocal microscopy and quantified using stopped flow analysis. For assessment of AQP1 dependent cell motility AQP1 knockdown cells were compared to AQP1 positive cells.
Results: AQP1 expression significantly changes cell permeability as measured by stopped flow analysis. This effect can be visualized by confocal microscopy. There are significant differences in cell motility depending on different levels of AQP1 expression and AQP1 localization.
Conclusions: Neuroblastoma cell permeability and motility is influenced by the different levels of AQP1 expression thus modifying their ability to move. The potential of tumor cell to adapt their behavior with changing AQP1 expression might significantly contribute to tumor cell migration and metastases.

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Role of SUV39H1 in ADAM17-mediated radioresistance

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Introduction: The therapeutic response of ionizing radiation (IR) is imparted by genomic instability and DNA damage. However, IR also triggers multiple intracellular signaling processes as part of IR-induced stress responses that lead to the secretion of various para- and autocrine factors. Here we investigated treatment-dependent secretion of these factors, which drive acquired rescue mechanisms and determine the overall radiation sensitivity of the tumor.
Methods: Secretome analysis was performed using antibody arrays. Secretion kinetics of selected factors were determined using ELISA across different established tumor cells and in murine blood serum, derived from irradiated tumor xenograft-carrying mice. Clonogenic survival and xenograft tumor growth delay assays were performed in response to IR in siRNA- or inducible shRNA-targeted tumor cell lines or in combination with small molecular agents.
Results: We identified amphiregulin and ALCAM as top hits of the IR-dependent secretome analysis in lung carcinoma cells. These factors are shed by the common upstream metalloprotease ADAM17 (A Disintegrin and metalloprotease domain 17). Irradiation induced a dose-dependent increase in the activity of ADAM17, which correlated with subsequent substrate shedding. siRNA- or inducible-shRNA-mediated silencing of ADAM17 or targeting of ADAM17 with the small molecular inhibitor TMI-005 suppressed IR-induced shedding of these factors, downregulated ErbB-signaling in target cells and enhanced IR-induced cytotoxicity in vitro and in vivo. Recently, we identified that targeting of ADAM17 also interferes with epigenetic mechanisms. It decreased the basal level of the histone methyltransferase SUV39H1 and abolished a short-term increase of the SUV39H1 protein in response to irradiation. Interestingly, SUV39H1 was downregulated to almost non-detectable levels in irradiated ADAM17 knockdown cells 24 hours after irradiation

in comparison to irradiated control cells. Surprisingly decreased amounts of residual gH2AX were detected in ADAM17-depleted cells after irradiation despite their increased radiosensitivity in comparison to wildtype cells, implying a defect in the DNA repair machinery and poining towards a potential mechanism of radiosensitization by ADAM17 targeting.
Conclusion: Our findings demonstrate that IR significantly activates ADAM17, which results in shedding of survival factors, growth factor pathway activation and contributes to treatment resistance in NSCLC cells. Additionally, our data point towards a novel link between ADAM17, regulation of the cancer cell epigenome and the DNA damage response. We demonstrate that the impact of targeting ADAM17 is more pleiotropic than just diminishing ErbB signaling and provide a sound rationale for positioning ADAM17 inhibitors as radiosensitizers to improve the treatment of NSCLC.

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A novel BRCA1-associated protein-1 isoform affects response of mesothelioma cells to drugs impairing BRCA1-mediated DNA repair

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Introduction: BRCA1-associated protein-1, BAP1, is a tumor suppressor involved in multiple cellular processes such as transcriptional regulation, chromatin modification and DNA repair by deubiquitinating histone 2A. BAP1 mutations are frequent in malignant pleural mesothelioma (MPM). Our aim was to functionally characterize a newly identified isoform of BAP1 and investigate the effects of its expression on drug sensitivity in MPM.
Methods: Expression of BAP1 isoforms was detected in MPM and normal mesothelium cell lines, tumor and non-tumor samples by qPCR. Histone H2A ubiquitination levels were analyzed by western blot after acidic extraction of core-histones. Subcellular localization of BAP1 isoforms was examined by immunofluorescence. MPM cell survival in response to PARP- and to dual PI3KmTOR-inhibitors was analyzed by in vitro assays.
Results: We have identified a novel alternative splice isoform of BAP1 (BAP-1delta) which misses part of the catalytic domain. Cells transfected with BAP1delta showed loss of deubiquitinating activity compared to full length BAP1. The expression of BAP1delta transcript is more abundant in nontumor compared to tumor samples. MPM cell lines expressing higher level of endogenous BAP1delta are more sensitive to Olaparib, a poly(ADP-ribose) polymerase 1 (PARP1) inhibitor, and this sensitivity is enhanced when Olaparib treatment is combined with GDC0980, dual PI3K-mTOR inhibitor, which induces downregulation of BRCA1.
Conclusion: These observations suggest that BAP1delta may regulate DNA damage response and drug sensitivity. It might therefore be relevant to investigate whether patients with high expression of BAP1delta may be responsive to PARP/PI3K-mTOR inhibitors.

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Investigating a novel potential ATM/ATR/DNA-PK phosphorylation site on the MET RTK as a new player in tumorigenesis and a link between MET addiction and radioresistance

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MET, the receptor tyrosine kinase (RTK) for hepatocyte growth factor, is a proto-oncogene mainly expressed in epithelial cells and involved in development, homeostasis and tissue regeneration. Deregulation of MET signaling has been reported in numerous cancers, leading to a broad interest in MET targeting for therapy. Additionally, there is emerging evidence that MET signaling extends to the DNA damage response (DDR) machinery and protects tumors from DNA-damaging agents such as ionizing radiation (IR). Consequently, inhibition of MET as a radiosensitizing means is under investigation. The detailed molecular mechanisms behind this MET-DDR crosstalk remain largely elusive and are at the center of this work.
A study of post-translational changes in a MET-addicted cancer cell line upon MET inhibition, IR or combination revealed a yet unknown phosphorylation site on MET. Intriguingly, this phosphosite is part of a consensus motif recognized by the master DDR kinases and thus could be a part of the link

between MET and DDR. Our results confirm that the phosphorylation of this site fluctuates in response to MET inhibition and that DDR kinases can target it. Functional characterization of this phosphosite revealed that it is not restricted only to DDR but plays a role also with regard to migration ability and anchorage-independent growth. More specifically, abrogation of this phosphorylation appears to downregulate pathways downstream of MET and to sensitize cells to DNA damage, suggesting a crosstalk between MET and the DDR machinery. Additionally, the loss of this phosphosite promotes anchorage-independent growth while reducing 2D migration ability, suggesting that this site and the DDR kinases have an impact on MET-mediated invasiveness. Based on proteomics data, we speculate that the impact of the new phosphosite on these aspects of tumorigenesis could originate in a signaling pathway involving the Ras-related nuclear protein (Ran) and MET.

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Accumulation of macrophages and mesothelial precursors cells is essential for tumor development in asbestos-exposed mice

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Mesothelioma development is associated with asbestos exposure but early steps of carcinogenesis are unknown. To assess the role of loss of homeostasis in the mesothelial environment during mesothelioma development, Nf2+/- mice were injected eight times with asbestos fibers every three weeks in the peritoneum and several parameters were assessed thirty-three and forty-two weeks after the first exposure. Mesothelioma developed in 11% of mice within the first time point, when macrophages and mesothelial precursors levels significantly increased into the peritoneal cavity, accompanied by a peak of IL-10, G-CSF, CCL2, IL-6, and VEGF, which tended to decrease at the later time point. CCL5, IFNg, GM-CSF and CXCL1 increased levels remained stable. Transcriptomic profile revealed that 1260 genes were commonly upregulated in tumor and inflamed tissue, including signature of deregulated NF2/Hippo pathway. However, one of the highest upregulated genes was Arg1. The presence of Arg1 positive cells and other markers associated with M2 macrophages was confirmed. Intraperitoneal injection of syngeneic mesothelioma cells reproduced this phenotype. Administration of clodronate liposome impaired macrophage and mesothelial precursor accumulation and tumor growth. Altogether, asbestos-induced loss of homeostasis in mesothelial environment, skewed toward accumulation of M2 macrophages and mesothelial precursors, is an essential step in mesothelioma development.

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Microenvironmental T-helper cells induce the expression of mutagenic AID in B-cell precursor acute lymphoblastic leukemia

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B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common childhood malignancy. Although 90% of pediatric BCP-ALL patients show long-term survival, relapsed patients are difficult to treat due to drug resistance. BCP-ALL cells at relapse often harbor additional mutations compared to cells at diagnosis. In mature B-cells, beneficial mutations in immunoglobulin genes are caused by T-helper (Th) cell induced expression of activation-induced cytidine deaminase (AID). Interestingly, AID seems to play a role also in the pathogenesis of BCP-ALL by inducing off-target mutations. The regulation of AID expression in BCP-ALL cells, however, is unknown. Since Th cells home to the bone marrow (BM), the microenvironment of BCP-ALL cells, we hypothesized that these Th cells contribute to the regulation of AID expression in BCP-ALL cells and thus to disease progression and drug resistance. To assess the potential of Th cells to interact with BCP-ALL cells, we co-cultured BCP-ALL cells with autologous Th cells from the BM and found increased expression of activation markers, indicating that microenvironmental Th cells may interact with BCP-ALL cells. Furthermore, we found that stimuli expressed by microenvironmental Th cells increased expression

of AID in BCP-ALL cells. IFNγ, the main cytokine expressed by Th cells in the bone marrow, however, did not affect the expression of AID. Preliminary experiments suggested that IFNγ increases the stability of nuclear AID and therefore potentially increases the activity of AID. Currently, we are investigating the role of AID as a potential cause for relapse-associated mutations. Therefore, our identification of microenvironmental Th cells as inducers of AID expression might open up new targets for treatment to avoid relapse-associated mutations.

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Wnt ligands control initiation and progression of HPV-driven SCC

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Human Papilloma Virus (HPV)-induced cutaneous squamous cell carcinoma (SCC) is the most common cancer among immunosupressed patients after solid organ transplantation (within 15 years 90% develop skin warts, 40% SCC). Despite certain indication suggesting HPVs as promoters of genomic instability during SCC development the exact mechanism, how HPVs induce SCC remains unknown. To investigate the molecular mechanisms driving HPV-induced skin cancer, we used a mouse model in which UV-induced overexpression of the human papilloma virus 8 (HPV8)-derived oncogene E6 in keratinocytes (K14-HPV8E6) results in progressive SCC. Expression profiling of UV-accelerated HPV-induced SCC revealed strikingly elevated secretion of Wnt ligands resulting in enhanced Wnt/b-catenin-signaling. Blocking the secretion of Wnt-ligands via Porcupine inhibitor LGK974 dramatically affects the initiation and progression of HPV-driven SCC. Whereas the initiation process fully depends on the activity of Wnt-ligands, blocking the Wnt-secretion in already established tumors promotes their differentiation accompanied with apoptosis of the tumour cells that happens on the expense of reduced stem cell signature, invasiveness and proliferation. Hence, for HPV-induced SCC the aberrantly enhanced Wnt/b-catenin signaling is required similarly as for non-viral types of SCC and key proteins promoting Wnt-secretion may represent a promising target for therapeutic intervention.

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Multiplexed Imaging Cytometry Analysis Toolbox

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Quantitative, systems biology studies are producing rich, but complex datasets that require new visualization tools to aid analysis and provide biologic insight. High-dimensional imaging cytometry measures cellular heterogeneity to an unprecedented depth, but relies on computational tools that disregard cell morphology and tissue organization. We developed miCAT (multiplexed image cytometry analysis toolbox), an open-source, cross-platform, modular computational workflow and platform for the coordination of spatial information with single cell analysis methods. miCAT combines multiplexed image visualization and cytometry analysis into a single user-friendly software. Here, we highlight miCAT's unique ability to define cell types and neighborhoods from 35-parameter mass cytometry images of human breast cancer.

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SOX10 as a therapeutical target for malignant melanoma

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Melanoma is a highly aggressive skin cancer that is responsible for 80% of deaths from skin cancer with only 14% of patients with metastatic melanoma surviving for five years. Despite the increasing number of clinical trials testing the efficacy of various pharmacological agents, the incidence and mortality rates due to metastatic melanoma continue to rise. Understanding the molecular mechanisms that cause melanoma progression will be crucial for developing novel drugs. Sox10 is a transcription factor crucial for the self-renewal of neural crest stem cells during development. Moreover, Sox10 promotes the formation and maintenance of giant congenital nevi and melanoma in mice and humans. Despite the fact that SOX10 might serve as a promising therapeutic target in melanoma, it is a transcription factor and

therefore, it will be difficult to target therapeutically.
It has been previously shown that Sox10 expression is upregulated by $Nras^{Q61K}$-mediated signaling, however, the precise mechanisms of this regulation remain to be characterized. To investigate the upstream signaling pathways regulating SOX10 expression, we have used a panel of human melanoma cell lines carrying $BRAF^{V600E}$ and/or $NRas^{Q61K}$ mutations (in collaboration with Department of Dermatology, USZ). We applied several TKI inhibitors currently used in clinics such as vemurafenib and dabrafenib (BRAF inhibitors), selumetinib (MEK inhibitor) and everolimus (mTORC1 inhibitor) in vitro in human melanoma cells lines and scored for changes in the expression of SOX10. Surprisingly, we did not observe any detectable changes in the expression of SOX10 when human melanoma cells were treated with clinically relevant TKI inhibitors. However, inhibition of GSK3 suppressed SOX10 protein in melanoma cell lines carrying either $BRAF^{V600E}$ and/or $NRas^{Q61K}$ mutations. Importantly, this effect was also seen in vemurafenib-resistant cell lines. These data suggest that Wnt/β-catenin signaling might regulate SOX10 expression in melanoma.

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The role of SOX10 in melanoma resistance

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Malignant melanoma is the most aggressive skin cancer and its incidence is steadily rising. Despite the recent success in treatment of melanoma patients with a variety of targeted inhibitors of the RAS-RAF-MAPK signaling pathway, the vast majority of patients develop resistance and succumb to the disease eventually. It has been hypothesized that stem cells are accountable for tumor relapse and recently this hypothesis has been supported by the experimental data obtained from genetically engineered mouse models of glioblastoma, colon cancer and prostate cancer. Here, we aim to address this hypothesis in melanoma using tissue biopsies, patient-derived melanoma cell lines as well as genetically engineered mouse models of melanoma (Tyr::$Nras^{Q61K}$Ink4a-/- model and $Braf^{V600E}Pten^{fl/fl}$Tyr-CreERT2 model). We have obtained several sensitive and resistant cell lines (in collaboration with the Department of Dermatology, USZ). The majority of BRAF-mutated cell lines acquired resistance due to the treatment with vemurafenib (BRAF inhibitor), while some of NRAS-mutated cell lines are resistant to binimetinib (MEK inhibitor). Sox10 is a transcription factor playing a crucial role in the self-renewal of neural crest stem cells as well as in the maintenance of giant congenital nevi and melanoma. Our preliminary data demonstrate that SOX10 is expressed at higher level in sensitive cell lines as compared to the resistant melanoma cells. Moreover, we show that upon treatment with inhibitors the expression of SOX10 is downregulated in sensitive cell lines. On the contrary, the resistant cell lines increase the levels of SOX10 expression upon treatment with inhibitors. To dissect the cellular mechanisms of resistance in vivo, we have generated xenografts from both cohorts of melanoma cell lines, sensitive and resistant. As a first step, we have analyzed the expression of melanoma-specific markers as well as the expression of SOX10. Surprisingly, based on our preliminary results, we do not observe any heterogeneity with respect to SOX10 expression. In xenograft tissue obtained from sensitive melanoma cell lines the expression of MART-1 (melanoma antigen recognized by T cells 1) and TYR (Tyrosinase), markers often used to diagnose melanoma in clinics, is distributed in a homogeneous manner. Interestingly, the xenograft tissue of resistant cell lines is characterized by completely different pattern of MART-1/TYR expression, with either patches of tissue showing positivity or only single cells expressing these markers. To further investigate the reasons underlying our observations also in vivo we are currently establishing mouse models, which will mimic the acquisition of resistance and therefore, will allow us to more precisely understand the molecular and cellular mechanisms of melanoma resistance.

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SOX10 as a therapeutic target in Clear Cell Sarcoma

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Clear Cell Sarcoma (CCS) is a very rare and aggressive subtype of soft tissue sarcoma, associated with a high tendency to relapse locally, metastasize distantly and with a poor overall survival. Most cases affect young adults between 20 and 40 years of age. CCS often arises next to tendons, aponeuroses and fascia on distal extremities. CCS is considered to be resistant to conventional chemotherapy. Neither targeted therapeutic options nor immunotherapeutic approaches have been successful, yet. The cells of origin

of CCS are neural crest derived. The defining molecular feature of CCS is either the recurrent translocation t(12;22) (q13;q12) or, in rare cases, t(2;22) (q34;q12), resulting in expression of EWSR1-ATF1 or EWSR1-CREB1 fusion genes, respectively. These fusion proteins are the transforming oncogenic event in CCS.

SOX10 is a transcription factor, which is essential in neural crest development, the self-renewal of neural crest stem cells and for the formation of melanocytes arising from the neural crest. Previous research showed that SOX10 plays also a very important role in the maintenance of melanoma and it might represent a therapeutic target. SOX10 is required for the cAMP-mediated activation of the M-MITF gene promoter in CCS by cooperating with CREB1 and ATF1 and it is therefore conceivable that SOX10 is a therapeutic target in CCS.

We first analyzed, whether SOX10 is expressed in CCS. We cultured primary cultures of melanoma as a positive control and a panel of CCS cell lines and assessed levels of SOX10 mRNA and protein. Both were readily detectable and expressed to similar levels as in melanoma. We next analyzed biopsies from patients by SOX10 immunohistochemistry (IHC). 5/5 samples showed a strong nuclear SOX10 staining. We are currently expanding this analysis in a collaboration with the EORTC and Prof. P. Schöffski, University Hospitals of Leuven, who have generated a tissue-microarray from samples from a clinical trial on CCS, which will be stained for SOX10. Staining will be quantified, grouped and correlated with clinical characteristics, as well as with other IHC markers. This will establish, whether SOX10 serves as a biomarker in CCS.

In preliminary functional experiments, we have knocked down SOX10 in the human CCS cell line KAS using lentiviruses expressing short hairpin RNAs targeting SOX10. Two different shRNAs reduced SOX10 protein expression compared to uninfected parental cells, or to cells expressing a non-silencing scrambled control shRNA. Both SOX10-shRNAs significantly reduced colony formation in long-term proliferation assays in vitro.

In order to evaluate the therapeutic potential of silencing SOX10, we are currently expanding our in vitro experiments. Experiments, in which we knock down SOX10 using short hairpin RNAs in our panel of CCS cell lines and assess effects on proliferation by performing colony formation assays and MTT assays, as well as induction of apoptosis by Annexin-V / propidium iodide co-staining, followed by FACS analysis, are ongoing. To assess the effects of SOX10 knock down in vivo, we are currently also establishing a CCS xenograft model in nude mice.

We have preliminary evidence that SOX10 might serve as a therapeutic target in CCS in vitro. In ongoing in vitro and in vivo experiments, we will now expand those analyses. This will firmly establish, whether SOX10 serves as a biomarker and is a preclinically promising therapeutic target in this aggressive orphan sarcoma.

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Targeting glioma stem cells with interferon (IFN)-β

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Glioblastoma is the most common malignant primary brain tumor in adults. Despite surgery, radiotherapy and chemotherapy, patients suffering from this tumor have a poor prognosis with a median survival still limited to approximately one year in population-based studies. Current standard of care is only moderately effective and patients eventually all succumb to their disease. Therefore, the main effort in the field consists in finding new and better therapies based on increasing knowledge of the complex tumor microenvironment and the signaling pathways that drive this devastating disease. A population of cells within the tumor with stem cell features, now commonly referred to as "glioma-initiating cells" (GIC), has been attributed a central role in the escape of glioblastomas from the current clinical approaches of radio- and chemotherapy.

We have recently demonstrated that long-term glioma cell lines (LTC) as well as GIC cultures express receptors for IFN-β, IFNAR1 and IFNAR2, and respond to IFN-β with induction of STAT-3 phosphorylation and MxA protein accumulation. Single exposure to IFN-β inhibits cell cycle progression, induces a minor loss of viability, and strongly interferes with sphere formation in GIC cultures. Modelling acquired IFN-β resistance in GIC in vitro induces a stable resistant phenotype defined by clonogenic survival assays which allows to identify resistance mechanisms in more detail. Transcriptomic and proteomic profiling of IFN-β-treated GIC in vitro already revealed candidate molecules potentially mediating these biological responses. We also examined the anti-glioma activity of type I interferons in clinically relevant orthotopic xenogeneic animal models in vivo. Treatment with the more stable pegylated IFN-β significantly prolongs survival in the LN-229 and ZH-161 mouse models. In vivo, IFN-β induces a significant increase in MxA expression in the tumor cells but does not change vessel density. Since IFN-β is widely used for the treatment of human patients with multiple sclerosis,

there is considerable knowledge on its safety in patients with brain disease, allowing for rapid translation of novel IFN-β-based strategies into the clinic.

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Characterization of novel mediators of pre-metastatic niche formation

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Metastasis is the leading cause of cancer-related mortality. This multistep process requires that disseminated tumor cells (DTCs) evade immune surveillance and adapt to a hostile environment in the metastatic organ. Evidence from mouse models and human samples shows that the primary tumor can orchestrate the production of pro-inflammatory molecules and infiltration of bone marrow-derived myeloid cells in the metastatic organs before the arrival of cancer cells, thus creating a pre-metastatic niche. It has been shown that the pre-metastatic niche is essential for the success of the metastatic process. However, the exact mechanism how the primary tumor promotes the formation of this niche and the exact step in the metastatic cascade where this process is crucial, are largely unknown.

To address this question we use an orthotopic model of stage IV triple negative breast cancer that preferentially metastasizes to the lungs (4T1). We observed that after injection of a primary tumor neutrophils and inflammatory monocytes progressively accumulate in the lungs and blood. As the number of myeloid progenitors increased in the bone marrow, we think that the primary tumor produces factors that impact on myelopoiesis. Surgical removal of the primary tumor completely abolished the changes in the immune infiltrate of the lungs proving that those modifications are primary tumor-dependent. Moreover, removal of the primary tumor at different times allowed us to trace back the timepoint when cells start to migrate from the primary tumor.

In addition, we are currently developing a model for dormant breast cancer metastasis, where orthotopically injected 4T07 tumor cells migrate to the lungs but do not produce macroscopic metastasis. Our future plans include to further characterize this model and compare the difference in the immune infiltrate of the lungs in the intra and extra-vascular compartments of both models. The comparison of 4T1 and 4T07 will enable us to further dissect whether the pre-metastatic niche is necessary only for the initial seeding of tumor cells or whether it is also necessary to the proliferation and survival of the DTCs in the target organ.

Once both models are sufficiently characterized, we will choose a time point when tumor cells are not yet detectable in the lungs and perform an unbiased analysis of transcripts and micro-RNAs. We expect to identify novel pathways of pre-metastatic niche formation that can be therapeutically relevant. In follow-up experiments, we will interfere with those selected pathways to try to prevent metastasis formation.

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TRAIL expressing NK cell recruitment by CD26/DPP4 inhibitor treatment suppress lung cancer growth

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Background: Lung cancer is the leading cause of death among cancers. There is broad evidence that immune cells are involved in the growth and development of these malignancies. CD26/DPP4 (dipeptidyl peptidase 4) is a transmembrane glycoprotein, that is constitutively expressed on hematopoetic cells, but also found on lung epithelial and endothelial cells. We found previously that the activity of CD26/DPP4 of lung cancer patients is four times higher than in normal tissue. Here, we tested if CD26/DPP4-inhibition is able to modulate lung cancer growth via TRAIL expressing NK cell activation in mice.

Methods: An orthotopic lung tumor model was employed by sc. injections of the mouse lung cancer (Lewis Lung Carcinoma (LLC)) and a human lung adenocarcinoma cell line (H460). These were developed in mice C57BL6 (n=18) and CD1-nude mice (n=20) respectively. The CD26/DPP4-inhibitor Vildagliptin was given in drinking water of 50mg/kg daily dose. Tumor growth was evaluated by wet weight of tumor mass at 2 weeks. Histological assessments included immunohistochemistry (IHC) of Ki-67, p-Histone3, CD3, B220, F4/80, NKp46, and γH2AX. IL-10, Arginase, IL-12, NKp46, NK1.1, IFN-g, TRAIL, and Perforin 1 were analyzed by RT-PCR and FACS. In vitro analysis

of γH2AX expression in the TRAIL treated LLC was performed by western blotting. For a proof of concept and as a loss of function experiment, macrophage ablation was performed by clodronate-liposome during Vildagliptin treatment and NK cells were deleted by IL-15 knockout.
Results: Vildagliptin treatment significantly reduced the tumor growth of both, LLC and H460 in mice. IHC showed macrophages (F4/80) and NK cells (NKp46) to be significantly increased by Vildagliptin within tumors, while TUNEL stain and IHC of T- and B cell infiltration did not show any difference. Gene expression levels of anti-inflammatory markers (IL-10, and Arginase) were unchanged, while the pro-inflammatory cytokine TNF-α, IL-12, and IL-15 were significantly elevated. The NK cell markers NKp46, NK1.1, IFN-g, TRAIL, and Perforin 1 were significantly upregulated within the tumor by Vildagliptin, indicating that inhibition of CD26/DPP4 recruits NK cells into the tumor. FACS revealed TRAIL expressing NKp46+ NK cells were found elevated by Vildagliptin treatment. Furthermore, we found enhanced γH2AX expressions in the lung cancer cell line by TRAIL treatment in vitro and in vivo tumor by Vildagliptin treatment. As the consequence of treatment, the proliferation rate (p-Histone3/Ki-67) was significantly down-regulated. Macrophage ablation with clodronate-liposome in Vildagliptin treated mice reversed the tumor size significantly. Deletion of NK cell significantly increased the tumor size compared to control.
Conclusion: The Inhibition of CD26/DPP4 decreased lung cancer growth in primary models of mouse and human lung cancer and increased inflammatory macrophages and NK cell cytotoxicity including TRAIL within those tumors. Furthermore, an increased expression of cellular stress marker γH2AX by TRAIL treatment in lung cancer cell lines suggests that NK cell derived TRAIL inhibited the lung cancer growth.

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Non-canonical Ubiquitination drives the DDR cascade: identification of new factors involved

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The involvement of E2 enzymes in ubiquitin (Ub) modification pathways reflects their central roles in processes like regulating protein degradation, function, and localization, thereby controlling the biology of the eukaryotic cells. Recently, different laboratories, including ours, highlighted the essential role of ubiquitination in the organization of the DNA damage response (DDR) after double-strand breaks (DSBs). These ubiquitination events are driven by Ub ligases, such as RNF8, which, together with Ubc13, modifies chromatin by K63-linked polyubiquitin by targeting the H1-type linker histones. This first ubiquitination event promotes the recruitment of RNF168 that, in complex with an unknown E2 conjugating enzyme, ubiquitinates histones H2A and H2A.X. We demonstrated that RNF168 induces non-canonical K27-linked ubiquitination of damaged chromatin, specifically targeting the novel site K13/K15 at the N-terminus of histones H2A and H2A.X .
Despite the constant stream of new insights, many important pieces of the complex puzzle of ubiquitination and the role of E2 enzymes in DDR, as critical players herein, remain missing. Here, we focused on the identification of the molecular machinery working with RNF168 in the generation of this peculiar Ub mark that alerts the cell to activate DDR in vivo by employing an in-depth E2-conjugation screen via biochemical and single-cell immunofluorescence analysis. Using this systematic approach, we were able to shortlist few E2s as potential candidates to work with RNF168, which are currently under characterization.
Given that E2s are key mediators of Ub chain assembly that can be selectively targeted by small molecules, in future it will be important to further expand our understanding of their function as essential cofactors, as well as to elucidate their relevance and contribution to disease pathogenesis.

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The interplay between neutrophils and CD8+ T cells improves survival in human colorectal cancer

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Purpose: Tumor infiltration by different T lymphocyte subsets is known to be associated with favorable prognosis in colorectal cancer (CRC). Still debated is the role of innate immune system. We investigated clinical relevance, phenotypes and functional features of CRC infiltrating CD66b+ neutrophils and their crosstalk with CD8+ T cells.
Experimental design: CD66b+ and CD8+ cell infiltration was analyzed by immunohistochemistry on a tissue microarray including >650 evaluable CRC samples. Phenotypic profiles of tissue infiltrating and peripheral blood CD66b+ cells were evaluated by flow cytometry. CD66b+/ CD8+ cells cross-

talk was investigated by in vitro experiments.
Results: CD66b+ cell infiltration in CRC is significantly associated with increased survival. Interestingly, neutrophils frequently co-localize with CD8+ T cells in CRC. Functional studies indicate that although neutrophils are devoid of direct antitumor potential, co-culture with peripheral blood or tumor associated neutrophils (TANs) enhances CD8+ T cell activation, proliferation and cytokine release induced by suboptimal concentrations of anti-CD3 monoclonal antibody (mAb). Moreover, under optimal activation conditions, CD8+ cells initially stimulated in the presence of CD66b+ cells show decreased expression of PD-1 "exhaustion" marker and are significantly less susceptible to apoptosis induced by T- cell receptor triggered re-stimulation. Importantly, combined tumor infiltration by CD66b+ and CD8+ T lymphocytes is associated with significantly better prognosis, as compared to CD8+ T cell infiltration alone.
Conclusions: Neutrophils enhance the responsiveness of CD8+ T cells to TCR triggering. Accordingly, infiltration by neutrophils enhances the prognostic significance of CRC infiltration by CD8+ T cells, suggesting that they might effectively promote antitumor immunity.

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Helicobacter urease–induced activation of the TLR2/ NLRP3/IL-18 axis protects against asthma

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Helicobacter pylori is a gram-negative bacterium that colonises the stomach. The infection of H. pylori is highly associated with peptic ulcer and gastric cancer. Epidemiologic data strongly indicates that since 1950's there has been an increase in incidence of allergic diseases, such as asthma and inflammatory bowel disease, in developed countries. However, this is accompanied by a decrease in infectious diseases, including H. pylori infection.
H. pylori primes the activation of multiprotein oligomer inflammasomes in dendritic cells through pathogen-associated molecular patterns urease B subunit, which is recognised by Toll-like receptors 2 (TLR2). This in turn initiates transcription of Nod-like receptor 3 (NLRP3) through transcription factor NF-κB. Subsequently, NLRP3 forms a complex with ASC and pro-caspase-1 and activates caspase-1 by autocleavage. Once pro-caspase-1 is auto-cleaved, pro-IL-1β and pro-IL-18 are processed into their active forms. IL-1β drives a pro-inflammatory response while IL-18 promotes regulatory Treg differentiation, as seen in patients with peptic ulcer and asymptomatic carriers, respectively.
Remarkably, urease-proficient H. pylori infection is able to prevent asthma through Tregs induced in the gut. These Tregs are believed to migrate to the lungs and secrete IL-10 along with dendritic cells to counteract allergic reactions. Urease mutant-infected mice displayed asthma symptoms when sensitised and challenged with house dust mite allergen, while wild type-infected mice do not. Moreover, the protection can be transferred to a naïve recipient from wild-type-infected donor through Treg extracted from the mesenteric lymph nodes.
Recently, our preliminary data has shown that dendritic cells up-regulate TLR2 after infection. The factor that causes this up-regulation is yet to be determined. Thus, the TLR2/NLRP3/IL-18 axis does not only have important implications for the understanding of asthma, but is also important for studying the interaction between H. pylori and the host.

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Antagonizing cross-talk of bFGF and TGF-β signalling controls tissue infiltration in medulloblastoma

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How medulloblastoma (MB), a metastatic paediatric tumour of the cerebellum, infiltrates the brain tissue is poorly understood. We hypothesized that growth factors secreted from the tumour microenvironment could trigger a motile and invasive tumour cell phenotype. To identify and target the key players of MB cell motility and invasiveness, we have established automated cell dissemination counter (aCDc), a screening platform to quantify tumour cell migration and invasion in high-throughput. aCDc is highly sensitive and it enabled for the first time a phenotype-based analysis of the growth factor susceptibilities of primary MB tumour and patient-derived xenograft cell cultures. We identified Fibroblast Growth Factor (bFGF) and its receptor FGFR1 as potent pro-migratory factors present in the tumour microenvironment

of MB and it promotes tumour cell infiltration through the adaptor protein FRS2. The function of FRS2 is antagonized by TGFβ, which induces a contractile, non-motile cell phenotype through ROCK activation and direct FRS2 repression. In the absence of TGFβ, activated bFGF-FRS2 attenuates ROCK and enables motility and invasiveness through Rac-PAK and ERK1/2. In contrast, at high concentrations of bFGF, TGFβ rescues the motility impeded via FGFR1-dependent negative feedback and triggers invasiveness.
This antagonistic crosstalk between bFGF and TGFβ signalling pathways tunes motile and invasive properties of brain tumour cells for tissue infiltration under varying environmental conditions and reveals novel drug targets.

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Novel ubiquitylation and deubiquitylation components controlling cellular responses to DNA damage: impacts on genome stability and links to cancer

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As the genetic information encoded in DNA represents the most important result of organisms' evolution, it is crucial to assure transmission of such information to the next generation. DNA double-strand breaks (DSBs) are prevalent drivers of genomic instability, with their generation and ineffective repair being hallmarks of many cancers and other human diseases. To prevent accumulation of harmful mutations, cells have evolved mechanisms to detect and repair DNA damages arising upon exposure to endogenous factors or exogenous agents such as radiation and mutagenic chemicals. Proteins of the so-called DNA damage response (DDR) network are tightly regulated by post-translational modifications (PTMs) that affect their activity, localization, interactions and DNA binding affinities. Among these PTMs, ubiquitylation and deubiquitylation have recently emerged as particularly crucial in DDR regulation, although the precise ways that they control DDR events is not yet known.
In this study we aim to elucidate novel DDR-related events controlled by ubiquitylation/deubiquitylation. Thus, we are carrying out a two-pronged approach, taking advantage of recent data obtained in the lab, identifying ubiquitin-system proteins with previously uncharacterized DDR links. By exploiting tools already available in the lab, we initially plan to extend such findings, focusing on validating the newly identified DDR connections and aiming to gain insights into the specific role and mechanism-of-action of the most promising ones by cell-based approaches and mouse genetic studies. In parallel, we intend to carry out new systematic screening studies of a collection of ubiquitin-component and DDR gene knockout cell lines exclusively available in the lab to profile their sensitivity to a set of around 100 compounds, including best-in-class approved and experimental anti-cancer drugs. We will also use a genetic "bar-code" DNA-sequencing based screening strategy on such cell lines as a highly versatile strategy to systematically assess the impact of gene knockouts on cell viability under different DNA-damaging conditions. The importance of the proposed project is supported by the established relevance of targeting ubiquitylation and deubiquitylation pathways that are frequently altered in cancer and other human diseases.

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Development and implementation of standards for oncogenomic data sharing

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With the growth of genomic data from rapidly advancing molecular profiling technologies, the "age of big-data" is greatly enriching our understanding of human malignancies. Analyses of both germline and cancer derived samples have shown a vast amount of genomic variations, occurring as inherited variants or as the result of somatic mutation events. As a result, a large 'knowledge gap" has been realised, reflecting the limited understanding of the impact of individual and compound genome variations.
A feasible approach to close this knowledge gap lies in the generation of a large number of genomic variation maps from healthy and disease related tissues, and in the comparison of these variations with biological, population related and environmental metadata associated with the respective samples and individuals. However, this approach cannot be addressed in any single study but requires the integrative analysis of many, distributed, international datasets. Nevertheless, such an approach is currently limited by the lack of consistent formats for data reporting and exchange as well as in the real and perceived threat to personal privacy, associated with the possible exposure

of both genome based information and associated metadata.
Recently, a group of leading scientists working in the areas of biology, medicine, computational research, data security as well as law and ethics founded the "Global Alliance for Genomics and Health" (GA4GH), with the aim to develop standards for the exchange of genome data and supporting information and the promotion of legal and ethics procedures for the use of this data in biomedical research.
As members of the GA4GH Data Working Group (DWG), we are collaborating in the development of modern, standardised data schemas, to facilitate annotation and mining of biological or biomedical attributes associated with physical or procedural objects, related to genome data. Our contributions to the development of GA4GH related standards and resources is based on our experience in genome and metadata curation, as expressed in our progenetix.org and arraymap.org cancer related data resources.
For the projects presented here, we utilise over 63'000 genomic copy number profiles and associated clinical annotations related to human cancer from our arrayMap resource, to create the first GA4GH schema compliant database of cancer genome variation data. Additionally, we present the implementation of a cancer genome "Beacon", part of a GA4GH driver project to promote federated sharing of genome variation data in an international context.
For the future, we are confident that our contribution will help to overcome the barriers of incompatible data structures and access protocols in cancer genomic research, to enable easy and secure collaboration and genomic data sharing between data providers and users.

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Identification of Specific Methylation Targets of the Serrated Pathway to Colon Cancer

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Around 25% of sporadic colon cancers arise via the serrated pathway. Most of them are proximal colon cancers with a CpG Island Methylator Phenotype high (CIMP-H). A well known target of methylation is the promoter of the MMR (mismatch repair) gene MLH1 leading to its loss of expression and consequent defect of the DNA repair system. Sessile serrated adenomas/polyps (SSA/Ps) are thought to be the precursors of CIMP-H/MMR-deficient colon cancers. We hypothesised that CIMP occurs early on in tumourigenesis, with detectable levels of methylation in SSA/Ps (i.e., proto-CIMP). The aim of our study was to test this hypothesis and exploit proto-CIMP for diagnostic purposes.
Forty-eight fresh proximal colon tissues were prospectively collected by endoscopy: 12 SSA/Ps and, for comparison, 12 conventional adenomas (precursors of non-CIMP-H colon cancers), including their matched normal mucosa. We employed a genome-wide analysis based on bisulfite sequencing of roughly 2.7 million CpG sites located mainly in gene regulatory regions. In addition, RNA-sequencing was performed on the same precancerous lesions to explore the relationship between DNA methylation and gene expression.
Principal component analysis revealed that SSA/Ps and conventional adenomas have a clearly distinct methylome. Although both types of tumours showed increased methylation of a large number of regulatory regions, this phenomenon was more pervasive in SSA/Ps, in terms of number and spreading of hypermethylated regions (i.e., proto-CIMP). For example, we only detected methylation in SSA/Ps in the EPM2AIP1 promoter that is shared with MLH1. The SSA/P-specific methylated regions are currently being verified and eventually will be implemented in a molecular diagnostic assay to facilitate pre-colonoscopic diagnosis of SSA/Ps and aid their precise histologic classification.
Similar to the methylation patterns, our data also indicates that SSA/Ps have a different transcriptome compared to conventional adenomas. However, and unexpectedly, while most of the dysregulated genes in conventional adenomas were down-regulated (in comparison to matched normal mucosa samples), SSA/Ps showed more up- than down-regulated genes in spite of proto-CIMP. This indicates that the correlation between methylome and gene expression is very complex. A method for integrative analysis of these diverse -omics data is currently underway to disentangle this relationship.

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The role of TIP5 in aggressive prostate cancer development and progression

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Prostate cancer (PCa) is the second most commonly diagnosed cancer and the third most common cause of male cancer-related deaths in developed countries. The lack of efficient therapies for advanced PCa remains a major health problem. A considerable limitation to identify efficient therapeutic targets is that PCa remains particularly resilient to classification into molecular subtypes associated with distinct disease outcomes.

Recent work of our laboratory determined that the epigenetic factor TIP5 is implicated in aggressive prostate cancer. TIP5 establishes epigenetic silencing at genes frequently repressed in metastatic PCa and is indispensable for survival and invasion of metastatic PCa cells. Remarkably, TIP5 is required for a subpopulation of PCa cells with stem-like features, which are considered to be the basis of castration resistance and metastatic disease, and thus an important target for effective therapy to fight PCa. Indeed, there is a certain consensus that PCa may origin from stem or progenitor cells. Interestingly, a diagnostic feature that is observed in human prostate cancer progression is the increase in luminal cells and loss of basal cells.

To determine how TIP5 is implicated in the development of prostate cancer, we have started to analyze whether and how TIP5 plays a role in prostate epithelia differentiation. Gene expression analysis of TIP5 in mouse prostate epithelium revealed significant enrichment of TIP5 in luminal cells compared to basal cells. These data were consistent with immunofluorescence analysis of prostate tissues, which showed that TIP5 is expressed in luminal cells. Expression analysis of CD49f+ prostate basal and CD24+ luminal cells from wild type and TIP5 KO mice revealed that TIP5 KO basal compartment increases the levels of p63, a marker for prostate basal stem cells, and Prostate Cancer Stem Cell Antigen (PSCA), a characteristic marker for transit amplifying cells, suggesting an impairment in basal to luminal cell differentiation. Consistent with these results, TIP5 KO mouse prostate tissue displayed an increased number of p63 positive cells and showed an altered prostate duct morphology. Remarkably, generation of organoids from TIP5 KO prostate epithelium cells was less efficient in comparison to WT cells and TIP5 KO organoids had an altered structure, indicating defects in proliferation and/or epithelial differentiation. Taken together these findings suggest that TIP5 is implicated in prostate epithelium differentiation process and the lack of TIP5 impairs stemness capacity of prostate progenitor cells by hampering their ability to differentiate into luminal cells and leading to their accumulation in basal compartment.

To determine the suitability of prostate organoids for ex vivo manipulation and to model PCa development, we established knockdown Pten expression in mouse prostate organoids by infection with lentivirus encoding a shRNA targeting Pten. Pten knockdown organoids had higher proliferation compared to control organoids, changed the phenotype into hyperplastic form and displayed an expression profile characteristic for PCa. We are currently applying this ex vivo PCa model to study the role of TIP5 in PCa development.

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Novel roles of Wnt5a in melanoma pathogenesis

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Melanoma is a type of skin cancer formed by transformed melanocytes. It is highly aggressive due to its malignant nature: metastasis can be formed early during disease progression. Once metastasized, patients have a 5-year survival rate of only 5% (1). Despite efforts on elucidating the biological nature of melanoma during the last decades, prognosis for patients with metastatic melanoma is still poor. A series of in vitro experiments indicate an important role of Wnt5a in affecting the motility and invasion of patient-derived melanoma cell lines (2, 3, 4). However, the influences of tumor microenvironment such as the interaction of the tumor cells with the immune system or other cell types are not taken into consideration in in vitro experiments. Further, the roles of Wnt5a during tumor initiation have not been studied so far due to the limitation of the system used. Determination of the roles of Wnt5a during tumor initiation, growth, metastasis and further investigation of the underlying molecular mechanisms in an in vivo system, which allows studying gene functions of melanocytes in their natural microenvironment, could bring new hope for melanoma patients. Using a murine melanoma model which resembles the human disease with respect to histopathology as well as molecular pathogenesis, we have identified novel roles of Wnt5a in melanoma initiation. These findings could bring new aspects in melanoma treatment.

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ArrayMap & Progenetix 2016: Oncogenomic Databases towards Personalized Medicine

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Screening for somatic mutations in cancer has become integral to diagnostic as well as target identification procedures. arrayMap is a curated oncogenomic resource, focusing on genomic arrays and copy number aberration (CNA) profiles. The underlying data has been extracted from NCBI's Gene Expression Omnibus (GEO), EBI's ArrayExpress, and, importantly, through targeted mining of publication data. Since its first release in 2012, arrayMap underwent improvements to facilitate meta-analysis of cancer related genome data and clinical use, such as the diagnostic validations as well as target evaluation for personalized therapeutic approaches.
For the 2016 arrayMap update, we expanded both the scope and depth of the database, as well as improved the metadata structure. In a systematic mining of genomic array data from GEO, we evaluated more than 120'000 annotation files. The individual data review yielded around 22'000 additional data sets related to somatic mutations in cancer associated samples (cancer specimen or associated reference profiles). Additionally, we were able to increase the database of publications assesssed for describing original cancer genome profiling experiments to now more than 3'000 individual articles. The result is a comprehensive and useful database containing information for aproximatedly 400 cancer types and 63'000 genomic array profiles.
Furthermore, the hierarchical representation of the data in individuals, biosamples and experiments and its association to metadata, such as geographic location of the study, allows for further meta-analysis. In ongoing studies, we will use our data collections to investigate temporal and geographic trends and biases in cancer genome research, to provide a clear view of the current oncogenomic research landscape and to identify knowledge gaps, to guide the direction of future studies.

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Mechanisms Underlying the Formation of Tertiary Lymphoid Structures in Cancer

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Lymphoid aggregates are able to form upon infiltration of lymphocytes into non-lymphoid adult tissues under autoimmune and chronically inflamed conditions, as well as in tumours. Some of such aggregates form an organised architecture with distinct B and T cell zones that resemble the structure of secondary lymphoid organs (SLO). These are referred to as tertiary lymphoid structures (TLSs). TLSs are also able to induce adaptive immunity similar to SLOs and, hence, could be beneficial in cancers by acting as on-site locations of tumour-specific immune response generation. This hypothesis is supported by the observation that tumour-associated TLSs correlate with prolonged survival of patients with different cancer types.
The aim of this project is to identify which factors are involved in the formation of TLS and to further investigate the function of TLS in mouse models of cancer. By studying models of spontaneous lung metastasis from Lewis lung carcinoma (LLC) and 4T1 breast carcinoma, we observed organized lymphoid aggregates in metastatic lungs derived from 4T1 but not from LLC tumours. Furthermore, we observed that in an intravenous model of LLC where C57BL/6 mice were also exposed to LPS as an additional inflammatory stimulus, LLC tumours still did not encourage TLS formation at the tumour border implying that they don't support or even prevent TLS formation. We also successfully established a short-term model of local LPS-induced TLS in the lungs for comparison and elucidation of tumour-specific mechanisms of TLS formation. In the future, RNA from 4T1 lung metastases and lungs treated with LPS will be analysed at different time points for expression of potential factors implicated in TLS formation –to capture different phases of TLS formation– and later the cell types responsible for expression of such factors will be studied and their roles functionally investigated. Lastly, LLC tumours were genetically modified to overproduce CXCL13 or lymphotoxins,

which are molecules implicated in secondary lymphoid organ formation, to promote TLS formation by this tumour in vivo. In the future, reporter strains of CXCL13 and CCL19 will be utilized to examine which cell types are responsible for early phases of TLS formation in these settings.

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Tumor-treating fields (TTFields) interfere with biological key properties of glioma cells in vitro

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Tumor-treating fields (TTFields) are low amplitude alternating electric fields which are supposed to exert anti-tumor effects by targeting dividing tumor cells while sparing cells in the brain not undergoing cell division. Although this novel therapeutic approach has shown encouraging results in phase III trials in glioblastoma, its biological effects on tumor cells have only been poorly understood.

Here, we investigated the effects of TTFields on glioma cells in vitro using the inovitro™ system that allows the application of TTFields to cell cultures. Exposure to TTFields potently induce autophagy and necroptosis and interfere with the migration and invasion of long-term glioma cell lines, but also of glioma-initiating. The combination of TTFields with irradiation or temozolomide (TMZ) reduced viability and clonogenic survival in an additive or synergistic manner. Further studies suggest that the O^6-methyl-guanine DNA methyltransferase (MGMT) status does not influence the efficacy of TTFields and TMZ-resistant glioma cells remain responsive to TTFields application, thus making TTFields particularly attractive for the majority of glioblastoma patients with tumors that are unlikely to benefit from TMZ treatment.

In summary, these findings demonstrate that the application of TTFields may interfere with various biological key properties of glioma cells and may allow for a more detailed clinical evaluation of TTFields beyond the clinical data available so far.

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Establishment of a patient-derived myelofibrosis xenograft mouse model

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Introduction: A growing number of patient-derived xenograft (PDX) mouse models have been developed over the past few decades that allow engraftment of human hematopoietic stem cell (HSC) malignancies. This is a powerful tool for investigating the evolution of HSC and leukemic stem cells, as well as disease heterogeneity. However, engraftment is often limited due to potential lack of supportive factors in the bone marrow (BM) microenvironment. This limitation facilitated the development of more advanced mouse strains that express human cytokines and growth factors that are needed for efficient human hematopoietic development in vivo. Myelofibrosis (MF) is a HSC disorder characterized by bone marrow fibrosis that has the potential to transform into acute myeloid leukemia depending on the clonal evolution of MF stem cells (MF SCs). However, the engraftment of MF SCs in PDX models is poor (Wang et al., JCI 2012). We hypothesized that the constitutive expression of human cytokines and growth factors in a PDX model may promote the development of the human MF clone in vivo. Therefore, we used next-generation mice that express human M-CSF, IL-3, GM-CSF, TPO, and SIRPαTg (MISTRG) in order to develop a pre-clinical MF PDX model.

Methods: Purified peripheral blood stem and progenitor (CD34+) cells were collected from MF patients and intrahepatically transplanted into sublethally irradiated newborn MISTRG mice. NSG mice (Standard PDX mouse strain) are used as controls. Each mouse strain is transplanted with the same patient sample. 5-9 weeks after transplantation mice are sacrificed and analyzed for human engraftment using flow cytometry and immunohistochemistry.

Results: Most patient samples so far were transplanted into MISTRG mice. We are currently in the process of also transplanting NSG mice as a comparison. Preliminary results show that four out of seven samples engraft in the BM of MISTRG mice with a total median human CD45+ cell engraftment of 23%. Over 60% myeloid differentiation was observed in the mice that showed engraftment. Overall, preliminary results suggest that the next-generation MISTRG mice support human MF engraftment.

Conclusions and outlook: MISTRG mice support unprecedented myeloid engraftment of human MF SCs in 57% of patient samples investigated so far. Immunohistochemistry will also be performed on the BM and spleen of these mice in order to check for fibrosis and megakaryocytes. In order to determine whether specific somatic mutations promote human MF engraftment in PDX models next-generation sequencing will be performed on transplanted patient samples. Moreover, clonal evolution in vivo will be tracked and correlated with disease progression in patients.

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Targeting cullin ubiquitin ligase leads to growths arrest in malignant pleural mesothelioma cells

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Introduction: Mutation of the tumor suppressor gene Neurofibromatosis type II (NF2) was detected in 30-40% of malignant pleural mesothelioma (MPM) patients. NF2 suppresses tumorigenesis in part by inhibiting Cullin4 ubiquitin ligase. Cullin4A (CUL4A) gene amplification and its' overexpression has been detected in MPM cell lines and tumors. We hypothesized that cullin4 is a potential treatment target for MPM. Cullins' activity can be blocked by the inhibition of neddylation, a post-translational modification for cullins. In this study we assessed the efficacy of pevonedistat, an inhibitor of protein neddylation, in MPM cells.
Methods: Thirteen MPM cell lines and 3 MPM primary cells grown in monolayer (2D) were employed to assess the efficacy of pevonedistat in vitro compared to normal mesothelial cells, using MTT assay. The expression of cullins was assessed by quantitative real time PCR and western blot. Cell cycle was analyzed by flow cytometry. Four cell lines were cultured in multicellular spheroid (3D) format and measured for viability by acid phosphatase assay.
Results: Across 13 MPM cell lines, 5 cell lines (38%) were sensitive to the treatment with pevonedistat (IC50<500nM). All sensitive cell lines overexpressed CUL4A in both mRNA and protein levels. The treatment induced G2 cell cycle arrest and accumulation of cells containing >4N DNA content, representing cells undergoing DNA re-replication. DNA re-replication is known to be mediated by the accumulation of a DNA replication licensing factor, CDT1. Indeed, higher CDT1 accumulation was detected in the sensitive compared to the resistant cell lines. All primary cells showed no CUL4A overexpression compared to normal mesothelial cells, nonetheless 2 of them were sensitive to pevonedistat. Interestingly, these cells exhibited higher levels of neddylated (activated) CUL4A and higher CDT1 accumulation following the treatment. Cell lines overexpressing CUL4A remained sensitive to pevonedistat when cultured in 3D spheroids.
Conclusion: Inhibition of cullins by pevonedistat induced growth arrest preferentially in MPM cells overexpressing CUL4A in 2D and 3D cultures. The major mechanism seems to be mediated by DNA re-replication induced by CDT1 accumulation. More investigation into the role of cullin ubiquitin ligase in MPM is ongoing.

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Mitochondrial Pyruvate Carrier 1 can regulate growth and differentiation in Melanoma

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In 1927, cancer cells were described to, independently of the presence of oxygen, energetically lean on glucose catabolism via glycolysis – Warburg effect (O. Warburg, et al., 1927). When located in hypoxic conditions, other cell types show the same metabolic switch as cancer cells. In fact, stem cells (hematopoietic, mesenchymal and neural) maintain their undifferentiated and proliferative properties by downregulating OXPHOS and increasing glycolysis (Suda, et al., 2011; Shyh-Chang, et al., 2013; Simon, et al., 2008). Moreover, hematopoietic stem cells (HSCs) require oxidative phosphorylation (OXPHOS) in order to differentiate (Jang, et al., 2007), suggesting that mitochondrial function is crucial in the differentiation process.
Recently, two independent studies pinpointed that the MPC (mitochondria pyruvate carrier) complex is responsible for the transport of pyruvate across the mitochondrial membrane (Bricker et al., 2012; Herzig et al., 2012). This discovery inspired the investigation of the role of MPC in the maintenance of the Warburg effect in cancer cells. Schell et al., reported, consequently, that preservation of the Warburg effect via decreased MPC activity is able to promote growth in colon cancer.

Accordingly, we show that inhibition or downregulation of the mitochondrial pyruvate carrier subunit 1 (MPC1), which is a key subunit of the MPC complex, downregulates mitochondrial function and further increases melanoma growth (in vitro and in vitro). Moreover, MPC1 expression correlates with melanocyte differentiation markers in vitro, in vivo and in patient samples. We suggest that, comparably with colon cancer, MPC1 downregulation increases growth and may influence the differentiation status of melanoma cells.

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Tumor enrichment by DNA-based flow-sorting for highly sensitive analysis of genomic evolution in matched samples of heterogeneous pulmonary squamous cell carcinoma

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Background: Lack of tumor purity is one of the main limiting factors in cancer research. Notably, the average purity of squamous cell carcinoma of the lung (SCC) is as low as 49% (1). Even if the required decreases with continuously improving next generation sequencing technologies (NGS), dilution with DNA from benign cells is still an unsolved issue ensuing in falsified results. This hurdle exacerbates especially the investigation of the intratumoral heterogeneity, where pure populations are desirable. In this study we demonstrate an approach to precisely isolate the aneuploid tumor populations and decipher the clonal evolution in 6 patients with matched primary and metastatic SCC tumor specimens.
Methods: Nuclei were extracted from multiple biopsies, frozen and FFPE, from SCC patients and were subjected to flow-sorting based on DNA content using DAPI. Subsequent genomic profiling by array comparative genomic hybridization (aCGH) and NGS (Ion Torrent Comprehensive Cancer Panel with an all-exon coverage of 409 cancer genes) was applied to the isolated aneuploid tumor cell populations. Diploid tumor cell population served as a control.
Results: Our proposed approach demonstrates successful enrichment of aneuploid tumor fractions in tumor specimens admixed with a variable proportion of benign tissue components. Shared and unique genomic break points indicate clonal relationship between tumor samples in aCGH analysis. Diploid population can be used as a negative control. NGS analysis supported observations in clonality between matched longitudinal tumors. Due to high purity of tumor cells low allelic frequency mutations from presumably small subpopulations become clearly detectable even in highly heterogenous tumors.
Conclusion: In this study matched primary and metastatic tumor samples of SCC were analyzed. To overcome the low purity in heterogenous tumor samples we sorted aneuploid tumor populations to identify the evolutionary progression. NGS and aCGH proved to be a strong combination to decipher clonal relationship between tumor samples. Our approach avoids contamination by admixed benign cells, increases the precision of genomic analysis and allows to reconstruct the clonal evolution of the disease.

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Tissue-specific mediation of metastatic formation by L-selectin on myeloid cells

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Bone marrow-derived myeloid cells have been identified as promoters of metastasis. L-selectin is a leukocyte homing receptor that is important for the initial capture of leukocytes to the activated endothelium. We sought to determine the function of L-selectin in metastatic progression. L-selectin-dependent monocyte recruitment to the metastatic emboli promoted experimental lung metastasis. Lower numbers of L-sel$^{-/-}$ myeloid cells correlated with reduced levels of cytokines during lung seeding. While a depletion of circulating monocytes diminished metastasis both in WT and L-sel$^{-/-}$ mice, adoptive transfer of monocytes restored lung metastasis. WT monocytes facilitated trans-endothelial migration of tumor cells, L-sel$^{-/-}$ monocytes showed no effect in vitro. We demonstrated that injection of tumor-conditioned media rescued experimental lung metastasis in L-sel$^{-/-}$ mice and that spontaneous lung metastases in the absence of L-selectin were not affected. Interestingly, spontaneous liver metastasis was increased in L-sel$^{-/-}$ mice. Similarly, increased experimental liver metastases were observed in

the absence of L-selectin. Among differences in leukocyte population within the liver, a significant reduction in the number of F4/80+ cells was observed in tumor-bearing L-sel$^{-/-}$ mice when compared to WT controls. Expression of anti-tumor cytokines (e.g. IFNγ, IL-12, IL-2) was reduced in the livers of L-sel$^{-/-}$ mice, thus more protumorigenic environment was likely promoting metastatic growth. Although L-selectin facilitates tumor cell extravasation and metastasis in the lungs through the recruitment of F4/80+ cells, the very same cells limit the tumor outgrowth in the liver changing the tissue microenvironment.

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Extravasation of metastatic melanoma cells across the lung endothelium: Differential roles of endothelial VCAM-1 and PECAM-1

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Spreading of primary melanoma to secondary sites is the main cause of melanoma related death. However, metastasis prevention remains an unsolved challenge in the treatment of melanoma patients. Metastasis formation is a multi-step process that includes the invasion of melanoma cells into the local tissue, intravasation into blood or lymph vessels, and extravasation at distant sites across the vascular endothelium into organ tissue. Cell adhesion molecules critically contribute to the formation of metastasis. In this study, we investigated the roles of endothelial VCAM-1 and endothelial PECAM-1 for melanoma cell extravasation across the lung endothelium. VCAM-1 is well known as an important trafficking cue for activated T cells across the inflamed endothelium with important clinical impact in the treatment of multiple sclerosis. PECAM-1 is involved in maintaining junctional integrity of the endothelium with rather limited knowledge about its role in trafficking. In this study, we modelled metastatic melanoma with mouse B78chOVA cells expressing mCherry fluorescent protein to clearly identify melanoma cells. B78chOVA cells are positive for the $\alpha 4\beta 1$-integrin ligand of VCAM-1 but devoid of PECAM-1. In vitro live cell imaging allowed us to elucidate the exclusive role of the VCAM-1/a4-integrin interaction in the arrest of melanoma cells on inflamed primary mouse lung endothelial cells (pMLuECs) under physiological flow. However, long term imaging showed that blockade of VCAM-1/a4-integrin only transiently reduced intercalation of melanoma cells into the endothelial monolayer. In contrast, PECAM-1 did not have any role in shear resistant arrest of melanoma cells on pMLuECs. However, the increased permeability of PECAM-1 deficient pMLuECs correlated to increased melanoma cell intercalation and diapedesis when compared to wild type pMLuECs. Taken together, our data suggest that targeting of a4-integrin could provide only a short-term transient benefit whereas preserving endothelial integrity might be of superior relevance in the prevention of melanoma metastasis formation.

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Modulation of immune cell trafficking into human colorectal cancer by gut microbiota

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Colorectal cancer (CRC) is a leading cause of cancer-related death. CRC infiltration by immune cells, including cytotoxic CD8+ T cells (CTLs), IFN-gamma-producing T-helper 1 cells (Th1), Foxp3+ regulatory T cells (Tregs) and CD16+ MPO+ neutrophils, is associated with favorable prognosis. However, chemokines driving these cell populations into the tumor site, their cellular sources and their microenvironmental triggers remain to be elucidated. We investigated the chemokine/chemokine receptor network promoting CRC infiltration by immune cells associated to favorable prognosis. CRC infiltration by immune cells was associated with defined chemokine gene signatures, including CCL5, CXCL9 and CXCL10 for cytotoxic T lymphocytes and T-helper 1 cells, and CCL17, CCL22 and CXCL12 for T-helper 1 and regulatory T cells. Most of these chemokine genes were expressed by tumor cells upon exposure to gut bacteria in vitro and in vivo. Indeed, chemokine expression levels were significantly higher in orthotopic xenografts than in intraperitoneal tumors, and were drastically reduced by antibiotic treatment of tumor-bearing mice. Importantly, in human CRC samples, extents of chemokine production and immune cell infiltration was significantly associated with bacterial loads.

Gut microbiota stimulates chemokine production by CRC cells, thus favoring T cell recruitment into tumor tissues.

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TRIM24 is an oncogenic transcriptional activator in prostate cancer

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The steroid hormone androgen mediates a wide range of developmental, physiological and malignant responses by acting as an agonist for the androgen receptor (AR). AR in turn functions as a nuclear receptor transcription factor and executes specific gene expression programs in an androgen-dependent manner. Since the growth of prostate cancer (PC) cells initially depends on androgen, cancer therapy uses hormone-deprivation approaches to reduce the levels of androgen. While PC initially responds to androgen-ablation, most tumors progress to a castration resistant (CR) state insensitive to treatment. Both the androgen-dependent and the CRPC state depend on AR, which is probably activated by alternative molecular pathways in CRPC in response to low androgen levels. Here, we assessed the role of the transcriptional co-activator TRIM24 in mediating AR-dependent gene expression and growth in CRPC. We show that recurrent PC-driver mutations in Speckle Type BTB/POZ Protein (SPOP) stabilize the TRIM24 protein, which promotes proliferation under low androgen conditions. TRIM24 augments AR signaling, and AR and TRIM24 co-activated genes are significantly up-regulated in CRPC. Expression of TRIM24 protein increases from primary PC to CRPC, and both TRIM24 protein levels and the AR/TRIM24 gene signature predict disease-recurrence. Analyses in CRPC cells reveal that the TRIM24 bromodomain and the AR-interacting motif are essential to support proliferation. These data provide a rationale for therapeutic TRIM24 targeting in SPOP-mutant and CRPC patients.

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Human DNA2 possesses a cryptic DNA unwinding activity that functionally integrates with BLM or WRN helicases

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Human DNA2 (hDNA2) contains both a helicase and a nuclease motifs within the same polypeptide. The nuclease of hDNA2 is involved in a variety of DNA metabolic processes. Little is known about the role of the hDNA2 helicase. Likewise, it was undefined how hDNA2 functions together with BLM and WRN helicases. We used bulk and single-molecule approaches to study the enzymatic behaviour of hDNA2 and its physiological protein partners including WRN, BLM and RPA. We show that hDNA2 is a processive helicase capable of unwinding kilobases of dsDNA in length. The nuclease activity prevents the engagement of the helicase by competing for the same substrate, hence prominent DNA unwinding by hDNA2 alone can only be observed using the nuclease-deficient variant. We show that the helicase of hDNA2 functionally integrates with BLM or WRN helicases to promote dsDNA degradation by forming a heterodimeric molecular machine (Pinto et. al., 2016). This collectively suggests that the hDNA2 motor promotes the enzyme's capacity to degrade dsDNA in conjunction with BLM or WRN and thus promote the repair of broken DNA.

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The potential role of HTATIP2 as a tumor suppressor in glioblastoma

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In a genome-wide DNA methylation analysis of human glioblastoma we have identified HIV-1 Tat interactive protein 2 (HTATIP2) to be aberrantly hypermethylated. The strong negative correlation between promoter methylation and gene expression incited us to investigate potential tumor suppressor function(s) of HTATIP2 in glioblastoma (GBM). HTATIP2 has been reported to bind to nuclear transport receptors of the importin-b family (HEAT repeats) and may thereby act as a regulator of nuclear import of cancer relevant proteins.

First we confirmed the functional relevance of DNA promoter methylation on HTATIP2 expression in GBM cell lines. The demethylating agent Aza-cytidine induced expression in the cell lines with a methylated HTATIP2 promoter. Modulation of HTATIP2 expression by ectopic expression or knock-down with siRNAs did not indicate a general effect on proliferation or anchorage independent growth in vitro.

Next we followed up on our observation that the DNA repair enzyme Methylpurine-DNA Glycosylase (MPG) displays cytoplasmic and not nuclear expression in a subset of GBM. MPG comprises a nuclear localization signal (NLS) "KKQRP" that corresponds to the classical monopartite NLS. This feature makes MPG a potential substrate for importins. Using confocal microscopy and ImageStream FACS we could show that ectopic expression of HTATIP2 increases the fraction of GBM cells with predominant cytoplasmic versus nuclear localization of MPG. Hence our preliminary experiments suggest that HTATIP2 expression may modulate cellular localization of MPG, and thereby indirectly affect the cellular response to DNA damage. MPG is a base excision repair (BER) enzyme that removes alkylated DNA bases, including N3-mehyladenine (N3meA) and N7-methylguanine (N7-meG) that account for over 70% of DNA adducts induced by temozolomide (TMZ). MPG expression plays a clinically relevant role in resistance to TMZ as we have reported previously. Hence, HTATIP2 may exert tumor suppressing function(s) by keeping cancer relevant proteins out of the nucleus that is abolished be its epigenetic silencing

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SMAD Signaling by TGF-β Superfamily Members Drives Simultaneous Melanoma Growth and Metastasis in vivo

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The malignant progression of melanoma requires neoplastic cells to either have the potential to shift genetically / epigenetically between proliferative and invasive phenotypes or express both phenotypes, simultaneously. Contrary to conventional "phenotype switch" model in melanoma, here we elucidate the augmented SMAD-dependent signaling by depleting the inhibitory protein SMAD7 in a murine melanoma model increase the number of tumors harboring malignant cells that are associated with high proliferation and invasion program. Furthermore, conditional deletion of Smad4, abrogating canonical SMAD signaling reverted this phenotype by preventing tumor proliferation and metastasis. These results point out the requirement of a pro-invasive / pro-proliferative ligands. Ligand screening revealed that BMP7 promotes melanoma cell proliferation and overrides the effects of pro-invasive TGFβ-2/NODAL at the populational level. Dual treatment of pro-proliferative / invasive ligands with SMAD7 knock down concurrently induce the expression of pro-invasive transcription factors in proliferating melanoma cells. These results show how SMAD-dependent signalling can cooperate to malignant transformation of the disease by conferring both proliferative and invasive phenotypes on melanoma cells.

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Xenovaccines and their adjuvants in murine model: a promising anti-cancer immunotherapy

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Immune system plays a critical role in the prevention of tumour development. The sophisticated interaction between cancer cells and the immune system is defined as cancer immunoediting. Unfortunately, during this dynamic process, malignant cells may acquire various mechanisms enabling them to escape the immune-mediated control and manifest as clinically-apparent cancer. Due to impaired and misbalanced anti-tumour immunity in cancer patients, immune response-modulating strategies, known as tumour immunotherapy, have been introduced into clinical practice. Among various types of cancer immunotherapy, therapeutic cancer vaccination is one of the most promising approaches, especially when combined with other immuno-

therapeutic strategies as well as cancer chemotherapy, radiation therapy and targeted therapies.

Therapeutic cancer vaccination aims at inducing and augmenting cytotoxic cellular immune responses that are able to overwhelm the cancer-driven immunosuppressive arm of antitumor immune response. Therapeutic vaccines exploit mostly dendritic cells (DCs) that are the main initiators and orchestrators of adaptive immune responses. DCs in this study are targeted in situ. Selection of proper tumour-associated antigens (TAAs) and induction of optimal DC maturation are the critical steps in therapeutic cancer vaccination.

One of the ways of in situ stimulation of antigen presenting cells (APCs) is intradermal application of protein TAAs. Polyvalent antigen sources, in clinical practice, are usually autologous (tumour material). Tumour cell lysate (TCL) contains a mixture of proteins resulting from induced lysis of tumour cells, which ensures a broad spectrum of target antigens. On the other hand, TCL contains not only immunogenic TAAs, but also various immunosuppressants naturally occurring in cancer cells. It is a clinical challenge to supplement TCL with appropriate adjuvants and use its rich antigen pool for immunogenic anti-cancer vaccination.

Another emerging source of antigens for polyvalent vaccines, on which this study concentrates, is xenogeneic tissue. Foetal and immune-privileged tissues, such as testis, share many antigen determinants with human cancer cells: the so called oncofoetal and cancer/testis antigens. Xenogeneic vaccines need to be tested for each individual cancer, since one type of vaccine will not impact every type of cancer. Furthermore, they also require adjuvants to potentiate their immunogenicity.

Experiments were conducted using xenogeneic foetal sheep lungs, adult sheep testis, whole chicken embryo and embrional rat brain tissue vaccines. Explored models included metastastatic/non-metastatic Lewis lung carcinoma (LLC) and non-metastatic B16 melanoma settings. Effectiveness of vaccines was measured in mice survival, $CD8^+$ T-lymphocyte presence in circulating blood, metastatic activity and primary tumour growth rate.

Most effective vaccine (foetal sheep lung vaccine) resulted in significantly improved survival in both LLC and B16 models. Furthermore, it proved efficient both as primary therapy, and as an adjuvant therapy following primary tumour removal (anti-metastatic effect for LLC setting). This effect was achieved due to significantly increased cytotoxic lymphocyte population in blood stream and limited metastatic spread in the lungs.

Secondary highlight of this study is the impact of adjuvants on effectiveness of xenogeneic vaccines. The same vaccine was studied in both LLC and B16 models using three different adjuvants: polyxidonium, cycloferon and poludan. Similarly, the same adjuvant was tested in different settings with different vaccines. This cross investigation revealed one adjuvant, which produced significant results in all experiments: polyxidonium.

This study concludes that xenogeneic vaccines are indeed a perspective treatment for oncological diseases. The vaccines combined with optimal adjuvants increased survival and limited metastatic activity of tumours in multiple investigated murine models.

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Identifying the cell of origin of neuroblastoma

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Neuroblastoma (NB) is a pediatric cancer of the peripheral nervous system, which occurs almost exclusively in infancy and early childhood. It is widely assumed that oncogenic mutations occur in the cells of sympathoadrenal (SA) lineage during prenatal and postnatal life, however, the exact cell of origin has not been yet characterized. MYCN amplification and activating mutations of ALK (anaplastic lymphoma kinase) are among the most common genetic alterations in human NB described so far. There have been a number of attempts to model NB development in mouse models. The targeted expression of ALK^{F1174L} and MYCN oncogenes driven by a tyrosine hydroxylase (Th) and dopamine beta-hydroxylase (Dbh) promoters resulted in NB development. However, these mouse models fail to faithfully recapitulate human NB, there is a lack of schwannian stroma, only minimal metastasis formation and most importantly, a failure of mimicking the spontaneous regression, one of the key features of NB in humans. Furthermore, the onset of disease is delayed and majority of mice develop NB at 2 months old, which does not correspond to the pediatric characteristic of this tumor. A possible explanation for these discrepancies might be the fact that the oncogene expression is induced in progenitor cells rather than stem cell population.

In order to identify the cell of origin of NB, we first traced neural crest stem cells (NCSCs) and different progenitor populations of SA lineage during embryonic development. Then, we induced oncogenes expression in these key cellular populations to assess whether these mouse models recapitulate better the features of human NB. We used several Cre mouse lines, where Cre expression is driven by promoters of genes what expression marks different levels of SA lineage differentiation, namely Sox10, Ascl1, Dhh and Dbh. Through recombination was allowed population-specific transcription of rosa26/tdTomato reporter, or oncogenes as rosa26/MYCN and ALK^{F1174L}.

Here, we performed lineage tracing different cellular populations in adrenal medulla (AM) and sympathetic ganglia (SG). We proved that SA progenitors, marked by Ascl1 expression, are actually multipotent as they give rise to both glial and chromaffin cells in AM and SG. Surprisingly, lineage tracing of glial progenitors, marked by Dhh expression, showed that some of the glial cells can give rise to chromaffin cells. While the induction of MYCN expression in Dhh-expressing cells did not result in developmental abnormalities, the expression of ALK^{F1174L} in the very same cellular population was lethal.
Lineage tracing of Dbh+ progenitors demonstrated that this population gave rise to only chromaffin cells in AM, indicating that Dbh gene is not expressed in SA progenitors but rather in the committed progenitors of chromaffin lineage.
The expression of either MYCN or ALK^{F1174L} in Sox10+ cells resulted embryonic lethality. The oncogenic activation of ALK^{F1174L} in NCSCs induced embryonic death at E12.5, followed by resorption of the embryo. The earliest phenotype had been observed between E10.5 and E11.5, characterized by enlarged portions of the embryo's body and defects of the development.
Concluding, we described two interesting cellular populations marked by Ascl1 and Dhh expression that are not committed in a specific lineage as Dbh+ cells are. These findings suggest the contribution of at least two common progenitors that give rise to the glial and SA lineages.

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Zrf1 depletion affects ERα signalling and causes an aggressive cancer phenotype

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Zuotin-related factor 1 (Zrf1) has been identified as an epigenetic regulator of gene transcription in stem cells and cancer. The genomic locus of Zrf1 (7q22–31) is commonly altered in several types of human cancers including breast cancer (Aloia L., 2015). According to the Oncomine database Zrf1 shows a reduced expression in breast cancer (Riberio JD., 2013). Furthermore, a recent study analyzing the antibody response in sera of breast cancer patients with tumors revealed that the Zrf1 antigen and auto-antibodies are potential tumor markers, which could facilitate early breast cancer detection (Dyachenko D., 2016). Based on these information, we aimed to explore Zrf1's function in breast cancer progression in detail.
We chose the estrogen receptor positive MCF7 cell line as a model, which represents the early stages of cancer with positive hormonal receptor properties. After generating MCF7 cells either expressing a non-specific shRNA (shNMC) or shRNA targeting Zrf1 (shZrf1), we performed an analysis of cell growth. Although Zrf1 knockdown cells grow slightly less compared to control cells, we didn't observe dramatic changes between the two cell lines. Next, we carried out both wound healing and transwell migration assays to investigate whether Zrf1 depletion affects the cell migration capacity of MCF7 cells. Compared to control cells, Zrf1 knockdown cells closed their wound faster and significantly migrated more. These data indicate that Zrf1 limits the cell invasion of cancerous cells into other tissues. To further assess this metastatic property, we checked the cell adhesion capacity of both cell lines using collagen coated plates. Compared to the control, Zrf1 knockdown cells showed less adhesive properties. Collectively, these data suggests a more aggressive cancer phenotype in MCF7 cells upon Zrf1 depletion in accordance with breast cancer patient results.
Estrogen is the major driver of breast cancer progression and its biologic activities are mediated by the nuclear estrogen receptor alpha (ERα). To gain more insight into the molecular mechanism, we checked ERα dynamics in control and Zrf1 knockdown cells. After serum starvation, we treated cells with either an ERα activator (17β-estradiol) or an ERα inhibitor (ICI 182,780) for 7 days and assessed cell growth and viability using MTT Assay. We found that Zrf1 knockdown cells are growing more compared to control cells after estrogen induction. Also, Zrf1 knockdown cells are more viable in the presence of ICI 182,780. To elucidate whether this phenotype is a consequence of increased ERα levels at its target genes, we analyzed early (4 hours) and late (48 hours) estrogen responsive genes with qRT-PCR. We found that both early and late responsive genes are upregulated in the knockdown background. In line with these results, we observed that ERα is co-precipitated with Zrf1 and that it is maintained at chromatin upon Zrf1 depletion. Taken together, our data imply that Zrf1 and ERα act together during the ER response and that in absence of Zrf1, ERα is stabilized on chromatin presumably driving estrogen mediated transcription

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Pharmacogenomics of cantharidin in tumor cells

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Cantharis vesicatoria (blister beetle) is highly toxic according to the Chinese pharmacopeia. Cantharis patches have been used in China and Europe for long time against various skin-related diseases. In this study, we investigated the cytotoxicity of the Cantharis ingredient, cantharidin, in 41 tumor cell lines (Oncotest panel) and compared the results with those of 60 cell lines of the National Cancer Institute, USA. We found profound activity at low micromolar concentrations (log10IC50 values between -6.980 and 5.009 M). Molecular docking analysis pointed out that cantharidin bound to protein phosphatase 2A (PDB ID: 3DW8) with higher affinity (-8.12 kcal/mol) than to protein phosphatase 1β (PDB ID: 1S70) (-6.25 kcal/mol). Using a PCR array for 84 apoptosis genes, up-regulated gene expression of caspase-1 and nerve growth factor receptor, down-regulated gene expression of Bcl-2 like protein 10, Fas ligand, and tumor necrosis factor-α were found out. 21 genes were found to significantly correlate with response of 60 tumor cell lines to cantharidin as shown by COMPARE analysis of microarray-based transcriptome-wide mRNA expressions. Hierarchical cluster analysis and chi-squared test provided the distribution of cell lines in the dendrogram according to their gene expression profiles, which predicted sensitivity or resistance to cantharidin ($P = 6.482 \times 10\text{-}5$). The compassionate use of Cantharis patches in two patients suffering from basalioma and Mycosis fungoides, respectively, considerably improved the diseases without signs of toxicity. In conclusion, cantharidin is a promising compound with potential to be used for cancer therapy.

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Comprehensive tissue biobanking as a resource to study cancer cell heterogeneity and its impact on precision medicine

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The identification of novel therapeutic agents effective against cancers that are difficult to treat is an important ongoing research topic. However, promising drug candidates developed through basic and translational research have repeatedly failed to show the anticipated responses in clinical testing. A major reason for this shortcoming may be the phenotypic and genetic heterogeneity within tumors which is not well represented in most pre-clinical studies as they often rely on immortalized cell lines and mouse model systems for human cancer research.
In order to provide more accurate and diverse models for precision medicine, we have recently extended our tumor tissue biobanking strategy by establishing primary cell cultures derived from surgical specimens from malignant human tumors sent to pathology for diagnostic purposes. In conjuncture with the large longitudinal collection of formalin-fixed tumor biopsies and fresh frozen tumor material, patient-derived tumor cell cultures constitute important research tools for functional cancer research and personalized medicine. Moreover, our comprehensive tissue biobank provides the basis to validate the primary cell cultures for their phenotypic and genetic resemblance to the original tumor. In particular, we want to address whether patient-derived cancer cell cultures recapitulate the complex morphological and genetic intratumour heterogeneity that is a feature of cancer types with an unfavorable prognosis such as renal cell carcinoma (RCC). We plan to employ microscopy-based single-cell identification, a recently developed technology that combines the image-based classification of cells with analysis of mutations on the single cell level. This will enable us to correlate specific molecular features to phenotypically distinct classes of cells. Investigating the intratumor heterogeneity in primary cell cultures at the single-cell level will provide us with important biological insights into tumor formation and its implications on stratification and treatment strategies. Thus, we envision the use of patient-derived cancer cell cultures to unravel important mechanisms responsible for therapeutic failure and cancer relapse in individual cells which will have a significant impact on future clinical developments and the success rate of cancer therapy.

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Intermolecular Trapping of ErbB2 Entails Pan-ErbB Inhibition and Overcomes Adaptive Resistance in ErbB2-Dependent Tumors

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ErbB2 (HER2/neu) is a receptor tyrosine kinase linked to malignancies of different origin and a validated target for therapeutic monoclonal antibodies and tyrosine kinase inhibitors. Nevertheless, the current targeted therapies against this oncogenic driver have not yet lived up to their potential, and the tumors invariably develop resistance due to adaptive capabilities of the ErbB oncogenic network. Compensatory mechanisms, which entail reactivation of the PI3K/AKT pathway during anti-ErbB2 treatment, often operate via relief of AKT-ErbB3 negative feedback thereby leading to upregulation and rephosphorylation of ErbB3. Recently, we have described yet another adaptation route leading to reactivation of the PI3K/AKT pathway, which acts independently of ErbB3 re-phosphorylation. This signaling bypass of phospho-ErbB3 operates in ErbB2-dependent (HER2-positive) cancer cells via RAS-PI3K crosstalk and is attributable to active ErbB2 homodimers. Consequently, blocking these compensatory mechanisms is predicted to potentiate the effect of incomplete ErbB2/3 blockade as it occurs during treatment by trastuzumab and pertuzumab, the current standard of care in therapy of ErbB2-positive breast cancer. In present work, we have carried out a systematic analysis of trastuzumab combination treatments to identify synergistic effects between blockade of ErbB2/3 receptors and inhibition of related signaling pathways. By this means, it was possible to convert this monoclonal antibody with rather moderate effects on growth of ErbB2-dependent tumor cells to a potent pro-apoptotic tumor targeting agent. Moreover, we have developed a novel class of biparatopic anti-ErbB2 Designed Ankyrin Repeat Proteins (DARPins), which effectively downregulate oncogenic ErbB2/3 signaling and exert tumoricidal activity as naked molecules without effector function or cytotoxic payload both in 2D- and 3D-cell culture models as well as in orthotopically xenografted animals. By creating an intermolecular trap with DARPin agents, which simultaneously target two distinct ectodomain epitopes of ErbB2, receptors adopt an inactive conformation with kinase domains incapable of productive interactions. Such a trapping obstructs signaling from all ErbB2 homo- and heterodimer complexes, thereby achieving a pan-ErbB inhibition. The ensuing dephosphorylation of both ErbB2 and ErbB3 results in persistent attenuation of downstream signaling and overcomes adaptive responses incorporated into the ErbB oncogenic network.

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NRP2 expression on immune cells: Relevance and function in T-lymphocytes

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Neuropilins (NRPs) are single transmembrane receptors with a short cytoplasmic tail. Due to the shortness of the intracellular part, NRPs function often as co-receptors with VEGFR or Plexins. There are two members in the Neuropilin-family, Neuropilin-1 (NRP1) and Neuropilin-2 (NRP2), which are conserved in all vertebrates.
The NRPs are known players in angiogenesis, lymphangiogenesis and axon guidance. Recently, our lab showed that NRP2 is involved in endocytosis, which is a crucial mechanism in many immune cells. NRP2 is highly expressed on different immune cells. In vitro studies showed the importance of NRP2 in the migration of native T-cells and dendritic cells. Also, the activation of T-cells via dendritic cells is NRP2 dependent. So far, there is no in vivo study revealing the exact function of NRP2 in the immune system. In our experiments, we analyze the immune response of NRP2 deficient immune cells in vivo. For that we used a lung inflammation model in Vav:cre-NRP2$^{f/f}$ mice. Conditional NRP2 knock-out resulted in a reduction of T-lymphocytes during inflammation in the lung. Especially, the amount of CD8+ T-lymphocytes in the BALF was significantly reduced. Ongoing work aims to unravel the specific function of NRP2 in T-lymphocytes using in vitro models.

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Investigating the role of tissue resident NK cells in liver metastasis

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Whereas surgical resection and radio- or chemotherapy can cure primary tumors, metastatic disease is largely incurable because of its systemic nature and the resistance of disseminated tumor cells to existing therapeutic agents. This explains why >90% of mortality from cancer is attributable to metastases. Metastases represent the final step of a multistep process, which involves dissemination of cancer cells to anatomically distant organ sites and their subsequent adaptation to foreign tissue microenvironments. Each of these steps involves crosstalk between tumor cells and hematopoietic cells, including lymphocytes.

Natural killer (NK) cells are immune effector cells, which act as a bridge between the innate and acquired immune system and can control tumors and virus-infected cells. We have recently found that NK cells don't seem to control established tumors but are essential in limiting metastatic seeding to lungs and especially liver.

Most of our current knowledge of NK cells is derived from studies of mouse splenic and human peripheral blood NK cells, referred to as "conventional" NK cells. However, NK cells are also present in other tissues and organs and recent data suggest that a significant proportion of hepatic NK cells differ from peripheral NK cells in terms of surface marker expression, cytokine profiles, and cytotoxic potential. More recently, a unique NK cell subset, termed liver-resident NK cells, was characterized in both mouse and human livers. The relative abundance and unique features of hepatic NK cells suggest a distinct function of these cells.

In this project we aim to examine whether and how conventional and tissue-resident NK cells in the liver are involved in controlling metastasis to the liver using preclinical models for spontaneous and orthotopic metastasis. We will perform flow cytometry based multidimensional analysis to characterize both populations.

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A novel tool for the study of the role of co-transcriptional R-loops in oncogene-induced DNA damage

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Genomic instability is a hallmark of cancer. A major cause of this instability is replication stress. Activated oncogenes induce the transformation of precancerous lesions to malignant tumors. They induce stalling and collapse of replication forks, which can lead to DNA double-strand breaks (DSBs). There are specific genomic loci, termed common fragile sites (CFSs) and early replication fragile sites (ERFSs), which are particularly susceptible to breakage under conditions of replication stress. These loci coincide with recurrent sites of chromosomal rearrangements in cancer. It has been proposed that oncogene-induced replication stress is caused by conflicts between perturbed replication forks and transcription complexes, which can give rise to genotoxic RNA:DNA hybrids or R-loops. R-loops are three stranded nucleic acid structures that form when the nascent RNA released from a transcribing RNA polymerase reanneals with the template DNA. This leaves the non-template DNA in single-stranded form making it highly susceptible to damage by genotoxins or nucleases.

We have developed a novel tool to investigate the molecular mechanisms involved in the formation and resolution of R-loops based on RNaseH1 - an endonuclease that resolves these structures by cleaving the RNA in the hybrid. We have prepared an inducible stable U2OS-T-REx cell line that upon doxycycline induction, expresses a catalytically-inactive mutant of human RNaseH1 (RNH1(D210N)) tagged with GFP. The mutation results in an enzyme that binds to but does not degrade hybrids. This tool enables us to visualize R-loops by fluorescence microscopy and isolate them through chromatin immunoprecipitation (ChIP).

Preliminary microscopy experiments showed that upon conditions promoting R-loop formation (for example: treatment of cells with diospyrin, a topoisomerase I inhibitor, or replication stress caused by aphidicolin or hydroxyurea), the level of chromatin-bound RNH1(D210N)-GFP dramatically increased compared to non-treated cells. By ChIP-qPCR, we could see enrichment of RNH1(D210N)-GFP at chromosomal fragile sites under conditions of replication stress. To analyse R-loop formation on a genome-wide scale, we performed ChIP-Sequencing and we could identify sites that formed R-loops

specifically upon replication stress. We would now like to extend our study to decipher the role of R-loops in mediating genomic instability upon oncogene activation. We are also interested to perform mass spectroscopy to identify proteins and their post- translational modifications at R-loops that may then serve as biomarkers of precancerous lesions.

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Neuropilin-2 modulates metastases promoting CXCR-4/CXCL-12 signaling in prostate cancer

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Neuropilin-2 (NRP-2), a non-tyrosine kinase receptor, has been shown to be involved in cancer progression and therapy resistance. We have already demonstrated that NRP-2 induces autophagy and anti-apoptotic pathways during stress in prostate cancer. In this study we aim to investigate the role of NRP2 as a prognostic marker in prostate cancer and its role during pro-metastatic CXCR4 signaling.

To analyze the effect of NRP-2 on CXCR-4 signaling we used human prostate cancer cell lines PC3 and LNCAP C4-2B. The expression of NRP-2 and CXCR-4 was detected by flow cytometry and ICC. NRP-2 was depleted by RNAi. Signaling cascades were analyzed by immunoblotting for ERK1/2 proteins. Invasion and migration was studied by Boyden chamber and chemotaxis assays. NRP-2 expression in human bone metastases was tested by standard immunohistochemistry. To analyze the prognostic impact of NRP2 expression in prostate cancer we executed standard NRP2 immunohistochemical staining in a TMA of 400 primary prostate cancer tissues and in bone metastases. The expression of NRP-2 correlates with cancer specific survival in prostate cancer patients. Furthermore, bone metastases of prostate cancer patients showed strong staining for NRP-2. NRP-2 and CXCR- 4 was co-expressed on all investigated cell lines. NRP-2 depletion by RNAi in PC3 cells abrogated the chemotactic migration towards the CXCR-4 ligand CXC-L12. Mechanistically, CXCL12/CXCR4 triggered MMP-9 expression was reduced after inhibition of NRP-2.

In conclusion, NRP-2 is a prognostic marker in prostate cancer patients and is highly expressed in prostate cancer bone metastases. Mechanistically, NRP-2 regulates the prometastatic CXCR-4/CXCL-12 signaling cascades by inhibiting MMP expression.

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Investigating the role of ISG15 in the regulation of genome integrity

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Ubiquitin (Ub) and ubiquitin-like (Ubl) modifications of proteins are pervasive mechanisms for controlling a number of essential cellular functions, including those regulating genome integrity. While the relevance of Ub and the Ubl SUMO has been already addressed in different processes regulating genome stability, such as DNA damage response (DDR) and DNA replication, the role of other Ubl modifications has been poorly characterized.

One of this Ubl is ISG15 (Interferon Stimulated Gene 15), which is strongly induced by IFN type-1 and mostly associated to the innate immunity where it plays a role in host defense. Noteworthy, ISG15 is also induced by different genotoxic stresses, is over-expressed in many tumors and it targets a number of factors involved in DDR and DNA replication, in a reaction called ISGylation.

My project aims to provide a detailed study on the relevance of ISG15 in the regulation of genome stability, by determining the role of ISG15 in DNA replication and by identifying and functionally characterizing potential novel targets of ISGylation involved in this process.

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Functional and structural dynamics of the bone marrow stromal microenvironment after cytoreductive therapies

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High throughput, continuous and tightly regulated production of mature blood cells takes place in bone marrow (BM) tissues during adulthood. Hematopoiesis is sustained by the proliferation and differentiation of hematopoietic stem and progenitor cells (HSPCs), which are critically regulated by signals emanating from stromal cells that form the BM microenvironment. The BM stromal compartment comprises different non-hematopoietic mesenchymal, endothelial and neural cells. Cytoreductive treatments such as ionizing irradiation and chemotherapeutic agents are the treatment of choice for multiple different haematologic and non-haematologic malignancies as well as for myeloablative conditioning regiments in BM transplantations. The cytotoxic damage and rapid killing of cycling cells elicited by myeloablative therapies have been extensively characterized in what pertains to their direct effects on hematopoietic cells. However, whether and to what extent these treatments target stromal BM cells is largely unknown. Here we have analyzed the dynamics of stromal cell populations upon myeloablation, the resulting microarchitectural effects on the BM microenvironment and the kinetics of regeneration of fully restored BM tissues post-injury. For this purpose we take advantage of unique 3D-microscopy imaging techniques developed in our laboratory in combination with customized computational tools for quantitative image-based analysis.

As previously reported, ionizing irradiation and 5-fluororuacil treatment led to a severe loss of HSPCs, which correlated with a profound decrease in the essential cellular components of HSPC niches, namely sinusoidal endothelial cells and mesenchymal stromal cells. Notably, the decline in stromal cell numbers was apparent 7 days after treatment and encompassed a major loss of structural integrity of the BM microenvironment. 3D imaging revealed massive sinusoidal dilation followed by the appearance of ruptures of the vessel walls. Both structural effects and decrease in cell numbers were partially reversed 14 days post treatment in a regenerative process that culminated at day 28 post-treatment. In addition, massive de novo differentiation of mesenchymal progenitors into adipocytes, lead to adipogenic infiltration of large regions of the BM, which became hematopoietically inactive. Of note, this process was fully reversible and virtually almost all adipocytes were cleared from BM tissues 56 days after myeloablation. To compensate for the impaired function of the BM, reversible extramedullary hematopoiesis could be observed at the time points of maximal BM damage.

Our observations demonstrate that the stromal BM microenvironment is highly sensitive to myeloablative therapies, which lead to massive alterations in BM microstructure. Of note, our observations further highlight that BM tissues are endowed with an intrinsic regenerative and self-organizing capacity that enables rebuilding of a fully functional tissue microenvironment after severe damage. We are currently investigating the inflammatory mechanisms that trigger injury as well as regeneration of a functional BM stromal infrastructure.

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Germinal centers underlie the prognostic benefit of tertiary lymphoid structures in lung squamous cell carcinoma patients and are negatively affected by steroids

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Tertiary lymphoid structures (TLS) form in chronically inflamed tissues including cancer and contain organized compartments of T cells, B cells, dendritic cells and lymphoid stromal cells[1]. TLS activate adaptive immune cells in autoimmunity and infection and correlate to improved survival in various cancer types[2]. This suggests that TLS induction in cancer could be a novel immunotherapeutic approach to enhance anti-tumour immunity, however not much is known about TLS formation and function in the context of human cancer[2].

Here we studied lung squamous cell carcinoma (LSCC) patients (n=138) and demonstrate that a high number of germinal centre (GC)-positive TLS is a significant positive prognosticator for disease-specific and progression-free survival independent from TNM staging and other clinical parameters (multivariate Cox regression p=0.001, hazard ratio=0.42, CI 95% 0.24-0.7).

By using multiparameter-immunofluorescence we saw that three distinct types of TLS are present in LSCC, which we think reflect sequential maturation stages: (1) clusters of B and T cells around CXCL13-expressing perivascular cells, (2) organized follicles containing differentiated follicular dendritic cells (FDCs), but no GCs, and (3) organized follicles with an ongoing GC reaction. We termed the latter two as primary follicle-like (PFL) and second-

ary follicle-like (SFL) TLS, respectively, in analogy to the functional states of lymph node follicles. By using a quantitative pathology approach we determined that in patients with low TLS numbers also their maturation towards SFL TLS stage is hampered (Mann-Whitney (MW) U test p=0.006) and the size of GCs is significantly decreased (MW U test p=0.000).
Stratification of patients based on whether they underwent neoadjuvant chemotherapy or not revealed that the prognostic power of TLS is confined only to the neoadjuvant treatment-naïve patients (n=87). In neoadjuvant chemotherapy (n=51) or neoadjuvant radiotherapy (n=4) treated patients the average numbers of TLS were not altered, however, the capacity of TLS to form GC was significantly impaired (MW U test p=0.002).
The genotoxic effects of chemo- and radiotherapy may negatively affect the rapidly proliferating GC B cells thus leading to GC shrinkage, however, all patients receiving neo-adjuvant treatments also concomitantly received corticosteroids to decrease therapy-associated side-effects. To evaluate whether the anti-inflammatory effects of steroids affect the number and maturation of TLS, we compared these parameters between groups of neoadjuvant therapy-naïve LSCC patients, who had no history of steroid treatment or were treated with systemic or local corticosteroids prior to surgery because of other comorbidities. We found that tumors of patients treated with corticosteroids had decreased TLS numbers and reduced GC size (MW U test p=0.006).
To characterise the TLS-associated tumour immune contexture we performed gene expression analysis in LSCC patients with high and low TLS numbers for various genes involved in immune functions and lymphoid neogenesis. As a result, CXCL13 and lymphotoxin beta were the most significant chemokines associated with TLS formation in LSCC. Further, several genes involved in adaptive immune responses like CD27, CD8A, CD4, CD20, IL-21, IGKC, PRF1 were upregulated in tumours with many TLS, while innate response genes like NKP46 and ITGAM (encoding CD11b) were not changed.
In conclusion we suggest that GC formation in TLS significantly contributes to protective anti-tumour immunity and that corticosteroids have a negative impact on TLS function. TLS induction possibly by a targeted delivery of CXCL13 and/or lymphotoxin to the lung tissue can provide a promising immunotherapeutic approach for LSCC patients.

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TNIK increases disease progression and stemness of colorectal cancer stem cells

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Colorectal cancer (CRC) is the third-leading cause of cancer-related death in men and women. Current therapies, such as radio- and chemotherapy, only target the fast dividing, differentiated tumor bulk cells, but not the more quiescent, therapy-resistant colorectal cancer stem cells (CSCs). Consequently, CSCs represent a major challenge in the treatment of CRC and new approaches specifically targeting CSCs are needed. The vast majority of CRC are driven by mutations that activate the Wnt-pathway. Therefore, targeting Wnt signaling in CSCs may be a potential to treat CRC. The Traf 2 and Nck-interacting kinase (TNIK) is a known activator of Wnt target genes and has been reported as an inducer of Wnt target gene transcription in human CRC. However, the role of TNIK in CSCs is unknown so far.
In this study we made use of oncogene-driven CRC mouse models that closely mimic the development of human CRC. In these models, tumor development is driven by the loss of APC and/or over-activation of Kras ($APC^{min/wt}$; $APC^{min/wt}$ x $Kras^{LSL\text{-}G12D/wt}$). To investigate the role of TNIK in CSCs, we generated crypt-derived intestinal cancer organoid cultures from TNIK-proficient and –deficient $APC^{min/wt}$ or $APC^{min/wt}$ x $Kras^{LSL\text{-}G12D/wt}$ mice. Intestinal crypts were isolated and cultured in vitro together with specific growth factors to select for malignant organoids. These so-called cancer organoids do not form crypt and villus structures and lack signs of differentiation.
We could demonstrate that the loss of TNIK reduces cancer organoid formation and CSC frequency in limiting dilution experiments in both CRC models. In addition, organoids from TNIK-deficient CSCs were enlarged in size, suggesting an increase in differentiation. Organoid-derived single cells can be transplanted subcutaneously to assess for tumor formation in vivo. TNIK-deficient cells showed attenuated capabilities to confer the disease into secondary recipients. Furthermore, TNIK-deficient tumors were more differentiated and less proliferative as analyzed by HE- and Ki67 stainings. These experiments imply that TNIK is a crucial factor in maintaining the number and function of CSCs. Of importance, normal intestinal homeostasis was not affected in TNIK loss of function mice. To demonstrate a human relevance of our findings, we made use of public available microarray datasets (Gene

Expression Omnibus databases) and could show that TNIK expression in CRC correlates with poor patient prognosis.
Conclusively, our results demonstrate that TNIK promotes stemness of murine CRC CSCs and identify TNIK as a promising therapeutic target for CRC.

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Novel strategies targeting the PI3K/AKT/mTOR signaling pathway in glioblastoma

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Glioblastoma is the most malignant of the gliomas, a class of tumors probably arising from neuroglial precursor cells, which are notoriously resistant to current therapies. Molecular genetic aberrations in the phosphoinositide 3-kinase (PI3K) / mammalian target of rapamycin (mTOR) pathway are very common in human cancers. Dysregulation of the PI3K signaling pathway is found in nearly all patients with glioblastoma, thus highlighting its importance as a therapeutic target.
We find that human glioma cells, including long-term cell lines (LN-18, LN-428, D247, LN-319, A172, U87MG, T98G, LN-308 and LN-229) and glioma initiating cell lines (T-325, T-269, ZH-161, S24 and ZH-305) show differential sensitivity to a novel, balanced dual pan-PI3K/mTOR antagonist (PQR309) assessed at the level of modulation of signaling pathways, loss of viability and inhibition of self-renewal capacity. Sensitive cell lines were characterized by higher p-AKT levels and efficient inhibition of key pathway members. We also explored the combination of PQR309 with the current standard chemo- and radiotherapy for glioblastoma in selected models. These studies disclosed no striking synergy and, importantly, no antagonism in any cell line. While the PI3K/mTOR pathway continues to pose major challenges to pharmacological targeting without causing intolerable toxicity, elucidation of a molecular signature associated with vulnerability to PI3K/mTOR pathway inhibition in the framework of this study may provide a significant clinical benefit.

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Chronic myeloid leukaemia progression in the bone marrow is fuelled by spleen resident leukaemia stem cells

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Background: Chronic Myeloid Leukemia (CML) is a myeloproliferative disorder of the hematopoietic system. CML is caused by the BCR-ABL translocation in hematopoietic stem cells, which results in the generation of a leukemia stem cell (LSC). Massive splenomegaly is a clinical hallmark of CML and it has been shown in previous studies that LSCs and leukemic progenitor cells are present in the spleen. However, their role in CML development is unknown. Therefore, this project aims to investigate role of the spleen as a potential independent niche for CML leukemia stem cells (LSCs) and its contribution to disease development and progression.
Methods: CML like disease was induced in mice by retroviral transfection of flow cytometry sorted Lineage- Sca-1$^+$ c-Kit$^+$ bone marrow cells (LSKs) with pMSCV-p210BCR/ABL-IRES-GFP. Transduced LSKs were then injected i.v. into recipient mice. Functionality of LSC isolated from bone marrow and spleen was assessed by FACS based cell cycle analysis, secondary transplantation assays and serial colony forming assays. Localization of CML stem and progenitor cells in the spleen was analyzed using confocal laser microscopy on optically cleared spleens.
Results: We were able to confirm and extend data demonstrating that there are large numbers of spleen resident LSCs. Confocal microscopy on optically cleared spleens revealed that splenic leukemia stem and progenitor cells exclusively localized within the red pulp. We further found an accumulation of leukemic CMPs in the spleen compared to the BM. Analysis of the functionality of spleen and BM LSCs revealed no difference in cell cycle distribution and serial colony formation capacity. However, we observed that splenic LSCs transmit disease more efficiently in secondary recipients compared to BM LSCs. In a next step we wanted to test whether the spleen contributes to the development of CML. Therefore, we performed splenectomies or sham operations on recipient mice prior to CML induction. Splenectomy significantly improved CML survival. Further we found a reduction of LSC and leukemic

progenitor numbers in the BM of splenectomized recipients.
Conclusions: These results strongly suggest the presence of an independent splenic LSC niche in addition to the well characterized BM LSC niche. The Splenectomy experiments provided evidence that the spleen directly contributes to the disease progression in the BM. Further experiments will aim to characterize the splenic niche components and the mechanisms by which the Spleen promotes disease progression in the BM.

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Epigenetic impact on genome stability: cancer-related shifts in miRNAome can affect DNA repair mechanisms and facilitate aberrant AID expression

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Genome instability, high mutation rate as well as epigenetic dysregulation are typical features of many cancer cells leading to their high variability and, as a result, to the tumour evolution. Our investigation aims to identify in what way the shifts in miRNAome can contribute to the DNA damage. MiRNA targets within gene transcripts were predicted in silico using the TargetScan software.
Our recent research shows that miRNAs miR-18, miR-19, miR-21, miR-23, miR-29, miR-155, miR-181, miR-206, miR-221/222 and miR-375, hyperexpression of which is essential for cancer cells, can target transcripts of around 60% of the genes encoding DNA repair enzymes as well as other DNA damage response proteins that are key elements of all DNA repair systems – base excision repair (UNG, SMUG1, MBD4, TDG, OGG1, NTHL1, NEIL1/2, APEX1, LIG3, APLF), direct reversal of damages (ALKBH3), repair of DNA-topoisomerase crosslinks (TDP1/2), mismatch excision repair (MSH2/3, MLH1/3), nucleotide excision repair (RAD23B, DDB1, RPA1/3, ERCC2, GTF2H1/2/3/5, CCNH, ERCC1/4/6/8, UVSSA), homologous recombination (RAD50/51/52, RAD51B/D, XRCC2, RAD54B, MRE11A, NBS1, RBBP8, EME1, GEN1) and non-homologous end-joining (XRCC6, PRKDC, LIG4, DCLRE1C, NHEJ1). Targets of the up-regulated miRNAs are also revealed in transcripts of genes encoding ATM kinase and Fanconi anemia proteins (FANCA/C/D2/E/F/G/I/M, BRCA2, BRIP1, RAD51C, BTBD12, FAAP20). Silencing of these genes facilitates the damage of antioncogenes and tumour suppressor genes as well as contributes to increase of mutation rate that leads to oncogene abnormalities and cancer cell variability underlying the tumour evolution and drug resistance acquisition [1].
Cytidine deaminase AID, encoded by AICDA gene, is a potent mutator, which activity leads to numerous translocations and mutations of oncogenes as well as to damages of antioncogenes in various types of cancer. It is clear that miRNA-mediated silencing of genes responsible for DNA repair, esp. base excision repair, facilitates the mutagenic ability of AID. AICDA expression is initiated in response to NF-kB pathway activity that is triggered by inflammatory signals, esp. TNF-a and IL-1b cytokines. In addition, tumour-related down-regulation of some miRNAs (e.g. miR-17 and miR-143) can contribute to the aberrant reactivation and hyperexpression of AICDA gene, because its transcript carries targets of these miRNAs. Also, down-regulation of miRNAs miR-15/16, miR-22, miR-31, miR-124, miR-125, miR-137, miR-140, miR-143, miR-145, miR-148/152, miR-199, miR-200, miR-203, miR-204 and miR-205 allows overexpression of genes encoding the factors NF-kB, SP1, HoxC4, TCF3, Stat6, Id2/3, which are AICDA transcription activators. Interestingly, tumours have indolent course if AID cannot be reactivated. For instance, MALToma cells hyperexpress miRNAs miR-155 and miR-150, high conservative binding sites of which are revealed in transcript of AICDA gene [2].
We speculate that AID can cause the target damage (endogenous gene knockout) of tumour suppressor genes (incl. genes of anti-onco-miRNAs) in result of mutations in single-stranded R-loop that arises during transcription or due to invasion of complementary non-coding RNAs into the DNA duplex. Therefore, carcinogenesis should be divided into following steps: 1) stage of epigenetic shifts; 2) genome destabilization and hypermutation start; 3) stage of mutation selection (tumour evolution and progression). It is indicative that oncogene abnormalities are not revealed in case of low-grade MALToma – without AID activity, this tumour is frozen at initial epigenetic stage for a long time.

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Evaluation of circulating microRNAs as non-invasive biomarkers in early diagnosis of ovarian cancer

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Background: Ovarian cancer (OC) is the seventh most frequent form of malignant diseases in women worldwide. Due to difficulties in detecting it, over 150.000 women die from it every year, placing it as the most lethal malignant gynecological disease in terms of mortality. However, over 70 percent of all ovarian cancer patients are diagnosed at a late stage disease (stages III and IV). Resulting, the development of sufficient early diagnostics is one of the big challenges in ovarian cancer research.
In earlier studies, microRNAs (miRNA) displayed promising potential as predictive and diagnostic biomarkers in various malignant diseases, including ovarian cancer. They play an important role in regulating mRNAs posttranscriptionally and therefore act as oncogenes and tumor suppressor genes.
Hypoxia and acidosis are known to play an important role in early tumorigenesis and tumor progression. We hypothesize that under these two conditions specific miRNA expression alterations could arise. These might have promising potential to indicate early tumor stages not only in vitro but also in vivo.
Methods: MiRNA expression levels of 15 miRNAs (let-7a, let-7d, miR-10a, miR-15a, miR-15b, miR-19b, miR-20a, miR-21, miR-25, miR-100, miR-103, miR-125b, miR-155, miR-191, miR-222) in the three ovarian cancer cell lines SK-OV-3, OAW-42, EFO-27. Each cell line was analyzed under three conditions: untreated, hypoxia and acidosis. We also analyzed the corresponding cell culture media under according to the same protocol, in order to evaluate secreted miRNAs as possible non-invasive screening-biomarkers for ovarian cancer. Quantitative expression analysis was performed by RNA isolation with Norgen´s RNA purification kit. Subsequently, microRNA expression levels of all analyzed samples were quantified using realtime-PCR.
Results: All 15 analyzed microRNAs could be detected in cell culture media. MiR-155, however, was only detectable intracellularly. MiRNA expression patterns of cell culture differ distinctly from miRNA expression patterns of cell culture media.
In vitro analyses revealed that the three microRNAs miR-15a , miR-21 and miR-125b show the highest expression levels in cell culture media whereas miR-10a, miR-19b and miR-100 showed the lowest expression levels in cell culture media among the fifteen investigated miRNAs.
Moreover, functional analyses demonstrated that the microRNA expression levels of eight microRNAs (let-7d, miR-15a, miR-15b, miR-19b, miR-100, miR-125b, miR-155, miR-222) undergo up- or downregulation in hypoxic or acidotic conditions, especially in cell culture media.
Conclusion: First, cell culture media analyses prove that microRNAs are secreted from cells into their surroundings. These findings show that the analyzed miRNAs qualify for possible circulating biomarkers in liquid biopsies of ovarian cancer patients. Second, we were able to show the large impact of hypoxia and acidosis on the expression levels of the 15 analyzed microRNAs. This supports our hypothesis that early tumorigenesis can be made visible regarding miRNA expression alterations.
Prospects: In order to demonstrate the feasibility to detect similar expression patterns in vivo, we analyzed the urine of 10 ovarian cancer compared to 10 healthy controls.
Seven urinary microRNAs showed significant differences between OC patients and healthy controls comparing their median miRNA expression levels. The four urinary miRNAs let-7a, let-7d, miR-10a and miR-15b showed significant downregulation in OC patients compared to healthy controls. In contrast, OC patients displayed significantly higher levels of miR-15a, miR-19b and miR-20a

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The Molecular Effects of Ginkgolic Acids on Breast Cancer Metastasis

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Ginkgolic acids (GA), a group of alkyl phenols found in crude extracts of Ginkgo biloba leaves, are known to have anticancer activity, but their mode of action is not well understood. Our aim in this study was to investigate the anti-migratory activity of seven GAs against breast cancer cells and determine the molecular mechanism behind this activity. The anti-migratory effect of the seven compounds as well as a mixture of GA's was investigated

by applying a wound healing assay with MCF-7 and MDA-MB 231 breast cancer cells. Resazurin assays were performed to test, whether cytotoxicity caused the anti-metastatic activity in these two cell lines. The effect on NF-κB signaling was investigated using the reporter cell line, HEK Blue Null1. Molecular docking was used to predict the binding energies and the site of interactions on SUMO-activating enzyme E1.Using a sumoylation assay, the effect of GA C13:0 on the sumoylation of NEMO was measured. The effect on the expression levels of urokinase plasminogen activator(uPA) and plasminogen activator inhibitor-1 (PAI-1) was determined by quantitative RT-PCR after treatment of MDA-MB 231 cells with two GAs (C15:0 and C13:0) and the GA mixture. All seven compounds and the mixture were found to inhibit wound healing in both cell lines at 25 µM. None of the compounds nor the mixture revealed cytotoxicity towards MCF-7 and MDA-MB 231 cells. GA C13:0 inhibited NF-κB activity in HEK Blue Null1cells. All seven GAsbound to the active adenylation site of the SUMO-activating enzyme E1 with high calculated affinities ranging from -10.28 to -12.27 kcal/mol. GA C15:0, GA C13:0 and the mixture significantly down-regulated the mRNA expression of uPA and PAI-1. We conclude that GA revealed considerable anti-migratory activity at non-cytotoxic concentrations, indicating anti-metastatic activity at low toxicity. This effect can be explained by the inhibition of NEMO sumoylation leading to reduced NF-κB activity, which in turn leads to the down-regulation of genes related to breast cancer metastases such asuPA and PAI-1.

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Niclosamide for the Treatment of Multidrug-resistant Cancers

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Multidrug resistance is a major problem that leads to failure of anticancer chemotherapy. Niclosamide, a known anthelmintic, is cytotoxic and cytostatic against cancer cells. Cytotoxicity was measured by resazurin using sensitive CCRF-CEM and multidrug-resistant CEM/ADR5000 leukemia cells. ROS levels were measured using flow cytometry. Protein targets of niclosamide were predicted using the DRAR-CPI software engine. Glutathione levels were measured using flow cytometry. Molecular docking for niclosamide on glutathione synthetase was performed using Autodock 4. COMPARE and hierarchical cluster analyses were run using NCI microarray database. Binding motifs for transcription factors promoters of deregulated genes were analyzed. NFAT promoter activity was quantified using a reporter assay. The cytotoxicity assay of niclosamide showed IC_{50} values of 0.675 ± 0.113 µM and 0.839 ± 0.089 µM in CCRF-CEM and CEM/ADR5000 cells respectively (Resistance ratio=1.24). ROS assay showed a dose-dependent increase in ROS levels. Glutathione synthetase (GS) was a predicted target of niclosamide. Niclosamide significantly reduced glutathione levels in a dose-dependent manner. Niclosamide docked on GS at the ATP- binding site with a binding energy of -9.40 kcal/mol. Genes involved in lipid metabolism correlated with cellular responsiveness to niclosamide. The dendrogram from cluster analysis showed five major branches with a significant difference in the distribution of sensitive and resistant cell lines ($p= 8.66 \times 10^5$). NFAT was predicted to bind to the promoters of the deregulated genes. Niclosamide significantly decreased NFAT activity in a dose-dependent manner. In conclusion, niclosamide is active against multidrug-resistant cancers. Inhibition of glutathione synthesis and NFAT signaling are two more mechanisms for the anticancer activity of niclosamide. Gene expression profiling can be used to predict the sensitivity or resistance of cancer cells to niclosamide.

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A new autocrine mechanism controlling cancer stem cell fate in breast cancer

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Over the last years it has been shown that many tumor types are organized as a hierarchy in which cancer stem cells (CSC) are at the apex. Recent data by a few research groups, including ours, demonstrate that CSCs are not only responsible for tumor development and resistance to therapy, but they are also the cell-of-origin of metastasis. Since over 90% of cancer-related deaths are due to metastatic disease, understanding the mechanisms that CSCs use in order to colonize secondary organs is of paramount importance to the field of cancer biology. We here identify Transforming

Growth Factor b induced (TGFBI) as an extracellular matrix protein that is essential to control mammary gland stem cell fate. TGFBI knock-out mice exhibit defects in mammary gland formation and decreased numbers of $Lin^{-}CD24^{+}CD29^{low}CD61^{+}$ luminal stem cells. In tumors, TGFBI is mainly expressed by metastatic stem cells and fibroblasts. However, injecting wt or ko tumor cells into wt and ko recipients demonstrates that TGFBI acts in an autocrine manner in metastatic stem cells. Moreover, limiting dilution assays demonstrate that TGFBI-/- mammary gland tumors have a 38-fold reduction in tumor initiating capacity and a dramatic decrease in metastatic colonization ability. Mechanistically, our results indicate that this is due to a decrease in stemness in this population due to impaired TGFb and Notch signaling. Interestingly, human data mining shows that TGFBI is a factor of poor prognosis in breast cancer and is significantly increased in claudin-low breast cancer cells, which are known to contain high numbers of CSC. Overall, these data reveal a new biological mechanism of CSC maintenance in breast cancer. Since TGFBI is barely expressed in healthy tissues in the adult, we think that it could potentially be exploited to treat breast cancer from a CSC perspective.

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Unveiling the Tumor Microenvironment and Immunophenotype Using Imaging Mass Cytometry

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Cancer is a complex disease of cells from different tissue origins characterized by uncontrolled growth and cell dissemination. During the last two decades, extensive data provides evidence that multiple "normal" cell types besides tumor cells, are essential to drive the growth and malignancy of tumor cells. These cells, together with Extracellular components, make up the so-called "Tumor Microenvironment" (TME). Imaging Mass Cytometry (IMC) is a recently developed technology by us that allows the simultaneous antibody-based detection of up to 52 proteins and their modifications in tissue sections. With this approach, it is possible, for the first time, to study multiple cell types, alongside with signaling pathways, extracellular matrix components, cell function surrogates, and lineage markers, in the original tissue context. Our analyses uncover relevant morphological and biochemical features of tumors both in mouse cancer models and human patients. Furthermore, by using systems-biology and computational analysis tools developed in our laboratory, new signaling loops, prognostic and diagnostic markers are being discovered. Different immune cell types are quantified and the degree of infiltration within tumor areas is measured, thus giving a comprehensive understanding of the immune response in the context of signaling and tissue architecture. Furthermore, our software tools enable three-dimensional reconstruction of tissues as well, thus providing for first time a full understanding of the spatial relationships amongst the different cell types involved in cancer growth. In conclusion, we show that IMC is an ideal tool for better understanding the TME and the mechanisms driving cancer, as well as for screening for novel biomarkers with clinical relevance.

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Nogo-A and S1PR2 as novel regulators of developmental and tumor angiogenesis in the CNS

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Glioblastomas are among the most common brain tumors, associated with a poor prognosis. One hallmark of glioblastoma growth is angiogenesis, the formation of new blood vessels. Classical approaches to target glioblastoma

angiogenesis – for instance using the anti-VEGF-A antibody bevacizumab (Avastin®) – have not led to the desired improvement of patient survival. Accordingly, the identification of novel regulators of brain tumor angiogenesis is of great interest.

There is emerging evidence that the patterning of neural and vascular networks in health and disease (e.g. brain tumors) are governed by common mechanisms, through guidance molecules acting on the neural growth cone as well as on the endothelial tip cell. The membrane protein Nogo-A is mainly expressed in the central nervous system (CNS) and has well-known functions as a very potent, myelin-derived neurite outgrowth inhibitor and as a regulator of axonal growth in the CNS. Recently, we have identified a new, unexpected function for Nogo-A as a negative regulator of angiogenesis in the developing CNS and the Nogo-A specific receptor in neurons has recently been identified as the Sphingosine 1-Phosphate-Receptor 2 (S1PR2). Therefore, we hypothesize that Nogo-A and S1PR2 may constitute a novel ligand-receptor pair regulating angiogenesis during developmental and tumor angiogenesis in the CNS.

So far, we have shown that Nogo-A was expressed in vicinitv of growing blood vessels and endothelial tip cells expressing S1PR2 during mouse and human brain development in vivo. At the functional level, the number of endothelial tip cells was significantly increased in Nogo-A$^{-/-}$ as well as in S1PR2$^{-/-}$ mice, indicating a negative regulatory role for this ligand-receptor pair in vivo. During mouse and human glioblastoma angiogenesis, Nogo-A showed a perivascular expression pattern whereas S1PR2 was expressed on the glioblastoma vasculature. In a mouse glioma model (GL-261), S1PR2 was upregulated within the tumor as compared to the surrounding brain tissue and in vicinity of brain tumor endothelial tip cells. Interestingly, increased Nogo-A expression correlated with a reduced vessel density in human glioblastoma.

Moreover, Nogo-A inhibited the adhesion, spreading, migration and filopodia- and lamellipodia of primary murine brain-derived microvascular endothelial cells (MVECs) as well as of human glioblastoma-derived MVECs in vitro. Interestingly, these effects were – at least in part – mediated by the Nogo-A receptor S1PR2. Mechanistically, we have obtained preliminary results indicating that Nogo-A-S1PR2 signaling interacts with VEGF-A-VEGFR2-Dll4-Jagged-Notch signaling in vitro.

In summary, our data support a negative regulatory role for Nogo-A-S1PR2 in mouse and human developmental brain- and glioblastoma angiogenesis.

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T-cadherin is a novel marlker of prostate cancer onset: correlation with cancer progression and effects on proliferation, invasion and angiogenesis

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Prostate cancer (PCa) represents the second leading cause of cancer-related death in men. T-cadherin is an atypical GPI-anchored cadherin recently detected in prostate tissue. AIM: to study T-cadherin gene and protein expression in PCa and its effects on PCa cells. METHODS: T-cadherin gene expression was analyzed in 550 PCa samples from the TCGA dataset, and by qPCR in 44 benign hyperplasia (BPH) and 57 PCa samples. Correlations between expression of T-cadherin gene, other genes and clinical characteristics of the patients were analyzed in the TCGA dataset using Qlucore software. T-cadherin protein expression was studied by IHH using PCa tissue microarrays. Effects of T-cadherin on proliferation, invasion and tumor angiogenesis were analyzed using BPH-1 and DU145 prostate cell lines transduced with lentiviral vectors to overexpress human T-cadherin gene. Angiogenesis was studied using endothelial spheroid assay and tumor xenografts in mice. RESULTS: TCGA and qPCR analysis demonstrated weak positive correlation of T-cadherin gene level with the Gleason score. A cluster of genes have been identified which correlate best with T-cadherin level during PCa progression. T-cadherin protein was drastically upregulated in PCa tissue comparing to BPH, the increase correlating with CK8 expression and being more prominent in early stage organ-confined tumors than in advanced hormone-resistant PCa. T-cadherin overexpression in BPH-1 cells promoted proliferation, invasion and growth of organoids in 3D gels, while overexpression in DU145 cells stimulated angiogenesis in vitro and in vivo.

CONCLUSION: T-cadherin protein is strongly upregulated in early PCa, correlates with tumor luminal/basal differentiation, and promotes proliferation and invasion of benign prostate cells. Therefore, T-cadherin may be a novel functional contributor and a marker of PCa onset and progression

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Gastrokine as a novel potential biomarker for premalignant pancreatic lesions

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) has one of the most dismal prognoses of all cancer types. Diagnostic techniques for early malignant and potentially curable lesions are limited, which shows an evident need for early detection methods. Gastrokine 1 & 2 (GKN1 & GKN2) are secreted proteins found almost exclusively in the gastric epithelium, where they are involved in gastric epithelial homeostasis and tumor suppression. So far nothing is known about gastrokine expression and function in other organs. A whole genome microarray analysis of a mouse with predisposition to pancreatic cancer showed a striking upregulation of the two gastrokines in the pancreas during carcinogenesis.
Methods: GKN1 & GKN2 mRNA expression was confirmed by qPCR in patient and mouse pancreas samples. The presence of GKN1 was verified by western blot and IHC in mouse pancreas. Pancreatitis in Gkn2-/- mice was induced by cerulein injection. Mouse pancreatic juice was analysed by proteomic analysis.
Results: Gastrokine was highly upregulated during the early stages of pancreatic carcinogenesis in the mouse and in peri-tumoral human pancreas tissue. Gastrokine was not found in the healthy pancreas nor in tissue with pancreatitis. Immunohistochemistry shows a specific GKN expression in tissue areas with premalignant PanIN lesions while it is absent in PDAC. ELISA and proteomic analysis in mice confirmed the secretion of GKN1 into pancreatic juice but not serum.
Conclusions: We identified for the first time specific gastrokine expression in premalignant lesions in pancreatic tissue. The secretion into the pancreatic juice during carcinogenesis could make gastrokine a potential biomarker for the detection of early pancreatic premalignant lesions.

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The Metastatic Role of SOX9 in Neuroblastoma

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Approximately 50% of neuroblastoma patients suffer from metastatic disease at diagnosis and require intensive treatment. Despite intensive therapy, the vast majority of patients still have a poor clinical outcome (5-year survival rate of 30% to 40%). Investigating the molecular basis of neuroblastoma metastasis is crucial to develop more effective therapies. The similarities between neural crest development and neuroblastoma progression have been recognized. Transcription factor SOX9 is involved in cell migration during neural crest delamination, and has also been implicated in formation and growth of various tumors. Currently, we know little about the role of SOX9 in neuroblastoma pathogenesis. Our hypothesis is that SOX9 plays a crucial role in neuroblastoma metastasis, and the goal is to investigate the function of SOX9 and to find out its dysregulation of downstream genes. In this study, expression level of SOX9 in neuroblastoma cell lines was examined by western blot analysis. Low SOX9 expressing cell line: IMR-5 was chosen to overexpress SOX9, while high SOX9 expressing cell: SK-N-AS was used to knock down SOX9. In in vitro study, overexpression of SOX9 in IMR-5 significantly enhanced cell migration, invasion and tumorigenicity. On the other side, reduction of these abilities was observed in SOX9 knockdown SK-N-AS. The result of tumorigenicity was also confirmed in in vivo orthotopic model: implantation of SOX9 overexpressing IMR-5 had more rapid tumor growth than its control, while tumor formation largely reduced in mice injected SOX9 knockdown SK-N-AS. Taken together, these results clearly indicate that SOX9 positively involves in cell motility and invasive ability of neuroblastoma cells in vitro, and promotes tumorigenesis both in vivo and in vitro model. In vivo metastasis is now under analysis. Moreover, to support clinical relevance, SOX9 expression in patient's tumor will be analyzed. Base on our study, we expect to further figure out the molecular mechanism how SOX9 boost metastasis which eventually provides more therapeutic targets in metastasis prevention of neuroblastoma.

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A BRCA2-derived cell-penetrating peptide inhibits RAD51-mediated repair of DNA double-strand breaks

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Protein-protein interactions (PPIs) play a crucial role in regulating many biological processes, and, thus, represent attractive targets for pharmacological intervention in cancer therapy. So far, inhibition of PPIs has been challenging because PPI interfaces commonly do not support binding of small drug-like molecules. In contrast, peptide-based inhibitors of PPIs are more potent but are otherwise difficult to deliver into cells. Cancer cells strongly rely on efficient mechanisms sensing and repairing different types of DNA damage to survive and proliferate. Therefore, peptide-based inhibitors of the BRCA2-dependent DNA double-strand break (DSB) repair pathway could significantly improve the efficacy of conventional anti-cancer therapies.
In this study, we designed a BRCA2-derived peptide capable of blocking BRCA2-RAD51 interaction in vitro and in cells. Importantly, we found that incubation of cells with the BRCA2 cell-penetrating peptide resulted in reduced RAD51 foci formation and hypersensitivity to DSB-inducing chemotherapeutic drugs. Taken together, our data establish a way to screen and optimize peptide-based inhibitors of PPIs essential for DSB repair that provide a basis for the development of new and improved cancer therapies.

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Bispecific binders trap the human epidermal growth factor receptor 2 (HER2) in an inactive state and thereby drive HER2-addicted cancer cells into apoptosis

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We have selected designed ankyrin repeat proteins (DARPins), a novel class of small binding proteins, binding to the extracellular part of the human epidermal growth factor receptor 2 (HER2), which is an important oncogene that is overexpressed in many tumors and promotes malignant growth via receptor-dimerization. By means of protein engineering, joining two HER2-binding DARPins with a very short peptide-linker, we created a particular bispecific DARPin construct that is able to intermolecularly connect two HER2 molecules by binding one at extracellular domain I and the other at extracellular domain IV, as determined by the crystal structures of the two particular DARPins in complex with their targets. This bispecific binding distorts the extracellular parts of the bound HER2-receptors such that the HER2 kinases are unable to productively dimerize with each other or other EGFR family members. The DARPin-induced conformational change thereby locks the HER2 molecules in an inactive state and prevents their signaling via the MAP-Kinase- and PI3K-Akt-pathways, which are vital for HER2-addicted tumor cells. As a result, DARPin-binding induces apoptosis via induction of the pro-apoptotic protein BIM and caspase-9 as could be shown in several HER2-addicted tumor cell lines and in tumor-bearing mice. Intriguingly, the cytotoxic effect of our DARPin construct is much stronger than that of the therapeutic antibodies trastuzumab or pertuzumab, binding HER2-domains IV or II, respectively. Furthermore our constructs inhibit both ligand-independent and ligand-induced signaling.

We envision that this work will help to define the molecular requirements for anti-tumor agents that can induce strong cytotoxic responses in HER2-addicted cancer cells.

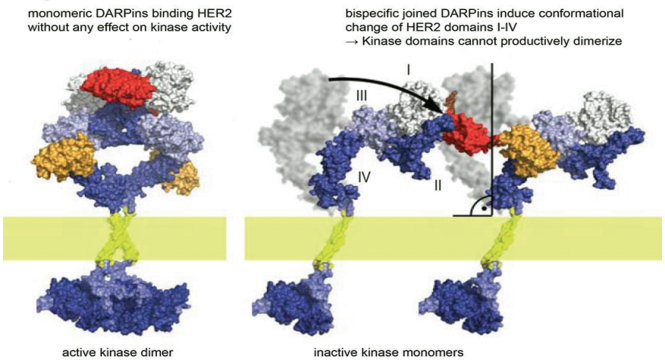

Illustration of the conformational changes induced by the bispecific joined HER2-binding DARPins (depicted in red and orange)

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LepR-expressing cells are key regulators for a sustained hematopoietic response during chronic inflammation

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The blood system is established and maintained by hematopoietic stem and progenitor cells (HSPCs) that are located in the bone marrow (BM) where they are surrounded by different types of supportive cells. Accumulating evidence suggests that important extrinsic regulatory mechanisms have evolved to maintain stemness of HSCs and guide HSPC differentiation into more mature blood cell lineages.

We set out to study how the BM microenvironment supports the hematopoietic system in adaptation to inflammation. Therefore, wild-type (WT) mice were stimulated with LPS or polyIC to mimic gram-negative bacterial or viral infection, respectively. Based on expression of CD45 and Ter119 to exclude hematopoietic cells in conjunction with Sca1, CD31 and CD140b, we are able to flow-cytometrically define several non-hematopoietic cell types in mouse BM: so-called PαS cells which were shown to have mesenchymal stem cell characteristics, a stromal cell population enriched for CXCL12-abundant reticular cells (CARs), and endothelial cells (ECs). These different cell types were isolated and their gene expression profiles were determined and compared to steady-state WT mice.

We found that Il6 is significantly and specifically up-regulated during LPS stimulation by the cell population enriched for CARs. This could be validated using qPCR and by injecting BM chimeric mice (WtàWt, Wtà $Il6^{-/-}$, $Il6^{-/-}$àWt, $Il6^{-/-}$à$Il6^{-/-}$) with PBS and LPS. We could show that IL6 protein is almost exclusively produced by non-hematopoietic cells. We then generated cell type-specific Il6-deficient mice by crossing LepR-Cre mice with $IL6^{fl/fl}$ mice, and could confirm that IL6 is mainly produced by non-hematopoietic cells that are enriched for CARs. To functionally study the regulatory role of IL6 on hematopoiesis during inflammation, WT and $Il6^{-/-}$ mice were injected with PBS or LPS over 3 weeks every other day. We analyzed phenotypically defined hematopoietic stem and progenitor cells as well as more mature myeloid cell types, and observed a significant difference in absolute cell numbers of these cell types in LPS-treated WT mice compared to LPS-treated $Il6^{-/-}$ mice. We could observe the same significant difference in absolute cell numbers when we repeated the experiment with LepR-Cre; $IL6^{fl/fl}$ mice and their LepR-Cre negative littermates. Moreover, the total number of colony-forming unit granulocyte (CFU-G) was significantly reduced in the BM of LPS treated $Il6^{-/-}$ mice and LepR-Cre; $IL6^{fl/fl}$ mice compared to control mice.

Our preliminary data suggests that CAR cell produced IL6 is essential for a sustained myelopoietic response during chronic LPS treatment. IL6 may act as a proliferative stimulus for the early HSPC compartment during infection to provide a reservoir of highly proliferative myeloid progenitors in order to counterbalance inflammation-driven cell losses.

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BRAFV600E Transduced Human CD34+ Cells Establish Aggressive Langerhans Cell Histiocytosis in Humanized Mice

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Langerhans cell histiocytosis (LCH) is the most common histiocytic disease in humans with a complex pathophysiology and unknown etiology. LCH has been recently reclassified from an inflammatory disease to a neoplastic disorder due to the high prevalence of mutations in the MAP kinase pathway. Recurrent mutation in BRAF kinase (BRAFV600E) was detected in more than 50% of patients. We recently showed that the BRAFV600E mutation can be detected in circulating CD34+ cells in LCH patients and that expression of the mutation in distinct hematopoietic compartments defines the stage and the clinical risk in LCH disease (Berres et al. J Exp Med 2014). Knowledge on induction and progression of LCH is limited due to appropriate models resembling the human disease. We here tested if xeno-transplantation of BRAFV600E transduced human CD34+ cells in immunodeficient mice would result in LCH.

Mice transplanted with human CD34+ HSPCs expressing BRAFV600E developed anemia and reduced WBC and RBC counts. Analysis of lymphoid and non-lymphoid organs demonstrated accumulation of ''atypical" BRAFV600E expressing dendritic cells in granuloma-like lesions. Using immunohistochemistry staining we observed the expression of LCH-associated markers, CD1a and CD207 to a varying extent in different organs. We did not observe

development of hairy cell leukemia. In vitro studies revealed that the expression of BRAFV600E blocks the commitment of erythroid progeny toward mature erythrocytes shown by lower number of BFU-E colony type and reduced CD235ahigh-expressing cells. Moreover, BRAFV600E expression seemed to increase monocytosis and generation of classical dendritic cells in short term liquid cultures, without affecting the proliferation potential of human HSPCs. We demonstrate that expression of BRAFV600E in human HSPCs induces LCH-like disease in MISTRG mice (i.e. mice that we developed which carry the human myeloid cytokines, knocked into the respective mouse loci; Rongvaux et al., Nat Biotech 2014). The involvement of a wide range of organs resembles high-risk LCH in humans and confirms our already reported data with aggressive LCH being a HSPC disease. Using this model system will further support basic understanding of LCH patho-biology and testing of targeted therapies in LCH.

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Intrinsic and extrinsic factors control hematopoietic stem cell aging

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Life-long self-renewing hematopoietic stem cells (HSCs) repetitively contribute to replenishment of mature blood cells. With aging HSCs show reduced self-renewal, less efficient bone marrow (BM)–homing capacity, and myeloid-skewed differentiation. Here, we tackle the questions what extrinsic and intrinsic factors determine HSC behaviour at cellular and molecular level. CFSE-labeled young or aged HSC-containing fractions (LKS) were transferred into steady-state young or aged recipients. Eight weeks BM analysis showed that young LKS proliferated faster than old independent on environment, while both young and aged LKS appear to be more dormant in old environment. To test biological function of HSC with distinct divisional history, quiescent or cycling LKS were isolated and transplanted into lethally irradiated mice. Dormant aged HSCs irrespective of the environment favour myelopoiesis. In contrast, cycling aged HSCs that had been exposed to young environment showed balanced lineage repopulation. To dissect aging-associated extrinsic factors, we performed antibody based protein arrays and transcriptome analysis with total BM of young versus aged animals. Expression levels of some inflammatory cytokines and myeloid differentiation factors were altered in aged BM. These factors drive young HSC towards proliferation/differentiation, while this effect is limited on aged HSCs, due to their increased quiescent state.

These findings demonstrate that extensive proliferative history imprints a cell dormancy program on HSCs that is associated with myeloid-biased differentiation, and that the differentiation program can be modulated via environmental cues. Understanding of how cell cycle and fate is determined and altered upon aging will open new avenues for regenerative medicine.

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Maintenance of human hematopoiesis in in vivo engineered human bone organs

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Hematopoietic homeostasis is maintained in a specialized microenvironment in the bone marrow (BM), so-called BM niche. Cellular property of hematopoietic stem cells (HSCs), i.e., self-renewal and differentiation, are tightly controlled to sustain lifelong blood production via extrinsic factors supplied by BM niche cells. While the cellular and molecular components of the human BM niche have been extensively studied in experimental animals, little is known about the human BM niche components.

To study human BM niche homeostasis, we took a developmental tissue engineering approach that allows to ex vivo generate a human cartilage template with human adult BM-derived mesenchymal stromal cells (MSCs), and to in vivo develop human bone organs, thereafter called "ossicles", through endochondral ossification (Scotti et al., PNAS 2013). Ex vivo generated ossicles were implanted into immune-deficient human cytokine knock-in mice (Rongvaux et al., Nature Biotechnology 2014), and 4 weeks later, a third party donor cord blood (CB)-derived human CD34+ cells were transplanted in order to reconstitute human hematopoiesis, following sub-lethal irradiation. Histological analysis showed that human MSC-derived ossicles developed a vascular network and a mature trabecular bone-like structure.

Flow-cytometric analysis at 2 month post CB transplantation showed comparable development of human hematopoiesis with phenotypic HSC in the human ossicles compared to mouse BM. Myeloid-colony forming unit (CFU) assays and serial transplantation showed a significantly higher frequency of hematopoietic stem and progenitor cells maintained in human ossicles than in mouse BM. This suggests that human ossicles can serve as niche to support human hematopoiesis.
Our findings indicate that bone organs in vivo developed by adult BM MSC can support engraftment and maintenance of allogenic human HSCs and hematopoiesis, proving functional human BM niche. The engineering of a heterotrophic human BM niche that is transplantable and genetically re-engineerable will serve as a platform that allows to studying physiology and pathophysiology of healthy and diseased human hematopoiesis in their optimal environment in vivo.

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Applying 3D quantitative microscopy to study global topography and cellular interactions in the bone marrow

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During adulthood, bone marrow (BM) cavities are the primary sites of production of vast amounts of mature blood cellular components from a rare population of hematopoietic stem cells (HSCs). Beyond their hematopoietic function BM tissues provide the necessary microenvironment for the initiation of immune responses and the maintenance of immunological memory. In addition to hematopoietic cells, the BM is also populated by a heterogeneous mixture of non-hematopoietic stromal cells of endothelial, mesenchymal and neural origin, which provide the necessary tissue infrastructure for hematopoiesis to unfold and play essential functional regulatory roles. For instance, HSCs reside in perivascular niches where they directly interact with BM sinusoidal endothelial cells and mesenchymal stromal cells, which provide necessary factors for HSC maintenance. Thus, a thorough understanding of the spatial distributions, structural dynamics and cellular interactions established by the diverse hematopoietic and stromal components within the complex landscape of BM tissues, is key for the generation of comprehensive models of healthy and malignant hematopoiesis.
In our laboratory we have recently developed protocols and advanced microscopy techniques that enable the 3D visualization of large volumes of BM tissues at an organ wide level and with cellular and subcellular resolution. Here we report the generation of customized computational tools, which allow to generate quantitative spatial information in an automatic and unbiased fashion, and the extraction of spatial statistics for rigorous analysis of cellular interactions. We have employed this newly developed software suite to describe for the first time the spatial distribution of the key components of the HSC niche, namely sinusoidal vessels and mesenchymal stromal cells. First, our data demonstrate that in general the quantitative contribution of BM stromal cells to the total BM cellular asset is substantially underestimated by widely employed flow cytometric techniques. Detailed topographical analysis revealed that the highly branched and dense sinusoidal vessel network occupies in average 18% of the entire BM volume, subsequently constraining the space available for cells to distribute. Indeed, through rigorous spatial statistics analysis it can be estimated that 95% of the BM space is contained within a distance of 22 µm from the nearest sinusoid. Nonetheless, density of mesenchymal stromal cells is significantly enriched in perivascular locations, pointing to a preferential interaction between these two key stromal components. Collectively our analyses provide a quantitative measurement defining microarchitectural organization of BM stroma in homeostatic conditions. The tools developed are currently being applied to study mouse models of acute myeloid leukemia and dissect the effects of disease progression in BM microenvironmental integrity

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The role of the RNF8/RNF168 pathway in replication and fork protection

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The DNA damage response (DDR) represents a crucial antitumor barrier in precancerous lesions, where it signals increasing levels of DNA replication stress. However, key players and mechanisms of the DDR have been mostly characterized in response to DNA double strand breaks (DSBs). In particular, the RNF8/RNF168 pathway is instrumental in the cellular response to DSBs. Upon DSB formation the E3 ubiquitin ligase RNF168, in conjunction with the ubiquitinating complex RNF8/UBC13, ubiquitinates histone residues in the vicinity of the break site, which leads to the recruitment of prominent repair factors such as BRCA1 and 53BP1(Schwertman et al. 2016). Several recent publications have implicated multiple homologous recombination (HR) factors - such as BRCA1/2 (Schlacher et al. 2011; Schlacher et al. 2012; Ray Chaudhuri et al. 2016) or RAD51 (Zellweger et al. 2015) - in replication fork protection and remodelling, upon drug induced replicative stress. This suggests a role for classical HR factors beyond their established functions in DSB repair. In this project we focus on the role of the RNF8/168 pathway in DNA replication and we report that several of its components assume an active function in replication fork remodelling and fork protection, even in the absence of genotoxic treatments. Our ongoing experiments suggest a pivotal role for classical DDR factors in the modulation of DNA replication, and may uncover crucial roles for the RNF8/168 pathway in determining the sensitivity of certain tumors to cancer chemotherapeutic regimens.

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Replication fork reversal triggers fork degradationin BRCA2-defective cells

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Besides its well-established role in loading RAD51 for double-strand break repair, the tumor suppressor BRCA2 protects stalled replication forks from nucleolytic degradation. Defects in the latter function were recently shown to underlie the sensitivity of BRCA2-defective tumors to chemotherapeutics, but how BRCA2 assists replication fork integrity remains elusive. Using DNA fiber spreading coupled to electron microscopic visualization of replication intermediates, we now report that fork degradation in BRCA2-defective cells targets reversed replication forks and is suppressed by genetic inactivation of replication fork reversal by RAD51 or ZRANB3 depletion. Unstable RAD51 filaments, as those induced by the Fanconi anemia associated RAD51-T131P mutation, allow fork reversal, but do not effectively protect reversed forks, thus exposing cells to fork degradation. Furthermore, PTIP depletion - which suppresses chemosensitivity and embryonic lethality associated with BRCA2 defects - restores normal levels of reversed forks in BRCA2-defective somatic and embryonic cells. We propose that RAD51 mediates fork reversal independently of BRCA2. However, BRCA2-dependent RAD51 loading on reversed forks is essential to protect them from nucleolytic degradation. As reversed forks are abundant upon chemotherapeutic treatments and in unperturbed ESCs, we propose reversed fork protection as BRCA2 essential role in both chemoresistance and early embryogenesis.

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Monitoring replication and repair of psoralen-induced interstrand crosslinks on human genomic DNA

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Interstrand crosslinks (ICL) represent highly cytotoxic lesions that covalently link two strand of DNA in a duplex, thus posing a threat to replication and transcription, two fundamental processes for life. ICL inducing agents are among the most widely used chemotherapeutics, but unfortunately cancer cells are able to repair ICL using Fanconi anemia and homologous recombination proteins, thereby evading or limiting cell death. ICL were long considered an absolute roadblock to the replication machinery, but a recent study has shown that replication machinery can efficiently traverse ICLs. Using a chemically modified psoralen we exploited single-molecule approaches (DNA fiber assays and visualization of replication intermediates by electron microscopy) and looked at the replication occurring in vicinity to an ICL (local replication) and replication occurring at a distance from ICL (global replication). Investigating global replication in different genetic backgrounds (i.e. upon inactivation of crucial Fanconi anemia factors) we found that fork-remodeling factors are immediately recruited to stressed replication forks, that fork reversal is a very frequent event even upon DIG-TMP treatment and that global fork slowdown upon ICL induction is dependent upon fork remodeling itself. We also developed Immuno Electron Microscopy (iEM) to map specifically ICLs on EM molecules: this will reveal architectural changes of forks facing ICLs and will assess a direct functional link between fork remodeling and ICL bypass or repair. These studies have the potential to provide mechanistic insight on ICL replication and repair and thus to clarify resistance mechanisms upon cancer chemotherapeutic regimens with ICL-inducing agents.

Charles Rodolphe Brupbacher Stiftung

Charles Rodolphe Brupbacher Foundation

Mme Frédérique Brupbacher

Portrait by Peter Cerutti

Charles Rodolphe Brupbacher Stiftung

Die Stiftung hat das Ziel, die Krebsforschung in der Schweiz und international zu fördern.
Wichtigstes Element ihrer Tätigkeit ist die Verleihung des Charles Rodolphe Brupbacher Preises für Krebsforschung, verbunden mit einem wissenschaftlichen Symposium in Zürich.

Die Stifterin

Frau Frédérique Brupbacher hat im November 1991 in Verehrung ihres Gatten, Charles Rodolphe Brupbacher, eine Stiftung mit Sitz in Vaduz errichtet. Die Stiftung verleiht alle zwei Jahre den Charles Rodolphe Brupbacher Preis für Krebsforschung an Wissenschaftler, die in der Grundlagenforschung herausragende Leistungen erbracht haben. Die Preisverleihung findet statt im Rahmen eines internationalen wissenschaftlichen Symposiums.

Auf Antrag der Medizinischen Fakultät ernannte die Universitätsleitung Frau Frédérique Brupbacher 2005 zum Ständigen Ehrengast der Universität Zürich, in Anerkennung der grossen Verdienste, die sie sich mit ihrem Altruismus und ihrem Engagement für die Krebsforschung erworben hat. Durch ihre Initiative und ihren persönlichen Einsatz konnte die Krebsforschung im Raum Zürich nachhaltig gestärkt werden. Am 20. Juni 2001 ernannte Präsident Jacques Chirac sie zum Chevalier de la Légion d'Honneur.

Charles Rodolphe Brupbacher Foundation

The mission of the Foundation is to foster cancer research in Switzerland and internationally.
The key element of its activities is the Charles Rodolphe Brupbacher Prize for Cancer Research which is awarded in association with a scientific symposium in Zurich.

The Founder

In honour of her late husband Charles Rodolphe Brupbacher, Mrs. Frédérique Brupbacher set up a foundation registered in Vaduz, Liechtenstein, in November 1991. The Foundation's mission is to present the biennial Charles Rodolphe Brupbacher Prize for Cancer Research to a scientist with internationally acknowledged meritorious achievements in the field of fundamental research. The Prize is awarded in the context of a scientific symposium.

The Executive Board of the University of Zurich appointed Mrs. Frédérique Brupbacher in 2005 as a permanent Guest of Honor of the University, in appreciation of her altruism and her engagement for cancer research. Through her personal committment, cancer research in Zurich has been significantly strengthened. President Jacques Chirac of France appointed her to Chevalier de la Légion d'Honneur.

Charles Rodolphe Brupbacher
1909 – 1987

Charles Rodolphe Brupbacher wurde am 5. Februar 1909 in Zürich als Bürger von Wädenswil geboren. Sein Vater, C.J. Brupbacher, war Inhaber einer Privatbank am Paradeplatz. Die Mutter, geborene Französin, legte grossen Wert auf eine zweisprachige Erziehung des Sohnes. Dies erklärt auch seine lebenslange, enge Beziehung zu Frankreich, zu dessen Geschichte und Kultur und seine dauernde, grosszügige Unterstützung der Ecole française und der Alliance française in Zürich. Sein jahrzehntelanger Einsatz für die Anliegen der französischen Kultur wurde mehrfach durch die jeweiligen Staatspräsidenten geehrt:

1961 Präsident Charles De Gaulle
Ernennung zum Chevalier de la Legion d'Honneur
1973 Präsident Georges Pompidou
Ernennung zum Officier de la Legion d'Honneur
1979 Präsident Valéry Giscard d'Estaing
Ernennung zum Commandeur de l'Ordre National de Merite

Schon früh zeigte sich bei Charles Rodolphe Brupbacher eine ausgesprochene Sprachbegabung; er beherrschte fünf Sprachen fliessend. Als musikalisches Wunderkind mit dem absoluten Gehör widmete er sich der Interpretation klassischer Musik und bedauerte zeitlebens, dass er auf eine Ausbildung als Konzertpianist verzichten musste. Charles Rodolphe Brupbacher besuchte die Schulen in Zürich und Paris.

Charles Rodolphe Brupbacher was born on February 5, 1909 in Zurich, as a citizen of Wädenswil. His father, C.J. Brupbacher, owned a private bank on Paradeplatz. His mother, a French citizen, placed great importance on a bilingual education for her son. This explains his lifelong, close relationship with France, its history and culture. This is also reflected by his continuous and generous support of the École française and the Alliance française in Zurich. Several French Presidents honoured his commitment to French cultural issues:

1961 President Charles De Gaulle
Election to Chevalier de la Legion d'Honneur
1973 President Georges Pompidou
Election to Officier de la Legion d'Honneur
1979 President Valéry Giscard d'Estaing
Election to Commandeur de l'Ordre National de Merite

At an early age, Charles Rodolphe Brupbacher showed a distinct talent for languages, and he spoke five of them fluently. As a musical prodigy with perfect pitch, he devoted himself to the interpretation of classical music. He regretted throughout his life that he had not been able to receive an education as a concert pianist. Charles Rodolphe Brupbacher attended schools in Zurich and Paris.

Mit 18 Jahren musste er auf Verlangen seines Vaters die Ausbildung am Gymnasium in Zürich und Paris aufgeben und eine Banklehre absolvieren. Anschliessend besuchte er ab 1929 immer wieder die Vereinigten Staaten, sowie Lateinamerika und trat so in Beziehung zu grossen Persönlichkeiten in führender Stellung.

Nach seiner Rückkehr in die Schweiz gründete er, als damals jüngster Bankier, mit 24 Jahren die auf Vermögensverwaltung spezialisierte Bank «Affida» am Paradeplatz in Zürich. Sein Erfolg war in hohem Masse seinen Geschäftsprinzipien zu verdanken. Dazu gehörte der Aufbau eines Informationsnetzes, welches ihn mit den wichtigsten finanziellen und politischen Zentren verband. Von grosser Bedeutung waren dabei seine detaillierten Kenntnisse der internationalen Rechtsprechung, der Nationalökonomie und ganz speziell auch von Währungsfragen. Nach 40jähriger Tätigkeit verkaufte er die Affidabank an die Schweizerische Kreditanstalt (Credit Suisse).

Auf Grund seiner umfassenden Kenntnisse wurde Charles Rodolphe Brupbacher 1938 von Prof E. Böhler in die Gruppe für Konjunkturbeobachtung der Eidgenössischen Technischen Hochschule (ETH) berufen. Als deren Mitglied nahm er auch an Besprechungen kriegswirtschaftlicher Probleme in Bern teil.

Als anerkannter Fachmann in Währungsfragen wurde Charles Rodolphe Brupbacher nach dem Kriege als einziger Beobachter aus der Schweiz zu den internationalen Währungskonferenzen eingeladen. Seine persönlichen Beziehungen zu wichtigen Politikern in den USA erlaubten es ihm, durch jahrelange, zähe Verhandlungen grosse schweizerische Guthaben zu deblockieren.

Auch bemühte sich Charles Rodolphe Brupbacher intensiv um die Probleme, welche sich bei dem Wiederaufbau der Montanindustrie zwischen Deutschland und den Alliierten entwickelt hatten. In diesem Zusammenhang wurde er von der französischen Regierung und der Regierung von Nordrhein-Westfalen zur Teilnahme an dem Treffen anlässlich der ersten Reise von General de Gaulle nach Deutschland eingeladen.

Schon im Jahre 1963 hat Charles Rodolphe Brupbacher an der ETH eine Stiftung zur Unterstützung von Studierenden auf dem Gebiet der Sozialwissenschaften gegründet, die seither laufend Stipendien vergibt.

Charles Rodolphe Brupbacher starb am 1. Januar 1987 und hinterliess seine Ehefrau Frédérique, die er 1953 geehelicht hatte.

At the age of 18, however, he had to give up his education at the Gymnasium (College) to undertake a banking apprenticeship. He visited the United States and Latin America in 1929 and frequently thereafter: first, for the purpose of training; later, to keep himself informed.

At the Paradeplatz in Zurich, at the age of only 24, he established the «Affida Bank», which specialized in asset management. His success was largely due to a committment to personal business integrity. His achievements included the setting-up of an information network that connected him with important financial and political centres. His detailed knowledge of international commercial law, of national economics and, especially, of currency policy were great assets. After 40 years, he sold the «Affida Bank» to Credit Suisse.

Based on his detailed knowledge, Charles Rodolphe Brupbacher was invited by Professor E. Böhler in 1938 to join a select group formed at the Swiss Federal Institute of Technology (ETH), which met to monitor the economy. As a member, he often took part in discussions in Bern of wartime economic problems.

As a recognised expert in monetary policy, Charles Rodolphe Brupbacher was the only observer from Switzerland to be invited after the war to the international currency conferences. His personal relationship with prominent politicians in the United States enabled him, through years of negotiations, to release major Swiss assets.

Charles Rodolphe Brupbacher also helped to attenuate problems which had developed between Germany and the Allies regarding the restoration of the coal and steel industry. In this context, he was invited by the Government of France and by the State of North Rhine-Westphalia to participate in the meeting on the occasion of General de Gaulle‘s first visit to Germany.

Already in 1963, Charles Rodolphe Brupbacher established a Foundation at the ETH with the objective of supporting students in the field of social sciences. Since then, the Foundation has continuously granted scholarships.

Charles Rodolphe Brupbacher died on January 1, 1987, survived by his wife Frédérique whom he married in 1953.

Stiftungsrat

Der Stiftungsrat verwaltet die Stiftung und vertritt sie nach aussen. Er trifft die Entscheide über Preisverleihungen und die begleitenden wissenschaftlichen Symposien.

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The Foundation Board administers the Foundation and determines policy. The Foundation Board makes final decissions regarding Prizes and Symposia.

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Wissenschaftlicher Beirat

Der Wissenschaftliche Beirat nominiert Kandidaten für den Charles Rodolphe Brupbacher Preis für Krebsforschung und empfiehlt Redner für die wissenschaftlichen Symposien.

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The Scientific Advisory Board nominates candidates for the Charles Rodlphe Brupbacher Prize for Cancer Research. The Advisory Board also suggests Symposia speakers.

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Alle Korrespondenzen und Anfragen bezüglich des Symposiums sind an das Wissenschaftliche Sekretariat zu richten.

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