5th EACR Conference

A Matter of Life or Death
Mechanisms, Models and Therapeutic Opportunities

12 - 14 February 2020
Bergamo, Italy

Scientific Programme Committee

Patrizia Agostinis, Peter de Keizer,
Simone Fulda, Verena Jendrossek, Marion MacFarlane

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Wednesday 12th February 2020

12:00  REGISTRATION ☕
      until 13:30

12:30  WELCOME LUNCH & TRADE EXHIBITION ☑️

13:30  CONFERENCE WELCOME

Scientific Programme Committee

SESSION 1: CANCER METABOLISM, AUTOPHAGY AND TUMOR MICROENVIRONMENT
Chair: Verena Jendrossek

13:40  THE EMBO KEYNOTE LECTURE
Sharon Tooze
The Francis Crick Institute, UK
*Molecular mechanisms of autophagosome formation*
Q&A: 14:10 - 14:25

14:25  Katja Simon
Kennedy Institute of Rheumatology, UK
*Autophagy in cell fate decisions in the immune & hematopoietic system*
Q&A: 14:45 - 14:55

14:55  Monica Vara-Perez
VIB – KULeuven, Belgium
Proffered Paper 1: “BNIP3 sustains HIF1α to promote melanoma growth and dissemination”
Q&A: 15:05 - 15:10

15:10  EXHIBITOR INTRODUCTIONS
60-second intro by each company

15:20  COFFEE BREAK & TRADE EXHIBITION ☕

15:50  Patrizia Agostinis
VIB – KULeuven, Belgium
*Autophagy in tumor angiogenesis*
Q&A: 16:10 - 16:20
16:20  **Almut Schulze**  
DKFZ, Germany  
“Understanding the complex role of lipid metabolism in cancer”  
Q&A: 16:40 - 16:50

16:50  Sarah-Maria Fendt  
VIB-KULeuven, Belgium  
“The role of metabolism in metastasis formation”  
Q&A: 17:10 - 17:20

17:20  **POSTER SPOTLIGHTS**  
3 top-scoring abstracts (posters 1-3) presented in 3 minute ‘flash talks’

17:30  **NETWORKING RECEPTION**  
An opportunity to make new connections over a drink and some light food

18:00  **POSTER DISCUSSION SESSION 1**  
*till 19:30*  
Posters 1-30 will be presented

**Thursday 13th February 2020**

08:30  **POSTER VIEWING & TRADE EXHIBITION**  
*until 09:00*  
Coffee / tea available

08:45  **INDUSTRY SPOTLIGHT - VECTORBUILDER**  
**Matthew Wheeler**  
“VectorBuilder - Advances in vector construction and virus packaging”

09:00  **KEYNOTE LECTURE**  
**René Bernards**  
NKI, Netherlands  
“Exploiting senescence for the treatment of cancer”  
Q&A: 09:30 - 09:45

09:45  **Nataly Kravchenko-Balasha**  
The Hebrew University of Jerusalem, Israel  
Proffered Paper 2: "Deciphering the intra-tumor and inter-patient signaling heterogeneity in cancer for the rational design of patient specific drug cocktails”  
Q&A: 09:55 - 10:00

10:00  **David Wallach**  
Weizmann Institute of Science, Israel  
“Novel insights into the functional roles of the signaling pathway for necroptotic death”  
Q&A: 10:20 - 10:30
10:30 **COFFEE BREAK & TRADE EXHIBITION**

11:05 **Marion MacFarlane**  
MRC Toxicology Unit, UK  
"Uncovering the Therapeutic Potential of BH3-mimetics in Patient-relevant Pre-clinical Models of Mesothelioma"  
Q&A: 11:25 - 11:35

11:35 **Samuel Sidi**  
Icahn School of Medicine at Mount Sinai, USA  
Proffered Paper 3: "FANCI Functions as a Repair/Apoptosis Switch in Response to DNA Crosslinks"  
Q&A: 11:45 - 11:50

11:50 **Dagmar Kulms**  
TU Dresden, Germany  
"Caspase-8 promotes progression of p53-proficient tumors"  
Q&A: 12:10 - 12:20

12:20 **POSTER SPOTLIGHTS**  
3 top-scoring abstracts (posters 31-33) presented in 3 minute 'flash talks'

12:30 **LUNCH & TRADE EXHIBITION**

13:00 **POSTER DISCUSSION SESSION 2**  
Posters 31-59 will be presented

**SESSION 3: SENESCENCE, CANCER AND THERAPEUTIC TARGETING**  
Chair: Patrizia Agostinis

14:30 **Manuel Serrano**  
ICREA Professor, IRB Barcelona, Spain  
"Cellular senescence as a driver of multiple human diseases"  
Q&A: 14:50 - 15:00

15:00 **Marco Demaria**  
ERIBA/UMCG, The Netherlands  
"Modulating senescence-associated phenotypes to prevent side effects of cancer therapy"  
Q&A: 15:20 - 15:30

15:30 **Olivier E. Pardo**  
Imperial College, UK  
Proffered Paper 4: "Targeting the S6 kinase, RSK4, prevents chemoresistance and metastasis in lung cancer"  
Q&A: 15:40 - 15:45

15:45 **COFFEE BREAK & TRADE EXHIBITION**
16:15 **Laura Conti**
MBC, University of Torino, Italy
Proffered Paper 5: “The immunotargeting of the xCT cystine/glutamate antiporter targets cancer stem cells and potentiates the efficacy of Her2-targeted immunotherapies in breast cancer”
Q&A: 16:25 - 16:30

16:30 **Peter de Keizer**
UMC Utrecht, The Netherlands
“Targeting Heterogeneity in Senescence against Aging and Cancer”
Q&A: 16:50 - 17:00

17:00 **Daniel Murphy**
CRUK Beatson Institute, UK
“Partners in crime: MYC, RAS and cell-extrinsic mechanisms of oncogenic cooperation”
Q&A: 17:20 - 17:30

17:30 **DISCUSSION FORUM**
Chair: Patrizia Agostinis, the panel of invited speakers will tackle this important question.
“Novel frontiers in regulated cell death and senescence: what is the desirable type of cell inactivation in cancer therapy?”

19:00 **CONFERENCE DINNER**
Optional ticketed dinner at Taverna Valtellinese

**Friday 14th February 2020**

08:30 **POSTER VIEWING & TRADE EXHIBITION**
Coffee / tea available

08:45 **INDUSTRY SPOTLIGHT - TECAN**

**Stefan Haberstock**
“Optimization of drug titration in cell based assays”

09:00 **MEET THE EXPERTS TALK**

**Silvia Formenti & Verena Jendrossek**
An interactive session with a focus on women in science where our two experts will share from their personal and professional perspectives on the path to scientific leadership.
SESSION 4: HARNESSING CELL DEATH AND CANCER VULNERABILITIES FOR CANCER THERAPY
Chair: Marion MacFarlane

09:45  Clemens Schmitt
MDC Berlin, Germany
"Post-senescence: a matter of life and death"
Q&A: 10:05 - 10:15

10:15  Verena Jendrossek
Universität Duisburg-Essen, Germany
"Novel opportunities for a biologic optimization of radiotherapy"
Q&A: 10:35 - 10:45

10:45  COFFEE BREAK & TRADE EXHIBITION

11:15  Serk In Park
Korea University College of Medicine, South Korea
Proffered Paper 6: "Mobilization of Monocytic Myeloid-Derived Suppressor Cells (MDSC) Is Regulated by Osteoblast Activation by PTH1R"
Q&A: 11:25 - 11:30

11:30  Brent Stockwell
Columbia University, USA
"Ferroptosis: mechanisms and therapeutic potential"
Q&A: 11:50 - 12:00

12:00  CLOSING KEYNOTE
Silvia C. Formenti
Weill Cornell Medicine, USA
"Radiotherapy to convert the tumor into an in situ vaccine"
Q&A: 12:30 - 12:45

12:45  CONFERENCE SUMMARY
Scientific Programme Committee

13:00  LUNCH & DEPART
EACR-Worldwide Cancer Research Meeting Bursary Award winners

Congratulations to the winners of the EACR-Worldwide Cancer Research Meeting Bursaries. Each winner received a full registration free of charge and funds of up to 500 Euros to assist with the cost of travel.

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Interactive activities

An important part of the EACR Conferences is the range of opportunities we aim to provide for participants to interact, discuss, reflect and build relationships and collaborations.

We hope you enjoy the dedicated interactive activities, which are listed below.

Networking Reception

Wednesday 12 February | 17:30-18:00

A complimentary buffet dinner will be served for all participants and exhibitors to enjoy on Wednesday evening. Why not take the opportunity to make new connections in an informal setting! The trade exhibition will be open during this time, and Poster Discussion Session 1 will follow at 18:00.

Discussion Forum

Thursday 13 February | 17:30-18:30

“Novel frontiers in regulated cell death and senescence: what is the desirable type of cell inactivation in cancer therapy?”

This session will provide an opportunity to discuss the role of regulated cell death and senescence elicited in response to various conventional or targeted therapeutic approaches, including but not limited to, immunogenic cell death and ferroptosis inducers, on inflammatory and immune responses. Chaired by Patrizia Agostinis, the panel of invited speakers will address which type of cancer cell death has the potential to increase the immunogenicity of cancer cells and overcome resistance mechanisms, what are potential adverse effects of therapy-induced cell death and senescence, and finally how this knowledge can be used to improve current and design novel therapeutic concepts.

This will be a forum for open discussion led by panel members Peter de Keizer, Silvia Formenti, Dagmar Kulms and Brent Stockwell. Participants are invited to ask their questions and contribute to the conversation.

Meet the Experts

Friday 14 February | 09:00-09:45

An interactive session with a focus on women in science where our speakers Silvia Formenti and Verena Jendrossek will share from their personal and professional perspectives on the path to scientific leadership. This session will take place in the lecture theatre, and will be a forum for open discussion where participants are encouraged to ask their questions.
Conference Dinner

**Thursday 13 February | 19:00, Taverna Valtellinese**

The Conference Dinner will take place on the final night of the meeting at the atmospheric Ristorante Taverna Valtellinese, a short walk from the conference venue. It will be an excellent opportunity for participants and speakers to get to know each other in a relaxed and informal environment. A four course meal will be served, with wine (or alternative) and coffee included in the ticket price.

Poster Spotlights

**Wednesday 12 February | 17:20 - 17:30 | Posters 1 - 3**  
**Thursday 13 February | 12:20 - 12:30 | Posters 31 - 33**

Six high-scoring abstracts have been selected to give Poster Spotlight presentations. These three minute ‘flash talks’ will be presented in the lecture theatre.

Poster Discussion Sessions

**Wednesday 12 February | 18:00-19:30 | Posters 1 - 30**  
**Thursday 13 February | 13:00-14:30 | Posters 31 - 59**

There are two dedicated 90-minute Poster Discussion Sessions in the programme. At these times, the presenters for that session should stand by their posters to discuss their work with other participants and invited speakers. As well as the dedicated sessions on Wednesday and Thursday, there will also be additional poster viewing opportunities each morning - coffee will be available from 08:30, and posters can be freely browsed.

Don’t forget to let us have your feedback about these activities in the survey we send after the conference!
We’d like to thank all our sponsors for their support, interest and enthusiasm.

**Premium Sponsors**

Join our Premium Sponsors **Tecan** and **VectorBuilder** at their Industry Spotlights.

**Thursday 13 February 08:45 – 09:00**

**Matthew Wheeler** VectorBuilder

“VectorBuilder - Advances in vector construction and virus packaging”

**Friday 14 February 08:45 – 09:00**

**Stefan Haberstock** Tecan

“Optimization of drug titration in cell based assays”
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Charles Swanton, United Kingdom
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THE EMBO KEYNOTE LECTURE

Molecular mechanisms of autophagosome formation

Sharon A. Tooze\(^1\)

\(^1\) The Francis Crick Institute, London, UK

Autophagy is a conserved lysosome-mediated degradative pathway required for cell survival, homeostasis and human health. Autophagy is carried out by membranes called autophagosomes which capture cytosolic cargo such as damaged mitochondria and deliver it to lysosomes. Autophagosome formation is a complex process that requires a cohort of autophagy proteins (ATG proteins) acting in a coordinated manner. This core machinery drives the formation of the phagophore, the nucleating membrane, and the nascent autophagosome. Under amino acid starvation this coordinated response is initiated by the transmembrane protein ATG9 and the serine/threonine ULK1/2 kinase. ATG9 trafficking and ULK1/2 activation sense signals which drive the initiation of the phagophore. Following initiation, the Class III PI3 kinase, and the PI3P effector WIPI2 mediate recruitment of ATG12-5-16L1, and the ATG8 family of proteins. ATG8s are required for cargo selection and maturation of the autophagosome. In an effort to understand the formation and maturation of autophagosomes we are studying the trafficking and function of ATG9, substrates of the ULK1/2 complex, and the selectivity of the ATG8 proteins. Our recent results will be presented which provide new insight into these processes.

This work was supported by the Francis Crick Institute (FC001187) which receives its core funding from Cancer Research UK, the UK Medical Research Council, and the Wellcome Trust.
Autophagy in cell fate decisions in the immune & hematopoietic system

Ghada Alsaleh¹, Hanlin Zhang¹, Susanne Kraft⁴, Dingxi Zhou¹, Christian Behrends⁴, Anna Katharina Simon³

Autophagy is a conserved major cellular degradation process that delivers unwanted bulk cytoplasmic material to the lysosome. It takes place in every cell at all times at basic level, however, it can be induced to recycle material when nutrients are scarce. In addition unwanted organelles and macromolecules are turned over via autophagy once they have been labeled for degradation. Our in vivo work has demonstrated that under physiological conditions autophagy determines cell fate: it prevents cell death and cellular ageing, and maintains the life span of long-lived cells in particular.

Our recent results also show that autophagy is key to normal differentiation of hematopoietic cells. Cellular differentiation requires remodeling of the cytoplasm and change of metabolism. Autophagy’s contribution to this process is the maintenance of mitochondrial quality and generation of ATP via fatty acid oxidation. We have also recently uncovered a novel pathway signaling for autophagy that relies on translation and is key to rejuvenation of the aging immune system. I will present our data on autophagy’s impact on the immune system, with a particular emphasis on differentiation, maintenance and aging in mouse and human.
BNIP3 sustains HIF1α to promote melanoma growth and dissemination

Monica Vara-Perez1,2, Matteo Rossi3,2, Chris Van Den Haute4, Maria Livia Sassano1,2, Sarah Maria Fendt3,2, Max Mazzone5,2, Patrizia Agostinis1,2

1 Cell Death Research and Therapy Group, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, BELGIUM, 2 VIB Center for Cancer Biology, Leuven, BELGIUM, 3 Cellular Metabolism and Metabolic Regulation group, Department of Oncology, KU Leuven, Leuven, BELGIUM, 4 Neurobiology and Gene Therapy group, Department of Neurosciences, KU Leuven, Leuven, BELGIUM, 5 Laboratory of Tumour Inflammation and Angiogenesis, Department of Oncology, KU Leuven, Leuven, BELGIUM

Abnormal stabilization and upregulation of Hypoxia Inducible Factor 1 alpha (HIF1α) is associated with poorer prognosis in melanoma. Emerging evidence underscores that the modulation of prolyl hydroxylases’ (PHDs) activity by cancer cell’s metabolic status is crucial for HIF1α stabilization. We previously reported that the HIF1α–responsive, pro-mitophagic, atypical BH3-only protein BNIP3 confers survival advantage to melanoma cells in vitro; yet, its role ‘in vivo’ remained unexplored. Transcriptomic and IHC analysis of melanoma patient samples showed that higher BNIP3 levels correlate with poorer survival. When injected in syngeneic mice, B16-F10 melanoma cells lacking BNIP3 (shBNIP3) displayed a severe tumor growth delay, compared to their matched control (shCntl) or autophagy-deficient melanomas (shATG5). Loss of BNIP3 in melanoma cells compromised mitochondria clearance (mitophagy) without blocking overall autophagic flux. In spite of their abnormal mitochondrial network, shBNIP3 cells reprogrammed their metabolism towards OXPHOS whereas ATG5-deficient cells became more reliant on anaerobic glycolysis. The OXPHOS metabolic dependence displayed by the BNIP3 deficient cells was enforced by a PHD2-mediated downregulation of HIF1α and of its main glycolytic and pro-angiogenic transcriptional program. Remarkably, expression of an undegradable HIF1α mutant largely rescued the metabolic phenotype and the delayed growth of the BNIP3 silenced melanoma cells in vivo. Mechanistically, we found that loss of BNIP3 boosted ferritinophagy in melanoma cells, which increased the level of free labile iron, an essential cofactor for the PHD2-mediated degradation of HIF1α. However, restoring HIF1α levels in shBNIP3 cells did not affect ferritinophagy, underscoring that modulation of ferritinophagy by BNIP3 is upstream to HIF1α signalling and not secondary to it. Melanoma TCGA data analysis corroborated that BNIP3 levels correlated with an elevated HIF1α signalling signature rather than with HIF1α transcript levels. Altogether, these data unravel an unexpected BNIP3-dependent feedforward loop fostering HIF1α-driven glycolysis and angiogenesis in early-stage melanoma.
Autophagy in tumor angiogenesis

Odeta Mece¹, Diede Houbaert¹, Jelle Verhoeven¹, Patrizia Agostinis¹

¹ Laboratory of Cell Death Research and Therapy, Department of Cellular and Molecular Medicine and VIB-KUL Center for Cancer Biology, KU Leuven, Leuven, BELGIUM

Autophagy is a major lysosomal pathway for the degradation and recycling of cytoplasmic components, with vital homeostatic functions. In cancer, autophagy has been shown to shape tumor growth and dissemination by regulating vital cell autonomous and non-autonomous processes affecting cancer cell-stromal cells interface. Our previous work highlighted that melanoma growth is delayed in mice harboring genetic deletion of ATG5 specifically in blood and lymphatic vessels (i.e. ATG5ECKO) suggesting that EC-associated autophagy may provide a conducive microenvironment favoring melanoma progression (Maes et al, Cancer Cell, 2014). Emerging evidence indeed indicates that the aberrant tumor vasculature supports cancer outgrowth, not only by promoting the hypoxic and pro-angiogenic status of the tumor, but also by actively regulating tumoral immune responses. To clarify the specific contribution of blood EC (BECs) or lymphatic EC (LECs)-associated autophagy in melanoma dissemination, we generated transgenic mouse models whereby ATG5 is specifically deleted either in BECs or LECs. Here I will present unpublished data underscoring the crucial role of EC-associated autophagy in inflammation- and tumor-driven (lymph)angiogenesis.
Almut Schulze

1 Division of Tumor Metabolism and Microenvironment, German Cancer Research Center (DKFZ), Heidelberg, GERMANY

Oncogene activation and loss of tumor suppressor function changes the metabolic activity of cancer cells to drive unrestricted proliferation. Moreover, cancer cells adapt their metabolism to sustain growth and survival when access to oxygen and nutrients is restricted, such as in poorly vascularized tumor areas.

Metabolic and transcriptomic analyses of isogenic colon cancer cell lines exposed to metabolic stress revealed that loss of p53 activates the sterol regulatory element binding protein 2 (SREBP2), which controls the expression of enzymes involved in the mevalonate pathway. This promotes the synthesis of ubiquinone, an essential electron transport molecule within the mitochondrial electron transport chain, which supports respiration and pyrimidine synthesis. Inhibition of the mevalonate pathway by statins selectively blocked pyrimidine nucleotide biosynthesis in p53-deficient cancer cells, leading to oxidative stress and induction of apoptosis. Moreover, we found that statins block intestinal hyperproliferation in an Apc/KrasG12D mouse model. Finally, we demonstrate that ubiquinone produced by the mevalonate pathway is limiting for the growth of p53-deficient tumor organoids. These results highlight the importance of the mevalonate pathway for maintaining mitochondrial electron transfer and biosynthetic activity in cancer cells exposed to metabolic stress.
The role of metabolism in metastasis formation

Sarah-Maria Fendt

† VIB-KU Leuven Center for Cancer Biology, Leuven, Belgium, BELGIUM

Metabolic rewiring is a hallmark of cancer cells. However, how nutrients drive the ability of cancer cells to rewire their metabolism is poorly defined. We are investigating the in vivo nutrient metabolism during metastasis formation to mechanistically understand how nutrients from the microenvironment enable cancers to progress from a local to a systemic disease. Using 13C tracer infusions in mouse models we find that nutrient availability shapes the metabolism and phenotype of cells and subsequently promotes the progression of cancer. Consequently, interfering with nutrient metabolism emerges as a promising therapeutic strategy against cancer. Taken together, our research highlights that nutrient metabolism is an important driver of cancer progression.
KEYNOTE LECTURE

Exploiting senescence for the treatment of cancer

Rene Bernards

Netherlands Cancer Institute, Amsterdam, NETHERLANDS

Conventional cancer therapy consists of using drugs at a maximum tolerated dose, which in advanced disease almost invariably leads to resistance. Synthetic lethal drug combinations can help delay onset of resistance, but a drawback from this approach is that toxicity of a given synthetic lethal drug pair can be prohibitive in reaching an effective dose. We have therefore focussed on a new iteration of synthetic lethality in which we give drugs sequentially rather than simultaneously, but retain the strong synergy between the two drugs. I will discuss how we can use sequential drug treatment to deliver a lethal “one-two punch” to cancer cells. In this scenario, we use the first drug to expose a major new vulnerability of the cancer cells that is subsequently targeted by the second drug. Senescence induction represents a very useful to induce a novel vulnerability for two reasons. First, senescence is a stable phenotype, which makes it possible to withdraw the first drug once senescence has been induced. Second, senescent cells have significant new vulnerabilities, both in terms of chromatin/transcription alterations and in terms of metabolomic alterations, which should be targetable. Examples of effective sequential drug treatments based on the induction of senescence in cancer followed by selective killing of senescent cells will be presented.
PROFFERED PAPER 2

Deciphering the intra-tumor and inter-patient signaling heterogeneity in cancer for the rational design of patient specific drug cocktails

Heba Alkhatib¹, Ariel M. Rubinstein¹, Swetha Vasudevan¹, Shira Stefansky¹, Amichay Meirovitz², Nataly Kravchenko-Balasha¹
¹ Bio-medical sciences department, Faculty of Dental Medicine, The Hebrew University of Jerusalem, Jerusalem, ISRAEL, ² Sharett Institute of Oncology, Hebrew University-Hadassah Medical Center, Jerusalem, ISRAEL

Cancer research is moving into the frontiers of how to assign the correct drug(s) to a given patient. We have recently demonstrated that using information-theoretic analysis of big proteomic datasets every malignancy can be mapped according to the protein network reorganization in each tumor, namely the patient-specific signaling signature (PaSSS). This PaSSSs can be utilized to rationally design personalized drug combinations. We have recently validated the approach in-vivo in several cancer types. For example we have demonstrated that in Triple Negative Breast Cancer (TNBC), for which not a single targeted therapy has been approved for the treatment, PaSSS-based targeted drug combinations work more efficiently than the clinically prescribed therapies. Moreover our predictions are highly selective: the predicted and very efficient combination for one TNBC is significantly less efficient for another TNBC and vice versa.

Lately we took a step forward and extended the approach to the single cell analysis. The single cell strategy resolves the intra-tumor molecular heterogeneity and allows to break down a tumor into distinct cellular subpopulations, and the altered protein networks associated with each subpopulation. We demonstrate how this strategy can be used in order to sensitize aggressive TNBC to radiotherapy (RT). Using mouse models and patient-derived TNBC tumors we show that two distinct subpopulations expand in response to RT. We demonstrate that simultaneous targeting of central proteins representing those subpopulations, Her2 and cMet, was essential in order to sensitize TNBC to RT and stop its growth. The presented strategy can be broadly applicable to clinical use.
Novel insights into the functional roles of the signaling pathway for necroptotic death

David Wallach², Seongmin Yoon², Ju Seong Jeon², Konstantin Bogdanov², Tae-Bong Kang¹
¹ Konkuk University, Chung-Ju , SOUTH KOREA, ² Weizmann Institute of Science, Rehovot, ISRAEL

Our knowledge of the physiological roles of programmed cell death lags far behind that of its mechanisms. We have detailed information, gained mainly in cells culture studies, of cellular components that cooperate in mediating programmed death. However, for several reasons, our current notions of the exact functional roles of the various death forms have remained largely speculative. Our state of knowledge of the signaling for necroptosis – a form of programmed death associated with rupture of the cellular membranes - is a good example for this deficiency. Our notions of the functional role of this set of signaling mechanisms are based, by and large, on just one set of data – records of inflammatory and tissue damaging processes in mice that occur consequently to knockout of the genes encoding either caspase-8 – an enzyme withholding the signaling for necroptosis, or of its adapter protein - FADD/MORT1. However, complete deficiency of either caspase-8 or FADD is unlikely to occur in real life. Moreover, each of the proteins in the signaling pathway for necroptosis also serves additional roles other than death induction. Potential clues towards clearer notions of the physiological roles of the necroptotic pathway can be gained from the fact that, unlike the case of apoptotic death that is exerted by cooperation of numerous molecules, necroptosis is mediated by a single effector protein – the pseudokinase MLKL. We still do not fully understand how does MLKL kill cells. Moreover, emerging knowledge indicates that, as in the case of the other signaling proteins in the pathway, cell death is just one of the functions that MLKL was destined to serve. Fathoming the mechanisms for the deadly function of MLKL and for the non-deadly ones will provide us with better tools for probing the exact functions served by the necroptotic pathway at different situations in vivo.
Uncovering the Therapeutic Potential of BH3-mimetics in Patient-relevant Pre-clinical Models of Mesothelioma

Xiao-Ming Sun¹, Gareth Miles¹, Ian Powley¹, Jonathan Bennett², Apostolos Nakas², Kelvin Cain¹, Marion MacFarlane¹

¹ MRC Toxicology Unit, Cambridge, UK, ² University Hospitals of Leicester NHS Trust, Leicester, UK

Malignant Pleural Mesothelioma (MPM) is an asbestos-related cancer; its clinical prognosis is poor - therefore, new approaches to target MPM are urgently required. Currently, routine drug discovery is carried out in 2D cell culture models. However, due to the stereo-complexity of the tumour microenvironment, the effect of drugs on tumour cells may be overlooked. Here we present a 3D drug efficacy evaluation platform, which employs freshly-resected tumour tissue cultured ex-vivo (Explants), where the contribution of the tumour microenvironment to drug efficacy can be properly evaluated. We have employed this platform to assess the ability of BH3-mimetics to promote apoptosis in live MPM tumours. Structure-based design of BH3-mimetics has produced compounds that can specifically inhibit individual members of the pro-survival BCL-2 family. Most mesothelioma cells express more than one member of the pro-survival BCL-2 family. Consequently, single agent treatment with BCL2/BCL-XL (ABT-737) or MCL-1 (S63845) inhibitor alone failed to induce apoptosis in mesothelioma cells, both in 2D (conventional) and 3D (Explant) culture systems. However, combinatorial treatment with ABT-737 and S63845 induced significant apoptotic cell death. Single-agent treatment had little effect on either mitochondrial ETC-driven oxygen consumption (OCR) or glycolysis. In contrast, the combination of ABT-737 and S63845 completely blocked uncoupler-stimulated OCR. These results recapitulate our previous findings, using either a metabolic switch (by inhibiting glycolysis with 2DG) or siRNA targeting to down-regulate MCL-1. Taken together, down-regulation of MCL-1, either by 2DG, siRNA or with a small molecule MCL-1 inhibitor permits ABT-737 to kill mesothelioma cells which are normally resistant to this agent. Our studies also show that molecular interactions centred on BH3-binding play a key role in mitochondrial bioenergetics. Since BH3-mimetics are currently being evaluated in the clinic, it is therefore important to delineate the role of pro-survival members of the BCL-2 family in regulation of respiratory chain function versus mitochondrial outer membrane permeabilisation and induction of cell death.
PROFFERED PAPER 3

FANCI Functions as a Repair/Apoptosis Switch in Response to DNA Crosslinks

Richa Shah¹, Jennifer Kernan¹, Anya van Hoogstraten¹, Yuanyuan Li³, Chelsea Chang¹, Ivy Mininger¹, Tony Huang², Agata Smogorzewska³, Ruth Thompson¹, Aneel Aggarwal¹, Samuel Sidi¹
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Defective DNA repair is a major driver of genomic instability and oncogenesis, events that are in turn efficiently countered by programmed cell death. Yet, surprisingly, DNA repair factors that can directly engage apoptosis in the event of repair failure have not been reported. Here we identify Fanconi Anemia (FA) Complementation Group I (FANCI) as such a “repair/apoptosis switch”, operating in the cellular response to DNA interstrand crosslinks (ICL). When facing such lesions, FANCI typically binds its partner in FA signaling, FANCD2, to direct ICL repair. We find that FANCI can also elect to interact with a pro-apoptotic factor, the death domain (DD) protein PIDD, in a manner that is mutually exclusive with FANCD2 binding by virtue of a shared binding site at the FANCI C-terminus. FANCI’s association with PIDD acts to enable ICL-induced, ATR-mediated phosphorylation of the PIDD DD, which triggers PIDDosome formation, caspase-2 activation and apoptotic cell death. Through a series of genetic and functional experiments in human cells and live zebrafish, we show that FANCI switches from repair to apoptotic signaling in the event of lack, failure or unavailability of DNA repair. Specifically, removing FANCP/SLX4 or other FA repair effectors, increasing ICL levels, or forcing damaged cells into mitosis (when FA repair is suppressed), all suffice to toggle the FANCI switch from the FANCD2 into the PIDD position. Finally, we identify FANCI monoubiquitination at K523 as a critical determinant of FANCI switch function, whereby a K523R FANCI mutant deficient in ICL repair engages PIDD in response to otherwise repairable levels of ICLs. These data identify a direct repair/apoptosis switch in vertebrate cells and point to a novel and highly efficient mechanism of genome maintenance.
Caspase-8 promotes progression of p53-proficient tumors

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Cytosolic caspase-8 is a well-known mediator of death receptor signaling and its suppression can drive necroptosis. While caspase-8 expression is lost in some tumors, it is sustained or increased in others, indicating a conditional pro-survival function of caspase-8 in cancer. Nuclear localization of caspase-8 has been sporadically reported in some cancers, however, the significance of this remains to be elucidated. Here we show that tumor cells harness caspase-8 activity in the nucleus to override the G2/M cell cycle checkpoint. Caspase-8 is upregulated and localized to the nucleus in multiple cancer types correlating with treatment resistance and poor outcome of patients. Nuclear caspase-8 fuels cancer progression by promoting mitosis of cells that are normally paralyzed and/or executed at the G2/M checkpoint in a p53-dependent manner. In the nucleus, caspase-8 cleaves and inactivates the ubiquitin-specific peptidase 28 (USP28) thereby preventing stabilization of p53. This event leads to de facto p53 protein loss, switching cell fate from G2/M arrest and apoptosis towards mitotic cell division. Our work identifies a non-apoptotic role for caspase-8 employed by cancer cells to overcome the p53-dependent G2/M checkpoint and provides a rationale targeting caspase-8 in p53 proficient cancers.
Cellular senescence as a driver of multiple human diseases

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A major advance in the field of ageing research has been the demonstration that senescent cells play a key role in aging and, even more importantly, the discovery of small pharmacological compounds that can kill senescent cells within the organism resulting in improved health. Upon tissue damage or stress, a substantial fraction of cells respond by adopting a cellular state known as “senescence”. Regardless of their initial cell identity, senescent cells share key properties; namely, global chromatin remodelling, robust proliferation blockade, and a massive pro-inflammatory secretome. The initial biological purpose of senescent cells is to orchestrate tissue repair, ultimately leading to their own disposal by the immune system and to their replacement by new, functional cells. This is the favorable, beneficial, face of cellular senescence. However, in certain contexts that are generally associated with chronic damage, degenerative processes, or organismal ageing, tissue repair is inefficient and senescent cells are not cleared. Indeed, senescent cells accumulate in many human pathologies including various fibrotic diseases, atherosclerosis, and neurodegenerative diseases. This is the detrimental, pathological, face of cellular senescence. Importantly, the last few years have witnessed the identification of small compounds that preferentially kill senescent cells, termed senolytic drugs. Such senolytic treatments in mice show an unprecedented therapeutic effect on the aforementioned diseases including lung fibrosis, atherosclerosis, and neurodegenerative diseases. I will present our contributions to the understanding of cellular senescence both in tissue repair and in pathological contexts.
Modulating senescence-associated phenotypes to prevent side effects of cancer therapy

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Most used anti-cancer therapeutic approaches are based on impairing mitosis and targeting highly proliferative cells. The non-specificity of these interventions often leads to transient and chronic side effects, and a general accelerated aging phenotype.

Cellular senescence - a complex stress response whereby cells lose irreversibly their capacity to proliferate – is potent tumor suppressive mechanism and a desired outcome of anti-cancer therapies.

Our data show that chemotherapy agents induce senescent cells in both mice and humans. However, chemotherapy-induced senescent cells develop a strong secretory phenotype (SASP) and contribute to chemotoxicity in mice including bone marrow suppression, cardiac decline, decreased physical activity and cancer relapse. Interestingly, chemotherapy treatment combined to genetic or pharmacological senolysis delays or prevents the onset of several pathologies.

We also show that similar to genotoxic chemotherapy, inhibitors of Cyclin-Dependent Kinases (CDK)-4/6 induce a p53-mediated senescence program. Interestingly, using RNAseq, cytokine arrays and in-tissue analyses we demonstrate that CDKi-induced senescent cells are characterized by a strongly reduced SASP. CDKi-induced senescent cells fail to promote paracrine detrimental effects, and do not lead to adverse effects in mice. Moreover, transient treatment with CDK4/6i in combination with chemotherapy is sufficient to reduce SASP and improve healthspan.

Together, our data suggest that modulating the senescence response is a potent strategy to limit the toxicity of ant-cancer treatments. This effect can be achieved either by eliminating detrimental senescent cells or by designing drugs that can promote stable growth arrest without strong paracrine effects. Importantly, the phenotypical characterization of these different senescent cells could predict toxicity and side effects associated to standard oncological drugs.
PROFFERED PAPER 4

Targeting the S6 kinase, RSK4, prevents chemoresistance and metastasis in lung cancer

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Lung cancers are mostly incurable due to early development of drug resistance and metastatic dissemination. Hence, novel therapies that tackle these two processes are urgently needed to improve clinical outcome. We have identified the S6 kinase, RSK4, as a promoter of drug resistance and metastasis in lung cancer. RSK4 expression was undetectable in normal lung but overexpressed in ~60% of lung cancers correlating with poor survival of adenocarcinoma patients. Silencing this kinase sensitises to therapy and hinders invasion in vitro and in vivo, while its overexpression has the opposite effect. Silencing RSK4 is associated with inhibition of NFkB activity through p300 activation. This is accompanied with decreased transcription of anti-apoptotic proteins such as BCL2, cIAP1 and cIAP2 and reversal of epithelial-mesenchymal transition. Lung cancer cells knocked-out for RSK4 showed delay in tumour formation, sensitisation to chemotherapy and decreased ability to colonise the lungs of immunosuppressed mice. To identify small-molecule compounds that selectively target RSK4, we performed a drug screen that revealed several floxacin antibiotics as potent RSK4 activation inhibitors. In particular, trovafloxacin reproduces all effects of RSK4 silencing in vitro and in vivo, both in xenograft models and in KRASV12G/p53⁻/⁻ GEMMs. Following determination of the crystal structure of RSK4 kinase domain, in silico docking, deuterium exchange mass-spectrometry and Markov transient analysis, we identify the binding site of trovafloxacins on RSK4 and propose a mechanism for the action of this compound. Finally, we show that patients undergoing chemotherapy and adhering to prophylactic floxacin in the large placebo-controlled randomised phase3 SIGNIFICANT Trial had significantly increased long-term overall survival. Hence, we suggest that RSK4 inhibition represents a novel therapeutic strategy for treating lung cancer.
The immunotargeting of the xCT cystine/glutamate antiporter targets cancer stem cells and potentiates the efficacy of Her2-targeted immunotherapies in breast cancer

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Despite marked advancements in its treatment, breast cancer is still the second leading cause of cancer death in women, due to relapses and metastases. Therefore, the development of combination therapies able to target key cancer-inducing or cell-sustaining pathways is needed. We have previously demonstrated that the cystine-glutamate antiporter xCT, which is overexpressed in mammary cancer stem cells (CSCs) and plays a key role in the maintenance of their redox balance, self-renewal, and resistance to ferroptosis and chemotherapy, is a promising target for breast cancer immunotherapy. In this study we developed a combined immunotherapy targeting Her2 and xCT in breast cancer using the Bovine Herpes virus (BoHV)-4 vector, a safe vaccine that can transduce cells in vivo and confer immunogenicity to tumor antigens.

Two BoHV-4 vaccines were developed: BoHV-4-RHuT, coding for a chimeric rat/human form of Her2, and BoHV-4-xCT, coding for mouse xCT. Transgenic BALB-neuT mice - which spontaneously develop rat Her2⁺ mammary tumors - were immunized six times with the single or combined vaccines, and the induced immune response and its effects on tumor growth, lung metastases and CSCs were analyzed.

Vaccination with BoHV-4-RHuT significantly slowed down mammary tumor growth, and its combination with BoHV-4-xCT significantly decreased lung metastases. This antitumor activity was mediated by the induction of cytotoxic T cells and of specific anti-Her2 and xCT antibodies that induce antibody-dependent cell cytolysis, hinder cancer cell proliferation and directly inhibit CSCs by inducing their ferroptosis.

Our findings demonstrate the effectiveness of the combination of Her2 and xCT immunotargeting in hampering breast cancer progression and metastasis, opening new perspectives in the management of Her2⁺ breast cancer.
Targeting Heterogeneity in Senescence against Aging and Cancer

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Aging and cancer are closely connected. Identifying processes that underly both might thus have broad benefits to society. As we age, our cells accumulate damage, which can eventually cause them to become “senescent”. Senescent cells cease to divide, but chronically secrete a wide range of factors that permanently alter their environment. As such, they are thought to impair tissue function and accelerate age-related diseases. In addition, they can also promote cancer progression, migration and therapy resistance by permanently enforcing a state we called “stem-lock”. Senescent cells and senescence-like cancer cells pose exciting candidates for therapeutic removal.

Today, I will discuss how we focused on the identification of different subtypes of senescence and how we think these play different roles in disease development. More specifically, I will highlight interaction between the damage-associated proteins FOXO4 and p53 as a pivot in senescent cell viability. Inhibition of FOXO4, or interference with its interaction with p53 using cell penetrating peptides could selectively eliminate senescent cells and target signs of aging in vivo. We further developed these compounds in an attempt to make clinical translation feasible.

As cancer cells that survived chemotherapy may develop a senescence-like response, we tested these improved compounds in models of therapy resistance. We found the FOXO4-p53 inhibitors to selectively eliminate therapy-resistant, but not primary, cancer cells in a broad sense. Together, this shows that, a) it may be possible to therapeutically restore healthspan once it has already declined and b) that therapy resistance in cancer may be overcome with the right anti-senescence drugs. It will now be crucial to dissect senescence heterogeneity and identify molecular mechanisms that dictate sensitivity or resistance. Our current research is focused on these mechanisms and preparing the translation of our FOXO4-p53 drugs to clinical trials.
Partners in crime: MYC, RAS and cell-extrinsic mechanisms of oncogenic cooperation

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MYC and RAS represent a well-established paradigm for oncogenic cooperation yet the mechanistic basis for this cooperation has remained elusive. Early results from overexpression studies in vitro strongly supported complementary roles for MYC and RAS: activated RAS can suppress MYC-induced apoptosis while MYC overexpression overcomes RAS-induced senescence. End of story, no? Well, not quite. For starters, MYC and RAS cooperate even when they’re expressed at levels that fail to strongly induce apoptosis or senescence, so mutual inhibition of cell-intrinsic tumour suppressive responses doesn’t fully explain their combined potency. Moreover, there is now overwhelming evidence that MYC is a downstream effector of the RAS pathway: KRAS activation increases MYC transcription, translation and protein stability, and genetic studies have shown that MYC is required for KRAS-driven tumourigenesis in multiple tissues. So, wherefore cooperation? Using endogenously expressed activated KRAS combined with modestly deregulated MYC, we have sought to re-examine the relationship between MYC and RAS to better understand their combined potency in vivo and in vitro. We have found an unexpected convergence on the regulation of immune visibility of developing cancers, distinct from PD-L1 expression, that complements tumour cell-autonomous mechanisms of oncogenic transformation and participates in the maintenance of established tumours. Our data suggest new opportunities for reversing immune evasion and enhancing anti-tumour immunity.
Post-senescence: a matter of life and death

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Cellular senescence is a terminal cell-cycle arrest program evoked by various stresses including activated oncogenes and DNA-damaging anti-cancer agents. Work including ours of the recent years has clarified that senescence is not a static endpoint but rather a dynamic state switch which requires active upstream signaling to be maintained; in turn, loss of senescence-essential gene capacities may permit cell-cycle re-entry out of a full-featured, deeply arrested senescence condition. Importantly, senescence-associated reprogramming into a latent stemness program will exert its tumor-promoting aggressivity upon senescence escape. At the meeting, we will present data that position this biological principle as an alternative mechanism to apoptotic insensitivity in clinical treatment failure, and discuss strategies to overcome its detrimental implications.
Novel opportunities for a biologic optimization of radiotherapy

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More than 50% of all cancer patients receive radiotherapy (RT) during the course of their disease, either alone or in multimodal treatment approaches. Yet despite technical innovations cure-rates remain unsatisfactory for common cancer types with common disease progression and high loco-regional failure-rates such as lung cancer or head and neck cancer highlighting the need for further improvements in RT practice. Success of radiotherapy is limited by intrinsic, acquired or microenvironment-mediated cancer cell radiation resistance as well as by adverse effects in radiosensitive normal tissues located in the radiation field. Therefore, there is high interest in the development of effective strategies for a biological optimization of RT by modulating critical biological processes that determine the radiation response of tumors and normal tissues for improving patient outcome.

The presentation will highlight findings obtained in a German consortium that combines efforts from radiobiology, radiation oncology, pathology, bioinformatics, and systems biology (ZiSStrans) for the identification of signaling networks and critical network regulators with impact on radiation sensitivity of cancer cells and normal tissues. that might be suited as biomarkers or molecular targets in order to improve the therapeutic ratio. The presentation will also discuss the potential use of identified signaling networks and their regulators as biomarkers of radioresistance or therapeutic targets for enhancing tumor radiosensitivity, protecting co-irradiated normal tissues, or both, and thereby improving the therapeutic gain of RT in future combinatorial approaches.

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PROFFERED PAPER 6

Mobilization of Monocytic Myeloid-Derived Suppressor Cells (MDSC) Is Regulated by Osteoblast Activation by PTH1R

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-- The abstract has been withheld at the request of the author --
Ferroptosis: mechanisms and therapeutic potential

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In my lab, we reported several years ago that several compounds induce a distinct form of iron-dependent oxidative cell death that we refer to as ferroptosis. We found that ferroptosis is distinct from apoptosis, necroptosis and classic necrosis on the basis of morphological, biochemical, genetic and inhibitor sensitivity data. In addition, we have elucidated mechanisms regulating ferroptosis, and examined the relevance of ferroptosis to a variety of diseases and therapeutic applications. We discovered that ferroptosis represents death by peroxidation of polyunsaturated fatty acid moieties in phospholipids, due to loss of activity of the lipid repair enzyme GPX4 and depletion of CoQ10, which can occur in a number of different ways. Most recently, we explored the cellular membranes that undergo peroxidation during ferroptosis using novel imaging tools, and discovered specific organelle membranes that are dispensable for ferroptosis. I will discuss recent mechanistic insights into how ferroptosis is regulated and executed, and new therapeutic strategies for triggering ferroptosis in numerous cancers.
KEYNOTE LECTURE

Radiotherapy to convert the tumor into an in situ vaccine

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Radiotherapy has revealed an ideal adjuvant to cancer immunotherapy, because of its ability to convert the irradiated tumor into an individualized, in situ vaccine. Radiation-induced DNA damage response (DDR) is sensed by the innate immune system and can contribute to immune rejection of tumors. When successful at immunizing, radiotherapy evokes T cell memory, and induces effects outside the treated field, defined as abscopal effects (responses at a distant, synchronous, un-irradiated established tumor or metastasis). In the clinical setting, however, abscopal effects are extremely rare, because of immune-suppressive characteristic of established solid tumors. Thus, strategies to exploit the pro-immunogenic effects of radiotherapy require combination with immunotherapy: preclinical metastatic cancer models successfully testing the combination of local radiotherapy and immune checkpoint blockade (ICB) have matured to clinical translation. Recently, preclinical and clinical evidence has emerged to define optimal radiation protocols to be used during immunotherapy. Specifically, the issue of radiation dose and fractionation seems to be particularly relevant to the success of abscopal responses (responses at a distant, synchronous, un-irradiated established tumor or metastasis). For instance, the role of Trex1 exonuclease has been elucidated in the dose-dependency of abscopal response (Nature Communications 2017; Jun 9;8: 15618), confirmed by clinical results in metastatic cancers. Moreover, recent evidence has demonstrated how as part of DNA damage response, tumor mutations can originate neoantigens that contribute to sustained immune responses in patients (Nature Medicine, 2018; 24;12:1845).
Maternal immunization against ALK: a new weapon against neuroblastoma

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Neuroblastoma (NB) is the most common extracranial solid tumor in infancy. Some germline and somatic mutations associated with NB have been identified as activating mutations of the anaplastic lymphoma kinase (ALK) oncogene. Because of the nature of NB, which can occur in the early post-natal life or even during fetal life, we evaluated the efficacy of a DNA vaccine against ALK in a spontaneous preclinical model of NB, harboring ALKF1174L mutation in association with MYCN amplification (ALKF1174L/MYCN mice) by means of maternal immunization (MI).

Pre-birth immunization against ALK leads to extended survival time and to a lower tumor growth kinetic in ALKF1174L/MYCN offspring born from ALK-ECTM-vaccinated mothers (ALK-ECTM offspring) as compared to controls born from control empty vector vaccinated mothers. Maternally derived anti-ALK antibodies were successfully transferred from mothers to newborns. Moreover, anti-ALK IgM were found in the sera of five- and six-week-old ALK-ECTM offspring, suggesting the induction of the pups’ own immune response against ALK. This effect could be due to the breast milk-mediated transfer of immune-complexes containing ALK, found in the milk of vaccinated mothers and in their offspring sera. Finally, MI against ALK induces a decrease in ALK expression in ALK-ECTM offspring’ tumor tissue.

Overall, these results indicate that MI against ALK induces an active immunization against this oncoantigen in the offspring, impairing tumor development and enhancing survival time in a preclinical model of NB.
2 – Poster Spotlight

NIPP1 controls cellular senescence in murine skin

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NIPP1, for Nuclear Inhibitor of Protein Phosphatase 1 (PP1), belongs to the large group of regulatory interactors of PP1 (RIPPOs) and is essential for cell proliferation and lineage specification. NIPP1 acts as a scaffold protein that targets a subset of phosphoproteins for regulated dephosphorylation by associated protein phosphatase 1 (PP1). We have recently found that adult mice lacking epidermal NIPP1 develop a stress response that results in chronic chemokine-driven skin inflammation and global body-hair loss. Here, we have subjected control (CTR) and skin specific knockout (SKO) mice to two-stage chemical skin carcinogenesis, using consecutively the tumor-initiating mutagen 7,12-demethylbenz[a]anthracene (DMBA) and the tumor-promoting phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA). Despite the epidermal hyperplastic phenotype, we found that SKO mice were nearly completely resistant to the DMBA/TPA-induced skin carcinogenesis. The resistance was associated with a reduced number of oncogenic Hras mutations, an increased DNA damage response and an increased expression of two rate-limiting DNA repair factors, namely the XPD helicase and the XPG endonuclease. Intriguingly, a comparative transcriptome analysis (RNA-seq) on the tail epidermis of CTR and SKO mice revealed a strong induction of genes that are linked to senescence in SKOs. These include genes encoding a host of senescence-associated secretory phenotype (SASP) factors, the epithelial cytokine thymic stromal lymphopoietin (Tslp), and the Cdkn2a transcripts p16Ink4a and p19ARF. ELISA also showed an increased expression of TSLP in serum of SKO mice. qRT-PCR confirmed increased levels of Tslp and Cdkn2a transcripts in NIPP1-deficient keratinocytes as well as a further increased expression of Cdkn2a in the epidermis of SKO mice after 4h of DMBA treatment. Taken together, these data suggest that a cellular senescence program is activated in SKO mice, which could explain the DMBA/TPA-induced carcinogenesis resistance. Currently, we are searching for the trigger that provokes senescence after epidermal loss of NIPP1.
3 – Poster Spotlight

**Metabolic targeting of Pancreatic Ductal Adenocarcinoma**

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Pancreatic Ductal Adenocarcinoma (PDAC) is one of the deadliest malignancies in the world and novel therapies are desperately needed. Even though there have been progress in understanding the disease and diagnosis methods and treatment options in the last decades, 5-year survival rate of PDAC, which is around 8%, has not changed. PDAC has two molecular subtypes, Basal-like (Quasi-Mesenchymal, QM) and classical (progenitor-like) which use different metabolic strategies for progression and survival in poorly oxygenated and nutrient deprived tumor microenvironment. In this work, we investigate metabolic vulnerabilities of PDAC subtypes and evaluate PDAC susceptibility to metabolic targeting. To investigate their metabolism, we have used established PDAC cell lines, Patient Derived Xenografts, primary cells and patient samples and applied gene and protein expression analysis (RNA-seq, microarray and multiplex immunofluorescence, respectively), targeted and non-targeted metabolomics (UPLC-MS/MS and MALDI) and functional metabolic analysis (Seahorse). Our findings indicate the QM subtype is branded with hypoxic features, extensively dependent on glucose metabolism and uses even glycolysis-derived lactate as a mitochondrial fuel. Differently, classical subtype is rich in different lipid species and actively uses Fatty Acid in mitochondrial oxidation. After challenging this subtype specific metabolism via genome editing and pharmacological approaches, we aim to suggest an effective, subtype-specific, patient tailored metabolic targeting for clinical trials.
The receptor tyrosine kinase Tyro3 protects human cancer cells from apoptosis

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The TAM subfamily (Tyro3, Axl, Mer) of receptor tyrosine kinases are implicated in a variety of cancers as primary tumour promoters or mediators of tumour chemoresistance. In particular, little is known about the potential oncogenic role of Tyro3, including its activation mechanisms and downstream signalling in cancer cells. Addition of the TAM ligand protein S (ProS1) to human head and neck cancer (SCC-25) and bladder cancer (MGH-U3) cells caused activation of Tyro3 and downstream Erk kinase through increased phosphorylation in western blot. ProS1-Tyro3 also supported cell survival upon serum-starvation or staurosporine-induced acute apoptosis in MGH-U3 cells as detected by MTS assay. Flow cytometry experiments revealed that siRNA knockdown of Tyro3 in MGH-U3 cells caused an increased proportion of cells undergoing apoptosis (29%) versus control siRNA (6%), as well as exacerbating staurosporine-induced apoptosis (Tyro3 knockdown 82%; control siRNA 56%). In addition, presence of exogenous ProS1 inhibited staurosporine-induced apoptosis in cells, further demonstrating the anti-apoptotic function of the ProS1-Tyro3 axis. The results of this study demonstrate that Tyro3 is linked to cancer cell survival both in ligand-dependent and independent manners. These new insights should enable a more precise and effective approach to therapeutic targeting of TAM signalling in cancers where they play major roles in growth, progression or chemoresistance.
Combining dose of methylglyoxal altering doxorubicin MDR cancer cell lines resistance: genetics & therapeutic in vitro studies

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Methylglyoxal (MGO) is a highly reactive α-ketoaldehyde that is an intermediate product of glycolysis, fatty acids metabolism and amino acids breakdown. It is a cytotoxic substance inducing protein modification oxidative stress and apoptosis through production of advanced glycation end products (AGEs), inducing oxidative DNA damage and the production of Reactive Oxygen Species (ROS) and alter signalling pathways promoting cell death. The overexpression of glyoxalase I in the cancer cells is suggested to confer these cells multidrug resistance property. Thus, glyoxalase I is believed to be the major cellular defence against MGO and AGEs cytotoxicity. Furthermore, it has been found that glyoxalase I contributes to the human leukemia cells resistance to antitumor agent induced apoptosis. These findings might lead to further development of an anticancer drug through targeting glyoxalase I system and a personalized chemotherapy. The use of MGO in high doses has been used as a potential therapy that leads to growth inhibition and cytotoxicity to tumors. The normal cellular concentration of MGO is 1-2 µM. The reported MGO cytotoxicity to tumor cells is as low as 10 µM. It has anti-proliferative function but on the other hand, it has been demonstrated to promote vascular smooth muscle cells proliferation. In a recent study, treating cancer cell lines with a combinational doses of doxorubicin and MGO resulted in a marked decrease in cell viability compared with doxorubicin or MGO alone. Synergistic effect of MGO to the anticancer treatments as MGO inhibits the doxorubicin efflux from the cancer cell lines and triggers ROS mediated programmed cell death. In this project, we have investigated the cellular death activity of treating the resistant cancer cell lines by combinational doses of DOX-MGO. In addition, we are investigating the genetic changes of the resistant cell lines after the exposure to the drugs treatments of DOX-MGO.
Rational therapeutic combinations with BH3 mimetics as new treatments for rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood and adolescence, accounting for 7% of pediatric cancers. Patients with refractory/relapsed RMS have a bad prognosis, combined with a lack of specific biomarkers that difficult the development of new therapies to treat high risk RMS patients.

When exposed to the right therapy, cancer cells die by apoptotic programmed cell death which is regulated by the BCL-2 family of proteins comprising effectors, activators, sensitizers and anti-apoptotic proteins. In response to anticancer treatments, cancer cells often present anti-apoptotic adaptations to ensure survival, like upregulating anti-apoptotic BCL-2 family proteins. To avoid cancer resistance to therapy, BH3 mimetics -small molecules that inhibit anti-apoptotic proteins- are being developed, and some approved for the clinic, to overcome the apoptotic blockade observed on resistant cells.

In this regard, dynamic BH3 Profiling (DBP), a functional predictive biomarker that measures net changes in mitochondrial apoptotic signaling (or increase in priming), predicts cell death days/weeks in advance and has already been successfully validated in vitro, in vivo and on patient samples. Furthermore, DBP can also detect anti-apoptotic adaptations upon treatment, guiding the use of BH3 mimetics to defeat resistance and avoid relapse.

By performing DBP in vitro, we were able to identify which treatments caused a higher increase in apoptotic priming after 16 hours, thus the most effective for RMS. These predictions were further confirmed by cell death analysis at 96 hours, concluding that DBP is a good assay to predict cytotoxicity in this disease, assessed by ROC curve analyses. Furthermore, we could observe a correlation between anti-apoptotic adaptations to treatments predicted with DBP and protein changes analyzed by western blot. Interestingly, based on these predicted adaptations, we described synergistic combinations with BH3 mimetics in vitro and we validated this strategy in vivo using a PDX model of RMS.
The impact of HDAC8 on neuroblastoma differentiation and treatment resistance

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Background: High histone deacetylase (HDAC) 8 expression levels have been identified as predictors of poor outcomes in neuroblastoma, the most common extracranial solid tumor in childhood. When neuroblastoma cell lines are depleted from HDAC8, these cells show neurite-like outgrowths, a sign of neural differentiation. However, this differentiation phenotype might also be linked with increased resistance towards anti-cancer treatment. We aim to elucidate the resistant phenotype of HDAC8i treatment surviving neuroblastoma cells. With this, we aim to identify novel vulnerabilities to induce apoptotic cell death in these resistant cells.

Methods: Treatment efficacy is detected through automated counting of viable (% viability) and dead (trypan blue positive) cells. A transcription factor profile is generated through gene expression analysis and validated by realtime PCR. In addition to the well-established HDAC8 inhibitor (HDAC8i) PCI-34051, we are testing novel HDAC8i with improved in vivo stability.

Results: The viable cell number substantially decreased upon treatment of BE(2)-C cells with HDAC8i for 6 days. The treatment surviving cells were resistant against repeated HDAC8i treatment. Gene expression analysis revealed a transcription factor profile linked to differentiation, senescence and apoptosis inhibition via deregulation of members of the BCL-2 family and the PI3K pathway. Cell viability assays demonstrated that the treatment of BE(2)-C cells with HDAC8i for 6 days and subsequent treatment with BCL2i, pan-BCLi or PI3Ki resulted in cell death with signs of apoptosis.

Conclusion/Outlook: This study explores the HDAC8i induced differentiation phenotype and its role in treatment resistance. The identification of novel vulnerabilities, such as PI3K and BCL2 allows the induction of cell death via the combination of HDAC8i in aggressive neuroblastoma cells cell. Further steps include the in vivo validation of the combination, the detailed exploration of the type of programmed cell death and the role of predicted HDAC8 substrates, such as SMC3 in this mechanism.
A novel isoform of Bcl-2 in MOLM-13 is selectively inhibited by doxorubicin resulting in cell apoptosis

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Upregulation of anti-apoptotic proteins and the modulation of autophagy in leukaemic cells often contribute to drug resistance. Doxorubicin (Dox) is used as part of the induction therapy in the treatment of several types of leukaemia. We evaluated the potential of Dox to modulate autophagy and apoptotic cell death in leukaemic and non-leukaemic cells.

Cell proliferation (using 5(6)-carboxyfluorescein diacetate succinimidyl ester) and death (using annexin V/propidium iodide) were studied using flow cytometric analyses after co-incubation with cells. Protein expression of Bcl-2 and Beclin 1 were quantified in treated and untreated cells using Western blot analysis.

Dox is a known topoisomerase inhibitor, and this effect was confirmed in our study as the drug potently inhibited cell division of MOLM-13 cells over a three-day period. Dox also caused a decrease in Beclin 1 protein levels in MOLM-13 cells (p < 0.05) without significantly (p > 0.05) affecting the protein levels in non-leukaemic monocytes in 48 h. Dox showed a greater ability to induce cell death in MOLM-13 cells than in non-leukaemic monocytes (p < 0.05). When compared to OCI-AML2, CML K562 and the non-leukaemic monocytes, it was found that all the cells expressed the ‘usual’ isoform of Bcl-2 (p26-Bcl-2-α). Interestingly, an additional and a novel Bcl-2 protein (p15-Bcl-2) was expressed in MOLM-13 (but was absent in the other cells tested) and this isoform was significantly inhibited by Dox (0.5, 0.75 and 1 µM; p < 0.01) without any significant effect on the p26-Bcl-2-α. It is not conclusive that the selective inhibition of p15-Bcl-2 isoform in MOLM-13 led to its selective cell death induction (compared to non-leukaemic cells) but it is worth further investigations. The reduction in Beclin 1 could indicate a modulation of autophagy in MOLM-13 cells but it is not clear whether this confers a protective or cell-death inducing effect.
Activation of Keap1/Nrf2 pathway increases glutathione levels and limits DHA- and ionizing radiation-induced cytotoxicity

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Dihydroartemisinin (DHA), a reactive oxygen species (ROS)-producing anti-malaria drug, shows promises in cancer treatment. Similarly, ionizing radiation exerts cytotoxicity through ROS production. Unfortunately, many cancer cells are resistant to radiotherapy due to improved anti-oxidative capacity. Here, we analyzed the cellular response of HCT-116 colon cancer and NCI-H460 lung adenocarcinoma cells to DHA (6.25-50 µM) and addressed the question whether radiotherapy could be improved by boosting ROS production with DHA. DHA induced cell death dose-dependently, but in HCT-116 cells at lower concentrations compared to NCI-H460 cells. Mitochondrial ROS production was dramatically increased 24h after treatment with DHA. Furthermore, iron-dependent lipid peroxidation and protein thiol oxidation were detected in response to DHA, suggesting ferroptosis induction in both cell lines. Treatment with DHA after irradiation decreased clonogenic capacity, as measured by colony formation assay, but treatment with DHA before irradiation was less effective. Gene expression and protein analysis show that the glutamate/cysteine antiporter SLC7A11 and the transsulfuration pathway controlling glutathione synthesis were upregulated in response to DHA to a greater extent in HCT-116 cells than in NCI-H460 cells. Similarly, glutathione levels were increased significantly in HCT-116 cells but only slightly in NCI-H460 cells. Our data suggest DHA-induced activation of the Keap1/Nrf2 pathway in HCT-116 cells and increased glutathione production which is under transcriptional control of the transcription factor Nrf2. In NCI-H460 cells, however, Keap1/Nrf2 pathway is constantly activated due to a mutation of Keap1. Our colony formation assays showed that pharmacological inhibition of Nrf2 improved sensitivity to DHA- and radiation-based therapy. Taken together, our data indicate that the protective Keap1/Nrf2 pathway can be permanently upregulated due to a mutation or acutely upregulated in response to DHA. Thus, Nrf2 inhibition can overcome endogenous as well as DHA-induced resistance mechanism and further improve DHA- and radiation-induced cytotoxicity.
A new application for anti-emetic drugs in cancer therapy: synergistic cytotoxicity of 5-HT$_3$ receptor antagonists and paclitaxel via microtubule and calcium-driven apoptosis

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The 5-HT$_3$ class of serotonin receptor antagonists was introduced into the clinic in the early 1990s, and was a valuable advance in combatting chemotherapy-induced emesis. The 5-HT$_3$ antagonists are rapidly absorbed and penetrate the blood-brain barrier. They are well-tolerated over a wide range of doses, with the most commonly linked side effects being headache and constipation, although QT prolongation was found with ondansetron at higher doses. These antagonists are also useful in a number of other fields, including schizophrenia and gastroenteritis.

We examined the in vitro effect of tropisetron and ondansetron on several cancer and normal cell lines, and found a selective cytotoxic effect for cancer cells, including melanoma, breast and colon. Additionally, the cytotoxicity was synergistic with paclitaxel.

We next studied the cell death mechanisms of the single agents and combinations. Ondansetron and tropisetron bound to the colchicine binding site of tubulin, preventing tubulin polymerization and causing a strong upregulation of calcium within the cell as early as 6 hours. There was no block in the cell cycle. This led to increased DNA fragmentation, caspase-3 activation, phosphatidylserine exposure, mitochondrial permeabilisation and nuclear condensation and fragmentation. In each case, these characteristics were more frequent in cells treated with a combination of paclitaxel and a 5-HT$_3$ antagonist. ERK activation was upregulated and NFkB downregulated by 5-HT$_3$ antagonists.

In conclusion, it is possible that 5-HT$_3$ antagonists, given to cancer patients as anti-emetics, may additionally increase the efficacy of cytotoxic treatments. We have shown strong synergism with one chemotherapeutic drug, paclitaxel. The 5-HT$_3$ antagonists may act by increasing levels of apoptosis via microtubule binding. Further studies will investigate whether this synergism can be replicated in in vivo settings at doses well-tolerated in patients.
Non-invasive bioluminescence imaging elucidates the role of ERK1/2 kinase in sustenance of autophagic flux during acquirement of chemoresistance

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Several kinases including ERK1/2 regulate autophagic flux, which plays critical role towards acquirement of chemoresistance in cancer cells. However, the capacity of autophagy in governing the dynamic development of chemoresistance is poorly understood. Such knowledge is critical to develop autophagy directed cancer therapy which also requires an autophagy specific reporter sensor for drug screening in pre-clinical mouse model. Here we elucidate the role of ERK1/2 in regulation of autophagic flux during acquirement of chemoresistance in ovarian cancer cells with a novel in-vivo autophagy sensor.

Higher LC3I-II turnover and p62 degradation post cisplatin-paclitaxel treatment, was specifically observed in early resistant cells (onset of resistance, ER) compared to sensitive or late resistant cells (extreme resistance, LR). Increased number of autophagic bodies and LC3\textsuperscript{+ve}/LAMP1\textsuperscript{+ve} puncta were observed in ER cells. These cells were also characterized by increased ERK1/2 activation, inhibition of which reduced autophagic flux by impeding autophagosome-lysosome fusion.

We designed a p62 (mtFL-p62) based autophagy sensor using a mutant thermostable firefly luciferase. Live cell monitoring of mtFL-p62 activity in drug treated ER cells showed reduced luminescence (0.32 fold), indicating an upregulated autophagic flux while increased luminescence in sensitive and LR (4.72 and 1.7 fold) cells post drug treatment signified low autophagic flux. Combinatorial treatment of cisplatin-paclitaxel with ERK1/2 inhibitor (U0126) or chloroquine in ER cells increased luminescence by 4.93 and 5.50 fold, respectively.

Non-invasive optical imaging of mice bearing tumour xenografts expressing mtFL-p62 exhibited gradual reduction in luminescence (0.45 fold) till 10 days by platinum-taxol treatment, while combinatorial treatment of U0126/chloroquine with cisplatin-paclitaxel significantly increased luminescence (2.2 and 2.9 fold respectively) by day 10 indicating a blockade in autophagic flux.

Altogether our data suggests that hyper-activated ERK1/2 regulates autophagy at the onset of chemoresistance. The reported novel sensor can be utilized for in-vitro and in-vivo monitoring of autophagic flux.
AKRs are responsible for metastatic melanoma cells resistance to Ferroptosis execution

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Human skin melanoma is one of the most aggressive and difficult to treat human cancer with increasing incidence over the years. In fact, the known resistance to almost all therapeutic regimens makes this cancer a global public health problem. Recently, a new designed immunotherapy-based therapeutic regimen has been introduced with encouraging results. However, the appearance of heavy side-effects together with acquired resistance to therapy encourages the research of alternative therapeutic strategies. Ferroptosis induction and execution was evaluated in metastasis-derived wild-type and oncogenic BRAF melanoma cells, and the process responsible for the resistance has been dissected at molecular level. Although efficiently induced in all cells, in an oncogenic BRAF- and ER stress- independent way, most cells were resistant to ferroptosis execution. At molecular level we found that: resistant cells efficiently activate NRF2 which in turn upregulates the early ferroptotic marker CHAC1, in an ER stress-independent manner, and the aldo-keto reductases AKR1C1÷3 which degrades the 12/15-LOX-generated lipid peroxides thus resulting in ferroptotic cell death resistance. However, inhibiting AKRs activity/ expression completely resensitize resistant melanoma cells to ferroptosis execution. Finally, we found that the ferroptotic susceptibility associated to the differentiation of melanoma cells cannot be applied to metastatic-derived cells, due to the EMT-associated gene expression reprogramming process. However, we identified SCL7A11 as a valuable marker to predict the susceptibility of metastatic melanoma cells to ferroptosis. Our results identify the use of pro-ferroptotic drugs coupled to AKRs inhibitors as a new valuable strategy to efficiently kill human skin melanoma cells.
Redox regulation of cell death signaling

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Reactive oxygen species (ROS) have been shown to play an important role in cellular signaling. However, too high ROS levels can irreversibly damage proteins and cause cell death, so the generation and degradation of ROS is tightly regulated. Apart from glutathione and catalase, the thioredoxin system plays an important role in ROS scavenging and cellular redox homeostasis. In addition, various cancer entities were found to have high ROS levels due to increased proliferation and dysregulated metabolic processes. This suggests that cancer cells strongly rely on antioxidant enzymes to counteract this oxidative environment, making the ROS regulatory network a promising target for cancer therapy. Here, we aimed to further elucidate the role of ROS in signaling events involved in cell death induction and analyze their potential in cancer therapy. We could demonstrate that inhibition of the thioredoxin system increases ROS levels and induces cell death in acute lymphoplastic leukemia cells. This cell death was dependent on the BH3-only protein NOXA and led to activation of the pro-apoptotic BCL-2 protein BAK followed by caspase cleavage. On the other hand, thioredoxin reductase inhibition led to apoptosis-independent cell death in murine embryonic fibroblasts, identifying BAK as a potential target for oxidative modification. This oxidation seems to be critical for cell death induction upon oxidative stress in this system. Overall, our findings provide novel insights into the regulation of oxidative cell death and highlight the potential of redox active compounds in cancer therapy.
**Tumorigenesis and Treatment Response Using Gallbladder Organoid**

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Introduction: Gallbladder cancer (GBC) is a lethal cancer with a dismal 5-year survival below 10%. An existing GBC animal model drives to tumor formation after three months, but with complex genetic driven cancers, a fast, reliable, and flexible model is required.

Method: Gallbladder organoids were generated from adult Kras<sup>G12D</sup> and C57Bl6 mice. CRISPR-Cas9 and Retroviral based systems were used to introduce genetic manipulations. The modified organoids were transplanted subcutaneously to C57Bl6 or NSG mice. The pre-clinical benefits of nanoliposomal irinotecan (Nal-IRI) were investigated by implanted organoid subcutaneously to C57Bl6 mice, once the tumors reached 150 mm³, mice were randomly treated with vehicle, Irinotecan 50mg/Kg, or Nal-Iri 50mg/Kg. Tumor growth was monitored until it reached the end-point criteria. Liquid chromatography was used to measure CPT-11 and SN38 concentration.

Results: Activation of Kras<sup>G12D</sup> coupled with the loss of p53 led to tumor formation, and additional loss of Pten accelerated tumor progression, whereas activation of Kras<sup>G12D</sup> only was not sufficient enough to induce tumor formation. Expression of ERBB2 S310F and V777L induced tumor development within 30 days, but not from ERBB2 wild-type. Tumors from both models resembled histological hallmarks from human diseases with an abundant of the stromal compartment. Nal-IRI prolonged the median survival of tumor-bearing mice compared to irinotecan and vehicle. Nal-IRI treated tumors had higher bioavailability of CPT-11 and SN-38 (the active metabolite of irinotecan (CPT-11)) compared to irinotecan and vehicle-treated tumors. Nal-IRI treated tumors showed the accumulation of CPT-11 in tumor cells compared to stromal cells.

Conclusion: The histological similarities of organoid derived tumors resemble the character of the human counterpart makes GB organoid an appropriate and excellent pre-clinical model to study gallbladder tumorigenesis. The feasibility of growing the tumor in a fully intact environment allows studying the interaction between the tumor cell and microenvironment in tumorigenesis and treatment response.
Characterization of the pro-tumorigenic function of UV-induced senescence in melanoma

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Melanoma is the most aggressive type of skin cancer, and exposure to ultraviolet (UV) light, is one of the major causing risk factors for its initiation and progression. Cellular senescence is a state of irreversible growth arrest accompanied by the secretion of several pro-inflammatory and pro-growth factors, a phenotype known as the senescence-associated secretory phenotype (SASP), which can promote several age-related diseases.

Senescent cells accumulate in the skin during aging and upon genotoxic stress, including ionizing and non-ionizing radiation (UVB). Our data suggest that ionizing radiation-induced senescent cells can induce melanoma progression in vivo. However, not much is known about UVB-induced senescence, a phenomenon that seems to physiologically occur in humans and to be a potential driver of melanomagenesis. Within this context, we aimed to characterize the phenotype of UV-induced senescence skin cells in vitro and ex vivo. Using whole-transcriptome sequencing of different types of UVB-induced human skin cells, we show here that cells exhibit a diverse SASP expression, with fibroblasts and keratinocytes expressing higher levels in comparison to melanocytes. Our analysis revealed different pathways and factors that are shared or not among different cell types, some of which can play an important role for melanoma development.

We also show the induction of senescent cells in vivo by treatment of an inducible melanoma and senescence reporter mouse model, the BRaf/PTEN/p16-3MR mouse. We are currently studying if elimination of senescent cells is negatively correlated with induction and progression of melanoma. Taken together, this project will shed light into the relationship between environmentally-induced senescence (via UVB) and tumorigenesis in order to better understand and potentially target molecular mechanisms by which senescent cells promote melanoma.
A cell-penetrating peptide based on Connexin43-Src interaction impairs glioma stem cell growth and promotes temozolomide effect in murine glioma models

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Gliomas are among the most aggressive cancers, with a median survival of 15 months. These tumors are composed of a heterogeneous population of cells that include many with stem-cell-like properties, called glioma stem cells (GSCs), which are highly tumorigenic and resistant to standard therapies, including chemotherapy and radiotherapy. To date, the most effective chemotherapy to treat glioblastoma is the alkylating agent temozolomide (TMZ). However, 50% of the patients treated with TMZ do not respond to the drug and the increase in survival for those that do respond is modest (from 12.1 to 14.6 months median survival).

GSCs express very low levels of Connexin43 (Cx43), the main gap junction channel-forming protein in astrocytes. Restoring this protein reverses the stem cell phenotype and reduces the tumorigenicity of these cells through the inhibition of the proto-oncoprotein c-Src. Cell-penetrating peptides containing the region of connexin43 that interacts with c-Src (amino acids 266 to 283; Tat-Cx43²⁶⁶-²⁸³) mimic the effect on c-Src activity, appearing as a promising therapeutic strategy against these malignant tumors.

We treated murine Gli261 glioma cells with Tat-Cx43²⁶⁶-²⁸³ and observed reduced migration and invasion, although it did not affect cell growth. Nevertheless, when GSCs were isolated from the global Gli261 population, we observed a decrease in proliferation and sphere formation upon treatment with Tat-Cx43²⁶⁶-²⁸³, as well as an impairment in tumor growth when these cells were implanted in C57Bl/6 mice, with the concomitant increase in survival. Our results suggest that this effect may rely on the higher expression and basal activity of c-Src in Gli261 GSCs compared to non-stem cells. Interestingly, although Gli261 GSCs are not sensitive to TMZ and continue to form spheres when treated with this drug, combined treatment with Tat-Cx43²⁶⁶-²⁸³ strongly reduced GSC proliferation, suggesting a role for Tat-Cx43²⁶⁶-²⁸³ in GSC sensitization to TMZ action.
Proline dehydrogenase expression and function in non-small cell lung carcinoma

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

Materials and methods: We characterized PRODH expression in NSCLC by immunohistochemistry and qPCR and tested if there was correlation between expression of PRODH and clinical features of the tumours or expression of other markers. We tested the cellular processes that are influenced by PRODH in lung ADC cell lines. To do so, we ectopically modulated PRODH expression in ADC tumour cell lines and performed a panel of phenotypic assays.

Results and discussion: We found PRODH immunostaining in the majority (70%) of lung ADCs. Patients with PRODH positive tumours had better overall survival than those with negative tumours. Protein staining was paralleled by high transcript levels, suggesting transcriptional regulation. We found that ectopic modulation of PRODH expression in some ADC cell lines favoured survival, whereas it led to a decrease in clonogenicity in other ADC cell lines. In some ADC cell lines PRODH expressing clones also showed a reduced 3D growth compared to control clones.

Conclusion: Our immunohistochemistry data support a possible use of PRODH immunostaining as a prognostic marker. However, further research is necessary to 1) identify molecular factors that act as modifiers of PRODH effects on ADC cell lines and 2) better define the downstream processes activated by PRODH in lung cancer cells.
Normal tissue protection by targeting radiation-induced senescence

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Incidence of head and neck tumors rises as consequence of alcohol and tobacco consumption as well as due to HPV infection. Despite advances in treatment of locally advanced head and neck squamous-cell carcinoma (HNSCC), the disease is marked by its aggressiveness and likelihood of recurrence. Therapy for HNSCC includes radiotherapy with concomitant chemotherapy. However, radiation-related complications have a severe impact on the quality of life. Moreover, tumors may develop an intrinsic radio resistance, lead to therapeutic difficulties in patients. Therefore, novel treatment strategies are currently under investigation to improve radiation treatment outcome in HNSCC while reducing normal tissue complications. In our previous work, we identified differentially regulated signaling networks in irradiated tumor and normal tissues. In the present study we analyze the functional role and therapeutic potential of network-candidates on a functional and molecular level with a first focus on pathologic aspects of senescence.

Short term treatment revealed decreased cell death levels of normal tissue cells upon radiation and concomitant SASP-inhibitor treatment in vitro. The SASP inhibitor also reduced radiation-induced senescence, normal tissue associated production of certain SASP factors. Additionally, limited vascular dysfunction at early time points (21 days) after radiation was observed in vivo in an animal model of radiation-induced pneumopathy. In current in vivo experiments, we investigate the potential of SASP-inhibitor treatment for long-term protection (25 weeks), particularly the development of radiation-induced lung fibrosis. Tumorigenic murine cells of the head and neck region were analyzed upon combined treatment, and showed a decreased ability to form colonies upon increasing inhibitor concentrations.

First results indicate that SASP-inhibitor treatment can protect normal tissue cells against the cytotoxic effects of ionizing radiation, without protection of irradiated tumor cells or even exerting small radiosensitizing effects on tumor cells. Mechanistic analyses are ongoing.

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The interplay between adaptive and innate immune reactions during double cytokine armed adenoviral therapy

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Despite a strong rationale and promising pre-clinical results, suboptimal clinical responses have shown that virotherapeutics have not reached its full potential as a cancer modality. Therefore, to overcome this challenge, a novel virus was designed; two cytokine coding genes were added to a 5/3 chimeric, cancer-specific adenovirus (Ad5/3-E2Fd24-hTNFa-IRES-hIL2, also known as TILT-123) in order to enhance T-cell activation during virotherapy. However, since the specific reactions to oncolytic viruses such as TILT-123 are poorly understood, our goal in this study was to identify the most important TILT-123 recognizing receptors and signaling cascades and to determine their effect on the tumor. It is previously known that cells can respond to adenoviral infection through several different receptors, called pattern recognition receptors (PRR). The activation of PRR’s and the subsequent engagement of downstream signaling cascades modulates the tumor microenvironment. The modulation is thought to happen through PRR-dependent production and release of cytokines and alarmins that activate tumor-infiltrating lymphocytes (TIL). Through this study, we have got insight into the complex network of reactions to virotherapy and revealed broadly applicable tools and mechanisms to enhance anti-tumor activities. These results are relevant to several different cancer types and patients. Furthermore, this study proves the importance of the innate immunity as an enabler of tumor-directed, adaptive immunity activation.
ATG4B inhibition for the sensitization of treatment-resistant HER2+ cancers

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Breast cancer is the 2nd leading cause of cancer-related deaths among Canadian women. Approximately 25% of breast cancers overexpress the human epidermal growth factor receptor 2 (HER2). HER2 facilitates the normal growth and development of breast cells but promotes rapid growth and cancer development when it is overexpressed. While there are currently therapies designed to specifically target HER2, treatment resistance occurs frequently in advanced and metastatic cases. To this end, our group previously uncovered a new positive association between overexpression of the HER2 protein and high levels of a protein called ATG4B. ATG4B functions as a cysteine protease in the cell survival process of autophagy. We showed that HER2 overexpression increases ATG4B protein levels, and ATG4B inhibition enhances the sensitivity of HER2+ breast cancer cells to HER2-targeting agents, like trastuzumab. To understand this novel relationship between HER2 and ATG4B, we are currently performing mass spectrometry-based proteomics to identify novel or altered ATG4B protein-protein interactions that exist when HER2 is overexpressed. To evaluate the therapeutic potential of ATG4B inhibition, we are also investigating novel ATG4B inhibitors and their applications as tools for improving treatment responses in HER2+ breast cancer cell lines and mouse models. Together, this research has the potential to identify new therapeutic targets and combination strategies that will improve the treatment responses and overall survival of HER2+ cancer patients, and validate ATG4B inhibition as a potential therapeutic approach for overcoming treatment resistance in HER2+ cancers.
Investigations on the effects of CD40 ligation in human bone cancer-derived cells

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Background: CD40, a non-classical TNFR family member, is expressed by a variety of cancer cells. Previously, our laboratory has shown that membrane-presented CD40L (mCD40L) causes extensive apoptosis in carcinoma-derived cells but not in normal epithelial cells, rendering CD40 a promising target for cancer therapy. We have investigated for the first time the effects of CD40 ligation in human bone cancer-derived cells, by studying the functional consequences of CD40 activation (ligation) and the underlying signalling pathways in human osteosarcoma- and Ewing’s Sarcoma-derived cell lines.

Results: From a panel of well-characterised lines studied, CD40 was highly-expressed by osteosarcoma U2OS, whilst from a group of Ewing’s sarcoma lines, it was moderately-expressed by RD-ES whereas SK-ES-1 expressed little but reproducibly-detectable CD40. Receptor ligation by mCD40L induced marked death in U2OS and to a lesser extent in RD-ES and SK-ES-1 cells, in comparison to soluble CD40 agonist that had much weaker effects. Death was accompanied by DNA fragmentation and experiments in U2OS cells showed that apoptosis is caspase-9 and caspase-3/7-dependent, thus implicating the intrinsic apoptotic pathway. mCD40L induced rapid up-regulation of TRAF1 and TRAF3 in U2OS cells, whilst functional inhibition of the JNK/AP-1 pathway nearly fully-abrogated CD40-mediated death. Results will also be presented from current studies on the nature of the apoptotic pathway and the intracellular signalling mechanisms of CD40 ligation in RD-ES and SK-ES-1 cells.

Conclusion: Our findings suggest that CD40 induces apoptosis in osteosarcoma cells by engaging TRAFs for signal transduction and implicates JNK/AP-1 in apoptosis. Interestingly, in comparison to osteosarcoma or other cancer types we have previously systematically-studied (e.g. colon or renal), Ewing’s sarcoma cells appear uniquely less-susceptible to CD40-mediated death. Our investigations therefore aim to not only understand the nature of CD40-killing in bone cancer-derived cells, but to also mechanistically-explain the lower efficacy of CD40-mediated apoptosis in Ewing’s sarcoma cells.
Role of microtubules in ovarian cancer cell death of after treatment with pitavastatin as anticancer drug

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Statins are commonly used in to treat hypercholesterolemia. We have previously shown that statins such as pitavastatin may be useful in the treatment of cancer. Stains inhibit small GTPases by blocking the production of isoprenoids required for their membrane localization. However, which GTPases are crucially affected by statins to cause the apoptosis of cancer cells is unclear.

To address this, we made use of publically available databases which compare the sensitivity of cancer cell lines to statins with gene expression. We tested 18 genes whose mRNA levels correlated with statin sensitivity and found that the protein levels of one of these, MAP7, also correlated with sensitivity to pitavastatin. MAP7 is a microtubule associated protein thought to be involved in regulating microtubule dynamics. MAP7 interacts with the carboxy-terminal domain of tubulin and stabilizes microtubules. We found that MAP7 is highly expressed in Ovcar-8 and Ovsaho ovarian cancer cells. Notably, in a panel of ovarian cancer cells, these cells are most sensitive to pitavastatin. Following knockdown of MAP-7 by RNAi, the sensitivity of Ovsaho to pitavastatin decreased more than two-fold compared to cells transfected with non-targetting siRNA. When Ovcar-8 & Ovsaho were exposed to pitavastatin at two different two concentrations (1 and 2 times the IC50 in cell growth assays) levels of tubulin measured by western blotting and immunocytochemistry were found to decrease. Microtubules can be anchored to the cell membrane by CNP (2',3'-Cyclic Nucleotide 3' Phosphodiesterase), a protein that is also located at the plasma membrane as a result of prenylation by geranylgeraniol and farnesol. Pitavastatin caused the loss of CNP from Ovcar-8 cells when assessed by both western blotting and immunocytochemistry. These data suggest that the effects of pitavastatin on CNP and hence microtubules might contribute to the cytotoxic effects of statins in cancer cells.
CDK8/19 Inhibition Accelerates Death of Chronic Myelogenous Leukemia Cells by Bcr-Abl Antagonists

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Targeted drugs marked a new era in cancer treatment, with imatinib mesylate (Gleevec) as the first and the most remarkable example of selective inactivation of Bcr-Abl chimeric tyrosine kinase vital for chronic myelogenous leukemia (CML) cells. However, resistance to Bcr-Abl inhibitors justifies the need for new therapeutic approaches. Resistance mechanisms include Abl mutations and an epigenetic activation of signaling cascades that maintain the malignant phenotypes. We demonstrated that imatinib and the 3d generation Bcr-Abl blocker PF-114 potently killed logarithmically growing K562 cell line (CML) (IC₅₀ = 0.25 µM and 6 nM, respectively). In striking contrast, the efficacy of PF-114 (but not as much of imatinib) dramatically decreased in a dense culture suggesting that irresponsiveness to PF-114 can emerge due to a protective paracrine regulation. Since imatinib is less selective than PF-114, the former drug can evade protective signaling from bystander cells by inhibiting the targets other than Abl. To circumvent this ‘cell cooperation dependent’ resistance we took advantage of CDK8/19 mediated transcriptional reprogramming whose selective inhibition with a non-toxic compound Senexin B (SenB) has been shown to potently sensitize tumor cell lines and murine xenografts to a number of chemotherapeutics. The K562 cells treated with combinations of SenB with imatinib and PF-114 escaped a G1 phase arrest and underwent apoptosis via caspase activation, cleavage of poly(ADPriboso)polymerase and the loss of mitochondrial membrane potential. At least 12 h of exposure to SenB and imatinib/PF-114 was sufficient to initiate death in logarithmic and dense K562 cells (compared to 24 h for imatinib/PF-114 alone) implicating quick transcriptional reprogramming (via CDK8/19 inhibition) into CML cell sensitization to Bcr-Abl inhibitors. This study was supported by the Megagrant (grant no. 14.W03.31.0020 between the Ministry of Science and Education of the Russian Federation and Institute of Gene Biology, Russian Academy of Sciences).
Hybrid peptide Tat-Ram13-triggered necrosis-like cell death in human leukemia cell lines depends on the expression of PTEN protein

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Background: Non-apoptotic cell death, such as necrosis, is a necessary physiological process. We previously found that a 25mer hybrid peptide (termed Tat-Ram13), which conjugates the Notch-1 intracellular fragment RAM-13 peptide with a cell-penetrating HIV-1 Tat peptide, induces caspase-independent cell death in multiple human leukemia cell lines. But this peptide did not affect healthy human T-cells and leukemia cell line TALL-1 at all. Morphological features of cell death resemble necrosis/necroptosis. To understand the molecular mechanisms underlying the necrosis-like cell death by the peptide, we searched the differently expressed molecules between Tat-Ram13-sensitive leukemia cell lines and the insensitive cells.

Methods: All leukemia cell lines were maintained in 10% FCS, RPMI-1640 medium. All peptides were synthesized by Fmoc chemistry using the PSSM-8 peptide synthesizer (Shimazu Co.) Leukemia cells were incubated with the peptides or anti-tumor compounds for 24h. Cell viability was estimated with the WST-8 reagent (Dojin Chem.). We determined the expression of the protein or mRNA by western blot or qPCR analysis, respectively.

Results and Discussion: Tat-Ram13 peptide killed almost the leukemia cells (Jurkat-T, CCRF-CEM, Molt-4, etc.) except T-ALL1 cells. Expression of necroptosis-associated proteins (RIPK1, RIP3, MLKL) did not correlate with the peptide sensitivity. Interestingly, the loss of PTEN protein is observed in all the Tat-Ram13-sensitive cell lines such as Jurkat-T, although TALL-1 cells constitutively express the PTEN protein. Correspondingly, phosphorylation of AKT-1, downstream signaling of PTEN, was activated in the sensitive ones. Jurkat-T cells overexpressing PTEN has significantly reduced the sensitivity of cell death rather than parent cells.

Conclusion: These data suggest that the PI3K-AKT signaling is a potential targeting pathway for the Tat-Ram13-induced leukemia cell death.
Neuroblastoma cells escape cell death by employing compensatory activation of EPO and NGF signaling

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Currently, several drugs are considered for neuroblastoma (NB) targeted therapy, but most of them have limited efficacy for the treatment of relapsed and refractory NB tumors. Here we modeled cytotoxic stress by targeting receptor tyrosine kinase KIT using shRNA. High KIT expression is a hallmark of NB cancer stem cells, which give rise to more aggressive tumors, and several drugs target KIT and are used for the treatment of other cancers. KIT knockdown by shRNA resulted in induction of apoptosis, cell cycle arrest, and decrease in proliferation, but also resulted in upregulation of growth factor signaling. We showed that several growth factors, such as NGF, BDNF, IL6, and EPO substantially stimulate the ability of NB cells to survive after KIT knockdown. NGF and EPO, but not IL6 or BDNF, also rescued the cells from imatinib (inhibitor of KIT, PDGFR, and ABL). Extensive mining of publically available transcriptome data revealed that high expression of EPO receptor (EPOR) is associated with worse survival in patients with NB, and EPOR expression is elevated in relapsed NB tumors. Next, we collected transcriptome data for 60 primary NB tumors and showed that EPOR expression and EPO signaling is upregulated in patients who had poor response to the therapy and in patients with metastases. We designed a novel bioinformatics approach based on a combination of gene set enrichment analysis, multidimensional scaling, and assessment of individual gene prognostic value to show that KIT and EPOR are involved in the same biological processes that might drive the NB progression, such as DNA repair, chromatin organization, mRNA splicing, and MAPK signaling. Lastly, using kinase translocation reporter, we showed that EPO- and NGF-induced cell survival is ERK-dependent and ERK inhibition greatly reduces the ability of NB cells to survive imatinib treatment. This work is supported by RSF grant N19-74-00120.
Targeting of the p38 MAPK-HO-1 axis by demethoxycurcumin triggers caspase-mediated apoptotic cell death in oral squamous cell carcinoma cells

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Introduction: Curcumin (CUR), a well-known curcuminoid from turmeric extracts, has potent anticancer activities, but it is relatively unstable and largely unabsorbed after oral ingestion. Demethoxycurcumin (DMC) is a CUR analogue with better stability and higher aqueous solubility than CUR after oral ingestion and has potential to treat diverse cancers including oral squamous cell carcinoma (OSCC). Inhibitor of apoptosis (IAP) proteins and heme oxygenase (HO)-1 play diverse roles in regulating cancer progression and were reported to participate in the anticancer effects of CUR. However, whether IAP and HO-1 are involved in the anticancer properties of DMC against OSCC remains unclear. The aim of this study was to investigate the anticancer effects and underlying mechanisms of DMC against OSCC.

Material and method: The effect and potential mechanisms of DMC against OSCC were explored by an MTT assay, flow cytometry, immunofluorescence, Western blot assay, apoptosis antibody array, and genetic knockdown by small interfering (si)RNA. The TCGA database was used to investigate the prognosis of DMC-targeted genes.

Results and discussion: DMC was demonstrated to suppress cell proliferation via simultaneously inducing G2/M-phase arrest and cell apoptosis. Mechanistic investigations found that downregulation of cellular IAP 1 (cIAP1)/X-chromosome-linked IAP (XIAP) and upregulation of HO-1 were critical for DMC-induced caspase-8/-9/-3 activation and apoptotic cell death. Moreover, treatment of OSCC cells with DMC induced activation of p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK)1/2, and only inhibition of p38 MAPK significantly abolished DMC-induced HO-1 expression and caspase-8/-9/-3 activation. Furthermore, analyses of clinical datasets revealed that patients with head and neck cancers expressing high HO-1 and low cIAP1 had the most favorable prognoses.

Conclusion: Overall, the current study showed that caspase-dependent apoptosis is induced by DMC through suppressing IAPs and activating the p38-HO-1 axis, which supports a role for DCM as part of a therapeutic approach for OSCC.
Association between mammaglobin A and autophagy in breast cancer cells

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Mammaglobin A (MGB1) is an important diagnostic and prognostic breast cancer marker. It shows high expression in tumor cells that is correlated with the severity of the symptoms and the prognosis for the patient. Autophagy is a catabolic process leading to the degradation of proteins, cell organelles, and cytoplasmic fragments. It was shown that it is a primary process, occurring physiologically in most cells of the human body, and can be induced or inhibited depending on current metabolic condition. It is also strongly related to the neoplastic transformation, and its modulation can provoke a bi-directional effect (anti- or pro-survival), also in relation to pathological cells. The identification of compounds that affect autophagy can help to improve the existing cancer strategies. Regarding breast cancer, the mechanisms regulating autophagy are associated with its molecular subtype, and thus with specific breast cancer markers. However, the process requires further intensive studies.

Two breast cancer cell lines MCF7 (ER+/PR+/HER2-) and MDA-MB-231 (ER-/PR-/HER2-) were treated with drugs used in systemic therapy of breast cancer that are also capable of modulating autophagy (tamoxifen, everolimus or doxorubicin) in various concentration ranges. The mammaglobin A and crucial autophagy marker i.e., LC3-II protein level were assessed using Western Blot. Interestingly, a correlation between MGB1 and LC3-II was observed, suggesting a crosslink between these two factors. This may be a very useful information in the context of planning breast cancer diagnostic and therapeutic strategies in relation to autophagy in tumors showing different molecular characteristics.

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FoxM1 inhibition promotes mitotic cell death in response to spindle poison chemotherapy

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Chronic cell proliferation is a hallmark of cancer, and therefore therapies targeting key pathways driving cell division have been largely explored. In particular, inhibition of the essential spindle microtubule (MT)dynamics with MT poisons, such as taxanes and vinca alkaloids, has been successfully used in the treatment of sólido cancers for over 25 years. However, drug resistance is often a side effect that has limited their clinical utility. Now that the mechanisms by which spindle poisons elicit mitotic arrest are understood, efforts are needed towards elucidating how cancer cells respond to this prolonged cell-cycle delay. Using high-content imaging and live-cell analysis to monitor long-term cell behavior in response to spindle poisons, we identified the transcription factor Forkhead box M1 as a molecular determinant of cell fate decision. Interestingly, even though FoxM1 is the major regulator of late cell cycle gene expression, FoxM1 acts through the modulation of the apoptotic pathway rather than the mitotic pathway of the competing networks model. Importantly, we identified a pro-apoptotic gene, whose expression levels increase with FoxM1 repression. We found that there are FoxM1-binding cis-regulatory elements in that pro-apoptotic gene that act to induce expression of a neighbor mitotic gene. Through this mechanism, mitotic cells are able to circumvent cell death signaling induced by cell rounding detachment. We thus uncovered a mechanism that besides its potential of being targeted through FoxM1 inhibition, to increase cancer cell death in response spindle poisons, it should be further investigated to understand drug resistance.
BH3 mimetic therapeutic combinations to improve pediatric leukemia treatment

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Leukemia is the most common cancer in kids accounting for approximately 25% of all cases. At present, 90% of all cases achieve complete remission while the remaining 10% correspond to relapsed or refractory tumors. Even if complete remission is achieved treatment regimens cause a wide range of secondary effects in children, including mental retardation, cardiac problems, secondary neoplasia, among others. There is an urgent demand for more effective and less toxic therapies, and better predictive biomarkers to assign them to every patient.

To answer this unmet need, we used Dynamic BH3 Profiling (DBP) to find new effective therapies using B-cell Acute Lymphoblastic Leukemia (B-ALL) cell lines. DBP is a novel functional assay that uses synthetic peptides to measure how close cancer cells are to commit apoptosis after treatment. Using a titration of synthetic BIM BH3 peptide, that mimics the pro-apoptotic function of the full-length protein, we can measure the increase of apoptotic predisposition or “priming” in treated cells compared to control.

However, cancer cells often acquire resistance to treatments by overexpressing or activating proteins that protects them against apoptosis, the so called anti-apoptotic BCL-2 family proteins. In this regard, by using specific synthetic peptides, DBP can also detect anti-apoptotic adaptations upon treatment, guiding the use of BH3 mimetics (small molecules that inhibit anti-apoptotic proteins and are currently explored in the clinic) to overcome resistance.

Using this strategy, we have identified multiple effective treatments against B-ALL cell lines that were confirmed by cell death analysis using Annexin V/PI staining after 72 hours of treatment. Furthermore, using DBP we could also identify anti-apoptotic adaptations that we could overcome by combining conventional chemotherapy or targeted therapies with BH3 mimetics. Using these combinations, we could reduce therapeutic dosing while maintaining or improving B-ALL cytotoxicity.
31 – Poster Spotlight

Generating “Signalome” cell lines: A novel approach to studying signaling pathways in cancer

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Resistance to chemotherapy and targeted therapies is one of the major challenges of current cancer research. Rewiring of signaling pathways within cancer cells in response to therapy is a common mechanism of innate resistance.

Most available methods profile signaling pathway activity either at the population level or at low-throughput single cell levels. We have established a novel high throughput system – the Signalome – which allows for live imaging of 12 different pathways, at single cell resolution, dynamically over time, all in one single well. We exploited this system to screen for the effect of 423 drugs on the cancer cells and their signaling pathways. Our results show that drugs with similar reporter activity profiles have similar targets and cluster together using unsupervised hierarchical clustering. Furthermore, we show that modulating retinoic acid receptor activity can impact vemurafenib treatment outcome, and a combination of retinoic acid and vemurafenib can increase cell cycle arrest in A375 BRAF-mutated melanoma cell line. Finally, we demonstrate that retinoic acid receptor pathway activity is a significant predictor of patient outcome in three independent melanoma patient cohorts.

Better understanding of the role of nuclear receptors and the retinoic acid pathway specifically in melanoma may help in designing novel drug combinations. Our signalome approach is applicable to a wide range of diseases and treatments to foster a deep understanding of the unique signaling network of each disease.
Evaluation of the efficacy of anti-CSPG4 targeting as a promising strategy for the treatment of osteosarcoma

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The chondroitin sulfate proteoglycan (CSPG)4 is a transmembrane proteoglycan scarcely detectable on healthy tissues, while highly expressed in several solid tumors in which it orchestrates multiple tumorigenic processes. For its properties, CSPG4 represents an attractive tumor associated antigen and there is a growing interest in establishing the potentiality of its targeting. As a step forward in this direction, we evaluated the feasibility of appointing CSPG4 as a good immunotherapeutic target for the treatment of osteosarcoma (OSA). Indeed, despite current therapeutic options, the outcome of metastatic OSA patients is still dismally poor and the development of novel strategies to counteract OSA progression and metastatization is urgently needed. First, we aimed to exploit CSPG4 involvement in OSA. We found a strong correlation between CSPG4 overexpression and a worse prognosis in both human and canine OSA patients. Anti-CSPG4 monoclonal antibodies (mAbs) and sera derived from anti-CSPG4 vaccinated canine melanoma patients enrolled in our previous clinical veterinary trial (Riccardo et al., 2014; Piras et al., 2017) proved to be effective in significantly inhibit both human and canine OSA cells proliferation, migration and osteospheres generation in vitro, potentiating the effect of doxorubicin. Overall, these results prompted us to investigate the potential of an anti-CSPG4 DNA vaccination strategy for the treatment of canine CSPG4⁺ OSA patients in a clinical veterinary trial. Naturally occurring OSA-bearing dogs are being enrolled and adjuvantly treated with an anti-CSPG4 plasmid. At this time, the anti-CSPG4 vaccination is demonstrating to be safe and immunogenic. Noteworthy, vaccine-induced antibodies succeed in hampering both canine and human OSA cell migration. On the basis of these results and of the high translational power of spontaneous canine tumors for human malignancies, we strongly believe in the potentiality of using the anti-CSPG4 vaccination for canine OSA patients management, to be eventually translated in a human clinical setting.
33 – Poster Spotlight

Specific interactions of BCL-2 family proteins mediate sensitivity to BH3-mimetics in diffuse large B-cell lymphoma

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The BCL-2 specific inhibitor, venetoclax/ABT-199 has exhibited remarkable clinical activity in nearly all cases of chronic lymphocytic leukemia. In more aggressive B-cell lymphoma like diffuse large B-cell lymphoma, responses to ABT-199 are less frequent, indicating that co-expression of related anti-apoptotic BCL-2 family proteins may limit activity. In this study we have investigated the roles of the BCL-2 proteins in diffuse large B-cell lymphoma cells using a panel of specific BH3-mimetics and identified subgroups of diffuse large B-cell lymphoma cells that exhibited marked and specific dependency on either BCL-2, BCL-X₉ or MCL-1 for survival. Sensitivity to BH3-mimetics was independent of genetic alterations involving the BCL-2 family and only partially correlated with protein expression levels. Instead, sensitivity to BH3-mimetics was associated with a sequestration of the pro-apoptotic proteins BIM, BAX and BAK selectively by the specific anti-apoptotic BCL-2 protein that was important for cellular survival. Treatment with ABT-199 displaced BAX and BIM from BCL-2, leading subsequently to BAK activation and apoptosis. In contrast, apoptosis induced by inhibiting BCL-X₉ with A1331852 was associated with a displacement of both BAX and BAK from BCL-X₉ and occurred independently of BIM. Surprisingly, the MCL-1 inhibitor S63845 induced mainly BAX-dependent apoptosis mediated by a displacement of BAK, BIM and NOXA from MCL-1. In conclusion, our study indicates that in diffuse large B-cell lymphoma, the heterogeneous response to BH3-mimetics is mediated by selective interactions between BAX, BAK and anti-apoptotic BCL-2 proteins.
Tnbc, non-tnbc patients and normal ones could be reliably distinguished by a subset of genes from Wnt-cascade

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We studied the database of gene expression of the patients with various types of mammalian cancer: tmbc (118 entries), non-tnbc (852 entries) and healthy women (112 entries). The database was provided by Center of translational studies in oncogaemotology of Geneva university (prof. Vladimir Katanaev). We aimed to reveal the inner sturcturedness of the database in terms of clustering comprising various groups of patients. We tried a number of linear clustering and classification techniques to reveal the patterns; no pattern was found by these methods. Next we changed for non-linear statistics and clustering based on elastic map technique. That latter stably and reliably distinguishes tmbc patients from non-tnbc and healthy patients. It was found that almost any subset of genes could be used to omplement the cluster pattern, and the patterns observed for different subsets are pretty similar and homologous. This fact reflects the redundancy of the genes network expressed in the data amount necessary to reveal the structure distinguishing tmbc patients from non-tnbc ones and healthy people. Of course, the network may not be claimed redundant from the point of view of the network function. Also, a minimal subset has been tried to find; several subsets pretend to be such entities.
Knockdown of Retinoblastoma Binding Protein 6 (RBBP6) affects telomerase activity in cervical cancer cells

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Cancer continues to be a major health problem worldwide, with increasing mortality rates in developing countries. Both the retinoblastoma binding protein 6 and telomerase are overexpressed in cancer cells. RBBP6 overexpression is associated with increased cancer cell proliferation whereas silencing RBBP6 leads to significant apoptosis induction. A crucial component of telomerase, hTERT is highly expressed in over 90\% of cancers. hTERT expression and increased telomerase activity are associated with tumor progression. As RBBP6 and hTERT both play a role in cancer progression, we investigated a possible correlation between RBBP6 and telomerase. Findings from this study reveal co-localisation of RBBP6 and hTERT around the nucleus of tumorigenic cervical cancer cells and non-tumorigenic human lung fibroblasts (MRC-5). This was confirmed by flow cytometry, which revealed that both normal and cancerous cell lines show high intracellular RBBP6 and hTERT levels. Silencing the RBBP6 gene by RNA interference significantly reduced telomerase activity. These results suggest for the first time a novel function of RBBP6 in contributing to telomerase activity.
Anti-cancer effect of curcumin on survival and expression of DNMT1 and CDH gene in MIAPaCa2 cell line

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Pancreatic cancer is a deadly malignant cancer, the 5-year survival rate of these patients is below 5%. DNMT1 is the major enzyme for methylation after transcription. Ecad play a role in cellular connectivity through extracellular domains, Loss of Ecad Protein, encoded by CDH1 gene, lead to loss of Intercellular Connections. The pharmacological effects of curcumin include inducing apoptosis, anti-cell proliferation, antioxidant and anti-angiogenesis are proved and this compound has the potential to be used in cancer prevention. The current study was performed in order to explore in vitro antitumor activity of curcumin in human pancreatic carcinoma cell line MIAPaCa2.DNMT1 and CDH1 genes expression were examined by quantitative real-time PCR. Finally, the effects of curcumin on viability and DNMT1 gene and CDH1 gene expression status were evaluated. MiaPaca-2 cell line was cultured in monolayers. The cells were treated with curcumin using different concentrations of 2, 5, 10, 20, 40, 80 μM for 24, 48 and 72 hours. Viability was checked by MTT assay and DNMT1 and CDH genes expression was evaluated by RT-PCR. Results: Our results indicate that the level of DNMT1 mRNA expression, In 24, 48 and 72 hours for 2, 5, 10, 20, 40 and 80 µM concentrations was decreased after treatment. Expression level of CDH mRNA, in 24 hours for 40 and 80 µM concentrations were increased. In 48 hours for 20, 40 and 80 µM concentration was increased. After 72 hours all of concentration was increased. Data obtained from MTT revealed antiproliferative effects of curcumin for 20,40,80 µM concentrations but not 2,5 and 10 µM. We conclude that cell viability and level of DNMT1 mRNA was decreased after curcumin treatment, and level of CDH mRNA was increased. So, these observations suggest that curcumin, a molecule with varied actions, as a supplementary could be developed into an effective chemopreventive and chemotherapeutic agent for pancreatic cancer treatment.
Rapid \textit{in vivo} validation of HDAC inhibitor-based treatments in pediatric nervous system tumors

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The survival rate among children with relapsed nervous system tumors continues to be poor, thus new therapeutic approaches identified by reliable preclinical drug testing models are urgently needed. Zebrafish are a powerful vertebrate model in preclinical cancer research. We describe a zebrafish neuroblastoma yolk sac and pediatric high-grade glioma brain xenograft model to evaluate efficacy and toxicity of HDAC inhibitor treatments. Larvae were engrafted with fluorescently labeled, genetically diverse, established cell lines and patient-derived primary tumor cells. Engrafted tumors progressed locally and disseminated remotely in an intact environment. We demonstrate, that combination treatments involving the standard chemotherapy, namely doxorubicin, and HDAC inhibitors substantially reduce tumor volume, induce tumor cell death, and interfere with tumor cell dissemination to the tail region. We confirmed our results by comparison to the patient data from our phase I/II vorinostat trial. We therefore claim that this model allows for fast and cost-efficient \textit{in vivo} evaluation of drug toxicity and responsiveness of primary and metastatic lesions to therapeutic interventions.
Inhibitory effect of transducible HIF-1α transcription modulation domain on Angiogenesis

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Hypoxia-inducible factor-1 alpha (HIF-1α) is an important transcription factor for angiogenesis that is essential for metastasis of solid tumors. However, the strategies to modulate angiogenesis using chemicals inhibiting the functions of HIF1α are still limited due to the extensive side-effects including low delivery rate and unspecific targeting. Here, a novel therapeutic strategy was developed to regulate the function of HIF-1α utilizing the nucleus-transducible form of Transcription Modulation Domain of HIF-1α (ntHIF1α-TMD) protein, which can be delivered effectively into the nucleus of cells. ntHIF1α-TMD is composed of HIF-1α-specific N-terminal domain and its DNA binding domain of HIF-1α, which serves as dominant negative competitor for HIF-1α-mediated transcription. ntHIF1α-TMD also contains Hph-1 Protein Transduction Domain (PTD) which can deliver HIF-1α-TMD to the nucleus in vitro and in vivo. ntHIF-1α-TMD significantly reduced the secretion of VEGF which is a key factor in angiogenesis that can induce metastasis of solid cancers. The severity and incidence of colon cancer (CT26) and lung cancer (LLC1) were alleviated by the injection of ntHIF-1α both. Taken these results together, ntHIF1α-TMD can be a novel therapeutic reagent for the treatment of several solid cancers associated with the overexpression of VEGF or HIF-1α with the reduced side effects.
The role of the long non-codin RNA HAS2-AS1 in breast cancer cells

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One of the most abundant constituents of extracellular matrix is hyaluronan (HA), a glycosaminoglycan synthesized by three transmembrane isoenzymes (HAS1, 2, 3).

In aggressive cancers, HA is abnormally accumulated in the tumour niche; although several studies report that HA favours tumour growth and spreading, it is not so clear the molecular mechanism(s) behind this cancer-promoting effect.

We recently described that the natural antisense transcript HAS2-AS1, a long non-coding RNA, is critical to control HAS2 gene transcription via epigenetic modifications, in aortic smooth muscle cells (as HA is involved also in atherosclerosis). Moreover, transcriptome analyses (www.mitranscriptome.org) revealed an upregulation of HAS2-AS1 in many human tumour biopsies respect healthy controls.

Therefore, the objective of this study is to better understand the role of HAS2-AS1 in breast cancer cells. We compared the behaviour of MDA-MB-231 and MCF7 cell lines after modulation of HAS2-AS1 expression, evaluating cell proliferation, migration and invasion. We also analysed the expression of HA related genes and receptors. Data revealed that HAS2-AS1 knockdown stimulated a malignant phenotype, as its abrogation increased cell motility and invasion, as well as the expression of several HA related genes and CD44 receptor.

Further analysis were conducted to understand the molecular mechanism at the basis of the changes observed. An intriguing cytoplasmic function of IncRNAs is their ability to bind miRNAs, creating a competition for the interaction between the miRNA, IncRNA and other regulatory targets (ceRNAs). In silico analysis revealed that HAS2-AS1 exon 2 transcript contains several putative binding sites for different miRNAs, among which miR186-3p. Indeed, luciferase assays confirmed the interaction between HAS2-AS1 and miR186-3p.

Altogether, these data suggest that the “sponge effect” of HAS2-AS1 is able to antagonise the function of miRNA186-3p on its downstream targets and could explain the presence of a malignant phenotype after HAS2-AS1 silencing in MDA-MB-231 cells.
Ionizing Radiation-Induced Mitochondrial Damage and the Mitochondrial Damage Response

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Mitochondria are central organelles coordinating metabolism, ATP production, calcium homeostasis and apoptosis allowing eukaryotic cells to either adapt to the environmental demands and survive or induce cell death after irreparable damage. Mitochondria are under dual control of nuclear and mitochondrial DNA (nucDNA, mtDNA). Giving the importance of ionizing radiation in the treatment of cancer, ionizing radiation (IR)-induced damage and repair of nucDNA were extensively examined over the last years. Efficient nucDNA repair was previously shown to correlate with increased resistance to ionizing radiation. The effects of IR on mitochondria and mtDNA, however, are insufficiently understood. Here, we analyze the effects of low-dose irradiation (4Gy) on mitochondria in glioblastoma cells. 1h after IR, PGC1a, a transcription factor regulating mitochondrial biogenesis, temporarily translocated to the nucleus. Flow cytometric analysis revealed an elevated mitochondrial membrane potential 24h after IR, that normalized later on. 6h after IR, mitochondria took on a rounded, donut-like shape, but started to elongate soon after. Analysis of mean mitochondrial length showed a temporary increase of mitochondrial length with a peak at 48h after irradiation with 4Gy. This suggests functional changes in mitochondria induced by IR. Using seahorse analyzer, we detected respiratory shut-down 6h after irradiation, that was slowly restored later on. Furthermore, EdU incorporation into mtDNA remained high immediately after IR but declined 48h later, indicating a reduction in mtDNA synthesis and/or repair. Ku80 and p53, both proteins involved in nucDNA repair, increasingly co-localized with elongated mitochondria 48h after IR, hinting at a potential role of these proteins in maintaining mitochondrial function and mtDNA. Finally, using an oxidation-sensitive fluorescent protein targeted to mitochondria, we could show that mitochondrial ROS production increased in response to IR after 72h. The extent in mitochondrial ROS production correlated with IR-induced cell death. Taken together, our data documented mitochondrial reaction to IR.
Combining PDT with PD-1/PD-L1 Blockade: Can We Find the Key to Enhanced Antitumor Response?

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Recently, immune checkpoint immunotherapy has shown promising clinical results. However, there is still a large number of patients who respond poorly; therefore, more potent combinational therapies are required. In this context, immunotherapy based on the modulation of immune checkpoints like programmed death-1 (PD-1) and its ligand (PD-L1) in combination with photodynamic therapy (PDT) may be an innovative therapeutic strategy for more resistant cancers. Herein, we employed polarity-tunable bacteriochlorin-based photosensitizers that absorb near-infrared light to compare the PDT efficacy after protocols targeted to tumor vasculature, endothelial cells or tumor tissue. We performed the analysis of the molecular mechanisms of PDT crucial for the generation of antitumor immunity and indicated that PDT-induced cell death might affect the integrity of the host tissue and develop acute (protocol-dependent) local inflammation, which in turn led to the infiltration of neutrophils and macrophages. Using the multitarget approach, it was possible to evaluate whether modulation of inflammatory mediators induced by PDT determines the mode of cell death, tumor microenvironment and the susceptibility to systemic PD-1/PD-L1. More importantly, we present that bacteriochlorin-based PDT in vivo contributed to an immunogenic environment in the tumor, which may enhance PD-1/PD-L1 checkpoint blockade therapy by stimulating a strong and long-term antitumor immune response. We demonstrated that optimized PDT regressed the growth of not only primary- but also distant tumors examined in bilateral syngeneic and pseudo-metastatic mouse models. Moreover, based on rechallenge experiments, we assessed the generation of immune memory able to prevent tumor relapse, similar to the functions of cancer vaccines. Finally, we suggest that the combination of PDT with PD-1/PD-L1 axis inhibition designed in the current study offers a new strategy for treating metastatic cancers with primary tumors accessible by PDT.

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A chimeric Human/Dog CSPG4 DNA vaccine reveals potential therapeutic effects in canine and human melanoma patients

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Malignant melanoma (MM) is the sixth most common cancer worldwide. With the advent of immune checkpoint inhibitors (CIs), the revolution of immunotherapeutic treatments for MM started. However, the overall response rate to CIs still ranged between 20%-45%, with mild to severe side effects. Therefore, there is still room for improvement. In this panorama, antigen-specific cancer vaccines rise up as an interesting opportunity and the Chondroitin Sulfate Proteoglycan (CSPG)4 stands out as a promising immunotherapeutic target, with low expression in healthy tissues, high expression in several solid tumors and a key role in cancer progression.

Thanks to the translational power of dogs as pre-clinical models for human malignancies and the CSPG4 over-expression in both human and canine MM, we demonstrated the safety and the clinical effectiveness of a xenogeneic human (Hu)-CSPG4 DNA vaccine in client-owned dogs with stage II-III surgically resected CSPG4⁺ MM. However, Hu-CSPG4 vaccine was barely effective in activating human T cells from healthy donors in vitro. Aiming at improving this approach and its possible translation to human patients, we generated a hybrid plasmid, derived in part from the Hu- and in part from the dog (Do)-CSPG4 sequences (HuDo-CSPG4).

Chimeric HuDo-CSPG4 immunization was strongly effective in mice. In canine patients with stage II-IV surgically resected CSPG4⁺ oral MM, the vaccination was safe and immunogenic. HuDo-CSPG4 vaccine-induced antibodies reacted against both Hu- and Do-CSPG4, showing a higher affinity and a strong anti-tumor potential as compared to Hu-CSPG4. Moreover, clinically, HuDo-CSPG4 was effective in increasing the overall survival of vaccinated canine MM patients as compared to controls. Lastly, data obtained in vitro with T cells from human donors suggested HuDo-CSPG4 could be more immunogenic than Hu-CSPG4.

These results provide the rationale to propose HuDo-CSPG4 vaccination for the treatment of canine CSPG4⁺ tumors, to be successfully translated in a human setting.
Dihydrolipoamide dehydrogenase regulates cystine deprivation-induced ferroptosis

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Ferroptosis is a new form of regulated cell death driven by iron-dependent lipid peroxidation. Glutaminolysis and tricarboxylic acid cycle are engaged in ferroptosis, but the relevant metabolic process remains unclear. Here, we examined the role of dihydrolipoamide dehydrogenase (DLD) in induction of ferroptosis in head and neck cancer (HNC) cells. The effects of cystine deprivation and sulfasalazine were tested in HNC cell lines. The effects of cystine deprivation and sulfasalazine as well as the silencing and overexpression of DLD gene were by cell death, lipid reactive oxygen species (ROS) production, mitochondrial iron and membrane potential, mRNA/protein expression, α-ketoglutarate dehydrogenase (KGDH)/succinate/aconitase assays, and mouse tumor xenograft models. Cystine deprivation induced ferroptosis via glutaminolysis. Cystine deprivation or import inhibition using sulfasalazine induced cancer cell death, lipid ROS and mitochondrial iron accumulation, which significantly decreased by siRNA or shRNA targeting DLD (P <0.01) but not by dihydrolipoyl succinyltransferase. The same results were found in an in vivo mouse model transplanted with vector or shDLD-transduced HN9 cells. Mitochondrial membrane potential and free iron, KGDH activity, and succinate content significantly increased with cystine deprivation or sulfasalazine (P <0.001). These were blocked by DLD siRNA or shRNA, which were rescued by resistant DLD cDNA. Cystine deprivation also caused iron starvation response and mitochondrial iron accumulation for Fenton reaction and ferroptosis. DLD mRNA levels were significantly lower in HNC than normal mucosa from the TCGA datasets (P <0.001). Taken together, our data support that DLD closely links to ferroptosis induced by cystine deprivation or import inhibition.
hTERT downregulation modulates adhesion and migration pathways in human breast cancer cell lines MCF7 and MDA-MB-231

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The key telomerase catalytic subunit hTERT (human telomerase reverse transcriptase) is overexpressed in more than 90% of cancers and is recognized as a useful marker of malignant transformation. Additionally, there is increasing evidence for several roles of hTERT in tumor cells metabolism that are not interdependent with telomere maintenance. Presumably, some of those non-canonical functions are associated with modifications of drug resistance, proliferation, and adhesion abilities of cancer cells that may lead to their survival dysfunction. The study aimed to assess the effect of telomerase downregulation on breast cancer cell sensitization to therapeutic drugs in the context of migration and adhesion.

We created a sufficient and effective lentiviral system for hTERT silencing. The experimental model was chosen base on the well-known fact that telomerase expression and activity are elevated in breast cancer cells. The study revealed that hTERT downregulation provoked a significant attenuation of cell survival. MTT and clonogenic results also showed that the reduction of telomerase activity led to cancer cell sensitization to doxorubicin. This effect was accompanied by a significant decrease in adhesion and migration ability (examined via functional assays) as well as by alterations in respective pathways. It was manifested by remarkable variation in the level of individual proteins (β1 integrin, paxillin, c-Src, FAK) together with the reduction of their phosphorylation. Importantly, we observed cell cycle alternations in both cell lines, followed by the diminishment of cell proliferation rate assessed during the longterm culture evaluation. The next step was to distinguish which cell death type occurred. We noted the accumulation of beta-galactosidase, i.e., the main senescence marker in MCF7 cells, while autophagy markers (↑Atg 5, ↑Beclin 1, ↑LC3 II/ LC3 I, ↓p62) were observed in MDA-MB-231. These results provide new evidence to support a non-canonical function of hTERT.

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Contribution of telomerase inhibitor TMPyP4 to cancer drugs resistance in breast cancer cells

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Breast cancer is the most common malignancy in women. Current therapies are based on chemotherapy that often provokes multidrug resistance (MDR) that is responsible for therapy failure.

One of the MDR mechanisms is associated with an active removal of cytostatic drugs from a target cell via ABC transporter. In some types of cancer, a weak response to therapy may be associated with an induced telomerase expression. However, not much is known about the potential association between telomerase activity and drug resistance. The aim of the study was to assess the influence of telomerase inhibitor (TMPyP4) on multidrug resistance in breast cancer cells in vitro. Two breast cancer cell lines were used as an experimental model: human breast adenocarcinoma MCF7 and its subpopulation that was resistant to doxorubicin i.e., MCF7/DOX.

Cytotoxicity assays revealed a significant decrease in both cell types survival when treated with TMPyP4 in a time- and dose-dependent manner. qPCR demonstrated that MCF7/DOX cells showed almost four times higher basal level of hTERT as well as an increased expression of ABC coding genes comparing to maternal cells. In both cell lines, a decrease in hTERT as well as in some ABC family members (including ABCC1 and ABCG2) was observed after TMPyP4 treatment in a concentration-dependent manner.

The studies revealed a significant role of TMPyP4 in increasing the sensitivity of resistant breast cancer cells to doxorubicin in vitro. Such observation might bring a new perspective in the context of using telomerase inhibitors as an element of an adjuvant therapy when combined with classic cancer drugs.

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Different relevance of the cystine/glutamate antiporter xCT in chemoresistance and cell migration depending on the mammary cancer subtype

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Breast cancer represents the major cause of cancer-related death in women worldwide, due to metastases formation and chemoresistance. These could be ascribed, at least in part, to the ability of cancer cells to adapt to high oxidative stress conditions. The cystine/glutamate antiporter xCT is a key player in redox homeostasis and is upregulated in many tumors, including breast cancer, where it has been associated to chemoresistance and cancer cell migration. However, it is unclear if xCT has the same relevance in different breast cancer subtypes and if patients would equally benefit from xCT-targeted therapies. Analysis in the CCLE encyclopedia revealed that xCT expression is significantly higher in human TNBC cell lines as compared to HER2+ cell lines. Consistently, 4T1 cells, a mouse model of human TNBC, express higher xCT levels as compared to mouse HER2+ TUBO cells. This determines greater resistance to doxorubicin treatment, but a higher sensitivity to sulfasalazine-mediated xCT inhibition in 4T1 cells. Furthermore, migration assays suggest a role for xCT in cancer cell migration in 4T1 cells, where it localizes on membrane protrusions on migrating cells, while xCT seems to reside within intracellular foci in TUBO cells. Migration and chemoresistance features are further strengthened in xCT-overexpressing 4T1 cells. Conversely, TUBO cells do not show the same characteristics upon xCT modulation, supporting the hypothesis that xCT plays a role in these mechanisms in a cell-type dependent manner. These data suggest that patients of different breast cancer subtypes would benefit in a different manner from chemotherapy and xCT-targeted therapies, possibly depending on basal xCT expression levels. We are currently performing experiment on newly generated xCTnull cell lines and mouse models to better elucidate the contribution of xCT not only at the tumor cell-autonomous level, but also in the interaction between cancer cells and tumor microenvironment.
Verteporfin synergizes the antiproliferative effect of tamoxifen and doxorubicin through inhibiting autophagy in breast cancer cell lines

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Background: Verteporfin is a benzoporphyrin agent with pleiotropic effects on different types of tumors. Among several mechanisms, verteporfin interferes with early stages of autophagy through inhibiting autophagosome formation. Autophagy has a dynamic and complex role in cancer; depending on the tumor characteristics and microenvironment.

Aim: To detect effect of verteporfin pre-treatment combined with tamoxifen on MCF-7 and doxorubicin on MDA-MB-231 breast cancer cell lines.

Methods: We used MCF-7 (ER+, PR+, HER2-) and MDA-MB-231 (ER-, PR-, HER2-) breast cancer cell lines to test our hypothesis. The effect of verteporfin pretreatment (at 1, 3 and 5uM) for 24 hours prior to tamoxifen treatment of MCF7 (at 0.1, 0.5, 1, 5 and 10 uM) was detected using MTT. The same experiments were carried out on MDA-MB-231 cell line (pre-treated with verteporfin and then treated with doxorubicin). Drug interaction was detected by CompuSyn software. Effect on autophagosome formation was tested after another 24 hours using a fluorescent autophagosome marker. In addition, autophagy-related proteins Beclin-1 and LC3-1A/1B expression was detected by WB using rabbit monoclonal antibodies.

Results: There was a synergistic effect of verteporfin and corresponding treatment on MCF-7 and MDA-MB-231 cell lines (3&5 uM), drug combination index <1. The effect was also significant at verteporfin 1uM pre-treatment on MDA-MB-231, but not MCF7 cell line. Autophagosome formation was reduced by verteporfin pre-treatment in both cell lines. Single treatment with tamoxifen increased expression of Beclin-1 and LC31A/1B (to less extent with doxorubicin). Combined treatment significantly reduced the expression of both proteins in both cell lines.

Conclusion: The antiproliferative effect of tamoxifen and doxorubicin is synergized by the autophagy inhibitor verteporfin in breast cancer cell lines. Verteporfin can be a potential adjuvant therapy to minimize the toxicity of both medications.
Cancer patients are effectively stratified with complete blood count data

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We studied clustering of cancer patients suffering from various oncological diseases except leukemia. Complete blood count records have been used, the data base enrolled 876 entries. Patients varied in age from 16 to 86, and exhibited almost equal sex distribution. To reveal an inner structure of the data body, K-means classification has been used. No reliable or meaningful classification has been observed, for K-menas. An implementation of elastic map technique revealed four clusters. We used 18 parameter of CBC excluding those with high (greater than 0.86) correlations. The clusters exhibit no relation to age, sex, or a type of a tumor and/or localization. We studied the cluster structure in terms of distribution of the patients with various level of the parameters of CBC, tracing filling of the map from minimal to maximal values of CBC parameters. It has been found that 14 parameters yield the gradual map filling (as a “running wave”), while 4 parameters yield almost homogeneous and random map filling (as a “starry-sky”). Gradually filling parameters are considered to be an evidence of Liebeg principle which shows the non-specific organism respond on the stress caused by cancer. Surprisingly, 4 parameters are not involved into the stress respond pattern, for oncological patients.
Extracellular full-length HMGB1 stimulates the nuclear accumulation of NFkB and p53 in lung cancer cell lines

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Many cellular stimuli result in the induction of both the tumor suppressor p53 and NF-kB. The activation of p53 is associated with the induction of apoptosis, in contrast, stimulation of NF-kB does promote resistance to programmed cell death. Both proteins are recognized as crucial players in many steps of cancer initiation and progression. We found that the High mobility group box 1 (HMGB1) protein can play the role of a signal molecule that provokes the independent accumulation of p53 and NFkB in the nucleus. We focused on NF-kB activity in the lung cancer cell lines (A549 and H1299) as a putative downstream target of HMGB1 signaling. Only full-length HMGB1 was able to increase cell motility and induces cytosolic to nuclear translocation of NF-kB in both lung cancer cell lines. The activation of p53 can be provoked by an increase in its concentration as a result of high-level transcription, by the transformation of the p53 protein to an active conformation or by its translocation from the cytoplasm to the nucleus. p53 can be activated by a wide variety of stress signals that a cell might encounter during malignant progressions, such as genotoxic damage, oncogene activation, and hypoxia. HMGB1 protein can play the role of a signal molecule that provokes the accumulation of p53 in the nucleus in A549 cells. Same as in the case of NFkB stimulation, only the full-length protein is able to induce the translocation of p53 from the cytosol to the nucleus and the effect was considerably strong and almost equal to that generated by the positive control actinomycin D. The truncated tailless form of HMGB1 was not functional. This supported the hypothesis that the C terminus plays an important role in regulating the properties of HMGB1.

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Grant 28
Targeting BH3-activated apoptosis: in vivo tumor reduction and in vitro synergism of gamma-tocotrienol and 13-cis retinoic acid in treatment of high-risk neuroblastoma

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Neuroblastoma, one of the leading pediatric solid tumor in the world has poor survival rate for the high risk type at 27-29% and the statistic has not changed in the past 15 years. This is due to failure of 13-cis retinoic acid (13cisRA) treatment to induce differentiation on remaining tumour cells post surgery and the tumour recurrs. Targeted inhibition of Bcl-2 has been shown to effectively attenuate neuroblastoma tumor growth, as these small molecule inhibitors act as BH3 mimetics, binding to the pro-apoptotic BH3 domain of Bcl-2 protein could be potential anticancer agent. Gamma-tocotrienol (γT3) is an intriguing molecule, besides acting as antioxidant, it has also shown to be involved in both mediating pro-apoptotic and anti-apoptotic signaling pathway via the Bcl-2 family protein. This maybe an important mechanism of action, as we know that the dysregulation of apoptosis is the hallmark of cancer whereby Bcl-2 family proteins are involved in the initiation of apoptosis process. In this study, we demonstrated that γT3 acts similarly like a BH3 mimetics and binds specifically to the BH3 domain of the Bcl-2 protein to induce cell apoptosis in SH-SY5Y neuroblastoma cell line. γT3 act synergistically with 13cisRA to halt tumour growth by binding to the pro-apoptotic BH3 domain of the Bcl-2 protein and initiate apoptosis in both SH-SY5Y and SK-N-BE9(2) cells. The ectopic xenograft study also demonstrated that γT3+13cisRA group reduce tumour size compare to control group and has greater tumour reduction compared to the known BH3 mimetic ABT-263 co-treatment with 13cisRA group. Our data suggested that combination treatment of γT3+13cisRA could beneficial to patients of high-risk neuroblastoma to decrease the chances of tumour relapse and increase post-surgery survival rate.
Effect of PKCε downregulation on telomerase in human glioblastoma cells

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Protein kinase C epsilon (PKCε) is characterized as a calcium-independent and phorbol ester/diacylglycerol-sensitive serine-threonine kinase (member of novel PKC isotype group). This protein is involved in many signaling pathways, including adhesion, migration, proliferation, secretion, differentiation, gene expression, and apoptosis. PKCe shows the greatest oncogenic potential among all PKC isozymes. Since it is also associated with tumorigenesis we decided to assess its contribution to the regulation of another cancer marker i.e. telomerase. The key catalytic subunit of this enzyme is hTERT (human telomerase reverse transcriptase) and it is overexpressed in more than 90% of cancers.

In the present study, we investigated the effect of knockdown of PRKCE gene coding for PKCε on the hTERT expression in glioblastoma cells in the context of apoptosis induction. Two human glioblastoma cell lines U-118 MG and U-138 MG were used as the experimental model. We performed siRNA-mediated knockdown of PKCε expression and evaluated hTERT gene expression level.

Our results showed that PKCe downregulation led to a decreased expression of the catalytic subunit of telomerase that was accompanied by protein level depletion. This effect was followed by significantly increased levels of Bax, caspase-3, and PARP-189kDa, proteins involved in apoptosis.

Conclusion: Our results identify an interesting link between PKCε and hTERT, which requires further investigation. Importantly, it implies potential novel therapeutic approach in the anticancer strategy.

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A small molecule targeted to the microtubule–kinetochore interaction induces an autophagic block by impairing autophagosome trafficking and fusion

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Drugs targeting the mitotic spindle are commonly used in cancer therapy and big efforts are currently undertaken to identify new targets among mitotic regulators. In a previous study, we identified SM15 as a small molecule able to bind microtubules at the interaction domain with the kinetochore protein Hec1. The compound showed high pro-apoptotic and anti-mitotic activities in in vitro and in vivo model systems (Orticello et al., Oncogene 2018). By using human tumor cell lines with different origin, we have then demonstrated that SM15 induces a significant increase in autophagic features, as revealed by the appearance of GFP-LC3B-II-associated autophagosomal puncta and by immunoblot detection of membrane-bound form of LC3.

Herein, we investigated the molecular and functional effects of SM15 on autophagic pathways in cancer cells. Combined treatment of SM15 with chloroquine, autophagosome maturation assay using RFP-EGFP-tandem fluorescent-tagged LC3B-II and immunofluorescence detection of lysosome markers demonstrated that SM15 blocks the fusion of correctly formed autophagosomes with lysosomes. Interestingly, autophagic arrest was associated with an impaired autophagosome trafficking, as shown by the lack of centrosomal enrichment of the vesicles. Biochemical and super-resolution microscopy experiments demonstrated a defective association of the centripetal motor protein dynein to microtubules in SM15-treated cells, which might be responsible for vesicle traffic impairment. Accordingly, we also detected an accumulation of Rab7-positive/LC3B-II negative vesicles. Overall, these findings indicate that SM15 could represent a promising agent for cancer treatment, not only by inducing cell death through mitotic catastrophe but also by affecting other pathways relevant to cell survival. Collectively, these results highlight the relevance of affecting multiple cancer-related pathways in the development of effective anti-cancer strategies.
Dual Blockade of RTK and Src abolishes the Cytokine-Driven Survival of Leukemia Cells

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Little is known about the molecular mechanisms of blood cancer therapy resistance. Receptor tyrosine kinases (RTK) KIT and FLT3 are overexpressed in the majority of acute myeloid leukemia (AML) patients, but RTK-inhibitors are not fully effective in the treatment of AML. Thus, we aimed at identifying novel regulatory circuits responsible for chemotherapy-induced death escape of leukemia cells.

We found that the treatment of leukemia cell lines with the cytotoxic agent Cytarabine and RTK-inhibitor Imatinib resulted in strong up-regulation of cytokine signaling with a notable increase in THPO, NGF and IL3 receptors (MPL, NTRK1, and IL3Ra) expression. Previously, we showed that NTRK1 overexpression is associated with core-binding factor (CBF) AML. The mining of publicly available datasets revealed MPL receptor up-regulation in CBF-AML. Interestingly, IL3Ra expression is high in all AML patients, but the role of IL3 in therapy resistance is unknown. We revealed higher levels of IL3Ra in AML patients with FLT3-itTD, suggesting its association with constitutively active RTKs. Among tested proteins, THPO and NGF show the most prominent protective action against Cytarabine and Imatinib when combined with IL3. We identified that for the survival of leukemia cells with activating mutations in RTK KIT IL3 alone is sufficient to protect cells from chemotherapeutic drugs. To identify key downstream regulators of NGF, THPO and IL3-dependent survival we treated leukemia cell lines with inhibitors of RTK downstream kinases - JAK2, MEK, and Src. JAK2, MEK or Src inhibitors (AG490, PD184352 or Bosutinib) enhanced Imatinib and Cytarabine-induced leukemia cell death, but only Bosutinib effectively blocked IL3-mediated survival. Thus, we provide novel evidence of IL3, NGF and THPO involvement in AML progression. Our findings suggest that combined targeting of RTK and Src could be beneficial in acute myeloid leukemia treatment.

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FoxM1 inhibition induces a pro-apoptotic gene signature in cancer cell anti-mitotic chemotherapy

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Mitotic targeting with microtubule (MT) poisons has for long been used in cancer chemotherapy. Despite extensive research to understand how MT poisons elicit distinct cell fate outcomes and how frequently resistance arises, the molecular mechanisms behind the response to chemotherapy remain elusive. Furthermore, cytotoxicity in untransformed cells and in relation to patients’ age is still unexplored. Using long-term phase-contrast time-lapse live cell imaging to track individual cell behavior, we observed increased sensitivity to MT poisons in elderly vs. young primary human dermal fibroblasts. Based on our previous observation that elderly mitotic fibroblasts exhibit global repression of genes regulated by the Forkhead Box M1 transcription factor, that primarily drives G2/M transition, we asked whether FoxM1 could account for the aging-associated sensitivity to anti-mitotics. Through both genetic and pharmacological inhibition of FoxM1 expression, we found cell fate decision in response to MT poisons to switch towards increased mitotic death vs. mitotic slippage. Transcriptomic analysis revealed several Bcl-2 family members to be regulated by FoxM1 and to impact in cell fate decision following prolonged mitotic arrest. Since FoxM1 is overexpressed in most solid tumors, being recognized as poor prognosis factor, a combinational treatment using FoxM1 inhibition plus MT poisons was tested in cancer cell lines. We found increased pro-apoptotic efficacy following the combinational treatment vs. single treatment. Our data discloses FoxM1 as a molecular determinant of cell fate in response to anti-mitotic chemotherapy, whose inhibition might act to increase mitotic death in cancer cells through the establishment of a pro-apoptotic gene signature similar to that observed in aged cells. This could be explored to decreased cytotoxicity and resistance side effects of anti-mitotic chemotherapy.
Amyloid Precursor Protein (APP) Promotes Tumorigenesis by Controlling Iron-mediated Oxidative Stress

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Virtually all living organisms depend on iron to perform basic cellular processes, such as cell growth and DNA synthesis. Cancer cells have to meet an increased iron demand and therefore rely on tightly regulated iron metabolism pathways to avoid excessive accumulation of free redox-cycling ferrous iron (Fe²⁺). The Alzheimer disease (AD) associated protein APP (amyloid precursor protein) exerts neuroprotective features that are intricately linked to iron metabolism, yet the functional attributes and downstream signaling pathways outside the nervous system remain unclear. Here, we identify that APP-deficient mouse embryonic fibroblasts develop Fe²⁺ overload that leads to oxidative stress, lipid peroxidation and DNA damage hindering cell growth, clonogenic survival and oncogenic transformation. In prostate cancer (PrCa) cells, knockdown of APP inhibits cancer proliferation, colony formation, anchorage-independent growth and renders them highly vulnerable against oxidative stress. Moreover, in PrCa patients high APP expression is associated with poor event-free survival. Through RNAseq and MAPK profiling, we discover p38MAPK stress signaling as a key mediator of oxidative stress-induced DNA damage in APP-deficient cells. Genetic ablation of p38a blocks the formation of toxic DNA double strand breaks and fully restores the APP-deficient phenotype. We reaffirmed this finding in clinical PrCa samples demonstrating that APP expression negatively correlates with p38MAPK activation. Collectively, we demonstrate that APP depletion results in pro-oxidative Fe²⁺ accumulation that effectively hinders oncogenic transformation and survival of PrCa cells.
Potential anti-melanoma activity of Imidazoline I1 receptor agonists

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Melanoma is the deadliest form of skin cancer. Despite the advancements in targeted BRAF and MEK therapy and immunotherapies, metastatic melanoma patients still have poor prognosis with a median survival of 9 months and a long-term survival rate of 10%. There is an urgent need for novel treatment modalities that target metastatic melanoma. Imidazoline I1 receptor (IR1, IRAS, NISCH) is a scaffolding protein that has been shown to be a tumor suppressor in breast cancer through regulation of cancer cell survival, motility and invasion. IR1 role in metastatic melanoma has not been investigated to date. Of importance, several IR1 agonists are clinically approved for treatment of hypertension. The aim of this study was to examine the IR1 expression in melanoma and the effects of IR1 agonists on melanoma cell viability. To confirm the target expression, we first determined IR1 levels in primary and metastatic melanoma patient samples by qRT-PCR and immunohistochemistry. We found that it is expressed in primary tumors and liver metastases and, to a lesser extent, in metastatic lymph nodes. Next, we examined the activity of IR1 agonists – rilmenidine, clonidine and moxonidine – in a panel of metastatic melanoma cell lines (HTB140, FemX-1, A375 and 518a2) and found that rilmenidine most potently inhibited cell viability. Notably, it was not toxic towards human dermal fibroblasts and keratinocytes. Furthermore, rilmenidine time- and dose- dependently induced cell cycle arrest in G2/M phase and consequent apoptosis. Our results imply that imidazoline I1 receptor is potentially novel anti-melanoma target, and that its already clinically approved agents have promising anti-cancer activity.
Melatonin induces apoptosis and autophagy of vincristine-resistant oral cancer cells through modulation of miR-34b and MAPK pathway

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Introduction: Multidrug-resistance was resistance of cells to a variety of drugs normally used in tumor treatment. The related mechanism of drug resistance in oral cancer has not been completely explained. Melatonin is an endogenously-produced molecule involved in active biological mechanisms including antiproliferation, oncogene expression modulation, antitumor invasion and migration, and anti-inflammatory, antioxidant, and antiangiogenic effects. Despite these numerous actions, the effect of melatonin on vincristine (VCR)-resistant human oral cancer cells still largely unknown.

Material and method: Cell growth was measured using an MTT assay. Cell autophagy was examined by acidic vesicular organelles (AVO) staining. LC3-II and mitogen-activated protein kinase (MAPK) pathway were evaluated using western blotting.

Results and discussion: In this study, we demonstrated that melatonin induces apoptosis and autophagy of VCR-resistant oral cancer cell; these actions are mediated by AKT, p38, and JNK pathway. Moreover, melatonin also inhibits ATP-binding cassette sub-family B member (ABCB)-1 and ABCB-4 expression in vitro and in vivo. In addition, melatonin decrease drug resistance and promotes VCR-resistant oral cancer cell execute apoptosis through upregulation of miR-892a and miR-34b-5p expression. The expression of miR-892a and miR-34b-5p are also related with melatonin-induced apoptosis, but not autophagy.

Conclusion: This is the first study to reveal the novel function of melatonin in activating autophagy and apoptosis, suggesting that melatonin could serve as a new and potential chemopreventive agent for VCR-resistant human oral cancer cell lines.
Effects of novel inhibitors of HSP90 and alvespimycin on breast cancer cells

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HSP90 is a molecular chaperon that is required for proper folding and maturing of different cell proteins. This high conservative protein interacts with more than 200 targets, including oncogenes and tumor suppressors. Thus, the inhibition of HSP90 in cells could lead to degradation of targets, and inhibitors can be used as antitumor agents.

Fluoroaryl dihydrobenzisoxazoles are novel perspective inhibitors of HSP90 chaperone with potentially high antitumor activity. In this project we synthesized a new series of compounds of this chemical class and screened them for the ability to kill breast cancer cells of various molecular subtypes, and analyzed the mechanisms of HSP90 inactivation as a major mechanism of cell death.

We measured cytotoxic activity of chemical agents by MTT-test and selected the lead compounds. Then we continued to study the antitumor activity of them and conducted a detailed molecular analysis of effects of these inhibitors. We demonstrated that leading compounds effected on the signaling pathways as PI3K/Akt/mTOR and NF-κB. In parallel with this investigation, the effect of 17-DMAG (alvespimycin), well-known HSP90 inhibitor, was analyzed. There were G2/M-stage arrest, increase of number of polyploid cells and p21 expression under 17-DMAG influence in HBL-100 cells expressing wild-type p53 protein. However, we observed the opposite effects on MDA-MB-231 cells with p53 mutation. Exactly, the decrease of p21 level and G1-stage arrest were demonstrated. It may be the result of MDA-MB-231 cells p53-status. Thus, p53-status may be important for realizing the therapeutic potential of new fluoroaryl dihydrobenzisoxazoles and 17-DMAG.

In the future, the lead new compounds are planned to serve as drug candidates for a (pre)clinical investigations.

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ROS and mitochondrial genetic abnormalities as novel biomarkers to predict drug efficacy

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Mitochondria are a primary intracellular site of ROS generation. Generally, mitochondrial genetic abnormalities (copy number change and mutations) have been reported in various cancer types, which might be associated with their elevated ROS levels compared to normal cells. Since excessive levels of ROS can trigger apoptosis, treating cancer cells with ROS-stimulating agents may enhance cell death. This study aimed to investigate the link between mitochondrial genetic abnormalities and baseline ROS levels and how they might influence cancer cells’ response to ROS-stimulating therapy.

Sanger sequencing was used to screen for mtDNA mutations and qPCR to measure mtDNA copy number (mtDNAcn) in one normal and four cancer cell lines. 3D structural modelling was used to assess the potential impact of non-synonymous mutations on protein structure and function within the electron transport chain. ROS levels were measured by plate-reader based assays and confocal microscopy using the H2DCFDA and MitoSox probes. Cells were treated with a conventional drug (cisplatin) and a mitochondria-targeting agent (dequalinium) separately and jointly. Cell viability and apoptosis events were detected using MTS assay and flow cytometry, respectively.

Our results showed a positive correlation between mitochondrial genetic abnormalities, intracellular ROS level and drug resistance. Larger numbers of mutations in the D-loop and complexes I/III coding regions were detected in cells with significantly higher ROS levels and greater drug resistance. Synergistic effect of both drugs was observed with ROS being the key contributor in cell death.

Our findings suggest that specific mutations in the complexes I/III coding regions could be efficient biomarkers to indicate response to ROS-stimulating therapy, and cancer cells with low intracellular ROS levels are more sensitive to the treatment. In addition, cancer cells carrying G13708A, A10398G, T11120C, C13802T and T14798C mutations might be more resistant to the treatment.
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