3rd EACR Conference
Making it Personal
Cancer Precision Medicine

5 - 7 November 2018
Bergamo, Italy

Scientific Programme Committee

Johanna Joyce, Richard Marais,
Daniel Peeper (Chair), Ze’ev Ronai

Programme Book
Making It Personal: Cancer Precision Medicine

Mass cytometry yields new insights into systems biology by dramatically increasing the amount of information obtained from each cell. The Helios™ system provides a robust way to measure over 40 protein parameters per single cell using proven CyTOF® technology.

In this session, Dr. Shahram Kordasti from King’s Health Partners-Cancer Research UK Cancer Centre will share his results using mass cytometry and multidimensional data analysis to identify two distinct subpopulations of regulatory T cells (Treg A and Treg B) with distinct phenotypes, gene expression, expandability and function in patients with aplastic anemia (AA) and myelodysplastic syndrome (MDS). This approach also identifies an immune signature that is predictive of response to immunotherapy at the time of diagnosis of AA or MDS, which may allow a more patient-specific approach to future treatment decisions.

Visit us at Stand 8
November 5–7, 2018
Bergamo, Italy

When: Tuesday, November 6
11:45 - 12:15

Venue: Centro Congressi
Giovanni XXIII

Presenters:

Amy Hamilton
Senior Marketing Manager,
Fluidigm Corporation
A Brief Introduction to Mass Cytometry

Shahram Kordasti,
MD, PhD
Senior Lecturer
KHP-CRUK Cancer Centre
Bench to Bytes: Translating Big Data to the Clinic
### Day 1 - Monday 05 November

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15.30 – 15.50
Ze’ev Ronai Technion Integrated Cancer Center, Israel
“From genes to microb(iota) and back”

16.00 – 16.20
Fatima Mechtia-Grigoriou Institut Curie, France
“Fibroblast heterogeneity in immunosuppression in breast cancer”

16.30 – 16.40
Oded Sandler Weizmann Institute of Science, Israel
Proffered Paper 3: “Integrative Precision Therapy for overcoming innate drug resistance”

16.45 – 17.30
MEET THE EXPERT
Andrea Califano Columbia University, USA
Keynote speaker Prof. Califano will share his perspective on recent advances in single cell sequencing, as well as current challenges in the field.

17.30 – 19.00
POSTER DISCUSSION SESSION 1 & TRADE EXHIBITION Hall Bar and Foyer
Prosecco & finger buffet. Posters #1-28 will be presented.

Day 2 - Tuesday 06 November

08.30 – 09.00
POSTER VIEWING AND TRADE EXHIBITION

08.40 – 08.55
INDUSTRY SPOTLIGHT - BIO-RAD Oggioni Lecture Hall
Marzia Del Re Pisa University Hospital
“Liquid biopsy from activating mutations to target expression: the horizon is expanding”

SESSION 2: IMMUNO-ONCOLOGY
Session Chair: Itay Tirosh

09.00 – 09.20
Inge Marie Svane CCIT Herlev Hospital, Denmark
“Adoptive cell therapy based on TILs in the era of check point inhibitors”

09.30 – 09.50
Shai Shen-Or Technion, Israel
“High resolution longitudinal immune profiling reveals a clinically meaningful metric from dynamics of healthy immune-aging towards an older adult homeostasis”

10.00 – 10.10
Richard Crispin Institute for Cancer Research, Norway
Proffered Paper 4: “Development of small molecules for targeting the down-regulation of PDL1 in the potential immunotherapeutic treatment of leukaemia”
10.15 – 10.45  ☕ COFFEE BREAK & TRADE EXHIBITION

10.45 – 11.05  Silvia Formenti  Weill Cornell Medicine, USA
Q&A: 11.05 – 11.15  “DNA Damage Response and Immune Rejection of Cancer”

11.15 – 11.35  Ilaria Malanchi  The Francis Crick Institute, UK
Q&A: 11.35 – 11.45  “New protagonists of the tumour microenvironment”

11.45 – 12.15  INDUSTRY SYMPOSIUM - FLUIDIGM
Amy Hamilton  Fluidigm Corporation, UK
“A Brief Introduction to Mass Cytometry”
Shahram Kordasti  King’s College London, UK
“Bench to Bytes: Translating Big Data to the Clinic”

12.15 – 12.45  ✕ BUFFET LUNCH & TRADE EXHIBITION

12.45 – 14.15  POSTER DISCUSSION SESSION 2
Posters #29-55 will be presented.

SESSION 3: DORMANCY & TOLERANCE
Session Chair: Richard Marais

14.15 – 14.35  Alberto Bardelli  Candiolo Cancer Institute, Italy
Q&A: 14.35 – 14.45  “Inactivation of DNA repair to improve immune surveillance”

14.45 – 15.05  Christoph Klein  University of Regensburg, Germany
Q&A: 15.05 – 15.15  “The dynamics of metastatic spread”

15.15 – 15.25  Silvia Marsoni  FIRC Institute of Molecular Oncology, Italy

15.30 – 15.40  Marija Buljan  ETH Zurich, Switzerland
Q&A: 15.40 – 15.45  Proffered Paper 6: “Identifying protein interactions relevant in cancer development”

15.45 – 16.15  ☕ COFFEE BREAK & TRADE EXHIBITION

16.15 – 16.35  Maria Soengas  CNIO, Spain
Q&A: 16.35 – 16.45  “MetAlert mice: a platform for whole-body imaging and pharmacological targeting of metastatic niches”
16.45 – 17.05  
**Eytan Ruppin**  NCI (NIH), USA  
“Harnessing genetic interactions to advance whole genome precision oncology”

**Q&A:** 17.05 – 17.15

17.15 – 18.00  
**PANEL DISCUSSION:** How can we make it personal?  
*Join the discussion on the opportunities and challenges for the field. The panel will share their thoughts, and there will be plenty of time for comments and questions.*

18.00 – 19.30  
**OPTIONAL WALKING TOUR OF BERGAMO**  
*From venue to Città Alta. For those who have pre-registered only.*

19.30  
**OPTIONAL CONFERENCE DINNER**  
*Taverna Colleoni*  
*For those who have purchased tickets.*

### Day 3 - Wednesday 07 November

08.30 - 09.00  
**POSTER VIEWING AND TRADE EXHIBITION**

**SESSION 4: RATIONAL COMBINATIONS**  
Session Chair: Daniel Peeper

09.00 – 09.20  
**Jean-Christophe Marine**  VIB-KU Leuven Center for Cancer Biology, Belgium  
“Preventing Therapy-induced Cancer Stemness”

**Q&A:** 09.20 – 09.30

09.30 – 09.50  
**Richard Marais**  CRUK Manchester Institute, UK  
“Making it personal in melanoma”

**Q&A:** 09.50 – 10.00

10.00 – 10.20  
**Paul Workman**  The Institute of Cancer Research, London, UK  
“Targeting non-oncogene addiction with HSF1 pathway inhibitors: From phenotypic screen to chemical probe and preclinical development candidate”

**Q&A:** 10.20 – 10.30

10.30 – 11.00  
**COFFEE BREAK & TRADE EXHIBITION**

11.00 – 11.10  
**Gabriele Picco**  Wellcome Sanger Institute, UK  
*Proffered Paper 7:* “Functional linkage of gene fusions to cancer cell fitness assessed by pharmacological and CRISPR/Cas9 screening”

**Q&A:** 11.10 – 11.15
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 and accommodation.

Kate Eason UK
Olívia Pontes Portugal
Chloé Prunier Netherlands
Mariya Shapovalova USA
Xing Xu Germany

11.15 – 11.35 Daniel Peeper NKI, Netherlands
Q&A: 11.35 – 11.45 “Towards rational combinatorial cancer treatment – a functional genomics approach”

11.45 – 12.15 CLOSING KEYNOTE LECTURE
Q&A: 12.15 – 12.30 David Solit MSKCC, USA
“Defining the actionable genome”

11.45 – 12.15 CLOSING KEYNOTE LECTURE
Q&A: 12.15 – 12.30
“Defining the actionable genome”

Scientific Programme Committee

12.30 – 12.45 CLOSING SUMMARY AND PRESENTATION OF AWARDS

12.45 – 13.30 LUNCH, TRADE EXHIBITION CLOSES & DEPART
We fund research into any type of cancer anywhere in the world.

From the world’s best research institutions and renowned specialists to unexpected and diverse projects by up and coming talent. We fund all types of research and for one very good reason – to gain a global perspective. Because research doesn’t happen in isolation. And the answers will not come from one scientist, in one lab, in one country. That’s why Worldwide Cancer Research are prepared for whatever it takes and wherever it takes us.

Worldwide Cancer Research is a charity registered in Scotland, No: SC022918
Interactive activities at the Conference

An important part of the EACR Conference Series 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.

Meet the Expert talk

**Monday 05 November at 16.45**

**Andrea Califano** Columbia University, USA

As a leading scientist in the area of single cell sequencing, Prof. Califano will share his perspective on the current state of the field. Methodologies and recent developments will be discussed, and there will be an opportunity for you to ask your questions.

Poster Discussion Sessions

**Monday 05 November 17.30 – 19.00** Posters 1-28 will be presented.

**Tuesday 06 November 12.45 – 14.15** Posters 29-55 will be presented.

The programme features two 90 minute Poster Discussion Sessions which will be an excellent opportunity to present and discuss the latest research. As well as these dedicated sessions, there will be additional opportunities to browse the posters throughout the meeting.

Two poster prizes of €100 will be awarded by Clinical and Experimental Metastasis for the best posters as selected by the judges.

Poster Viewing

**Tuesday 06 November 08.30 – 09.00**

**Wednesday 07 November 08.30 – 09.00**

Tuesday and Wednesday morning will begin with an optional poster viewing slot. Participants are invited to use this time for further discussion in the poster areas, but presenters are not required to be by their posters at these sessions. Coffee and tea will be available.
Panel Discussion

Tuesday 06 November 17.15 – 18.00

The panel, comprised of invited speakers, will tackle the topic “How can we make it personal?”. Time will be allocated for open questions, and all participants are invited to share their thoughts. The session promises to be a highlight of the meeting.

Walking Tour of Bergamo

Tuesday 06 November 18.00 – 19.00

Join this social event to explore the fascinating city of Bergamo on foot, led by expert local guides. The tour will leave directly from the conference venue after the conclusion of the programme, and will finish in the centre of the ancient Città Alta, a UNESCO World Heritage Site.

For participants who signed up in advance.

Conference Dinner

Tuesday 06 November 19.30

The Conference Dinner will take place on the final night of the meeting at the Ristorante La Taverna Colleoni, in the centre of historic Bergamo. 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.

For participants who have purchased a ticket only.

Don’t forget to let us have your feedback about these activities in the survey we will send after the conference!
Meet the Exhibitors

Bio-Rad Laboratories Ltd
Website: www.bio-rad.com
Contact: contact_italy@bio-rad.com
Represented at the conference by: Marco Bianchi and Carola Cassani

Describe Bio-Rad Laboratories in 5 words or less
Bio-Rad drives the innovation

Tell us a little bit about Bio-Rad Laboratories
Bio-Rad Laboratories is a world leader in providing products for the life science research and diagnostic markets. In our Life Science Group, we build the industry leading solutions for oncology research, including the highly sensitive Droplet Digital™ PCR technology and our new technology for single-cell sequencing, the ddSEQ™ Single-Cell Isolator.

Why are you attending the conference? Who would you like to meet at the conference?
We want to play a key role in the Translational Research area especially in the immuno-oncology field by offering innovative and useful technologies that help accelerating the discovery process to obtain faster and better results. We would like to meet KOL, PI and Biotech PM.

Fluidigm
Website: www.fluidigm.com
Contact: info-europe@fluidigm.com
+33 1 60 92 42 40
Represented at the conference by: Amy Hamilton and Roberto Spada

Describe Fluidigm in 5 words or less
Intuitive, intelligent, inspirational and collaborative

Tell us a little bit about Fluidigm
Fluidigm is committed to empowering the cancer community with research tools for the comprehensive interrogation of immune cell function and the tumor microenvironment. Using proven mass cytometry and microfluidic technologies, we provide workflows to identify cell signatures and characterize changes in cellular phenotypes at single cell resolution.

Why are you attending the conference? Who would you like to meet at the conference?
Researchers from academic institutions, clinical research laboratories, and pharmaceutical, biotechnology companies worldwide.
Genomix4Life S.r.l
Website: www.genomix4life.com
Contact: info@genomix4life.com
Represented at the conference by: Maria Ravo and Giovanna Marchese

Describe Genomix4Life in 5 words or less
Efficient, innovative, creative

Tell us a little bit about Genomix4Life
Our mission is to identify new diagnostic tools in the field of personalized medicine, creating know-how and effective approaches to improve human health. Genomix4Life is a leading provider of genomic services and solutions, in medicine, forensics, animal husbandry and veterinary, agrifood and all other fields where these technologies find useful applications.

Why are you attending the conference? Who would you like to meet at the conference?
We are attending this conference mainly for networking purposes, to meet the keynote speakers and advise them about the broad range of analyses we can offer, to create connections with researchers and with potential customers interested in our services.

Nonacus
Website: www.nonacus.com
Contact: info@nonacus.com
Represented at the conference by: Chris Sale and David Hughes

Describe Nonacus in 5 words or less
Innovative solutions for non-invasive diagnostics

Tell us a little bit about Nonacus
Nonacus means ‘no needles’, everything designed at Nonacus facilitates non-invasive diagnostics. We have developed a complete workflow for cfDNA analysis, from our blood stabilisation tubes to cfDNA extraction to our NGS target enrichment method. Please visit us to discuss your project needs.

Why are you attending the conference? Who would you like to meet at the conference?
Do you have a challenging project and need some advice or are just starting to experiment with cell-free DNA then please come and visit our stand 7 to discuss: blood collection, cfDNA extraction from plasma, CSF or saliva and targeted NGS on cfDNA or CTC’s.
The organisers wish to express their appreciation for the support provided by sponsors at the EACR Conference *Making it Personal: Cancer Precision Medicine*. Their interest and enthusiasm for the conference has enabled the organisers to provide an impressive scientific programme.

**Elite Sponsor**

FLUIDIGM

**Premium Sponsor**

BIO-RAD

**Classic Sponsors**

GenomiX 4Life

NONACUS

Advancing Non-Invasive Healthcare

**Grant**

The Company of Biologists

Roche

This activity has been supported by a grant from F. Hoffman-La Roche Ltd, which has had no control over the educational content of this activity.
We are pleased to announce that Fluidigm will not only be exhibiting at the conference but also inviting participants to join their Industry Symposium.

**Tuesday 06 November, 11.45 – 12.15**

**Amy Hamilton** Fluidigm Corporation, UK  
“A Brief Introduction to Mass Cytometry”

**Shahram Kordasti** King’s College London, UK  
“Bench to Bytes: Translating Big Data to the Clinic”

**Industry Spotlight - Premium sponsor**

**Tuesday 06 November, 08:40 – 08.55**

**Marzia Del Re** Pisa University Hospital  
“Liquid biopsy from activating mutations to target expression: the horizon is expanding”

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**EACR Industry Partners**

The European Association for Cancer Research gratefully acknowledges the organisations that support the Association as Industry Partners. Industry Partners offer ongoing support to the EACR and provide the means for the Association to develop important initiatives. The EACR Conference Series is an example of this.
Use of targeted inhibitors in precision cancer medicine is largely predicated on the identification of actionable oncogene mutations. Yet, only ~25% of human malignancies present with actionable alterations, and only 5% to 10% of all patients benefits from targeted therapy in terms of progression free survival. Most critically, even among patients who initially respond, a majority will eventually relapse with drug-resistant disease. Thus, there is urgent need for complementary precision cancer medicine approaches that focus on protein targets representing individual and synergistic tumor vulnerabilities, independent of their mutational status.

To address this challenge, we have developed network-based methods for the systematic identification, validation, and pharmacological targeting of a new class of therapeutic targets. These targets comprise Master Regulator proteins, whose concerted aberrant activity within tightly regulated modules (tumor checkpoints) is responsible for the mechanistic implementation and maintenance of specific tumor cell states. We have identified and validated tumor checkpoints for multiple tumor types, from glioblastoma and lymphoma to breast and prostate adenocarcinoma and shown that they implement complex regulatory bottlenecks, whose genetic or pharmacologic inhibition abrogates tumor viability in vitro and in vivo and can modulate immunoevasion. Finally, we have developed methodologies that leverage large-scale drug-perturbation assays to systematically identify drugs and drug combinations whose mechanism of action is specifically effective in abrogating tumor checkpoint activity on an individual patient basis. To systematically evaluate this approach, we have opened a novel N-of-1 study, which has already enrolled more than 100 patients, across 14 tumor subtypes, who have progressed on multiple lines of therapy. Therapeutic value of computationally predicted therapies is first evaluated in patient-derived xenografts (PDX) and/or organotypic cultures and ultimately used to guide patient therapy. So far, of 39 drugs prioritized by this approach across seven diverse malignancies, 23 produced objective response in PDX models and four out of five treated tumors had clinical response in patients.
A unified model integrating cellular states and genetics for glioblastoma

Cyril Neftel¹, Julie Laffy², Mariella Filbin¹, Mario Suva¹, Itay Tirosh²

¹ Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA, ² Weizmann Institute of Science, Rehovot, ISRAEL

Glioblastoma is an incurable malignancy that remains poorly understood. Heterogeneous genetic, epigenetic and developmental programs are thought to drive glioblastoma, but their precise characterization remains challenging. Here we propose an integrative approach to model glioblastoma, combining single-cell RNA-sequencing of 28 tumors with bulk genetic and expression analysis of 401 specimens from the TCGA. We find that malignant cells in glioblastoma exist in four main cellular states that recapitulate distinct steps of neural development, are influenced by the tumor micro-environment, and exhibit plasticity. Importantly, we show that while each glioblastoma contains similar cellular states, their relative frequency varies between tumors and is influenced by chromosomal aberrations that each influence a particular state. By providing a roadmap of the cellular programs of malignant cells in glioblastoma and their modulation by genetic drivers, our work proposes a unifying model for glioblastoma.
Proffered Paper 1

Spatial Transcriptomics Analysis of Castration-Resistant Prostate Cancer

Maja Marklund\textsuperscript{3}, Niklas Schultz\textsuperscript{4}, Emelie Berglund\textsuperscript{3}, Jonas Maaskola\textsuperscript{3}, Stefanie Friedrich\textsuperscript{1}, Joseph Bergenstråhle\textsuperscript{3}, Firas Tarish, Anna Tanoglidi\textsuperscript{2}, Yao Liu\textsuperscript{3}, Patrik Ståhl\textsuperscript{3}, Erik Sonnhammer\textsuperscript{1}, Thomas Helleday\textsuperscript{4}, Joakim Lundeberg\textsuperscript{3}

\textsuperscript{1} Department of Biochemistry and Biophysics, Stockholm University, Science for Life Laboratory, Solna, SWEDEN, \textsuperscript{2} Department of Clinical Pathology, University Uppsala Hospital, Uppsala, SWEDEN, \textsuperscript{3} Department of Gene Technology, School of Chemistry, Biotechnology and Health, Royal Institute of Technology (KTH), Science for Life Laboratory, Solna, SWEDEN, \textsuperscript{4} Division of Translational Medicine & Chemical Biology, Karolinska Institutet (KI), Science for Life Laboratory, Solna, SWEDEN

Almost all men with advanced metastatic prostate cancer (PCa) respond initially to hormonal therapy, while the treatment stops working and resistance emerges after time, leading to a lethal stage of the disease called castration-resistant prostate cancer (CRPC). Preclinical studies have implicated androgen receptor (AR) signaling as the primary driver of CRPC. A greater understanding of biomarkers of response as well as mechanism of resistance is urgently needed. Analysis of tumor gene expression could enhance our understanding of this. So far, there have been very few studies including the subclonal architecture of tumors pre- and post-treatment. These studies have mainly focused on AR activity, whereas it is likely that multiple genes are involved in treatment failure. Here, we present a spatially resolved transcriptome approach (Ståhl et al. Science 2016), adapted to core needle samples before and after anti-androgen treatment for metastasized prostate cancer. We reveal multiple distinct resistant clones arising within the same prostate during the course of treatment. Notably, we also observe several shared clones between patients in response to treatment, including genes that are both non- and AR- dependent. Further, AR-loss in stromal cells has shown to correlate with more aggressive cancer and CRPC, and our analysis delineated full gene expression profiles of stroma cells lacking AR activity compared to stromal cells still having AR.

Our results indicate that a spatially resolved molecular analysis of gene expression in prostate cancer needle biopsies has the potential to improve diagnostics, by allowing a more defined categorization of the tumors. This would enhance our ability to prevent resistance while improving the accuracy of personalized medicine.
Despite its success in several clinical trials, cancer immunotherapy remains limited by the rarity of targetable tumor-specific antigens, tumor-mediated immune suppression, and toxicity triggered by systemic delivery of potent immunomodulators. Here, we present a proof-of-concept immunomodulatory gene circuit platform that enables tumor-specific expression of immunostimulators, which could potentially overcome these limitations. Our design comprised de novo synthetic cancer-specific promoters and, to enhance specificity, an RNA-based AND gate that generates combinatorial immunomodulatory outputs only when both promoters are mutually active. These outputs included an immunogenic cell-surface protein, a cytokine, a chemokine, and a checkpoint inhibitor antibody. The circuits triggered selective T cell-mediated killing of cancer cells, but not of normal cells, in vitro. In in vivo efficacy assays, lentiviral circuit delivery mediated significant tumor reduction and prolonged mouse survival. Our design could be adapted to drive additional immunomodulators, sense other cancers, and potentially treat other diseases that require precise immunological programming.
From genes to microb(iota) and back

Ze’ev Ronai\textsuperscript{2,1}
\textsuperscript{1} Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA, \textsuperscript{2} Technion, Israel Institute of Technology, Haifa, ISRAEL

Personalized medicine can be defined based on genetic and epigenetic signatures, or their combination. I shall discuss two examples for epigenetic-based studies focused on the ubiquitin ligase RNF5, in breast cancer and in melanoma, that helped guide personalized medicine.

RNF5 is an E3 ubiquitin ligase which has been implicated in the control of misfolded protein, through its role in ER associated degradation (ERAD). Our studies in breast cancer derived cell lines identified that RNF5 binds to misfolded SLC1A5 and SLC38A2, two of the glutamine carrier proteins, leading to their ubiquitination and degradation. Treatment of breast cancer cells with chemotherapy (i.e. Taxanes) causing ER stress, results in degradation of SLC1A5/38A2 and inhibition of glutamine uptake, resulting in reduced mTOR activity, activation of autophagy and cell death programs. While preserved in about 30% of BCa tumors, this mechanism is lost in large fraction of BCa, including triple negative BCa, resulting in high level of glutamine and resistance to taxane-based therapy. Reducing the level of glutamine in these tumors, enables their response to taxanes (i.e. paclitaxel), finding that directed multicenter clinical trials led by Calithera Biosciences, for BCa, which stratify tumors that exhibit high level of glutamine or glutamine carrier proteins for paclitaxel treatment. Personal marker-based medicine.

In a more recent study we discovered that mice lacking RNF5 limit growth of tumors, including melanoma and colon cancer, owning to an effective anti-tumor immunity, engaging toll like receptors and dendritic cells, that are initially activated at the intestinal milieu. Further, the anti-tumor immune response was found to be dependent on gut microbiota composition seen in the \textit{Rnf5}\textsuperscript{-/-}mice. Co-housing of WT and \textit{Rnf5}\textsuperscript{-/-}mice abolished anti-tumor immunity and tumor rejection phenotypes. We identified components of the unfolded protein response as driver of gut microbiota composition and anti-tumor immunity, and confirmed altered UPR signaling in responders to immune checkpoint therapy of mice and men.
Fibroblast heterogeneity in immunosuppression in breast cancer

Ana Costa¹², Yann Kieffer¹², Anne Vincent-Salomon¹², Fatima Mechta-Grigoriou¹²

¹ Institut Curie, Inserm, Paris, FRANCE, ² Stress and cancer lab, Paris, FRANCE

Tumors are complex ecologies that are affected by numerous stromal factors that dampen or enhance the effects of genetic epithelial alterations. CAF constitute one of the most abundant stromal components in solid tumors (Toullec, EMBO Mol. Med., 2010; Mateescu, Nat. Med. 2011; Lefort, Oncogene, 2017). By studying 6 stromal markers concomitantly and combining various approaches, we identified 4 CAF subsets (CAF-S1 to CAF-S4) in breast cancers (Costa, Cancer Cell, 2018). We confirmed their existence in ovarian cancers (Givel, Nat. Commun, 2018), showing the relevance of our findings in distinct cancer types. CAF-S2 and CAF-S3 are non-activated, while CAF-S1 and CAF-S4 are myofibroblasts. CAF-S3 accumulate in healthy tissues. LumA breast cancers are enriched in CAF-S2 cells, HER2 in CAF-S4 and triple-negative in CAF-S1 and CAF-S4. We found that CAF-S1 fibroblasts promote immunosuppression through a multi-step mechanism. CAF-S1 attract T CD4+CD25+ T lymphocytes, enhance their survival, stimulate their differentiation into CD25HighFOXP3High and promote the capacity of regulatory T cell to inhibit T effector proliferation. We uncovered several molecules, expressed by CAF-S1 fibroblasts, which are involved in the different steps of the CAF-S1-mediated immunosuppressive activity (Costa, Cancer Cell, 2018; Givel, Nat. Commun, 2018).

Recent publications of the lab:
Gentric, Cell Metabolism, In press
Costa A., Cancer Cell, 2018 Mar 12;33(3):463-479.e10
Givel AM, Nature Communications, 2018, Mar 13;9(1):1056
Lefort S, Oncogene. 2017, Mar 2;36(9):1211-1222
Gruosso T, EMBO Molecular Medicine. 2016 May 2;8(5):527-49
Proffered Paper 3

Integrative Precision Therapy for overcoming innate drug resistance

Oded Sandler6, Nancy Gavert6, Nitzan Friedlander6, Nitzan Tal6, Adi Jacob-Berger6, Ehud Zigmond6, Chen Varol6, Dennie Tompers Frederick4, Genevieve Marie Boland4, Kevin Shee5, Michal Barzily-Rokni1, Sangeeta N. Bhatia3, Keith Flaherty4, Ravid Straussman6

1 Broad Institute of MIT and Harvard, Cambridge, USA, 2 Geisel School of Medicine at Dartmouth, Hanover, USA, 3 Health Sciences and Technology/Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, USA, 4 Massachusetts General Hospital Cancer Center, Boston, USA, 5 The Research Center for Digestive Tract & Liver Diseases, Tel-Aviv Sourasky Medical Center, Tel Aviv, ISRAEL, 6 Weizmann Inst. of Science, Rehovot, ISRAEL

Precision anti-cancer therapy, where targeted drugs are tailored to tumor specific genetic abnormalities, has remarkably improved response rates over the last decade. Unfortunately, the immediate response to treatment is frequently sub-optimal due to innate resistance, resulting in partial rather than complete eradication of cancer cells. As the tumor microenvironment (TME) is known to contribute to innate resistance, we aimed to expand the concept of precision anti-cancer therapy by simultaneously blocking patient-specific TME secreted factors that can mediate innate drug resistance.

To characterize the effect of the TME secretome on innate drug resistance, we performed 200 high-throughput screens evaluating the effect of 321 TME factors (e.g. growth factors, cytokines, chemokines, hormones) on the response of 60 cancer cell lines (8 different cancer types) to 35 clinically relevant anti-cancer drugs. We found that the effect of TME factors on drug resistance may be drug-specific or tissue-specific. We also demonstrated that targeted therapies were more susceptible to the effect of TME-factors vs. cytotoxic therapies. Using available databases, as well as immunofluorescence staining, we demonstrated that TME factors that can mediate drug resistance are highly variable between patients. High variability exists even in tumors with the same origin and/or driver mutations, making the case for TME-specific therapy. Lastly, using in vitro co-culture systems and ex vivo 3D tumor models, we demonstrated the superiority of precision treatments integrating data from both the somatic mutation landscape of the tumor as well as its microenvironment makeup.

We thus propose Integrative Precision Therapy (iPT) as a promising approach to increase initial drug response by reducing innate resistance, which might ultimately lead to improved overall survival.
Meet the Expert

Andrea Califano¹
¹ Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY, USA

The ability to study tissues at the single cell level promises to transform our ability to investigate tissue heterogeneity, cell-cell communication processes, and lineage differentiation. Specifically, this supports two main objectives: first, the ability to avoid averaging over multiple cells, representing different transcriptional states. Second, the ability to dissect cells representing fundamentally distinct tissues with significant lineage-mediated epigenetic differences. However, while single cell analysis has been very effective in allowing identification of cell sharing similar transcriptional states, as revealed by genome-wide clustering algorithms, the ability to study biology at the single cell level is still dramatically hampered by the low depth of sequencing provided by current technology. In addition, with an average of 200K mRNA molecules per cell, achieving greater sequencing depth is unlikely to provide greater resolution and rather results in detecting the same mRNA molecule over and over (PCR artifacts). As a result, on average, only 20% of expressed genes are detected by one or more reads on the corresponding mRNA and most of them by a single read, also known as “gene dropout.” In this session, we will discuss some of the methodologies for single cell profiling and perturbation, including droplet and well-based methodologies, as well as methodologies for single cell CRISPRi perturbation, such as Perturb-Seq and CROP-Seq. In addition, we will discuss some of the core algorithms used for single cell clustering and analysis, including recent development in network-based methodologies that address gene-dropouts by using multiplex reporters for the activity of >6,000 proteins, thus resulting in a completely quantitative assessment of single cell protein activity.
Adoptive cell therapy based on TILs in the era of check point inhibitors

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TIL ACT approach can mediate complete and durable responses in 10%-20% of patients with metastatic melanoma, and can also yield clinical responses in other selected types of solid tumors. The presentation will include data on predictive markers for response, the role of neo-epitopes, immune escape mechanisms, as well as data on TIL ACT in anti-PD1 refractory patients and TIL ACT in combination with check point inhibitors.
High resolution longitudinal immune profiling reveals a clinically meaningful metric from dynamics of healthy immune-aging towards an older adult homeostasis

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Immune responses generally decline with age. We used multiple ‘omics’ technologies to capture population- and individual- level changes in the human immune system of 135 healthy adult individuals of different ages sampled longitudinally over a nine-year period. We observe a high inter-individual variability in the rates of change of cellular frequencies that correlate with baseline values, allowing identification of steady state levels towards which a cell subset converges and the ordered convergence of multiple cell subsets towards an older adult homeostasis. These form a high dimensional trajectory of immune-aging (IMM-AGE) that describes a person’s immune status better than chronological age. We show that taking an individual’s immune age into account increases the power to predict clinical outcomes. IMM-AGE score is associated with clinical outcomes.
Proffered Paper 4

Development of small molecules for targeting the down-regulation of PDL1 in the potential immunotherapeutic treatment of leukaemia

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The front-line treatment of many leukaemias relies on aggressive chemotherapy and targeted therapies, and while effective, patient survival remains unsatisfactory, highlighting the need for new therapeutic options. Recently, using high-throughput, competition-based screening, our lab discovered a handful of compounds that can induce the rapid degradation of c-Myc, a favourable target often considered ‘undruggable’. c-Myc is a critical transcription factor, deregulated in many types of cancer and essential for cancer cell proliferation, survival and immune-invasion. The expression of several immune-suppressing receptors, such as programmed death ligand 1 (PDL1), are regulated by c-Myc and important for cancer cell evasion from the immune system. Our data highlights how the small molecule, DJ34, can rapidly induce post-translational degradation of c-Myc in AML cells. DJ34 treatment also activated p53 and induced apoptosis, cell cycle arrest and cell differentiation, which inhibited survival of primary leukaemia cells ex vivo and in a zebrafish PDX model. Crucially, however, we show DJ34 treatment can directly down-regulate PDL1 cell surface and total cell expression in AML cells, important for T-cell recognition (‘Don’t See Me’ signal). Importantly, DJ34 induced proteasomal degradation of c-Myc, likely through specific disruption of c-Myc protein mis-folding yet bypassed the requirement for Cullins, which are frequently inactivated in leukaemia. We hypothesise that our small molecule compounds that can trigger c-Myc degradation have the potential for dual action in both initiating cancer cell apoptosis and promoting anti-cancer immune response. Further, we now begin to explore whether DJ34 may also prove effective in many ‘Myc-addicted’ cancers, i.e. those cancers were c-Myc is an essential oncogene required for survival and progression.
DNA Damage Response and Immune Rejection of Cancer

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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. 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 setting of clinical cancer, however, abscopal effects are extremely rare, because of immune-suppressive characteristics of established solid tumors (Curr Probl Cancer 2016;40;25-37). Thus, strategies to exploit the pro-immunogenic effects of radiotherapy require combination with immunotherapy: experiments in several syngeneic mouse models that mimic the setting of advanced cancer have demonstrated the promise of combining radiation with immune checkpoint blockade (ICB) (Clin Cancer Res. 2005;11:728-734). Radiation can compensate tumors with a low mutational load, by inducing de novo T cell priming to multiple tumor antigens and therefore, achieve responses in the absence of pre-existing neoantigens. Anecdotal clinical examples confirming the preclinical data (Trends Cancer 2016:2,6:286-294). Currently, multiple clinical trials are exploring optimal regimens of radiotherapy and immunotherapy, with some initial success. The issue of dose and fractionation seems to be particularly relevant to abscopal responses. A mechanism underlying the dose dependence of abscopal response was recently elucidated (Nature Communications 2017; Jun 9;8:15618). In mice bearing bilateral TSA murine breast carcinoma, when combined with ICB a single dose of 20 or 30Gy achieved comparable in field control to that of a regimen of 8GyX3 fractions, but only the fractionated regimen induced abscopal responses. Radiation-generated double strands (ds) DNA fragments reach the cytoplasm of irradiated cells where they are “sensed” by the cGAS/STING pathway (cGAS=cyclic GMP-AMP synthase and its adaptor protein STING= stimulator of interferon genes, aka transmembrane protein 173 – TMEM173). cGAS binds cytosolic dsDNA to initiate interferon (IFN-I) responses upon STING stimulation, resulting in dendritic cell recruitment and cross-priming of effector T-cells, the key steps to convert the tumor into an in situ vaccine. When tested in multiple carcinoma murine and human carcinoma cells lines and tumors, as the radiation dose per fraction increased, cytosolic dsDNA was found to accumulate to a threshold above which induction of three prime repair exonuclease 1 (Trex1) occurred, an enzyme that degrades cytoplasmic DNA. Single doses in excess of 10-12Gy induced Trex1 to rapidly degrade cytosolic dsDNA, the substrate for cGAS/STING. As a result, signaling to induce IFN was abrogated, impairing RT-induced abscopal effects. Consideration to these findings suggests that a hypo-fractionated regimen, ideally with 3-5 doses of less than 10-12 Gy each, should be used when radiotherapy is combined with ICB immunotherapy.
New protagonists of the tumour microenvironment

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Cancer cells behaviour is strongly influenced by their surrounding cellular environment, which makes the characterization of the local tumour microenvironment (or niche) a fundamental question in tumour biology. To date, a direct investigation of the early cellular changes induced by metastatic cells in the surrounding tissue in vivo is difficult to achieve, especially at early micro-metastatic stages. We have developed a strategy whereby metastatic cancer cells directly label their neighbouring cells, allowing identification of metastatic niche cells within the whole tissue from early micro-metastasis to late macro-metastasis. We have used this strategy to dissect the early breast cancer metastatic niche within the lung and uncovered a remarkable local lung epithelial regenerative response. The perturbed lung epithelial cells fuel cancer cell growth, which is further enhanced by changes in niche myeloid cells. Strikingly, lung alveolar epithelial cells in the niche show stem-cell features with multi-lineage differentiation potential. We confirmed the abundance of metastatic-promoting factors within the local tissue environment and observed Wnt signalling activity in the parenchyma of the lung metastatic niche. Additionally, we report WNT1 inducible signalling pathway protein 1 (WISP1) as a new factor involved in the protumorigenic activity of epithelial niche cells. In summary, here we describe a novel labelling system that enables spatial resolution of the metastatic microenvironment and, by applying this strategy to dissect the early lung metastatic niche, we highlight the potential of this methods as a platform for new discoveries.
Inactivation of DNA repair to improve immune surveillance

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When metastatic cancers are challenged with targeted agents almost invariably a subset of cells insensitive to the drug emerges. As a result, in most instances, targeted therapies are only transiently effective in patients. Strategies to prevent or overcome resistance are therefore essential to design the next generation of clinical trials. How can we overcome the near-certainty of disease recurrence following treatment with targeted agents?

Addressing this question means considering as a target not “only” individual oncogenes but also the evolving nature of human tumors. We used colorectal cancer (CRC) as a model system to test the hypothesis that by understanding tumor’s evolution, the emergence of drug resistance can be controlled. We find that to have long-term efficacy, the use of targeted therapies must take into account the continuous evolution of cancer cells, that is to say, therapies must adapt to tumor evolution. Another approach is to unleash the ability of the immune system to recognize drug resistant cells. We tested this possibility in syngeneic mouse models of CRC sensitive to targeted therapies. Our findings indicate that inactivation of DNA repair mechanisms and manipulation of mutational loads can trigger immune surveillance and prolonged therapeutic responses. We postulate that rationally combined targeted and immuno-therapies can restrain tumor evolution, and can limit the emergence of drug resistance thus leading to long-term responses.
The dynamics of metastatic spread

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Mutation, selection and adaptation are - by convention - thought to occur primarily within, and to a lesser degree, outside the primary tumour. However, we previously noted in breast cancer and melanoma that metastatic dissemination occurs often early and that advanced tumour stages seed relatively fewer cells. This indicates that metastatic founder cells may lodge and evolve considerable periods of time outside the primary tumour. However, this generates a plethora of questions: How do cancer cells survive at distant sites? From which disseminated cancer cells descent metastatic colonies? Can genomic evolution occur during dormancy in quiescent cells? Does the environment trigger progression of early-disseminated cancer cells or do late-arriving cancer cells take over incipient metastatic colonies? We try to address these questions by analysing single disseminated cancer cells isolated at various time points of disease. Our data suggest that available models and endpoint analyses – such as the comparison of primary tumours and metastases - are insufficient to reflect disease progression in patients. Therefore, we have to carefully consider the clinical and evolutionary stage of an individual disease when we try to address the underlying mechanisms.
Proffered Paper 5

Charting individual metastases evolution in HER2 amplified metastatic colorectal cancer patients undergoing dual EGFR/HER2 therapeutic blockade

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We previously reported the role of HER2 amplification in metastatic colorectal cancer (mCRC), and shown in HER2-amplified mCRC avatars that a dual EGFR/HER2 blockade is required for optimal therapeutic results (Bertotti et al., Cancer Discovery 2012; Nature 2015). Recently, we translated these findings in the HERACLES trial and documented an objective response rate of 30% in HER2+ patients refractory to standard therapies including anti-EGFR antibodies (Sartore-Bianchi et al., Lancet Oncology 2016). However, secondary resistance occurs in most of the cases (Siravegna et al., Cancer Cell 2018). To identify the evolution of individual metastases during HERACLES and ascertain mechanisms associated with clinical resistance we deployed a multidisciplinary approach involving analysis of clinical data, genomic profiling, imaging, sequencing of cell-free circulating tumor DNA (ctDNA) and phylogenetic and pharmacogenomic profiles of tumor tissues and cell models obtained at warm autopsy (DONUM, NCT03385980). These integrated studies revealed unforeseen organ and metastases specific evolutionary patterns. Remarkably, when standard radiologic assessments (RECIST) documented progression, response to HER2 blockade was often retained in individual lesions. Genomic and functional analyses were performed on post-mortem samples and cell models from 8 metastases of a single case unveiling lesion-specific evolutionary trees and mixed sensitivity to the therapy previously administered to the patient, paralleling the responses assessed in radiological re-evaluations. Of immediate clinical relevance, we found that a simple blood assay would have identified the sensitive patients. Finally, exploiting whole exome data from individual lesions we designed a metastases-specific NGS panel. When the latter were coupled with T-Cell Receptors profiles (corresponding to metastases-specific T-Cells infiltrates) we were able to concomitantly track in blood the evolution of neoplastic and T-Cells during several rounds of therapy. Blood-based analyses also revealed a linear correlation between lesions’ size and contribution to plasma ctDNA pool, an information which was previously not available in metastatic colorectal cancers.
Proffered Paper 6

Identifying protein interactions relevant in cancer development

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By detecting significant mutation patterns, cancer genomics studies have provided an unprecedented view into cellular pathways and processes relevant for cancer development. However, the likely effects of the observed genomic changes are often not clear and orthogonal data is needed to assist their characterization. With a goal to systematically investigate properties of protein residues mutated at a rate significantly higher than the one of their surrounding regions, we developed an R/Bioconductor package *DominoEffect*, which detects and annotates such protein hotspots (MSB 2018). When applied to pan-cancer mutation data this approach identified 180 hotspot amino acid residues. We found that these residues associated both with oncogenes and tumor suppressors, and many of the affected proteins could exist in the on and off states in the cell. Within oncogenes, hotspots often mapped to regions that regulated protein interactions. We further integrated mutation signatures with publicly available interaction data and were able to identify functionally related neighborhoods that assisted understanding of mutations’ likely effects. Next, with this rationale in mind, we generated and investigated interaction network of human kinases, the dominant protein family among cancer drivers. By using affinity purification coupled to mass spectrometry, we were able to gather data on interaction partners of more than 300 soluble protein kinases. We integrated into this network the information on known cancer drivers and genes frequently mutated among cancer patients. In this way, we identified a dozen of kinases whose interaction neighborhoods were strongly enriched in proteins associated with cancer. This provided cellular context for several of the frequently mutated proteins and it included instances where the bait kinase itself was not in the Cancer Gene Census. Overall, our integrated analyses of genomics, proteomics and interactome data identified known and highlighted novel protein complexes and processes of relevance in cancer development and progression.
MetAlert mice: a platform for whole-body imaging and pharmacological targeting of metastatic niches

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Metastatic dissemination of cancer cells is a complex process invariably associated with neo-vascularization. Intriguingly, while the presence of malignant cells in lymph nodes is a defining parameter in tumor staging, the specific contribution of the lymphatic vasculature to tumor progression and drug response remains largely unknown. This has been in part due to the scarcity of markers and amenable models for whole-body imaging of neolymphangiogenesis in vivo. Vegfr3 is an attractive “lymphoreporter” as its expression is strongly downregulated in normal adult lymphatic endothelial cells, but gets activated under pathological situations such as inflammation and cancer. Here we exploit immunocompetent and immunodeficient Vegfr3-lymphoreporter mice in the context of malignant melanoma, the most lethal form of skin cancer. We will show how Vegfr3 imaging revealed pre-metastatic niches activated before the onset tumor cell colonization, and guided in the identification of tumor-secreted pro-metastatic drivers. Moreover, we will present the versatility of our Vegfr3-reporter mice to follow metastatic relapse after surgical excision of primary tumors, as well as to screen for antimetastatic compounds. In particular, we will discuss dsRNA-based nanoparticles with a potent anti-melanoma activity in vivo derived from a three-pronged action: (i) direct tumor-self killing by autophagy, (ii) selective targeting of the lymphangiogenic vasculature, and (iii) activation of innate immunity programs. Importantly, our Vegfr3-reporter mice are not limited to melanoma, as they can be used as a cost-effective “MetAlert” platform for gene discovery and pharmacological testing in a broad spectrum of cancer types.
Harnessing genetic interactions to advance whole genome precision oncology

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Much of the current focus in cancer research is on studying cancer driver genes. In search for new and effective cancer drugs, this has been translated into searching for ‘actionable’ mutations in these genes, aiming at their therapeutic targeting. However, identifying novel genetic interactions occurring between cancer genes may open new drug treatment opportunities across the whole cancer genome. This talk will focus on studying the utility of two fundamental types of genetic interactions: The first are the well-known Synthetic Lethal interactions, describing the relationship between two genes whose combined inactivation is lethal to the cell. The second type are the much less studied Synthetic Rescues interactions, where a change in the activity of one gene is lethal to the cell but an alteration of its SR partner gene can rescue cell viability. I shall describe a new approach we have developed for the data-driven identification of these two types of genetic interactions by directly mining patients’ tumor data. Applying it to analyze the Cancer Genome Atlas (TCGA) data, we have identified the first pan-cancer genetic interaction networks shared across many types of cancer, which we then validated via existing and new experimental \textit{in vitro} and \textit{in vivo} screens. We find that: (a) Synthetic Lethal interactions offer an exciting venue for personalized selective anticancer treatments enabling the prediction of patients’ drug response and providing new selective drug target candidates, and (b) targeting Synthetic Rescue genes can mitigate resistance emerging to primary cancer therapy. Importantly, these results are obtained via an unsupervised approach and derived directly from patient data, thus they are more likely to have a significant translational impact.
Preventing Therapy-induced Cancer Stemness

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The inability to eradicate metastasis, the major source of cancer-related deaths, is the most important challenge faced by modern oncologists. Cancer therapeutics used to treat advanced metastatic diseases are incapable of killing all cancer cells, leaving behind a reservoir of surviving cells, known as the minimal residual disease (MRD), from which relapse -almost invariably- emerges. Incompleteness of any tumoricidal treatment is commonly explained by the (pre)-existence of cells harboring drug resistant-conferring mutation(s), owing to high genetic intra-tumor heterogeneity. There is, however, increasing yet underappreciated evidence that the surviving cells are not just static bystanders. Dynamic nongenetic reprogramming allows active adaptation to the treatment (stress). Our recent work has shown that (i) residual cancer cells can induce robust protective responses and acquire diverse drug-tolerant features, including Cancer Stem Cell (CSC) properties, and highlighted (ii) the major contribution of therapy-induced stemness in tumor recurrence.

In particular, we applied single-cell RNA-sequencing to malignant cells isolated from BRAF-mutant patient-derived xenograft (PDX) melanoma cohorts exposed to concurrent RAF/MEK-inhibition. We identified distinct drug-tolerant transcriptional states, varying combinations of which co-occurred within MRDs from PDXs and biopsies of patients ON treatment. One of these exhibited a Neural Crest Stem Cell (NCSC) transcriptional program largely driven by the nuclear receptor RXR. An RXR antagonist mitigated accumulation of NCSCs in MRD and delayed the development of resistance. These data identify the NCSCs as key drivers of resistance and illustrate the therapeutic potential of MRD-directed therapy. They also highlight how gene regulatory network architecture reprogramming may be exploited therapeutically to limit cellular heterogeneity, a key driver of disease progression and therapy resistance.
Making it personal in melanoma

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The melanoma genomic landscape is dominated by ultraviolet radiation (UVR)-induced mutations, but their significance in disease progression is unknown. We classified 419 melanoma genomes from the TCGA and 100 primary melanomas and identified 10 UVR-driven recurrently mutated genes that predict patient survival. To elaborate this study, we examined the signature we described in our mouse melanoma model which is driven by oncogenic BRAF and UVR. We found that the same 10 genes are mutated in mouse UVR-driven tumours and again they are predictive of survival. Moreover, in the mouse model this gene signature was imprinted by short wavelength UVR, and as few as 4 rounds of exposure to UVR were sufficient to accelerate melanomagenesis. Our mouse model therefore recapitulates the cardinal features of human melanoma, so we used the model to investigate responses to immunotherapy (IT), because although IT drugs have revolutionised melanoma patient care, robust biomarkers to guide treatment are still lacking. We observed heterogeneous responses to anti-PD-1 therapy in our mice, but tumour mutation or neo-antigen load, and tumour clonality did not predict to response. Similarly, CD8+ T cell infiltrate, PD-1 ligand 1 (PD-L1) expression, and expression of components of the IFNγ pathway did not correlate to response. Concordant results were observed in patients who received first-line anti-PD-1 therapy. However, we did identify a 26-gene based proliferation score that predicted response to anti-PD-1 therapy in patients with 80% accuracy, and also predicted response in our mice with 86% accuracy. Thus, we have developed a proliferation score that can identify patients who may benefit from first-line anti-PD-1 therapy.
Targeting non-oncogene addiction with HSF1 pathway inhibitors: From phenotypic screen to chemical probe and preclinical development candidate

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Extensive efforts have been focused on the discovery of therapeutic agents that act by targeting the products of oncogenes to which cancer cells become dependent or ‘addicted’. While this approach has been highly effective, leading to the approval of numerous drugs for personalized or precision medicine, it is clear that acquired or de novo resistance to such agents is common and in addition that many cancers lack oncprotein targets for which targeted agents have been developed. Moreover, in many cases the key oncogenic changes are not readily druggable with small molecules. As an alternative approach, Elledge and colleagues conceptualized a strategy of targeting ‘non-oncogene addiction’, whereby therapeutic targets are not themselves products of mutated oncogenes, but rather are proteins that are required by cancer cells to mitigate the effects of various intrinsic and extrinsic oncogenesis-associated cellular stresses (Solemni et al Cell 130 986-988 2007; Luo et al Cell 136 823-837 2009). Furthermore, Elledge and co-authors also envisaged drug combinations that would act on two or more orthogonal non-oncogene addiction targets to prevent or overcome drug resistance. Prominent among such envisaged non-oncogene addiction targets are proteins involved in managing proteotoxic stress, including the molecular chaperone HSP90 and the transcription factor Heat Shock Factor 1 (HSF1). Our laboratory has worked extensively on the discovery and development of HSP90 inhibitors. While these agents have shown promising activity in the clinic, we and others demonstrated that an on-target limitation of HSP90 inhibitors is the activation of the HSF1-mediated Heat Shock Response pathway, which reduces the apoptotic effects of HSP90 blockade (Powers et al Cancer Cell 250-262 2008). Accordingly, we set out to discover inhibitors of the HSF1 stress pathway which is frequently essential to maintain the oncogenic state (Dai et al Cell 30 1005-18 2007). Since HSF1 is a ligandless transcription factor with poor druggability, we used a phenotypic screen to identify a novel series of highly potent and selective bisamide HSF1 pathway inhibitors. These were optimized by our multidisciplinary drug discovery team to produce a chemical probe (Cheeseman et al J Med Chem 60 180-201 2017) and subsequently a preclinical development candidate. I will describe the discovery and therapeutic activity of our bisamide HSF1 inhibitors and the search for their molecular target(s), one of which is cupin family member pirin. I will also describe our work on the design and properties of a proteolysis-targeting chimera (PROTAC) derivative of our bisamides (Cheeseman et al 60 180-201 2017). Our preclinical development candidate shows potential for therapeutic activity in ovarian cancer and multiple myeloma as well as other tumour types.
Proffered Paper 7

Functional linkage of gene fusions to cancer cell fitness assessed by pharmacological and CRISPR/Cas9 screening

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Abstract withheld at the author’s request.
Towards rational combinatorial cancer treatment – a functional genomics approach

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For a long time, advanced-stage melanomas were refractory to the available therapeutic options, but developments in the current decade have begun to offer better perspectives for patients. The small molecule inhibitor vemurafenib, specifically targeting the mutant BRAF\textsuperscript{V600E} kinase, was the first standard of personalized care for patients diagnosed with mutant BRAF metastatic melanoma. Although this compound initially reduces tumor burden dramatically, eventually most melanomas become resistant and progress on treatment. This occurs by the acquisition of additional mutations or other alterations, most of which reactivate the mitogen-activated protein kinase (MAPK) pathway. Although further suppression of BRAF-MAPK signaling by the inclusion of MEK inhibitor delays resistance, eventually most patients relapse.

The clinical outcome of late-stage melanoma patients has greatly improved also thanks to the recent availability of T cell checkpoint blockade, primarily by CTLA-4 and PD-1/PD-L1 antibodies. But still, large patient groups fail to (durably) benefit from these treatments, underscoring the continuing need for developing novel therapeutic modalities.

Therefore, in spite of these new clinical perspectives, there is a dire need to identify new targets amenable to therapeutic intervention, which ought to be applied in rational combination settings. We use function-based, genome-wide experimental strategies to develop rational combinatorial cancer treatment, targeting both cancer and immune cells. By screening for novel therapeutic targets and predictive biomarkers, we aim to achieve more durable clinical responses for patients. On the one hand, we are increasing our understanding of how cancer cells rewire their signaling networks, to expose and exploit new pharmacologically tractable tumor susceptibilities, also in the context of immunotherapy. On the other, we are manipulating various cell types from the patient’s own immune system to boost their specific cytotoxicity towards tumor cells. With these approaches, we are developing new rational combinatorial therapies, which simultaneously eliminate the patients’ tumor and harness their immune system.
Tumor molecular profiling is now a standard of care for many cancer types. The existence of recurrent targetable alterations across distinct histologically defined tumor types, coupled with an expanding portfolio of molecularly-targeted therapies, demands flexible and comprehensive approaches to profile clinically significant genes across the full spectrum of cancers. This talk will review the MSKCC experience from a large-scale, prospective clinical sequencing initiative utilizing a comprehensive assay, MSK-IMPACT, through which we have compiled matched tumor and normal sequence data from a unique cohort of more than 30,000 patients with advanced cancer. Using these data, we identified clinically relevant alterations and mutational signatures that were shared among common and rare tumor types. Patients were enrolled on matched clinical trials at a rate of ~11%. Pathogenic germline mutations, some of which are predictive biomarkers of drug response, were also identified in a significantly higher proportion of patients than expected and suggest that matched germline sequencing to identify mutant alleles associated with increased heritable risk should be considered in all patients with advanced cancer. To enable discovery of novel biomarkers and deeper investigation into rare alterations and tumor types, all results will be made publicly accessible through the AACR GENIE initiative.
Prognostic value of IL-17, TGF-β, IL-22, VEGF, IL-10, and IFN in gastric cancer patients treated by hyperthermic intraperitoneal chemotherapy (HIPEC)

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Background: Peritoneal carcinomatosis is detected in more than 30% of patients with advanced gastric cancer (AGC), and almost 60% of deaths are caused by peritoneal dissemination. Several molecules and receptors may be associated with peritoneal dissemination and explore the clinical possibilities of candidate biomarkers. The main aim of presented study was to determine molecular tumor profiling and prognostic value of novel biomarkers.

Patients and Methods: 120 patient with AGC treated by HIPEC and 120 healthy individual. Invetigation of potential biomarkers (IL-17, IL-22, TGF-beta, VEGF, EGFR etc) in association with progression and prognosis of disease (UICC stages, TNM PG etc). Blood for biomarkers analysis was investigated by immunofluorescence and ELISA methods.

Results: In fact, all cytokine concentrations are different in healthy and cancer patients. However, only IFN increases after surgery, while other cytokines decrease or not change. Also, we have seen that TGF-β and VEGF are very high in plasma samples (p <0.001), both markers were very high in patients required HIPEC treatment:

<table>
<thead>
<tr>
<th>parameters</th>
<th>Healthy</th>
<th>Before HIPEC</th>
<th>After HIPEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-17 pg/ml</td>
<td>11.7±5.9</td>
<td>30.5±10.1</td>
<td>33.4±15.2</td>
</tr>
<tr>
<td>IL-22 pg/ml</td>
<td>15.7±1.4</td>
<td>19.2±3.7</td>
<td>25.4±5.1</td>
</tr>
<tr>
<td>TGF-β ng/ml</td>
<td>59.4±9.7</td>
<td>523.5±30.5</td>
<td>208.7±17.3</td>
</tr>
<tr>
<td>VEGF pg/ml</td>
<td>67±76</td>
<td>225±102</td>
<td>346±85</td>
</tr>
<tr>
<td>IL-10 pg/ml</td>
<td>3.97±0.78</td>
<td>20.5±24.1</td>
<td>16.7±21.2</td>
</tr>
<tr>
<td>IFNgamma pg/ml</td>
<td>9.4±7.6</td>
<td>34.1±15.7</td>
<td>54.3±18.1</td>
</tr>
</tbody>
</table>

Conclusions: High level of TGF-β and VEGF indicates on high probability of cancer spread potential. New prospective studies will be necessary to evaluate the prognostic effect of novel biomarkers. The determination of these novel biomarkers gives us possibility to find molecular predictors of clinical response and definition of molecular subtypes of gastric cancer with distinct clinical behavior.

This work has been supported by Shota Rustaveli Georgian National Science Foundation.
Natural Killer cells from colorectal cancer patients are endowed with pro-angiogenic/pro-metastatic phenotype and functions by up-regulating Angiogenin and the MMP9-TIM2 axis

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Natural Killer (NK) cells are effector lymphocytes involved in tumor immunosurveillance. In solid malignancies, tumor associated (TANK; peripheral blood) and tumor infiltrating (TINK) NK cells have compromised functions. We previously reported that NKs from Non-Small Cell Lung Cancer (NSCLC) acquire the decidual-like CD56\textsuperscript{bright}CD16\textsuperscript{-}VEGF\textsuperscript{high}PlGF\textsuperscript{high}IL-8\textsuperscript{-}IFNg\textsuperscript{low} phenotype and that TGF\textsubscript{b} is a relevant orchestrator in NK angiogenic switch. We also found that NKs from malignant pleural effusions are endowed with decidual-like/pro-amngiogenic phenotype and functions. Here, we functionally and molecularly characterize TINK and TANKs from blood and tissue samples of colorectal cancer (CRC) patients, a tumor type where inflammation and angiogenesis have clinical relevance, compared to NK isolated from control and non-oncologic inflammatory bowel disease patients.

NK subset distribution and cytokine profiling were performed by multicolor flow cytometry, using peripheral blood and tissue samples from CRC patients, for surface antigen and cytokine profiling characterization. Conditioned media (CM) from FACS-sorted NKs were used either for secretomic profiling using antibody membrane arrays or in functional in vitro angiogenesis assays.

CRC TINKs/TANKs showed decreased activation marker NKG2D, impaired degranulation activity, a decidual-like NK polarization toward the CD56\textsuperscript{bright}CD16\textsuperscript{dim/-}CD9\textsuperscript{-}CD49\textsuperscript{+} subset. TINKs and TANKs supernatants induced endothelial cell proliferation, migration, adhesion and formation of capillary-like structures in vitro. It has been reported that dNK release proangiogenic factors and metastasis-associated (MMP-9, TIMP-1-2) proteins.

We describe for the first time the expression of angiogenin and MMP2, MMP9, TIMP1 and TIMP2 by CRC derived NK cells. This could be a phenotype relevant to their invasive capabilities and pro-angiogenic function. STAT-3/STAT-5 activation was observed in TANKs, and inhibition of the STAT5 pathway by pimozide, an antipsychotic drug, reduced proangiogenic factor VEGF and angiogenin production and capillary-like structure formation, but did not affect the levels of TIMP-1, TIMP-2 and MMP-9.
Correlation between the biological functions of molecular hydrogen (H2) and cancer

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Oxidative stress in the cell result from the oxidizing potential of excess reactive oxygen species (ROS). Acute oxidative stress may result from various conditions, among several others causes, such as vigorous exercise, inflammation, ischemia and tissue transplantation. Chronic oxidative stress is related to the pathogenesis of many lifestyle, related diseases, aging and cancer. Elevated rates of ROS have been detected in many cancers. It has been demonstrated a strong correlation between ROS and cancer heterogeneity which may contribute to modulate therapy. It is well known that heterogeneity is affected by increasing levels of ROS. Tumour cells express increased levels of antioxidant proteins to detoxify from ROS, suggesting that a delicate balance of intracellular ROS levels is required for cancer function. ROS causes oxidative DNA and protein damage, as well as damage to tumour suppressor genes. It has been clearly demonstrated that H₂ reduces oxidative stress, protects DNA, suppresses genetic mutations and cancer cell growth, by effective action on angiogenesis. H₂ inhibits tumour growth by reducing oxidation products. H₂ induces cancer cell death apoptosis and act as suppressing the spread of cancer metastasis. Moreover, H₂ helps anti-cancer drugs and protects from radiation therapy. H₂ improve the quality of live for radiation and chemotherapy patients and increases survival rates of cancer patients. More recently, it has been suggested that hydrogen water enhances 5-fluorouracil induced inhibition of colon cancer and a recent study has demonstrated a protective effect of hydrogen-rich water on liver function of colorectal cancer patients treated with Folfox 6 chemotherapy. H₂ can modulate signal transduction across multiple pathways, but its primary molecular targets have not been determined. Examining critical overlapping signalling molecules would help map cross talk among critical pathways. To fully explain the biological functions of H₂, its molecular mechanisms of action must be clarified.
Role of Integrin β1 as a Biomarker of Stemness in Head and Neck Squamous Cell Carcinoma

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Signaling between cancer stem cells (CSC) and their extracellular matrix has a crucial role in CSC progression and maintenance. However, mediators of this signaling pathway in head and neck squamous cell carcinoma (HNSCC) are largely unknown. Here, we explored whether integrin β1, which is one of the key regulators of the communication between cells and their microenvironment, affected the stemness of HNSCC cells. We examined self-renewal capacity, chemoresistance, and xenograft tumorigenicity after knockdown of integrin β1 in primary HNSCC cells. In addition, we studied the role of focal adhesion kinase (FAK), an intracellular downstream molecule of integrin signaling, in influencing stemness of HNSCC. The relevance of Notch1 and integrin β1 interactions in HNSCC cells was also examined. Finally, immunohistochemical analysis was carried out to test whether the coexpression of integrin β1 and Notch1 in the samples from HNSCC patients correlated with their survival. Targeting integrin β1 in HNSCC cells inhibited self-renewal, chemoresistance, and in vivo tumor-forming capacity. Treatment with an inhibitor of FAK decreased self-renewal capacities and expression of various putative stem cell markers (Oct4, Sox2, and Nanog) in a dose-dependent manner. Moreover, knockdown of integrin β1 decreased the expression of Notch1 and its target genes (Hey1 and Hes1). Notably, HNSCC patients demonstrating simultaneous expression of integrin β1 and Notch1 in their tissue samples had significantly worse survival rate. In conclusion, integrin β1/Notch1 axis has a significant role in the regulation of stemness in HNSCC.

KEYWORDS: Head and neck cancer, Cancer stem cell; Biomarker; Integrin β1
Comparative oncogenomics and iterative mouse modeling identifies combinations of driver genes and drug targets in \textit{BRCA1}-mutated breast cancer

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\textit{BRCA1}-mutated breast cancer is primarily driven by DNA copy-number alterations (CNAs) containing large numbers of candidate driver genes. Validation of these candidates requires novel approaches for high-throughput \textit{in vivo} perturbation of gene function. We therefore developed genetically engineered mouse models (GEMMs) of \textit{BRCA1}-deficient breast cancer that permit rapid introduction of putative drivers by either retargeting of GEMM-derived embryonic stem cells, lentivirus-mediated somatic overexpression or \textit{in situ} CRISPR/Cas9-mediated gene disruption or base editing. We used these approaches to validate \textit{Myc}, \textit{Met}, \textit{Pik3ca}, \textit{Pten} and \textit{Rb1} as \textit{bona fide} drivers in \textit{BRCA1}-associated mammary tumorigenesis. Iterative mouse modeling and comparative oncogenomics analysis showed that MYC-overexpression strongly reshapes the CNA landscape of \textit{BRCA1}-deficient mammary tumors and identified MCL1 as a collaborating driver in these tumors. Moreover, MCL1 inhibition potentiated the \textit{in vivo} efficacy of PARP inhibition (PARPi), underscoring the therapeutic potential of this combination for treatment of \textit{BRCA1}-mutated cancer patients with poor response to PARPi monotherapy.
Protecting the cytotoxic T cell repertoire for immunotherapy by rescuing T cells from Activation-Induced Cell Death

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One of the most remarkable properties of antigen-specific T cells is their ability to undergo rapid and massive clonal expansion in response to antigenic stimulation. This hallmark of the adaptive immune response is essential to effectively clear pathogens. However, whereas a small subset of CD8+ T cells form an immunologic memory to resort to, the vast majority of antigen-specific T cells will undergo activation-induced cell death (AICD). As important as this process is to balance pathogen or tumor clearance and autoimmunity, it can have dire consequences in the context of immunotherapy, during which an effective anti-tumor response can be blunted by the depletion of tumor-reactive T cell clones from the T cell repertoire. AICD has also ramifications for currently applied ex vivo expansion protocols of (tumor-infiltrating) T cells for adoptive T cell transfer. The repeated stimulation, required to generate therapeutically relevant T cell numbers, is accompanied by significant amounts of cell death and thus loss of potentially therapeutically relevant T cell clones.

These considerations suggest that an understanding of the mechanism of AICD may contribute to improvement of T cell therapies. To systematically disentangle the mechanisms that govern AICD, we performed a CRISPR/Cas9-based genome-wide knockout screen in primary murine CD8+ T cells. For modelling AICD in vitro, naïve splenic CD8+ T cells were differentiated into effector T cells. Once they reached a non-proliferating state, antibody-mediated stimulation was used to mimic antigenic-stimulation, thereby efficiently inducing activation and concomitantly AICD. This unbiased approach not only allowed us to verify pathways that are known to be involved in AICD, such as FAS and NFκB signaling, but also novel genes, ranging from surface proteins to epigenetic modifiers.
Adaptive rewiring of oncogenic signaling in response to MTOR blockade in pancreatic cancer

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Background: Pancreatic ductal adenocarcinoma (PDAC) still carries a dismal prognosis with overall five-year survival of 8% and currently used therapies need urgent improvement. Although pre-clinical data show that the PI3K-AKT-MTOR pathway is a relevant pathway for therapeutic intervention, clinical trials have failed so far. Therefore, detailed molecular knowledge on how PDAC escape PI3K-AKT-MTOR inhibition is needed.

Methods: By the use of a dual-recombinase system, which is based on the flippase-FRT (Flp-FRT) and Cre-loxP recombination technologies, we generated a murine PDAC model allowing the genetic analysis of MTOR functions in tumor maintenance and adaption of PDAC cells to the loss of MTOR expression. RNA-seq data were analyzed to find pathways relevant to cope with MTOR deletion. Cross-species validation and pharmacological intervention studies were used to recapitulate genetic data and to develop novel combination therapies. Viability and clonogenic assays were used to validate novel combination therapies.

Results: Blocking MTOR genetically as well as pharmacologically results in adaptive rewiring of oncogenic signaling with activation of ERK- and AKT- pathways. In addition, analysis of RNA-seq data demonstrated activation of the pro-survival NFκB signaling pathway. In contrast to ERK- and AKT-activation, which occur with latency, activation of NFκB target genes was already detected six hours after MTOR inhibition, an effect blocked by BET inhibitors (e.g. JQ1 or OTX-015). Consequently, MTOR inhibitors (e.g. INK-128) and BET inhibitors synergize in human and murine PDAC models.

Conclusions: Our data demonstrate that MTOR inhibitor induced adaptive expression of NFκB target genes can be blocked by BET inhibitors. MTOR- and BET-inhibitor combination therapies should be further developed in PDAC.
Semaphorin 6A regulates a novel mechanism of resistance to Cetuximab in colorectal cancer

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Semaphorins are a large family of conserved secreted or transmembrane proteins, initially identified as axon guidance cues and later involved in the regulation of diverse biological processes, from angiogenesis to immune response, to cancer.

In particular, Semaphorin 6A (Sema6A) is a transmembrane family member known to control the development of central nervous system, but poorly studied in other contexts. Notably, Sema6A has been reported recently to sustain the viability BRAF-mutated melanoma cells, suggesting that it can regulate cancer cell behavior.

We focused our attention on the role of Sema6A in colorectal cancer (CRC), on the basis of gene expression profiling of CRC xenopatients. In fact, only a subset of all CRC lacking activating mutations in KRAS, NRAS, BRAF and PI3KCA oncogenes is sensitive to EGFR blockade with Cetuximab (CTX), and we found that CRC responsiveness to therapy was significantly associated with high levels of Sema6A expression (p value: $6.3 \times 10^{-6}$). Importantly, upon Sema6A knock-down in CTX-responsive cells we observed a ten-fold reduction of therapeutic efficacy, strongly suggesting that this semaphorin can regulate CRC susceptibility to anti-EGFR targeted therapies.

Sema6A silencing did not affect EGFR activity per se, but apparently induced the activation of alternative signaling cascades that ultimately sustained the viability of cancer cells challenged with anti-EGFR therapy.

Moreover, a high-throughput transcriptomic analysis of Sema6A-silenced cells indicated that this semaphorin controls the expression of several genes implicated in pivotal functions in cancer cells. The functional validation of candidate effectors is ongoing.

This study addressed for the first time the role of Sema6A in colorectal cancer, and unveiled a novel and unexpected functional importance of a semaphorin in cancer cell responsiveness to targeted therapy. The ongoing analysis of the implicated molecular mechanisms is likely to reveal previously unknown effectors of semaphorin signaling.
Identifying combinatorial targeted therapies for acute myeloid leukemia patients

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AML is the most common form of acute leukemia in adults and is classified in 14 different groups depending on its genetic makeup. Despite this heterogeneity its current standard of care is common for all the groups and has remained largely unchanged for the past 20 years. This treatment is far from optimal, with a five-year survival rate of approximately 27%. Furthermore, weak and elderly patients do not qualify for chemotherapy and typically enter palliative care. A precision medicine strategy could help to find specific, individualized treatment for each AML patient, leading to improved quality of life and survival rates.

Drug-driven precision medicine approaches directly test the sensitivity of primary cancer cells from individual AML patients and healthy donors to a panel of anticancer drugs to select the most effective drug for each patient. However, single-drug based targeted therapies usually only have short-term effects and patients almost universally relapse due to development of resistance. To overcome this problem, we are currently systematically screening for synergizing drug combinations.

We have established a high-throughput screening platform for AML, where we test the ex vivo drug sensitivity to a panel of 65 pair wise drug combinations. We aim to identify synergistic drug combinations for each patient that comes to our clinic. This will help us determine whether specific subgroups of patients respond with a similar dynamic to certain pairs of drugs, and find biomarkers that predict treatment response for future treatment of AML patients.
Exploring the molecular roles of Tip60 in breast cancer, and the potential therapeutic effectiveness of targeted inhibitors

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Cancer cells maintain genome integrity and avoid programmed death, while harbouring increased DNA damage. Maintaining genome stability following DNA double strand breaks (DSB) involves a complex signalling cascade, regulated by key acetylation events, with sustained genomic damage leading to tumourigenesis. The essential Lysine acetyltransferase (KAT) Tip60 regulates both gene expression and the DSB response, though Tip60-dependent acetylation and activation of the key signal transduction enzyme ATM. While Tip60 protein dysregulation in breast cancer has been examined, here we perform a detailed and comprehensive investigation and analysis using a large cohort of primary patient samples.

The role of Tip60 protein in breast cancer was explored using a breast cancer tissue microarray (>200 individual patient samples) and quantifying key criteria (Tip60 cellular location, staining intensity and percent cells stained). Widespread dysregulation in all breast tumours was found, including significant associations of specific Tip60 staining patterns with key clinicopathological characteristics.

To further explore the molecular role of Tip60 in breast cancer, we utilised our previously developed Tip60 inhibitor, TH1834 (Gao et al, Sci Rep. 2014). TH1834 exploits inherent weaknesses of tumours (compromised DDR pathways), allowing selective targeting of cancer cells. TH1834 allows precise and tuneable targeting of Tip60 activity (not otherwise possible, as homozygous knockouts induce cell death). TH1834 is revealing new subtype specific molecular roles for Tip60, in the DDR and other cellular processes, which modulate malignant transformation in breast cancer cells. Additionally, we explore and discuss the effectiveness, and mechanisms of action of a new generation of targeted Tip60 inhibitor.

KATi are an exciting new potential therapeutic (alone or in combination), specifically targeting tumour cells inherent, dysregulated, molecular mechanisms without affecting surrounding normal tissues.
Different classes of *HOX* Genes cooperate with oncogenic *NRAS* to establish acute myeloid leukemia both *in vitro* and *in vivo*: common signatures of gene upregulation by *HOXA1* and *HOXB7*

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We showed that *HOX* genes in different classes cooperate with oncogenic *KRAS* and *NRAS* to induce AML through in vivo screening system. The finding is clinically relevant because overexpression of *HOX* genes and *RAS* mutation(s) are frequently observed in AML patients. Since developing cancer therapeutics targeting *RAS* or *HOX* itself has been unsuccessful, we looked into downstream target genes of *HOX* transcription factors in our AML system which includes simultaneous expression of *HOX* and oncogenic *RAS* in murine bone marrow cells and regulated expression of *HOX*. The results showed that *HOX* genes prevent final differentiation of leukemic cells and both *HOXA1* and *HOXB7* upregulates common set of target genes in cDNA array as well as RNA Sequencing analysis. The system we described will be useful in developing novel drug targets for leukemia therapy.
Breast adipose tissue-derived mesenchymal stromal cells promote breast cancer chemoresistance

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Mesenchymal stromal cells (MSCs) which naturally recruit to the tumor cell mass and become important part of the tumor microenvironment (TME), significantly influence biological properties of the tumor cells, including their response to chemotherapeutic agents. During chemotherapy MSCs are exposed to chemotherapeutics alongside the cancer cells. Such drug-primed MSCs might then induce tolerance of breast cancer cells (BCC) towards these drugs. Unravelling the mechanism and molecules which are responsible for the MSC-mediated refractoriness of BCC against chemotherapeutics can lead to new potential therapeutic targets in the TME and thus restore the chemosensitivity. To decipher through which mechanisms the MSCs confer chemoresistance on BCC, we studied direct and indirect co-cultures of NLR-MDA231, NLR-T47D and NLR-JIMT with drug primed MSCs. Two sources of MSCs were used and evaluated in this study: healthy donor- and breast cancer patient-derived MSCs. Both groups of MSCs were pre-treated with commonly used agents, paclitaxel, cyclophosphamide and doxorubicin. MSC secretome analysis after treatment showed upregulation of IL-6, IL-8, SerpinE1 and CXCL1. Although the secretome was altered, the chemosensitivity of tumor cells exposed to the drug-primed MSC conditioned media was not changed substantially. More profound effect was observed in the direct co-cultures leading us to a conclusion that the effect is mediated via active cell-to-cell contacts rather than through secreted factors. To confirm that the drug priming of MSCs plays a key role in mediating the chemoresistance, breast cancer cells were co-cultured either with drug-pretreated or untreated naïve MSCs. The tumor cells were substantially more resistant to doxorubicin when co-cultured with MSCs which were previously pre-treated with the same drug compared to MSCs which have not been affected by the drug beforehand. The same effect was observed with MSCs obtained from healthy donors as with MSCs acquired from the breast fat tissue of carcinoma patients.
Analytical Validation of Multiplex Biomarker Assay to Stratify Colorectal Cancer into Molecular Subtypes for Personalised Medicine

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In colorectal cancer (CRC) only a handful of biomarkers are utilised for clinical decision making. In metastatic CRC (median survival only 24 months), only RAS and possibly BRAF mutation status are assessed – presence of these mutations rules out more than half of patients from receiving targeted therapy that can increase median survival by 6 months in wild-type patients. Of those who do receive targeted therapy, only approximately 40% will respond.

Transcriptomic molecular subtypes have proved their value as biomarkers for treatment decisions, e.g. in breast cancer. We published transcriptomic subtypes of CRC that have differential prognoses and responses to therapies, subsequently corroborated by independent groups. The nCounter platform (which is FDA-approved for subtyping other cancers) could allow subtyping of CRC patients in a cost/time efficient manner not currently feasible with alternative technologies (e.g. RNAseq or qPCR), allowing validation of subtypes’ value in prospective clinical trials.

We present the analytical validation of this assay in 4 independent cohorts (n=343). Firstly, our original gold-standard 786-gene classifier was refined to 38 key genes using a consensus of nine gene selection methods, and a lower-cost modified protocol adopted. We then benchmarked our assay versus the gold-standard protocol, gene set, and platform (RNAseq/microarray). We found that protocol has a negligible effect on assay outcome, concordance between platforms/gene sets is high (87%/93%, respectively), and area under the curve (AUC) for classifiable samples was 0.94.

Finally, we analysed matched fresh-frozen and FFPE samples (n=58), and show that subtyping is successful in FFPE. However, we find that tumour cell enrichment is an important consideration in the comparison of fresh-frozen and FFPE subtyping results, and that macrodissected FFPE may be the preferable RNA source for this assay despite characteristically low-quality RNA. Overall, this assay enables transcriptomic-based personalised medicine in CRC, potentially benefitting patients in the future.
The AP-1 complex regulates AXL expression and determines sensitivity to PI3Ka inhibition in esophagus and head and neck squamous cell carcinoma

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AXL overexpression is a common resistance mechanism to anti-cancer therapies, including the p110a isoform specific inhibitor of the phosphoinositide 3-kinase (PI3K), BYL719, in esophagus and head and neck squamous cell carcinoma (ESCC, HNSCC respectively). However, the mechanisms underlying AXL overexpression in resistance to BYL719 remained elusive. Here we demonstrated that the AP-1 transcription factors, c-JUN and c-FOS, regulates AXL overexpression in HNSCC and ESCC. AXL and c-JUN expression is correlated in HNSCC patients, and in HNSCC and ESCC cell lines.

Silencing of c-JUN and c-FOS expression in tumor cells reduced AXL expression, and enhanced sensitivity of human papilloma virus positive (HPV¹⁰⁹) and negative (HPV¹⁰⁸) tumor cells to BYL719 in vitro. Blocking of the c-JUN N-terminal kinase (JNK), using SP600125, in combination with BYL719 resulted in down-regulation of AXL expression, and potently inhibited mTOR pathway. Synergistic anti-proliferative effect was detected between BYL719 and SP600125 in 15 tumor cell lines in vitro. In-vivo, this drug combination induced tumor growth arrest in cell line and patients-derived xenograft models, and in syngeneic head and neck cancer models. Collectively, our data suggests that JNK inhibition in combination with anti-PI3K therapy is a new therapeutic strategy that may be tested in HPV¹⁰⁹ and HPV¹⁰⁸ HNSCC and ESCC patients.
Combination treatments with marine cytotoxin DL33-3 overcome resistance to Bcl-2 inhibitor Venetoclax in AML cell lines

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Acute myeloid leukemia (AML) is one of the main causes of death related to leukemia, with 70% of patients either not responding to treatment or eventually relapsing to secondary AML. Standard therapies achieved little improvement in the last decades, and new drugs or drug combinations targeting the resistant fraction of patients are actively researched.

B-cell lymphoma 2 (Bcl-2) and myeloid cell leukemia 1 (Mcl-1) are two major anti-apoptotic proteins frequently deregulated in cancer. Bcl-2 is the target of recently discovered BH3 mimetic ABT-199 (Venetoclax), which was approved by FDA for chronic lymphocytic leukemia treatment, and is under evaluation for AML treatment. However, single agent resistance to this drug is rapidly emerging. Recently, combining Bcl-2 inhibitors with compounds downregulating Mcl-1 has been proposed as a new strategy to kill resistant AML cells.

We show here that DL33-3, a bromotyrosine compound derived from the marine sponge Suberea Clavata, differentially affects proliferation and viability of a panel of AML cell lines. By performing Western Blot analyses, we show that DL33-3 induces a rapid downregulation of Mcl-1 in AML cell lines, which is paralleled by proliferation decrease. We then investigated the mechanism of action of DL33-3 and showed that this compound induces ER stress in U-937 cells, as proved by increased PERK phosphorylation, appearance of the spliced form of XBP1 and increased CHOP mRNA levels.

Moreover, by performing combination treatments of DL33-3 with ABT-199, we show that this marine compound allows to efficiently overcome resistance to ABT-199 in Bcl-2/Mcl-1 positive AML cell lines. Importantly, healthy CD34⁺ cells were less sensitized by DL33-3 than AML cell lines. Overall, these data show interesting anti-proliferative and sensitizing activities of a brominated sponge-derived compound, which could be exploited for rational combination treatments of AML.
Advancing personalized theranostics by targeting CD133 in aggressive variant prostate cancer

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Aggressive variant prostate cancer (AVPC) accounts for approximately 33% of all prostate cancer-related deaths and is characterized by visceral metastasis, minimal responses to current therapies, and poor overall prognosis. Novel targeted theranostics are urgently needed for earlier detection and treatment of AVPC. The transmembrane glycoprotein, CD133, has been frequently used to identify prostate cancer stem cells, however, targeting CD133 for diagnostics and therapeutics has remained limited due to complexities with CD133 structure and function.

Human single-chain variable fragment (scFv) phage display was used to identify an scFv (HA10) that preferentially recognizes a glycosylation-independent epitope on CD133. HA10 was analyzed by flow cytometry and cloned into a rabbit immunoglobulin (IgG) scaffold for immunohistochemistry and a human IgG for in vivo imaging. A near-infrared (NIR) dye was conjugated to the human IgG to assess the imaging potential of HA10 in mice bearing CD133\textsuperscript{pos} and CD133\textsuperscript{neg} tumors.

HA10 accurately detected CD133 better than a commonly used commercially available antibody using flow cytometry. Immunohistochemistry analysis with HA10 showed intense CD133 staining in patient-derived xenograft models originating from non-AR driven metastatic prostate cancer with neuroendocrine differentiation and liver biopsies from a patient who failed conventional therapies. Similarly, mice receiving an intravenous injection of 1 nmol of NIR-HA10 exhibited selective tumoral uptake in CD133\textsuperscript{pos} CWR-R1 tumors after 5 hours post-injection; the signal intensity was highest after 24 hours, and remained in CD133\textsuperscript{pos} tumors for up to 144 hours.

Our data clearly demonstrate that our novel scFv, HA10, can effectively distinguish between CD133\textsuperscript{pos} and CD133\textsuperscript{neg} tumor tissue in vitro and in vivo. Future experiments seek to use \(^{89}\)Zr-labeled HA10 to demonstrate its utility as a PET imaging agent. Using a targeted nuclear medicine based approach will facilitate a less invasive and more personalized diagnostic for AVPC and may also suggest HA10’s potential as a radioimmunotherapy agent.
USP7 inhibition drives senescence in metastatic melanoma

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Cellular senescence is a potent tumor-suppressive mechanism that irreversibly arrests cell proliferation. Thus, a promising strategy for metastatic melanoma treatment could be to target molecules, whose inhibition triggers a senescent phenotype. Recently Deubiquitylating enzymes (DUB), which remove degradative ubiquitin signals from protein substrates, represent attractive targets in cancer therapy, being frequently dysregulated in human cancer. The DUB ubiquitin specific protease 7 (USP7) targets proteins involved in many cellular processes. Consistent with its multiple functions, USP7 is upregulated in several cancers, but its role has never been investigated in metastatic melanoma.

A tissue microarray analysis of primary melanomas has shown that USP7 can be a prognostic factor in melanoma. USP7-knocked down (KD) cells display a senescent phenotype, accompanied by a G1 block of the cell cycle in vitro. Moreover, a strong reduction of tumor growth is shown in vivo. USP7-KD enhances the ERK signaling and regulates FOXO4 stabilization, suggesting a crucial role of USP7 in mechanisms controlling proliferation. We have shown that USP7 silencing downregulates the mTOR cascade, inferring that USP7 regulates oxidative stress in mutated cells. USP7 interacts and promotes the stabilization of DNMT1, the major and ubiquitously expressed DNA methyltransferase. By mass-spec analysis, we have shown that DNMT1 is one of the most downregulated proteins upon USP7-KD and that its reduction is an early event during senescence induction. Preliminary data indicate that P005091 (USP7 inhibitor) treatment recapitulates USP7 silencing in vitro and in vivo in our PDX-derived cells, thus suggesting that a combination of drugs targeting known melanoma drivers and the multiple pathways in which USP7 is involved in can be explored in melanoma cells.

In summary, this study provides mechanistic evidence for a pro-tumorigenic role of USP7 in melanoma, suggesting that its depletion can represent an alternative therapeutic option.
The potential role of the autoimmune regulator (Aire) in tumorigenesis

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Autoimmune regulator (Aire) is a unique transcriptional regulator that induces promiscuous expression of thousands of tissue restricted antigens (TRAs) in medullary thymic epithelial cells (mTECs), a step critical for induction of immunological self-tolerance.

On the molecular level Aire doesn’t function as a classical transcription factor, as it does not bind specific DNA sequences, instead it primarily localizes to transcription start sites (TSS) and/or enhancer regions. Results from our lab demonstrate that Aire utilizes a rather unconventional mechanism for transcriptional activation, which involves activation of the DNA damage response (Chuprin et al; submitted). Specifically, Aire induces formation of DNA damage foci in mTECs, characterized by phosphorylation of histone H2AX (e.g. gH2AX), and physically localizes to these DNA damage foci. Given that DNA damage is one of the key mediators of tumorigenesis, the above data raise a question whether Aire may possess oncogenic activity.

To test this hypothesis, we generated a transgenic mouse model with inducible and ubiquitous expression of Aire and a heterozygous mutation (R172H) in the main tumor suppressor gene - p53. These transgenic mice were injected at 8 weeks of age either with sub-lethal dose of doxycycline (4ug/ml) to induce Aire expression or a control vehicle and followed for ~30 weeks. Interestingly while several mice with ectopic-Aire expression developed sarcoma tumors in the bladder and abdomen at 30 weeks, all mice with no ectopic expression of Aire were tumor free. As accumulating and non-resolved DNA damage is one of the key triggers for cancer development, these results suggests that Aire may possess potential oncogene activity and that ectopic expression of Aire in tissues other than the thymus may lead to cancer development.
Strategic precision medicine through computational analysis of high-throughput drug sensitivity screening for targeted therapy of resistant acute myeloid leukemia patients

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AML is the most common form of acute leukemia in adults and is classified into 14 different groups depending on its genetic makeup. The heterogeneity of AML is defined by a diverse genetic landscape and is one major challenge in finding an effective treatment option for patients that do not respond to standard treatment. Current AML treatment remained unchanged for the past 20 years and is applied across all 14 different groups not considering the genetic diversity of this disease.

Therefore, we aim to identify the characteristics properties behind response and resistance of individual patients, who might not only develop resistance to standard treatment, but also to targeted therapy. The ability to predict resistance and identify strategic and personalized treatment options for a group of individual patients is of substantial significance.

We have screened a group of patients by assessing the sensitivity of primary patient-derived cancer cells ex vivo from individual AML patients and healthy donors to a panel of anticancer drugs. We observed that only a group of cancer cells showed response to a number of drugs. However, the other group did not show any therapeutically relevant response to the most effective drugs indicating the potential of resistance in an eventual treatment. We are approaching this therapeutic issue through a systematic screening of synergizing drug combinations. We have been able to develop computational methods for personalized single and/or combinatorial high-throughput drug screens, including randomized dispensing and automated deconvolution of big data for further methodological downstream analysis.

We further aim to map potential genetic alterations and identify through multidimensional data potential biomarkers for drug response or resistance and the implementation of automated high-throughput screening and computational analysis of big data in the hope not only to predict response and resistance, but also identify strategic treatment options for individual AML patients.
Anterior gradient 2 protein inhibits autophagy in colorectal cancer via attenuation of AMP-activated protein kinase

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There is growing epidemiological evidence indicating relationship between diabetes mellitus and increased incidence of colorectal cancer. The preferred initial and most widely used pharmacological agent for type 2 diabetes is metformin, which in parallel reduces risk of colorectal cancer and improves patients’ prognosis. AMP-activated protein kinase (AMPK) seems to be tightly associated with the beneficial metabolic effects of metformin serving as a cellular energy sensor activated in response to a variety of conditions depleting cellular energy levels, such as nutrient starvation (especially glucose), hypoxia and exposure to toxins that inhibit the mitochondrial respiratory chain complex. We studied the effect of metformin on colorectal cancer cell lines exposed to 5-FU and oxaliplatin alone or in combination with metformin with respect to the expression of AGR2, other recently emerging player involved in colon carcinogenesis. In cells expressing no AGR2 we observed significantly higher levels of phosphorylated AMPK indicating that the presence of AGR2 may interfere with the metformin dependent activation of AMPK resulting in attenuation of autophagy.
Biomarkers distinguishing a subtype of aggressive prostate cancer from indolent and identification of potential new treatments

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For recurrent and metastatic prostate cancer (PCa), androgen-deprivation therapy (ADT) and agents targeting its receptor mostly produce a favorable clinical response. Unfortunately, the majority of these patients will experience a recurrence within 5 years and run out effective therapies. Therefore, developing progression-associated biomarkers for PCa will distinguish patients in need of more aggressive treatments from patients unlikely to have a recurrence. We previously identified that metastatic progression of PCa is mediated by autonomous binding of galectin-4-O-glycan to cancer cells. More recently, we identified galectin-4 expression is upregulated during the PCa progression \textit{in vivo} after ADT, in association with the increased biosynthesis of mucin-type O-glycans. Together, we characterize a novel mechanism that overexpression of galectin-4 feedforward upregulates the specific O-glycosylation pathway synthesizing a protein-conjugated trisaccharide (sialyl-T antigen) as its binding site. Particularly, the O-glycan modifies multiple receptor tyrosine kinases expressed in PCa cells thus promoted various oncogenic processes, including tumor growth, epithelial-mesenchymal transition and cancer stem cell (CSC) properties. Data of this study further indicate that high-level expression of galectin-4 and MYC-regulated C1GALT1 in the clinical PCa specimens distinguishes a subtype of aggressive PCa from indolent tumors. Patients with double-positive tumors are at high risk of recurrence and metastasis \textit{vs.} double-negative tumors; and whose overall survival was 2 to 6 years in 36/231 subjects (16\%) \textit{vs.} >10 years in 111/231 subjects (48\%). Using knockin approach, we tagged the sox9 gene transcription with \textit{iRFP} and \textit{Renilla luciferase} genes at the 3'-UTR in the metastatic PCa cells (22Rv1-M4). Expression of the reporter genes in 22Rv1-M4 cells is exploited as the bioassay to guide our purification of phytochemicals that inhibit the sox9 transcription and CSC phenotype in PCa cells. Among the several candidate plants, we have shown oral BP3-6 inhibited the metastatic recurrence of PCa in a nude mice model.
Stress-induced block of adipogenic differentiation of bone marrow MSCs affects their functions

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High constitutive levels of autophagy and p53 expression are important characteristics of human bone marrow-derived mesenchymal stromal cells (MSCs). Recently, we found that multipotent MSC underwent spontaneous adipogenic differentiation after a few weeks in normoxic culture. Activity of autophagy and cell cycle machinery in bone marrow-derived adipocytes (BM-A) and non-differentiated MSCs were similar. However, prolonged stress dramatically reduced autophagy levels, inhibited differentiation into adipocyte lineage, and induced drastic morphological changes, like increased nucleus-to-cytoplasm ratio. In parallel, defected autophagy was accompanied by p53 disappearance and detachment of cells.

While changes in morphology, inhibition of p53 pathway and autophagy perturbation are among the main characteristics of MSC transformation, we wanted to know how stress-induced block of terminal differentiation of MSCs would affect their functions. We decided to direct our efforts towards migration under different stress stimuli as well as under p53 and autophagy inhibition. To this aim we used p53 inhibitors Nutlin-3 and PFT-α, autophagy inhibitors Baf-1 and CQ. We found that p53 or autophagy inhibition changes MSC morphology, blocks migration, and induces cell detachment and apoptosis of MSCs in a time- and dose-dependent manner. Long-term exposures of MSCs to stress conditions like starvation, or hypoxia - or a combination thereof - counteracted MSC migration. Interestingly, migration properties of BM-A were similar to unstressed MSCs. Our data show a strong impact of stress-dependent events on MSC functions and are therefore highly relevant to mesenchymal stem cell plasticity.
Application of Recombinant Variable Heavy Chain Domain of a Cell-Penetrating Anti-DNA Antibody for the Delivery of Biomolecules into Cancer Cells

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For diagnosis and therapy of diseases including cancer, targeting candidate intracellular proteins using efficient vehicles is important. For the delivery of protein modifiers inside cells, fragments of cell-penetrating antibodies can be applied as useful vehicles. We studied the applicability of single domain antibodies as delivery vehicles for mammalian cells. The properties of the recombinant VH single domain of a cell-penetrating monoclonal anti-double stranded DNA antibody were analyzed using flow cytometry, confocal microscopy, cell proliferation assay, and cell cycle analysis in various mammalian cell lines. The VH domain penetrated into various cell lines in a time- and dose-dependent manner, although the internalization efficiency varied. The domain was localized in the nuclei as well as the cytoplasm of living cells. It was also internalized into cells mainly through the clathrin-mediated endocytosis pathway. We tested further its efficiency in delivering specific biomolecule(s) using the conjugates of the VH domain and small interfering RNA (siRNA) for the testicular nuclear auto-antigenic sperm protein (tNASP). It was found that the siRNA was successfully delivered by the VH domain into cancer cells, and knockdown effects from the delivered tNASP-siRNA were observed. The levels of the RNA transcript and protein of tNASP were decreased and the down-regulated tNASP inhibited cell proliferation and caused G0G1 phase arrest of the cell cycle. These results indicate that the recombinant 2C10 VH domain could be applied as an efficient vehicle capable of delivering valuable biomolecules into the cytoplasm or cell nuclei for clinical uses.
Ameliorating the Cytotoxic effects of Cisplatin and Methotrexate via Specific inhibition of ABCG2 and ABCB5 in Primary Brian Tumors

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Background: For nearly seven decades since the cancer chemotherapeutic resistance was first experimentally documented, it still poses a major challenge in cancer treatment. Such failures are correlated with the existence of several factors including the overexpression of ABC-transporter proteins (ABC-TPs). Evidences of their strong association with chemotherapeutics’ resistance are growing and using specific inhibitors to target ABC-TPs will improve overall clinical outcomes.

Methods: After brain tumors’ surgical resection, tissues were dissociated using 0.4mg/mL of collagenase type-1a(Gibco) made up in HBSS (Invitrogen). The dissociated cells were grown in DMEM-F12(Gibco) media with 10% FBS (Invitrogen), 1% penicillin-streptomycin and grown in humidified incubator at 5% CO\textsubscript{2}. For drug treatment, Cisplatin and Methotrexate were used in combination with Verapamil and Pantoprazole (inhibitors for ABC-TPs) at serial dilutions from 200\textmu M to 0\textmu M. For ABC-TPs’ detection, standard immunofluorescence protocol was used involving Anti-ABCG2 antibody (1:50, Abcam:ab207732) and Anti-ABCB5 antibody (1:100, Abcam:ab203120).

Results: The growth inhibition assays have revealed IC\textsubscript{50} for Methotrexate and Cisplatin as 2.2uM and 100uM respectively; however when Verapamil and Pantoprazole were used in combination with the cytotoxic drugs, the IC\textsubscript{50} values were reduced that is for Methotrexate+ Verapamil, it was 1.5uM while for Cisplatin+ Pantoprazole it reduced to 50uM. These drug assays-derived concentrations were used for detection of ABCG2 and ABCG5 using immunofluorescence technique that also revealed reduction of ABC-TPs in brain cancer cultures, indicating the ameliorating effects of ABC-TPs’ inhibitors when used in combination of cytotoxic drugs.

Conclusion: The anticancer activities of cytotoxic drugs have been enhanced using the inhibitors for ABC-TPs in brain tumors. Since the ABC-TPs are involved in cancer chemotherapeutic resistance, specific inhibitors to target these transporter proteins will advance the field of precision medicines that will ultimately develop novel strategies to upsurge clinical effectiveness of the existing anticancer drugs and to open new avenues for novel drug discovery.
Tertiary Gleason, grade groups and periostin in aggressive prostate cancer

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Background: Tertiary Gleason patterns are reported with increasing frequency in relation with disease recurrence (Epstein et al 2012). We have previously reported association between periostin, Skp2 and Slug in aggressive prostate cancer. We decided to verify association of tertiary Gleason and grade groups with expression of these and other selected proteins in our patients.

Material and methods: Formalin-fixed paraffin-embedded tissues of 101 prostate carcinomas were stained immunohistochemically for E-cadherin, beta-catenin, vimentin, Skp2, Slug, Ki67, p53, androgen receptor, PSA, periostin, versican, and scored. Slides were reviewed for the presence of tertiary Gleason (worse than the primary and secondary grade, patterns 4 and 5). Carcinomas were classified into localized, advanced and metastatic groups, and ISUP 2014 Gleason grade groups (GG1-5, Pierorazio et al 2013 and Epstein et al 2016).

Results: Tertiary Gleason was recognized in 22% of radical prostatectomy cases, it was more frequent in advanced and metastatic tumors (p<0.01). It also positively correlated with total preoperative PSA (Rs=0.303, p=0.003), Ki67 (Rs=0.209, p=0.045) and periostin stromal expression (Rs=0.276, p=0.008) while negative correlation was observed for membrane-localized beta-catenin (Rs=-0.211, p=0.035). Grade groups were in negative association with E-cadherin (Rs=-0.203, p=0.045) while with nuclear Skp2 and periostin stromal expressions showed positive association (Rs=0.338 and 0.269, p=0.001 and 0.008, respectively). Periostin stromal positivity correlated with versican stromal expression (Rs 0.368, p<0.001) and stromal versican correlated with stromal vimentin (Rs=0.332, p=0.001).

Conclusions: For the first time, we show significant association of tertiary Gleason and grade groups GG4/5 with periostin, Ki67, Skp2 and beta-catenin. Regression models in larger cohorts are further needed to accurately identify potential aggressive prostate cancer requiring immediate treatment.

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A phase II, open-label, multicentre study to assess the anti-tumour activity of Afatinib in patients with activating epidermal growth factor receptor mutation (EGFRm) from Circulating Tumor DNA (CtDNA)

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The treatment efficacy of afatinib was assessed in patients with lung cancer harboring EGFRm which were detected from CtDNA. Primary objective was to prove overall response rate (ORR) in response evaluable population, and the secondary endpoints were progression free survival, overall survival and safety. EGFRm analyses for CtDNA and tumor DNA were performed by PANA Mutyper® EGFR kit (Panagene, Korea).

A total of 340 patients were screened for this trial. Tumor genotyping showed 24.5% (81/331), while CtDNA showed 20.5% (68/331) of positivity to detect activating EGFRm (exon 19 deletions, exon 21 and exon 18 point mutations). Among 81 subjects with tumor DNA EGFRm positive subjects, 48 showed EGFRm in their CtDNA (59% sensitivity). Types of EGFRm were identical between tumor DNA and CtDNA in 48 subjects. Among 21 subjects enrolled in this trial, 11 subjects had EGFRm only in CtDNA (tumor DNA EGFR wild or unknown, Group 1), and 10 subjects had same EGFRm in their CtDNA and tumor DNA (Group 2).

Afatinib (40mg) was initiated in 21 (female: 17, adenocarcinoma: 20) subjects with mean age of 68.5 years. Dose modifications were made in 13 subjects (62%). Partial remission was observed in 13 subjects, stable disease in 6 subjects, and response was not evaluated in 2 subjects (ORR : 68.4%). There was no significant difference in ORR between Group 1 (80%) and Group 2 (55.6%). As of July 25 2018, treatment is ongoing in 15 subjects, progression was confirmed in 3 subjects and 1 withdrew consent, 2 discontinued treatment due to serious adverse events (SAEs). A total of 5 SAEs including 2 subjects with possible drug induced lung disease were reported.

In conclusion, afatinib showed favorable efficacy in subjects with NSCLC harboring EGFRm in their CtDNA.

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A new ALK isoform transported by extracellular vesicles confers drug resistance to melanoma cells

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Background: Drug resistance remains an unsolved clinical issue in oncology. Despite promising initial responses obtained with BRAF and MEK kinase inhibitors, resistance to treatment develops within months in virtually all melanoma patients.

Methods: Microarray analyses were performed in BRAF inhibitor-sensitive and resistant cell lines to identify changes in the transcriptome that might play a role in resistance. siRNA approaches and kinase inhibitors were used to assess the involvement of the anaplastic lymphoma kinase (ALK) in drug resistance. The capability of extracellular vesicles (EVs) to transfer drug resistant properties was investigated in co-culture assays.

Results: We report a new mechanism of acquired drug resistance involving the activation of a novel truncated form of ALK. Knock down or inhibition of ALK re-sensitised resistant cells to BRAF inhibition and induced apoptosis. Interestingly, truncated ALK was also secreted into EVs and we show that EVs were the vehicle for transferring drug resistance.

Conclusions: To our knowledge, this is the first report demonstrating the functional involvement of EVs in melanoma drug resistance by transporting a truncated but functional form of ALK, able to activate the MAPK signalling pathway in target cells. Combined inhibition of ALK and BRAF dramatically reduced tumour growth in vivo. These findings make ALK a promising clinical target in melanoma patients.
T cell activity and cytotoxicity is modulated by the surrounding extracellular matrix

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Degradation of the extracellular matrix (ECM) surrounding tumors is an essential part of invasive cancer growth and it is accompanied by the deposition of a different tumor-specific ECM. The density of the tumor ECM correlates strongly with poor prognosis in many types of cancer including breast cancer and colon cancer. Previous investigations of the underlying mechanisms have mainly focused on the regulation of cancer cell proliferation and invasiveness. The relationship between the tumor ECM and tumor-infiltrating T cells has not been investigated yet. In this study we have investigated if a high ECM density can modulate the immune environment in tumors and influence the ability of T cells to kill cancer cells.

Using a 3D cell culture system, we have investigated if T cells respond to the density of the surrounding ECM. T cells cultured in different ECM densities displayed significant changes in cellular proliferation, and whole-transcriptomic analysis revealed that this cell type indeed responds to the ECM density. The changes induced by a high-density ECM involved a clear downregulation of cytotoxic activity markers and upregulation of regulatory T cell markers. The chemokine profile of the 3D cultured T cells was also dramatically altered by the density of the ECM.

To examine if the observed transcriptional changes had any functional implications, we cultured T cells isolated from a dissected melanoma in ECMs of various densities. Subsequently, these T cells were extracted and assayed for their ability to kill an autologous melanoma cell line. Consistent with the observed transcriptional changes, T cells that had been cultured in a high density ECM demonstrated a much reduced cytotoxic activity.

This study suggests the existence of a new and conceptually different mechanism regulating T cell activity, which could have great importance in cancer, and potentially could be targeted in order to improve immunotherapy efficiency.
A case report of a patient with metastatic prostate cancer treated with combined treatment of hormone therapy and Sasang type specific-herbal medicine

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The Sasang Constitutional Medicine (SCM) is used as complementary treatment for cancer in Korea. The SCM is a kind of typological personalized medicine in traditional Korean Medicine which categorized people into four types, namely, Tae-Yang type, So-Yang type, Tae-Eum type, and So-Eum type. It provides type-specific tailored herbal medicine and dietary regimen.

We report a case of 68-year-old Tae-Eum type male patient diagnosed with stage IV prostate adenocarcinoma (T3bN1M1b, Gleason score 8) with multiple bone metastasis in spine and suspected renal cell carcinoma on August 24, 2017. He was treated with anti-androgen therapy (daily bicalutamide 50mg) and tailored herbal medicine (G0501) and dietary regimen as complementary treatment since September 12, 2017, replacing leuprorelin injection, a luteinizing hormone-releasing hormone agonist considered to be the standard primary treatment for metastatic prostate cancer. G0501 is Tae-Eum type-specific tailored prescription consisting of mainly Puerariae Lobata, Ulmus Pumila, Typha Angustifolia, Purunus Mandshrika, Leonurus Sibiricus, Ganoderma lucidum, and Phellinus linteus. The patient was also received radiotherapy for 2 weeks as adjuvant therapy. After 6 months of the treatment, the patient’s prostate-specific antigen (PSA) decreased from an initial value of 562.65 to 1.17 ng/mL. Radiologic examination and bone scan showed that prostate volume was decreased from 33 cc to 17 cc, multiple bone metastases were reduced, and no significant lymph node enlargement was observed. Spinal pain and urinating problems due to bone metastases were also significantly improved. After 8 months of the treatment, the PSA level was found to be 0.204. This case suggests that SCM might be helpful in prolonging the survival and improving symptoms of the patient with metastatic prostate cancer as complementary treatment during hormone therapy. Additional follow-up is required to confirm the prognosis of this patient.
Down-regulation of HAI-2 promotes human non-small cell lung carcinoma cell invasion and metastasis via up-regulation of pericellular proteolysis.

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Dysregulation of pericellular proteolysis usually plays a role in human cancer cell invasion and metastasis. Isolation of a cell-surface protease system is an important issue for mechanistic study and therapeutic target identification for non-small cell lung carcinoma metastasis. In this communication, we employed Immunohistochemistry of a tissue array and TCGA database to assess the correlation between serine protease inhibitors (SPIs) and lung adenocarcinoma progression. We identified hepatocyte growth factor activator inhibitor-2 (HAI-2) was down-regulated following the lung adenocarcinoma progression, which was related to poor survival and tumor invasion. We further isolated a serum-derived serine protease, plasmin, to be a novel target of HAI-2. Down-regulation of HAI-2 promotes the cell-surface plasmin activity, epithelial mesenchymal transition (EMT), and cell motility. HAI-2 can suppress plasmin-mediated activations of HGF and TGF-β1, EMT and cell invasion. In addition, down-regulated HAI-2 increased metastasis of lung adenocarcinoma via up-regulating plasmin activity. Thus, HAI-2 functions as a novel inhibitor of plasmin to suppress lung cancer cell motility, EMT and metastasis.
Immunogenic properties of cancer resistance mutations

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Cancer immunotherapy has achieved remarkable successes in recent years, particularly in the treatment of some advanced solid tumours with checkpoint inhibitors. Despite these successes, our understanding of the interplay between the immune system and nascent or established tumours is still unsatisfying. A recent paper [Marty et al. Cell 2017] suggested that driver mutations exhibit low immunogenicity in the general population, a property that would allow them to evade the immune response in the early stages of cancer development. Here, we analysed immunogenicity of resistance mutations that emerge in patients treated with targeted therapies such as, for example, tyrosine kinase inhibitor therapy. To this end, we downloaded a list of 225 missense resistance mutations from the COSMIC website. We generated all amino acid peptides of length 8 to 11 that include the resistance mutations with each peptide matching the canonical gene sequence except for the mutated amino acid representing the resistance mutation. We then predicted the affinity of each peptide (using NetMHCpan) to all HLA class I alleles among 1,278 1000G individuals and use this as proxy for mutation immunogenicity. We calculated a mutation-specific population-wide immunoscore by weighing the affinity scores for each HLA-type by the allele frequency in our dataset. We observed that resistance mutations are more or, depending on the filters we apply, as immunogenic as passenger mutations and generally more immunogenic than mutations that are highly recurrent in TCGA (i.e. likely drivers). We suggest that this ‘weakness’ could be of interest for combined targeted therapy-immunotherapy treatments.
The use of protein arrays in targeted therapy – differences in signaling between neuroblastoma tumor samples and cell lines

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Neuroblastoma is the most common extracranial solid tumor in children and accounts for approximately 15\% of pediatric cancer deaths. This disease is characterized by its extreme heterogeneity. Some cases show aggressive phenotype with poor therapy response, whereas others may mature or undergo a spontaneous regression without therapy. The most important features used for the prediction of clinical behavior are age, \textit{MYCN} status, ploidy, allelic deletions on chromosome arms 1p and 11q and TrkA/B phosphorylation status.

Both TrkA and TrkB are receptor tyrosine kinases activated by neurotrophins NGF, BDNF, NT3 and NT4. TrkA expression was described in favorable neuroblastoma and correlates with patient survival. On the other hand, TrkB activity is connected with a poor prognosis and can be targeted by specific inhibitor Larotrectinib.

The phosphorylation of 49 different RTKs and 26 downstream signaling molecules was examined by the Proteome Profiler Human Phospho RTK/MAPK Array Kit (R&D System). mRNA level of the most often activated molecules was determined by quantitative real-time PCR. Western blotting was used for the detection of both total and phosphorylated proteins of interest.

We have examined 17 neuroblastoma samples from 14 patients, 17 primary and 2 established neuroblastoma cell lines. Our results identified significant differences in the expression and activity of Trk family receptors as well as other important signaling molecules. The most interesting finding was the difference between patient’s samples and cell lines in terms of TrkA/B phosphorylation. These results suggest that whereas TrkA/B might serve as good prognostic markers and therapeutic targets in patients with neuroblastoma, their use in 	extit{in vitro} studies is limited due to the absence of their ligands and therefore the lack of phosphorylation in these conditions. This indicates that data regarding different signaling markers (e. g. Trk kinases) obtained from cell lines must be interpreted carefully.

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Kinase inhibitor library screening identifies synergistic drug combinations effective in sensitive and resistant melanoma cells

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Melanoma is the most aggressive and deadly form of skin cancer with increasing case numbers worldwide. The development of inhibitors targeting mutated BRAF (found in around 60% of melanoma patients) has markedly improved overall survival of patients with late-stage tumors, even more so when combined with MEK inhibitors targeting the same signaling pathway. However, invariably patients become resistant to this targeted therapy resulting in rapid progression with treatment-refractory disease. The purpose of this study was the identification of new kinase inhibitors that do not lead to the development of resistance in combination with BRAF inhibitors (BRAFi), or that could be of clinical benefit as a 2nd line treatment for late-stage melanoma patients that have already developed resistance. We have screened a 274-compound kinase inhibitor library in 3 BRAF mutant melanoma cell lines (each one sensitive or made resistant to 2 distinct BRAFi). The screening results were validated by dose-response studies; different tools were applied to investigate and quantify potential synergistic effects of drug combinations. Eight inhibitors targeting Wee1, Checkpoint kinase 1/2, Aurora kinase, MEK, Polo-like kinase, PI3K and Focal adhesion kinase killed melanoma cells synergistically when combined with a BRAFi. Additionally, combination of a Wee1 and Chk inhibitor showed synergistic killing effects not only on sensitive cell lines, but also on intrinsically BRAFi- and treatment induced-resistant melanoma cells. Interestingly, chronic combined treatments with several of these drugs, did not lead to emergence of resistance.

Here, we have identified new, previously unexplored (in the framework of BRAFi resistance) inhibitors that have an effect not only on sensitive but also on BRAFi-resistant cells. These promising combinations, together with the new immunotherapies could be an important step towards improved 1st and 2nd line treatments for late-stage melanoma patients.
Biomechanical characteristics of head and neck cancer cells as a potential markers for tumor progression and metastatic potential

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Head and neck tumors are made of cancer cells and surrounding stromal cells with diverse genetic and metabolic features called intra-tumoural heterogeneity. Whereas most of cancer tissues are usually mechanically stiffer than normal tissue, some studies have disclosed that cancer cells themselves are, on the contrary, more pliable than normal cells. It has been shown that cell stiffness determined by atomic force microscopy (AFM) could be used as a marker for cancer progression and metastatic potential. Different cancer types display distinct cell stiffness and a connection between attenuated cell stiffness and the increased invasion capacity was also observed. Furthermore, changes in cytoskeletal architecture induced by stress were shown to significantly influence biomechanical features of cancer cells. Because cellular bio-mechanical characteristics including cell stiffness are very important for cell motility, changes in cytoskeletal architecture and consequent change in cell stiffness, cell dry mass, and motility could be an important secondary effect of many cytostatic drugs. In this study, we separated CD90-positive cells from tumour tissue of head and neck cancer patients and tested the hypothesis that their cell stiffness is in association with ability of these tumour cells to form lymph node metastasis in head and neck cancer patients and with chemoresistance. Cell stiffness of CD90-positive tumour cells derived from patients with stage of lymph node metastasis N0-1 was significantly higher than cell stiffness of CD90-positive tumour cells derived from patients with stage N2-3. Moreover, we found out that cisplatin caused a significant increase in cell stiffness in cisplatin-treated CD90-positive tumour cells compared to non-treated counterparts. This results indicate that attenuation of cell stiffness in CD90-positive tumour cells could be promising predictor of metastatic potential and therapy response in head and neck tumours.

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hMENA regulates Gas6/Axl axis in the dialogue between tumor cell and cancer associated fibroblasts

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Deciphering the aberrant communication between tumor cells and stroma, is essential for the development of new therapeutic targets.

The identification of phenotypical and functional cancer-associated fibroblast (CAF) subtypes, the most abundant stromal cells, is relevant to develop microenvironment-related anti-tumor treatment, due to the dynamic reciprocity between tumor cells and CAFs, via paracrine and juxtacrine pathways.

We have previously demonstrated that hMENA, a member of the ENA/VASP family, actin-regulatory proteins, and the epithelial-(hMENA11α) and mesenchymal-(hMENADv6) specific isoforms influence a number of intracellular signaling pathways involved in cancer cell proliferation, survival, invasion and epithelial mesenchymal transition (EMT). Of clinical relevance, we have suggested that the pattern of hMENA isoform expression in tumor cell compartment has prognostic value in early stage non-small cell lung cancer (NSCLC) and in pancreatic ductal adenocarcinoma (PDAC), both tumors with an abundant fibrotic stroma.

Herein we demonstrate that hMENA/hMENADv6, overexpressed in CAFs with respect to normal fibroblasts, regulate CAF activation and identify a subset of CAFs with pro-tumoral functions in NSCLC and PDAC. Notably, we define a novel function of hMENA in the regulation of tumor cell-CAFs cross-talk, via the modulation of the Gas6-Axl signaling axis.

hMENA/hMENADv6 regulate Axl expression in tumor cells, crucial in EMT, drug resistance and immune evasion. Tumor cells overexpressing hMENA/hMENADv6 secrete factors which up-regulate hMENA/hMENADv6 in CAFs leading to the secretion of the Axl ligand Gas6, favouring the invasiveness of Axl-expressing NSCLC and PDAC cells. We found that a high hMENA/Gas6/Axl gene expression signature is associated with a poor prognosis in PDAC patients.

Our findings indicate that the pattern of hMENA isoform expression in both tumor cells and CAFs may reveal tumor mesenchymal traits which may identify tumor subtypes for tailored therapies.

The hMENA/Gas6/Axl network may represent a novel prognostic and therapeutic target in NSCLC and PDAC patients.
3'UTR KRAS shortening as genetic biomarkers in glioblastoma

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3'UTR shortening has been established as a key mechanism of oncogene activation and has demonstrated promising potential as a prognostic marker. Generally, rapidly proliferating cells preferentially express mRNAs with shortened 3'UTRs. As a general mechanism, the shortening of 3'UTRs enables key genes to escape microRNA repression, thus leading to higher expression and promoting proliferation. The KRAS gene is an important regulator of cellular proliferation and its 3'UTR region has recently attracted attention as genetic biomarkers predictive for prognosis, diagnosis and treatment of cancers. mRNA transcript variants of the KRAS gene differing in the lengths of their 3' untranslated regions (UTRs) are reported but their function is still largely unknown. In contrast to the well-studied SNP and MRE, the properties and dynamics of the emerging shortening 3'UTR KRAS remain elusive. Moreover, recent reports indicate that brain tissue possesses the longest 3'UTRs. Mutations in KRAS are rare in human gliomas and particularly rare in WHO grade III and IV gliomas in adult patients. Interestingly, expression of activated HRAS in breast cancer cells results in significant changes in the 3'UTR expression pattern toward shortening (64%) as well as lengthening (36%). We investigated whether 3'UTR shortening can produce biologically relevant stratification of glioblastomas. Here, we report transcriptional analysis with specific primers for long and short transcripts of the KRAS gene at 3'UTR region. We measured differential expression of three transcript variants respectively in size of 5.2 kb, 1.2 kb and 2.3 kb across a panel of primary and established cultures of human glioblastoma. Glioblastoma multiforme samples exhibited significantly higher KRAS mRNA levels compared to established cultures. Moreover, expression of long transcript variant correlates with reduced aggressiveness in primitive tumours and mitogenic stimulation induced preferentially this variant in almost all samples. Further analysis will determine its potential role as genetic biomarker in predicting cancer outcome.
Characterisation of breast cancer adipose tissue-derived mesenchymal stromal cells and their role in cancer malignancy

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Tumors are considered as an organ-like structures than just a clonal expansion of mutant cells. There is a great deal of evidence that points to the stroma as a major regulator of tumor progression and contributor to the risk factors determining tumor formation. An important component of breast tumor stroma is adipose tissue - the rich source of mesenchymal stromal cells (MSC), which can have critical role in cancer progression via activation by tumor cells.

We analysed properties of the MSC isolated from breast adipose tissue in order to better understand how malignancy affects mesenchymal precursors in healthy tissue. We analysed four groups of adipose tissue patient´s samples. The first group is composed of adipose tissue collected from healthy women undergoing reduction mammoplasty. In the second and third group we analysed MSC from adipose tissue adjacent to premalignant or malignant lesions, and in the fourth group, MSC derived from malignant lesion of BRCA positive tumors. We have shown that breast cancer-derived MSC harbour significant expression changes compared to healthy MSC and influence also tumorigenicity of cancer cells in vivo connected with changed composition of subcutaneous tumors. We have previously shown, that MSC-secreted factors are able to increase migration potential and mammosphere formation in breast cancer cell lines cultivated in the presence of MSC-secreted factors. Our experiments showed also that cisplatin exposed healthy MSC were able to produce factors that turn on the changes in stemness and resistance of tumor cells by increasing both the ALDH positivity and expression of CD24-/CD44+/EpCAM+ cell surface markers in tumor cells.

Identification of key functional changes of MSC presented in tumor microenvironment, and understanding of conditions in which MSC enhance tumor growth and metastasis, may help to find ways for normalizing tumor microenvironment and regulating disease progression.
Canonical Wnt/β-catenin Pathway is a Critical Biomarker Associated with Invasion and Lymph Node Metastasis in Head and Neck Squamous Cell Carcinoma

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Background: Cancer stem cells (CSCs) are involved in tumor invasion and metastasis, but determinants that regulate this process in head and neck squamous cell carcinoma (HNSCC) are largely unknown. We investigated whether the canonical Wnt/β-catenin signaling pathway, a regulator of stemness in CSCs, acts as a modulator of invasion and lymph node metastasis (LNM) in HNSCC.

Methods: In vitro and in vivo loss of function experiments against canonical Wnt/β-catenin signaling were performed to evaluate its role in HNSCC cells. Slug was evaluated as a downstream protein in Wnt/β-catenin-mediated invasion. Wnt/β-catenin and Slug expression levels were examined in 119 HNSCC tissue samples to study the relevance of these proteins in LNM and prognosis of patients post-treatment.

Results: In vitro β-catenin expression suppression decreased migration and invasion of HNSCC cells. Using in vivo mouse orthotopic LNM model, decrease in LNM was observed with mitigated β-catenin expression. Slug expression upregulation mediates invasion and metastasis by canonical Wnt/β-catenin activation. Co-expression of β-catenin and Slug is the major predictive factor of LNM and survival rate in HNSCC patients.

Conclusions: Canonical Wnt/β-catenin/Slug pathway significantly contributes to tumor invasion and LNM. Its blockade may be a treatment strategy for LNM and tumor recurrence in HNSCC.
Reproductive history, breast cancer risk and optimal starting age of screening

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Background: Reproductive factors and familial risk contribute substantially to the risk of breast cancer (BC), which is the most incident cancer among women globally. We assessed BC risk by reproductive profile in women with and without a family history of BC to determine the optimal starting age of screening, which is lacking in the literature.

Method: We used the Swedish Family-Cancer Database (FCD), the world’s largest of its kind. The analysis included 207,705 invasive BC patients diagnosed from 1958 to 2015, of whom 27,903 had family history of BC.

Results: Higher parity and early age at first birth were associated with reduced BC risk among both women with and without a family history of BC. Women with older age at first birth and lower parity had higher BC risk, yet could be screened earlier. Among women with no family history of BC, nulliparous women attained same level of risk as 50-year old women in the general population at age 48 years (two years earlier screening), whereas women with a first birth before age 25 years and parity≥4 attained the same level of risk at the age 60 (10 years later). Among women with a family history of BC, nulliparous women could be screened 10 years earlier and those with 1-2 children and first birth at age ≥30, 11 years earlier; women with age at first birth before 25 and parity≥4, five years earlier.

Conclusion: Our study for the first time provides practical information on how many years earlier (or later) women with certain reproductive factors, such as parity and age at first birth, should be screened considering their family history of BC. This is an important step toward a personalized BC screening instead of the current one-size-fits-all policy, in which mass screening starts for all women at the same age.
Lower miR-195 and -497 levels in circulating exosomes correlate with menigioma progression via upregulation of GATA4

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Meningioma is the most common primary brain tumour and it is classified as benign (WHO I, ~60%), atypical (WHO II, ~35-40%) and anaplastic/malignant (WHO III, ~1-3%). The 3-year recurrence rate for WHO I menigioma is ~40% and it is much greater in WHO II and III.

To date, menigioma classification is based on histopathological characterization and no circulating biomarkers have been identified to predict tumour progression yet.

We performed in silico studies, which highlighted that cyclin D1 and E1 are regulated by the miR-15 family members (miR-15-16-195-497). Gene expression studies showed that miR-195 and -497 are downregulated in WHO II and III samples compared to WHO I (p=0.002), via upregulation of the transcription factor GATA binding-protein 4 (GATA4), highly overexpressed in KT21-MG1 (WHO III) compared with WHO I cells (Ben-Men-1 and tumour-derived cells, p<0.0001). This result was consistent in tissues (p=0.0001), where GATA4 expression was higher in WHO II and III compared to WHO I. Lentiviral-mediated RNA interference of GATA4 in KT21-MG1 led to an increase in the expression of miR-195 and -497 (p=0.0006) and a decrease in targeted cyclin-mRNA expression (p=0.0083).

MicroRNA are small non-coding RNAs released from tumour cells into the blood stream via exosomes, carrying the potential to be used as liquid biopsies. Consistent with our gene expression analysis in cells and tissues, miR-195 and -497 were lower in exosomes-cargo isolated from blood serum of WHO II and III menigioma patients compared to WHO I (p=0.004).

Overall, these data show that miR-15 family downregulation in WHO III menigioma cells and tissues is driven by overexpression of GATA4, suggesting that it may contribute to tumour progression, which is characterized by higher expression of cyclin D1 and E1. In addition, these data suggest that miR-195 and -497 are circulating prognostic biomarkers of menigioma progression.

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Zinc finger protein is suppressed by fibroblast that promotes malignancy of activated prostate cancer through JAK/STAT signaling pathway

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Prostate cancer (PCa) is the most frequently occurred cancer and common cause of cancer mortality of male. Current treatment relies on targeting androgen receptor signaling by hormone deprivation. However, tumors are heterogeneous and hormone depletion often results in the selection of drug resistant, highly metastatic tumor that survive despite the targeted tumor therapy. Currently there are no biomarkers that distinguish benign PCa from aggressive PCa. Therefore, there is an urgent need for such a biomarker that can be used to diagnose aggressive PCa and detect early.

Metastatic prostate cancer is affected by tumor microenvironment stromal cells, especially fibroblast are the key cell in the PCa tumor microenvironment, which induces tumor growth and metastasis. Activated fibroblast secretes the soluble factor such as cytokines, chemokines and growth factors, which makes PCa more aggressive. In this study we identified IL-6 secreted by fibroblast suppress Zinc finger protein (ZNF) as a novel suppressor of metastatic PCa. ZNF is to be known as a tumor suppressor gene in carcinogenesis. Here, we found ZNF affects diverse signaling including cell cycle, apoptosis and angiogenesis. The loss of the protein has been related to increased proliferation, invasiveness and motility, and resistance to apoptosis in prostate cancer cells. Furthermore, clinical data analysis from the metastatic PCa patients reveals that the expression of this genes was negatively correlated with the increase of Gleason score. Surprisingly we founded a novel role of ZNF as a regulator of the tyrosine phosphorylation of JAK/STAT pathway through the tyrosine phosphatase axis.

In conclusion, ZNF may suppress the important JAK/STAT pathway in metastatic PCa, and our study supports the targeted tumor therapy potential of ZNF in for this highly aggressive tumors. Ultimately loss of ZNF expression detected is a promising and valuable biomarker for PCa aggressiveness and metastasis in the personalized care of PCa.
High-content Functional Drug Profiling for Paediatric Precision Medicine

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Over 600 relapsed paediatric cancer cases have been analyzed by next generation sequencing and microarray-based technologies within our paediatric precision oncology platform (INFORM) at KiTZ, Heidelberg. While druggable targets can be identified in 50% of cases, remaining patients lack actionable alterations, indicating highest unmet medical need. We propose that direct functional drug response profiling of patient-derived cancer cells will provide additional key information for precision paediatric oncology.

To establish a standardized ex-vivo drug response profiling platform, a set of genetically defined paediatric 3D-tumor cell cultures (n=7) was selected based on known molecular characteristics and vulnerabilities with known clinical benefit, serving as positive control for drug responses. Spheroids were cultured in 384-well plates and screened towards a drug library consisting of 23 clinically approved drugs in 5 concentrations, including predicted active drugs for each model.

Drug responses were assessed after 72 h by quantitative high-content confocal imaging of spheroids with the Opera Phenix microscope (20x objective), using fluorescent dyes to detect for mitochondrial integrity, apoptosis and nuclear condensation complemented by metabolic readouts for cell viability. Quality controls, dose response curves and drug sensitivity scores were defined using analysis pipelines at FIMM.

Effective drugs predicted by the molecular characteristics could be confirmed, i.e. response to HDAC inhibitor panobinostat in a MYCN-amplified neuroblastoma model and response to BRAFV600E inhibitor vemurafenib and to MEK1/2 inhibitors (selumetinib, trametinib) in a BRAFV600 mutated BT40 model. This demonstrates the predictability of ex-vivo drug response profiling in paediatric oncology.
In conclusion, we have established a quantitative functional platform for image-based profiling of drug efficacies which can be translated to 3D-spheroids of patient-derived short-term cultures. Functional data integrated with molecular profiles of the tumor sample to identify most effective single-agent or combination treatments for individual patients will serve as basis for future clinical trial design in paediatric precision medicine.
Combining Lovastatin with Trastuzumab Improves the Targeting and Treatment of HER2+/CAV1+ Breast and Gastric Tumors

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Clinical evidence demonstrating the benefit from strategies that selectively target and inhibit the human epidermal growth factor receptor 2 (HER2) has resulted in the development of highly effective anti-HER2 therapies. Among these, anti-HER2 therapeutic antibodies (e.g. trastuzumab and pertuzumab), antibody-drug conjugates and molecular imaging agents including radio- or fluorescently-labeled antibodies have changed the prognosis and outcomes for breast and gastric cancer patients.

The effective treatment of HER2+ tumors with trastuzumab is premised on the binding of the antibody with HER2 at the cell membrane. We have identified that caveolin-1 (CAV1) – a protein present in cholesterol-rich structures at the cell membrane, mediates HER2 internalization and reduces HER2 availability at the cell membrane for binding with trastuzumab.

We found that CAV1 expression is inversely correlated with membrane-localized HER2 protein in various cell lines and patient tumor samples. CAV1 overexpression using CRISPR activation plasmid, dramatically reduced cell-membrane-localized HER2. Conversely, the half-life of membrane-localized HER2 was increased after CAV1 depletion using small interfering RNA (siRNA), which resulted in improved membrane binding of trastuzumab. Having established the inverse correlation between HER2 and CAV1, we used lovastatin – an inhibitor of the enzyme HMG-CoA reductase to deplete membrane cholesterol content and thus membrane-localized CAV1. Acute, intermittent treatment of cells with lovastatin led to a dramatic increase in HER2 localization at the cell membrane, an effect that is rescued by the addition of mevalonate. Intriguingly, pretreatment of mice bearing HER2+/CAV1+ tumors with pharmacologically-relevant doses of lovastatin increased and accelerated tumor uptake of radiolabeled trastuzumab allowing earlier delineation of tumors via PET imaging. Finally, lovastatin administration increased the efficacy of trastuzumab therapy by inhibiting the growth of HER2+/CAV1+ cell line, as well as patient-derived xenografts. Collectively, these preclinical data suggest a potential benefit from combining lovastatin with trastuzumab to improve the therapeutic efficacy and molecular imaging in HER2+/CAV1+tumors.
Identifying Collateral and Synthetic Lethal Vulnerabilities within the DNA-damage Response

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Genomic deletion in DNA-damage response (DDR) genes is an important driver of tumorigenesis, frequently accompanied by collateral heterozygous deletion of chromosomally neighbouring passenger genes. While deletion of these passenger genes is typically tolerated due to functional redundancy, cancer cells can become selectively vulnerable to inhibition of the remaining copies of these genes. Such collateral vulnerability can be exploited in synergy with synthetic lethality (SL) wherein the inhibition of genes that functionally compensate the DDR loss induces lethality.

We developed a computational method to identify genes inducing collateral vulnerability (genes co-deleted with DDR genes) and/or SL (genes deleted in a mutually exclusive manner to DDR and its co-deleted genes). Using AffinityPropagation, we identified gene clusters that are collaterally deleted (‘CD clusters’) with eight DDR genes (ATM, BRCA1, BRCA2, CDH1, MSH2, MSH3, PTEN and TP53) across >6,000 tumours from TCGA. We developed an edge-swapping method to identify genes that are deleted in mutually exclusive fashion to these clusters. We identified >600 CD clusters that formed 425 cluster pairs containing at least one SL gene pair. Of these, 125 pairs were validated using gene-essentiality (GARP) scores from siRNA-based knockdown screens on breast cancer cell lines [1]. We found FXR2 within the TP53 CD-cluster in agreement with existing literature [2], and BCL2 was predicted SL to the TP53-cluster. Inhibition of either BCL2 or TP53 in cell lines lacking the other showed the lowest GARP scores (highest cell death) compared to cells wild type for at least one of the genes (median BCL2⁻/⁻ TP53⁺/⁺ < -1.0, but median BCL2⁺/⁺ TP53⁻/⁻, BCL2⁻/⁻ TP53⁻/⁻ or BCL2⁻/⁻ TP53⁺/⁺ > 0). We found similar GARP evidence for PTEN-DYNC1L1 and PTEN-WDR48. Our method thus identifies novel vulnerabilities with potential therapeutic implications for DDR-defective cancers.


New chromene-based molecules as microtubule destabilisers and pro-apoptotic agents in cancer

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Cancer is a devastating disease worldwide, with millions of diagnoses per year and many people living with this disease. Breast cancer is the most frequent type of malignancy in women and due to the increasing cancer incidence, research in this area has been growing at the same rate. The chromene scaffold has been identified in several compounds with anticancer activity. The substitution pattern highly influences the mode of action and synthesis of new derivatives and is an important element in the search for improved drug candidates. This work addresses the synthesis of new chromene derivatives with enhanced anticancer activity.

New chalcone and chromene derivatives were synthesized in good yield through simple and effective reactions using innocuous solvents such as water and ethanol and high yielding aldol condensations. These compounds were tested on breast cancer cells (MCF-7 and Hs578T) and breast non-neoplastic cells (MCF-10A). After determination of IC\textsubscript{50} values and selectivity index, specific assays were performed to analyse the potential of these compounds.

Generally, compounds with halogenated substituents presented enhanced activity compared to those with methoxy or methyl groups. The bromine atom was often present in the bioactive molecules that proceeded to the final assays and showed to be promising candidates. A particular chromene acted as a cell migration inhibitory agent and triggered regulated cell death associated with G\textsubscript{2}/M cell-arrest and microtubule destabilization. Finally, combining these compounds with the currently used chemotherapeutic drug doxorubicin, potentiated its antitumor effect and decreased the cytotoxicity in non-neoplastic cells, anticipating a potential reduction of side effects. Therefore, we have developed an efficient apoptotic-inducer and microtubule destabilizer agent that can potentially be applied to future cancer therapy.
In vivo study of breast cancer cellular dormancy at the molecular level

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Breast cancer (BC) metastasis is linked to cellular dormancy, a state in which cells do not cycle and are resistant to chemotherapy. Between 36-56% of BC patients have dormant disseminated cells in their bone marrow that can potentially relapse and form metastases at any time. This poses cellular dormancy as a major clinical problem.

We have provided depth characterization of the dormant phenotype of the D2.OR mammary cancer cell line (Naumov, 2002) in the 3D matrigel on top (MoT) assay. We first confirmed the dormant phenotype by injecting D2.OR in the mesenteric vein and quantifying liver metastasis development. As opposed to proliferative mammary cancer cell lines, D2.OR does not form macrometastasis and often remains single non-proliferative cell. As first described by Barkan (2008), when cultured in vitro, D2.OR proliferate on plastic (2D) but become non-proliferative in MoT conditions. We confirmed that under dormant conditions, D2.OR cells lack Ki67 proliferation marker and do not demonstrate signs of apoptosis. Importantly, these cells can revert the proliferative arrest once re-plated in 2D condition. RNA-seq profiling of D2.OR in 2D and 3D conditions was performed to identify genes and biological processes associated with the dormant phenotype. We identified gene sets related to transcription, translation and cell cycle progression being downregulated in D2.OR cells when comparing the 2D vs 3D condition confirming its dormant behavior. Conversely, gene sets related to lipid and alcohol metabolism were upregulated in the D2OR when comparing 2D vs 3D corroborating another study realized on G0 dormant-like cells by Salony (2016). Finally, we identified several genes that were significantly upregulated in dormant cells. Of these, 2 genes could re-activate and induced proliferation of dormant cells upon knockdown, suggesting their role in dormancy induction. We are currently using in vivo models to validate these results.
Liquid biopsy in cancer precision medicine: a comprehensive workflow for mutanome identification in cancer patients

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Highly fragmented, double stranded DNA molecules, called cell-free DNA (cfDNA), are typically released from the tumor mass as a result of cell death into the bloodstream and represent a promising minimally-invasive diagnostic parameter in Oncology. Indeed, a fraction of cfDNA carries the same genomic aberrations and mutations of the tumor and/or metastases and can be used to screen for tumor-derived mutations. Here we describe a pilot study on a new complete workflow to identify the whole spectrum of gene mutations of the tumor, the “mutanome”, comparing normal vs cancerous tissues and cfDNA from different biological fluid of the same patient.

Tissues samples from 10 colon cancer patients were collected with plasma, urine and saliva from the same patients. Specimen collection procedures, preservation methods and protocols for nucleic acids extraction from microbiopitic samples, paraffin-embedded biopsies and biological fluids have been evaluated and optimized, together with different NGS library preparation protocols from low amounts of intact and degraded RNA and DNA. In addition, exome libraries were prepared from tissue and circulating DNAs using several enrichment methods (amplification and capture). The best exome enrichment strategy was selected based on the percentage of PCR duplicates, the number of in- and off-target reads and the coverage achieved for individual genes. For data analysis, an ad hoc bioinformatics pipeline was developed.

Results led to a seven days sample-to-report workflow able to identify somatic tumor mutations from needle and liquid biopsies and FFPE samples. Bioinformatics analysis identified particularly relevant mutations in common between tissues and liquid biopsies, demonstrating the power and effectiveness of this analytical strategy.

FAK pharmacological inhibition affects hepatocellular carcinoma growth and interferes with stemness markers of liver cancer stem cells

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Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide. Heterogeneous nature of HCC morphology and behaviour may depend on adult hepatocytes, hepatoblasts and a subset of liver cancer stem cells (LCSCs). However, pharmacological approaches remain limited and currently available drugs can trigger resistance phenomena in many patients by LCSCs, such as Sorafenib. We recently unveil a novel unprecedented role of focal adhesion kinase (FAK) in HCC growth, thus identifying a new crucial targetable network for anticancer therapies.

Aim: Here, we pointed to investigate the anti-tumorigenic effects of different pharmacological inhibitors of FAK on HCC cells and the effect on LCSC stemness.

Methods: FAK pharmacological inhibition was obtained by treating HCC cells with different chemical drugs (Sorafenib, PND-1186, TAE-226 and PF-431396). While, FAK stable silencing was obtained by transduction of shRNA lentiviral particles in HCC cells.

Results: HCC cells were treated with different concentration of inhibitors of FAK for 48h, to establish the half-maximal inhibitory concentration (IC50) for all drugs, evaluating: cell viability (XTT assay) and cell proliferation (DELFIA technology); the amount of total/phosphorylated form of FAK (Western Blotting) and apoptosis and cell cycle (cytofluorimetry). We found that inhibition of FAK Tyr397-phosphorylation with drugs reduce the viability and growth rate of HCC cells in a dose dependent manner. Combination IC50-based therapy with Sorafenib and specific FAK inhibitors significantly reduced the proliferation rates of HCC cells without increasing cell toxicity. Moreover, our preliminary data demonstrated that both FAK silencing and pharmacological inhibition reduced the expression of stemness markers in HCC cells, including NANOG, OCT-4 and PSMD10.

Conclusion: In summary, our results demonstrate that FAK pharmacological inhibition could alter the HCC growth and may overcome resistance to Sorafenib by still undefined mechanism that could involve the direct or indirect effect of FAK inhibition on the expression of LCSC markers.
Temporal stratification of breast cancer patients using laboratory tests and clinical features

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Early prognosis, better patient stratification and personalised treatment are crucial key elements for curing cancer. Breast cancer is a highly heterogeneous disease and usually divided into molecular subtypes according to the hormone-receptor and HER2 mutational status. However, some patients within subtypes often do not respond to treatment, showing the need for better stratification. We analysed temporal data consisting of standard laboratory tests for thousands of breast cancer patients and included data from January 2006 to June 2016 that mainly consisted of: lab tests, clinical notes, coding of diseases and drugs for around 3 million people. In this cohort around 30,000 were diagnosed with a breast cancer diagnosis. We took inspiration from network biology approaches usually applied to single-cell sequencing, to build a pipeline for clustering patients using lab tests as continuous values. The most important step of the pipeline was the use of the PhenoGraph method. In our setting, the key step in the PhenoGraph approach is to convert the lab data to a graph that represents the phenotypic similarities between patients. Then, the approach calculates the Jaccard coefficient between nearest-neighbor sets, and builds an undirected graph from the weighted links and it identifies communities using the Louvain method on the graph. The analysis of multiple lab tests and time points, showed that traditional lab test data are able to discriminate several clusters of patients according to different disease progression and other specific temporal trends. The approach was able to identify putative and novel subgroups that could increase our knowledge about breast cancer subtypes. In a clinical setting, the introduction of lab test results for a new patient into the method, could contribute to the early prognosis and identify the subgroup the patient belongs to earlier and could help in the decision-making process leading to the right treatment.
Biomarker-Driven Molecular Imaging of Aggressive Prostate Cancer Using the PEG10 Promoter

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Biomarker-driven molecular imaging of prostate cancer (PCa) can improve early diagnosis, aid in the development of tailored therapeutic regimens and monitor patient response to therapy. Our biomarker-driven imaging strategy relies on harnessing the transcriptional specificity of the PCa-associated gene PEG10 to drive the expression of a reporter gene for molecular imaging. Normally expressed at high levels only in the placenta, the PEG10 protein has been shown to be over-expressed in a non-androgen receptor (AR) driven form of prostate cancer that is currently incurable called aggressive variant prostate cancer (AVPC). In addition to AVPC, we recently discovered that PEG10 is also over-expressed in cell lines possessing AR splice variants. This discovery dramatically expands the number of patients who can benefit from PEG10-driven molecular imaging. In order to exploit the cancer specificity of PEG10 for molecular imaging, we developed a plasmid-based imaging construct uses the PEG10 promoter to drive the expression of near-infrared fluorescent protein or herpes simplex virus thymidine kinase for PET imaging. We have demonstrated for first time that this approach can be used to image subcutaneous xenograft tumors in vivo by the systemic delivery of the imaging construct using a nanoparticle-based delivery system. The efficiency the imaging construct in vivo was further enhanced by the addition of a synthetic advanced two-step transcriptional amplification system inserted downstream of the PEG10 promoter. Significantly, this strategy was able to non-invasively image a tumor model with poor vasculature longitudinally for nearly one week. Our biomarker-driven imaging approach has promising clinical translation potential due to the strength and specificity of PEG10 for AVPC. This gene therapy imaging approach could serve as a tool for detecting aggressive and recurrent prostate cancer as well as for monitoring the disease.
Combined approach for the analysis of copy number alteration heterogeneity in circulating tumor cells

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Background. Whole genome sequencing (WGS) of circulating tumor cells (CTCs) represents a non-invasive approach to study tumor progression and treatment resistance in clinical practice. In particular, copy number alteration (CNA) analysis offers a way to investigate the presence of heterogeneity between different groups of single CTCs. Since standard analysis pipelines for such a purpose are still lacking we aimed at developing a bioinformatic approach allowing to obtaining biologically and clinically useful information.

Methods. We collected 57 CTCs from 18 patients with advanced biliary tract cancer. Recovered cells were subjected to whole genome amplification (WGA) employing the Ampli1Ô WGA kit. Amplified DNA passing the quality control check were processed by low-coverage WGS (0.1X) using the IonTorrent Ion S5Ô system. WGS sequences were aligned to the human reference genome (hg19) using tmap aligner tool on Torrent_Suite 5.4.0 for CNV prediction by Control-FREEC 11.0. Clustering analysis was used to group CTCs as a function of CNA logRatio values considering firstly whole genome and thereafter genes with variance > 0.95 percentile. Using frequency alteration analysis, significant altered regions among all CTCs were identified and thereafter subjected to clustering analysis-validation in order to evaluate their role in distinguishing different groups of cells.

Results. Whole genome clustering analysis revealed a difficult-to-manage level of heterogeneity among CTCs. Frequency alteration analysis increased the informative content on heterogeneity through the identification of the most significantly altered regions among all CTCs. A Fisher’s exact test and its subsequent clustering analysis-validation identifies the genomic location and corresponding genes distinguishing CTCs from patients with different disease features.

Conclusion. CNA analysis of CTCs represents a promising way for obtaining information on the evolution of the disease under treatment pressure. In particular, the developed bioinformatic pipeline based on combination of clustering and frequency analysis identifies genes and pathways specific for distinct clinical categories.
Multimodal fiducial markers for surgical guidance

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Abstract withheld at the author's request.
Optimal starting age of screening for family members of prostate cancer patients: An example of precision medicine in early detection

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Background: Family history is the strongest risk factor for prostate cancer. However, current prostate cancer screening guidelines lack personalized evidenced-based guidance regarding the best age of screening for men with different types of family history of prostate cancer.

Objective: To provide evidenced-based optimal starting age of screening for family members of prostate cancer patients.


Method: The nationwide Swedish Family-Cancer Database, which linked information from the multi-generation register, national censuses, Swedish Cancer Registry and death registry, includes all Swedes born after 1931 and their biological parents, totaling >16 million individuals with >2 million cancer patients. 10-year cumulative risks were calculated for individuals with different types of family history.

Results: Out of a cohort of 8,200,846 PC-free men 309,487 developed PC during the follow-up (up to 58 years). The cumulative risk increased with age and numbers of affected relatives. Moreover, the earlier diagnosed age of FDRs, the higher risk in other family members. At age 50 (usual starting age of mass screening for prostate cancer), the 10-year cumulative risk for men from the general population was 0.85%, which was equivalent to the risk of 45, 46, and 44 years old men with one twin brother, brother or father affected with prostate cancer, respectively. Optimal starting age of screening for those with one affected first-degree relative (FDRs) diagnosed before age 50 was 8 years earlier than the general population; one FDR diagnosed after age 60, 4 years; two FDRs before age 50, 13 years; two FDRs after age 60, 6 years.

Conclusion: Our study for the first time provides evidence-based risk-adapted guidance for the optimal starting age of screening for family members of prostate cancer patients.
A novel drug-screening platform for experimental and human metastasis

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In this study, we have developed a new medium-throughput screening platform (METPlatform) based on ex vivo organotypic cultures to identify drugs targeting established metastasis. Based on its poor prognosis, limited knowledge of the molecular characterization and historical exclusion of these patients from clinical trials, we focus on brain metastases. We hypothesize that besides contributing to increase the limited therapeutic options available, this approach will allow us to identify critical molecular mediators for the viability of macrometastases. Our initial screen containing 114 FDA-approved or in clinical trials compounds has allowed us to validate various hits in experimental brain metastases derived from different cancer types, evaluate the effectiveness in the presence or absence of the microenvironment and at different stages of brain colonization (micrometastases versus macrometastases). Interestingly, METPlatform also allows to simultaneously evaluate potential toxic effects that drugs can exert on normal brain components, thus allowing to generate more relevant preliminary data and optimize the selection criteria to identify the best positioned anti-metastatic drugs. We have selected a drug from our hits that targets heat shock protein 90 (HSP90) for further in vivo validation and we have shown that it efficiently decreases brain metastasis burden as well as extracranial lesions in an aggressive lung adenocarcinoma model. Additionally, HSP90 is highly expressed by cancer cells in 44% of human brain metastases from different primary tumors including most common sources of brain metastasis (melanoma, lung and breast adenocarcinoma). Lastly, we have validated our top hit targeting HSP90 in human brain metastases organotypic cultures. HSP90 inhibition dramatically impairs tumor cell proliferation independently of the primary tumor origin, proving the potential of METPlatform as a personalized medicine drug-screening platform.
Oncogenic signaling of FGFR2 relies on truncation of its C-terminal tail

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A variety of human cancer entities including breast and gastric carcinoma harbor genetic aberrations of the fibroblast growth factor receptor 2 gene (FGFR2), presumably resulting in oncogenic activity of FGFR2. In similarity of these observations, using a transposon-based insertional mutagenesis screen in mice, we previously identified Fgfr2 to be a candidate driver of invasive lobular breast carcinoma (ILC). Through mapping of transposon insertions, we now show that most insertions in Fgfr2 cluster to the intron upstream of the last exon (E16), which results in truncated Fgfr2 expression lacking E16. Consistently, ILC formation in mice is faithfully induced only through mammary-specific expression of truncated Fgfr2$^{−E16}$, while full-length Fgfr2 expression has little effect on tumorigenesis. The molecular consequences of FGFR2$^{−E16}$ are strong potentiation of PI3K signaling and phosphorylation of S6 kinase, as compared to full-length FGFR2. Moreover, progressive deletion of the Fgfr2 3'-end and site-directed mutagenesis of E16 identifies a five-amino-acid motif highly critical for the suppression of both, S6 kinase phosphorylation and tumorigenesis in vivo. In human carcinomas, FGFR2 aberrations are frequently comprised of either fusion to a 3'-partner or amplification. Interestingly, fusions usually result in C-terminal FGFR2 truncation, while FGFR2 amplicons produce alternative transcripts lacking the last exon, both reminiscent of Fgfr2$^{−E16}$ oncogenicity. Indeed, in human tumor cell lines harboring FGFR2 fusions or amplifications, specific targeting of C-terminally truncated FGFR2 using RNAi strongly inhibits growth of these cells. Silencing full-length FGFR2, however, does not interfere with cell growth. Thus, we uncover a novel paradigm in oncogenic FGFR2 signaling that involves truncation of the C-terminal tail and propose therapeutic strategies that should target specific FGFR2 variants rather than blocking total FGFR2 signaling.
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