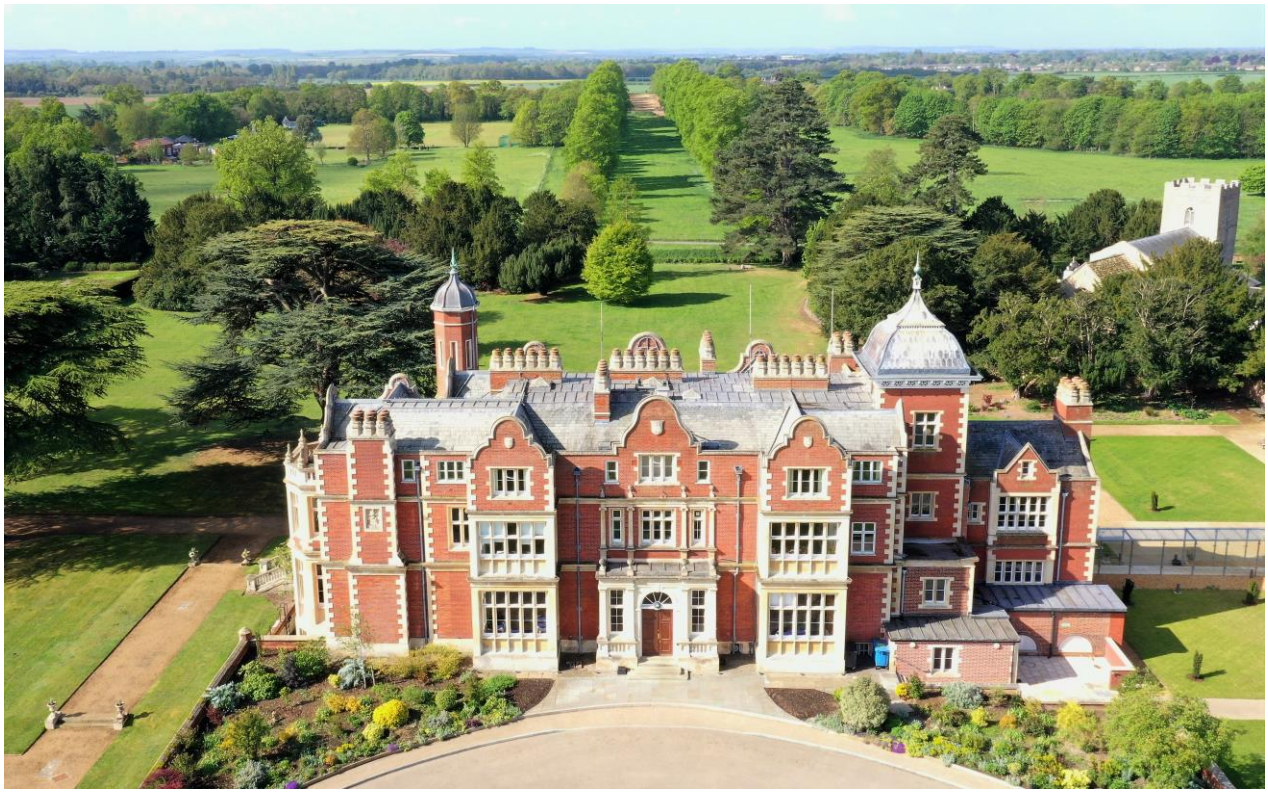
eulife

2019 SCIENTIFIC MEETING

Signalling & Gene Regulation in Health & Disease

BABRAHAM INSTITUTE | CAMBRIDGE, 18-19th NOV 2019

eulife



The Babraham Institute, Cambridge, UK

EU-LIFE Science Meeting: an inclusive event

As an organisation and community that values and promotes inclusivity, the Babraham Institute is committed to ensuring that the events we host are inclusive. Therefore in order to ensure that everyone enjoys this meeting and feels they can participate fully, all participants are required to adhere to this Code of Conduct which outlines both expected and unacceptable behaviour.

Behaviour that is expected of all participants

All participants should both in person and online:

- behave professionally,
- adhere to mutually-acceptable forms of address and greeting, with consideration for the meeting's participants as a whole,
- treat everyone with respect and dignity,
- help create an inclusive and safe environment.

Behaviour that is unacceptable

Harassment, intimidation or unacceptable behaviour includes but is not limited to:

- offensive or unwanted gestures, behaviour, or comments, including over social media,
- making threats or bullying,
- sexualised images or content, unwelcome sexual attention, or stalking,
- any behaviour that has the effect or intent of intimidation, loss of dignity or creation of a hostile environment.

Conduct normally becomes harassment if it persists once it has been made clear that the behaviour is regarded as offensive but a single incident may amount to harassment if it is sufficiently serious.

What to do if you experience or witness unacceptable behavior

Please notify a member of the organisational team as soon as you are able; they can be identified by the rainbow sticker on their name tags. Staff will be able to assist those experiencing unacceptable behaviour to feel safe for the duration of the conference.
You can also report a breach of the Code of Conduct at a later date in confidence by emailing the Institute's HR manager Michelle Barthelemy (michelle.barthelemy@babraham.ac.uk).

Consequences of failing to follow the Code of Conduct

Once staff are made aware of any unacceptable behaviour, those responsible will be asked to cease their behaviour immediately. Depending on the nature and severity of the incident they may be:

- required to avoid all interaction with or physical proximity to the individual(s) who was/were affected by the behaviour,
- no longer permitted to participate in talks,
- subject to an official complaint raised by the Institute with the individual's organisation,
- required to leave the event immediately.

References https://www.frontiersin.org/articles/10.3389/fmars.2016.00103/full; https://confcodeofconduct.com/; https://nam2019.org/lancaster/code-of-conduct

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Contents

Organisation Team

At the Babraham Institute
Susan Buttress, Cheryl Smythe, Emily Boyce and Simon Cook

At the EU-LIFE office
Montserrat Diaz Padilla, Ana-Belén Fernandez Llorente and Marta Agostinho

Introduction

This is the sixth and last meeting of the EU-LIFE scientific meetings series. With a yearly frequency during the first years of the EU-LIFE alliance, these meetings aimed at strengthening the scientific ties among researchers from our member institutes, with a particular focus on translational research. We foresee that in future our vibrant community will continue gathering researchers together in many other ways such as PhD student retreats, postdocs retreats, joint scientific workshops, training courses and visiting programmes – as it is already happening.

EU-LIFE scientific meeting series:
Biology of Cancer: bridging basic and translational research (Barcelona 2014), Epigenetics and Disease (Copenhagen 2015), Inflammation and Immunity in Health and Disease (Vienna 2016), Principles of Homeostasis (Berlin, 2017), Precision Medicine (Paris 2018), "Signalling & Gene Regulation in Health & Disease" (Cambridge, 2019).

About the Venue

The meeting will be held in the Cambridge Building at the Babraham Research Campus, postcode CB22 3AT. The Babraham Campus is about 30 minutes from London Stansted by taxi and similarly about 20-30 minutes from Cambridge City Centre. Should you need a taxi while in Cambridge, Panther Taxis is a local company 01223 715715. You can pre-book taxis with Cambridge Connections for more competitive prices.

Luggage can be safely stored at Babraham and Duxford.

Emergency contact on the day of arrival

Should you need to get in touch with us please phone +44-1223-494262.

Social Events

Dinner at Duxford Imperial War Museum

The meeting dinner and party will be held at the Duxford Imperial War Museum. Buses will be available to transport you from Babraham to Duxford and then transport you afterwards from Duxford to Cambridge city centre or back to Babraham Institute.

Transportation & Departure

Transport to Babraham 19th November

Please meet the bus on Parkside by Cambridge Fire Station (where bus drops off on previous evening) at 08:15 on 19th November.

Departure

If you would like us to pre-book a taxi for you to Cambridge station or an airport, please send Susan Buttress your travel details including flight departure time, flight number and destination. Please note that participants are required to cover their own travel costs including return taxis.

Agenda

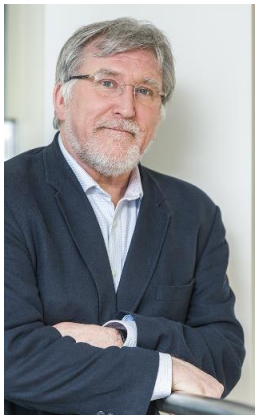
DAY 1_ MON 18NOV 2019		
TIME	TOPIC	PRESENTING
01:00-02:00PM	*Arrival and Lunch*	---
01:45	Welcome address	**Michael Wakelam**, Director BI
SESSION A	Chairs - Michael Wakelam & Sarah Ross	
02:00-02:45 PM	Precision Cancer Treatment Driving Success in the Clinic	**Susan Galbraith**, AstraZeneca
02:45-03:15 PM	KRAS-targeted precision medicine drug combination strategies in pancreatic cancer	**Markus Vähä-Koskela**, FIMM
03:15-03:45 PM	Flash Poster Session	
03:45-04:30 PM	*Coffee Break* & Posters – **kindly sponsored by PhoreMost Ltd**	
04:30-05:00 PM	A new developmental module involving an Alk5/Snai1-dependent EMT at the core of vertebral axial elongation	**Moises Mallo**, IGC
05:00-05:30 PM	B Cell Receptor Signalling Regulation by Non-coding RNAs	**Marek Mraz**, Ceitec
05:30-06:00 PM	Cooperation between signalling mutations and transcription factors in T-cell leukemia	**Jan Cools**, VIB
06:00-06:30 PM	Interconnecting autophagy and translational control	**Lisa Frankel**, BRIC
06:40 PM	Shuttle Bus to Duxford Air Museum	

DAY 2_ TUE 19NOV 2019		
TIME	TOPIC	PRESENTING
08:15 AM	Shuttle Bus departure	
SESSION B	Chairs - Courtney Hanna & Melanie Eckersley-Maslin	
09:15-09:45 AM	Tracking the dynamics of chromatin states in tumor cells at single-cell resolution: response and resistance to cancer therapies	**Celine Vallot**, Curie
09:45-10:15 AM	Tissue-specificity of alterations in cancer	**Martin Schaefer**, IEO
10:15-11:15 AM	Selected short talks from submitted abstracts	**Johanna Grinat**, MDC **Tobias Hoffmann**, CRG **Charlène Estrada**, Curie **Johannes Bigenzahn**, CeMM **Jonathon Coats, Sarah Ross**, BI
11:15-11:30	Group photo	
11:30-12:00 AM	*Coffee Break* & Posters	
12:00-12:30 PM	Single-cell Sequencing of Epigenetic Landscapes and Induced Perturbations	**Christoph Bock,** CeMM
12:30-01:00 PM	Structural variation in the 3D genomic era: Implications for disease and evolution	**Dario Lupiáñez**, MDC
01:00-01:30 PM	A single-molecule understanding of transcriptional bursting	**Tineke Lenstra**, NKI
01:30-02:15 PM	*Lunch*	
SESSION C	Chairs - Pam Lochhead & Michelle Linterman	
02:15-02:45 PM	Commercial Research Session	**Chris Torrance**, Phoremost Ltd **Katherine Ewings**, CRUK, TDL **Richard Sainson**, Kymab
02:45-03:15 PM	Modulation of G-quadruplexes by RNA helicase DDX1 is required for antibody gene rearrangements in B lymphocytes	**Claudia Ribeiro de Almeida**, BI
03:15-03:45 PM	Charting the epitranscriptome in native RNA molecules	**Eva Novoa**, CRG

03:45-04:45 PM	New biology and therapeutics from RNA structure and modification	**Eric Miska**, Gurdon Institute, University of Cambridge
04:45 PM	Close of meeting *Coffee* & Departures	**Simon Cook**, Group Leader, BI

Welcome

Michael Wakelam, Babraham Institute Director



Dear EU-LIFE Science Meeting Participant,

A warm welcome to you and in particular to those of you from beyond Babraham. I am delighted that we have so many participants from each of the EU-LIFE institutes. It is especially important in the current UK political climate to have this opportunity to welcome and meet with our European friends and colleagues to reinforce that we at Babraham want to continue to collaborate and work together irrespective of our membership of the EU. The Babraham Institute is an international organisation with a quarter of our staff coming from 25 other countries – without this overseas expertise and talent we would not be able to deliver research excellence.

For the first time at an EU-LIFE science meeting, we are also joined by colleagues from the commercial sector. The Babraham Institute benefits from a unique environment where it is co-located with over 60 SMEs. Working with colleagues from industry brings unique perspectives and opportunities and I would encourage you all to explore those opportunities over the next couple of days.

I would also particularly encourage the more junior participants to ask questions and engage in scientific debate; we actively encourage questioning by our junior researchers at Babraham as it is an important part of their training as critical thinkers and the visibility that questioning brings can lead to career development opportunities.

Finally my sincere thanks to all of you for your participation, especially our speakers. And huge thanks also to Montserrat Diaz Padilla, Ana-Belén Fernandez Llorente and Marta Agostinho at the EU-LIFE office, and Babraham's Susan Buttress, Cheryl Smythe, Emily Boyce and Simon Cook for all the efforts in organising our next 2 days of scientific discussions.

Sincerely,

Speaker's Abstracts

1. Susan Galbraith, AstraZeneca

Precision Cancer Treatment Driving Success in the Clinic

Understanding drivers of cancer and designing the right clinical trials is fundamental for developing drugs that will have the greatest chance of success in the clinic. This presentation will describe recent clinical data demonstrating the success of targeted therapies including EGFR tyrosine kinase inhibitors and PARP inhibitors.

Although targeted therapies are improving patient outcomes, cancer is a complex and evolving disease and resistance remains a key challenge. At AstraZeneca we are focussing on moving our drugs into earlier stage disease when tumours are less heterogeneous and developing new therapies to target emerging resistant populations. I will discuss how we are employing next-generation translational science to drive understanding of clinical mechanisms of resistance to current therapies, and the potential for multi-drug platform studies to identify new combination therapies to overcome this.

Biography

Trained as a Clinical Oncologist in the United Kingdom. She studied Medicine at Manchester and Cambridge Universities. She was admitted to Membership of the Royal College of Physicians in 1992, and then trained in Clinical Oncology in London. She gained Fellowship of the Royal College of Radiologists in 1997. She then completed a PhD at the University of London involving translational work on a vascular-targeting agent.

Susan joined the Clinical Discovery Oncology group at Bristol-Myers Squibb in 2001. Susan was closely involved in the in-licensing of ipilimumab from Medarex, elotuzumab from PDL, the acquisitions of Adnexus and Medarex and research collaborations with Exelixis. She held increasing levels of responsibility becoming VP for Oncology and Immunology Early Development, and then latterly taking on responsibility for the Clinical Biomarker team.

Susan joined AZ in September 2010, as Head of the Oncology Innovative Medicines group (iMed) responsible for Oncology Small Molecules Discovery and Early Development. Since then the Oncology iMed has moved 3 programs into Phase 3 trials; olaparib (now approved in USA and EU), selumetinib, and AZD9291 a mutant-selective EGFR inhibitor.

2. Markus Vähä-Koskela, FIMM

KRAS-targeted precision medicine drug combination strategies in pancreatic cancer

Prson Gautam[1], Markus Vähä-Koskela[1], Hanna Ojala[1], Elina Parri[1], Matilda Juusola[2], Lotta Mäkinen[2], Sanna Vainionpää[2], Hanna Seppänen[2], Tero Aittokallio[1] and Krister Wennerberg[1,3]
1 Institute for Molecular Medicine Finland, University of Helsinki, Finland
2 Dept. Gastrointestinal surgery, Helsinki University Central Hospital and University of Helsinki, Finland
3 Biotech Research & Innovation Centre, University of Copenhagen, Denmark

Pancreatic ductal adenocarcinoma (PDAC) is a particularly challenging form of cancer with a 5-year survival rate of 5% and resistance to nearly all treatments. Ninety-five percent of all PDAC cases are mutated in KRAS, which initially suggested the disease could be treatable if KRAS or its downstream signaling can be effectively targeted. To date, however, KRAS itself remains “undruggable”, and although the downstream MAPK pathway and PI3K pathways can be targeted, rapid compensatory signaling and complex stromal interactions create a vastly heterogeneous disease that requires individual tailoring of treatments and novel combinations.

To begin addressing these issues, we profiled vulnerabilities of 30 low passage pancreatic cancer cell lines to a collection of 526 clinically relevant small molecule anti-cancer agents alone and in combination with inhibitors of RAF/MEK/ERK or PI3K/AKT/mTOR. As expected, single inhibitor responses were only growth static or non-selectively toxic. On the other hand, one of the most promising selective cell-death-inducing combinations involved MEK1/2 inhibitor trametinib and mitosis/microtubule-targeting agents, such as vincristine. Using mutation patterns, EMT status, and basal level transcriptomes, we attempted to extract molecular hallmarks of sensitivity and resistance to this combination. Intriguingly, the expression levels of INKA1, a tumor suppressor and endogenous inhibitor of PAK4, correlated with response, suggesting PAK4 to be an important negative regulator of cell death triggered by the combination. We are currently detailing the functional roles of these regulators and their potential as biomarkers for patient stratification using PDAC patient-derived organoids and stromal co-culture models.

Biography

Markus’ research interests are in cancer systems biomedicine. Tumors are able to avoid destruction and survive even under harsh onslaught by harnessing a mixture of central and individual resistance mechanisms, fine-tuned by the tumor microenvironment and individual physiology. Working for the past 10+ years in multiple institutes, he has constructed advanced experimental assays with which to study these mechanisms in detail, including personalized organoid-stromal co-cultures for pancreatic and gastric cancer and novel long-term cultures of primary myeloid leukemia. He has probed cancer resistance mechanisms using combinations of small molecule targeted inhibitors, oncolytic viruses, checkpoint inhibitors, bi-specific antibodies and immune cells, revealing unique resistance hierarchies and vulnerabilities with translational potential. His current aim at the Institute for Molecular Medicine Finland (FIMM) is to integrate response data from the functional assays with underlying molecular and cellular biomarkers, including mutations, transcriptomes and cell surface proteins, across large patient cohorts for a systems level understanding of cancer resistance mechanisms.

3. Moises Mallo, IGC

A new developmental module involving an Alk5/Snai1-dependent EMT at the core of vertebral axial elongation

During vertebrate embryonic development body structures result from the activity of cells collectively known as axial progenitors. It is well known that these cells undergo an epithelial to mesenchymal transition (EMT) to generate mesodermal tissues, a process known as gastrulation. Using single cell transcriptional analysis we have now found that axial progenitors also undergo an alternative type of EMT, essential for extending the body axis beyond the trunk. This EMT differs from that associated with gastrulation in several fundamental ways. First, it is incomplete (cells keep various degrees of epithelial features); second, the resulting cells keep progenitor activity similar to that of the original epithelial cell; third, it is triggered by the convergence of Snai1 and Alk5 (Tgfb-RI) activities. These characteristics are closer to those associated with metastatic activities than to developmental processes. Therefore, in addition to its role in normal development and its possible involvement in congenital malformations, like spine bifida, this EMT provides a tractable in vivo model for metastatic activity.

Biography

Moises obtained a degree in General Medicine and Surgery at the University of Santiago de Compostela (Spain) in 1987. Soon, he left clinical praxis to engage in Scientific Research. At this stage he performed a PhD in Biochemistry and Molecular Biology at the University of Santiago de Compostela (Spain), concluded in 1991. He then moved to the laboratory of Thomas Gridley at the Roche Institute of Molecular Biology in Nutley, NJ (USA), where he performed his postdoctoral work studying the mechanisms of mammalian embryonic development, mostly focusing on the formation of the craniofacial region. In 1995, he moved to the Max Planck Institute of Immunobiology in Freiburg (Germany), where he began his work as an independent researcher, still studying different aspects of mammalian development. In 2001, he moved to the Instituto Gulbenkian de Ciência in Oeiras (Portugal) to lead the research group "Patterning and Morphogenesis" and to head the Transgenics Unit. Here, his studies have focused on different aspects of pattern formation in the vertebrate embryo, most particularly the layout of the basic body plan, now including an evolutionary component. For his research he uses a wide variety of genetic tools using the mouse as the model system.

4. Marek Mraz, Ceitec

B Cell Receptor Signalling Regulation by Non-coding RNAs

Katerina Musilova[1,2], Katerina Cerna[1,2], Sonali Sharma[1], Vaclav Seda[1,2], Gabriela Pavlasova[1,2], Jan Oppelt[1], Daniel Filip[1,2], Veronika Sandova[1,2], Laura Ondrisova[1,2], Sarka Pospisilova[2], Eva Vojackova[1], Sonali Sharma[1], Pedro Zeni[1], Jiri Mayer[2], Marek Mraz[1,2]

1 Molecular Medicine, CEITEC Masaryk University, Brno, Czech Republic

2 Department of Internal Medicine, Hematology and Oncology, University Hospital Brno and Faculty of Medicine MU, Brno, Czech Republic

B Cell Receptor (BCR) signalling is fundamental for the maturation, survival, and proliferation of B cells, and B cell malignancies frequently harbour mutations in this pathway or complex non-genetic deregulation of BCR signalling. This is underscored by the remarkable clinical effect of inhibitors targeting BCR-associated kinases BTK and PI3K, especially in chronic lymphocytic leukaemia (CLL). The differences in BCR signalling propensity contribute to variable prognosis in CLL and other "mature" B cell malignancies, and it has been shown that non-coding RNAs such as miR-150, miR-155, miR-34a or miR-17-92 play an important role in this process. The talk will focus on novel roles of microRNAs (miRNAs) in fine tuning the propensity of BCR signalling during microenvironmental interactions of B cells, and also the related changes during therapy with BCR inhibitors or classical DNA-damaging therapeutic drugs. This will include novel data on MYC-regulated and p53-regulated miRNAs acting as regulators of PI3K pathway. The data indicate that miRNA-induced changes in "tonic" and/or antigen-induced BCR signalling might be of key importance for the development and therapy resistance of B cell neoplasms.

Funding: This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 802644). This work was supported by the Ministry of Health of the Czech Republic, grant no. NV18-03-00054 and no.16-29622A. All rights reserved. Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601); MH CZ - DRO (FNBr, 65269705), MUNI/H/0865/2016, MUNI/A/1105/2018. Contact: marek.mraz@email.cz; ceitec.cz/mrazlabxxxxx

Biography

Dr. Marek Mraz is studying chronic lymphocytic leukemia (CLL), which is the most common cell leukemia among adults in the Western world and remains an "enigma" in modern hematology. Dr. Marek Mraz is interested in the regulation of B-cell receptor (BCR) signaling pathway in malignant B lymphocytes, which is known to affect the pathogenesis of CLL and other B cell malignancies. He is best known for his work describing the role of non-coding RNAs, mainly microRNAs, in BCR pathway and also the contribution of CD20 (target of rituximab) to BCR signalling regulation. He conducted post-doctoral work with Prof. Thomas KIpps (UCSD), and currently he is a group leader at the CEITEC Masaryk University and at University Hospital Brno (Czech Republic; ceitec.cz/mrazlab). In 2018 he was awarded the prestigious ERC grant.

5. Jan Cools, VIB

Cooperation between signalling mutations and transcription factors in T-cell leukemia

The NUP214-ABL1 fusion is a constitutively activated tyrosine kinase that is significantly associated with overexpression of the TLX1 and TLX3 transcription factors in T-cell acute lymphoblastic leukemia (T-ALL). Using a transgenic mouse model we have demonstrated that NUP214-ABL1 can directly cooperate with TLX1 in driving T-ALL development. Using integrated ChIP-seq, ATAC-seq and RNA-seq data, we showed that TLX1 and STAT5, the downstream effector of NUP214-ABL1, co-bind poised enhancer regions, and cooperatively activate the expression of key proto-oncogenes such as MYC and BCL2. Inhibition of STAT5, downregulation of TLX1 or MYC, or interference with enhancer function through BET inhibitor treatment caused reduction of target gene expression and induction of leukemia cell death. These data provide insight in how oncogenic signaling cooperates with deregulated transcription factors to drive leukemia development.

Biography

Jan Cools obtained his PhD degree in 2001 from the university of Leuven (Belgium) with a study on chromosomal defects in leukemia. From 2001 to 2003 he continued his research on the genetic causes of leukemia in the laboratory of Dr Gary Gilliland at Harvard Medical School (Boston, USA). After return to Belgium, he was promoted to assistant professor in 2005 and to full professor in 2009 at KU Leuven. In 2008, Jan was also appointed as group leader of VIB, a life sciences institute in Flanders. The laboratory of Jan Cools studies the genetic complexity of leukemia and uses that information to develop novel models of leukemia development and novel treatment strategies. Jan received an ERC starting grant and an ERC consolidator grant for T-cell leukemia research. He has served as a board member of the European Hematology Association and is now editor-in-chief of a new open access hematology journal: HemaSphere (journal of the European Hematology Association).

6. Lisa Frankel, BRIC

Interconnecting autophagy and translational control

Ribosomes are key genetic interpreters which ensure accurate decoding of the genome in space and time. While the existence of specialized ribosomes is increasingly recognized, we currently lack understanding of how ribosomes are degraded. In my lab, we are actively investigating the mechanisms governing ribosome turnover by the process of autophagy in mammalian cells. Autophagy is an evolutionarily conserved degradation pathway involving sequestration of cytoplasmic components within a double-membrane vesicle, the so-called autophagosome. Through fusion with lysosomes, autophagosomes deliver their cargo for degradation by lysosomal hydrolases. Autophagy plays a critical role in the response to stress, where it ensures degradation of damaged or harmful substrates and promotes nutrient recycling in order to maintain cellular homeostasis.

Autophagy can be highly selective, and we have established several lines of evidence demonstrating the specific accumulation of ribosomes in autophagosomes under different conditions of cellular stress using a variety of imaging- and biochemistry-based approaches. By tagging small- and large ribosomal subunits with a pH-sensitive fluorophore, we have developed an assay which allows us to monitor the delivery of ribosomes to the lysosome by live-cell confocal imaging. Using this readout, we have run high-throughput screens under different stress conditions to identify novel regulators of this process. I will discuss the data from these and similar investigations from my lab.

Biography

Born and raised in Toronto, Canada and currently living in Copenhagen, Denmark, I have trained at the University of Copenhagen and McGill University, Montreal, where I specialized in molecular and cellular cancer biology. During my PhD and postdoctoral work, at the Biotech Research and Innovation Centre (BRIC), I obtained a strong background in the field of RNA biology, including non-coding RNAs, RNA-binding proteins and translation. In addition, I have worked extensively with molecular regulation of the autophagy process. I am currently group leader and associate professor at the Danish Cancer Society Research Centre (DCRC). Our research is especially focused on the functional interplay between RNA, translation and autophagy and the biological importance of this cross-regulation.

7. Celine Vallot, Curie

Tracking the dynamics of chromatin states in tumor cells at single-cell resolution: response and resistance to cancer therapies

The dynamic nature of chromatin and transcriptional features are expected to participate to tumor evolution, particularly in the context of response to cancer treatment and acquisition of resistance. Yet, the contribution of epigenetic plasticity to cancer cells remains unclear and means to target it are still rather non-specific and inefficient, mostly due to the lack of relevant cellular models and in vivo datasets. We have recently achieved the mapping of histone marks at single-cell resolution in human breast tumors, enabling the investigation of the dynamics of chromatin marks, and its contribution to tumor evolution. Using in vivo models of acquired resistance to cancer treatment, our recent data indicate that resistance to tamoxifen or chemotherapy may be associated with the emergence of an epigenetic subclone, characterized by a specific histone mark profile. More generally, the research projects of the group aim for a better understanding of the mechanisms of non-genetic selection, with the objective to design strategies to enhance or restore sensitivity to cancer treatments.

Biography

I am a group leader at Institut Curie, since 2017, and a tenured Scientist at CNRS since 2013. My group works on the dynamics of chromatin features in breast cancer and we develop both computational and experimental single-cell approaches to do so.

8. Martin Schaefer, IEO

Tissue-specificity of alterations in cancer

Our lab studies how mutations or epigenetic modifications of the DNA transform a healthy cell into a cancer cell. This transformation is an evolutionary process in which cells undergo alterations that promote competition and survival of the fittest. Critical to this evolution is on the one hand side the role of the tumor environment as it modulates mutational processes and the fitness effect of each DNA alteration. On the other hand, tissue-specific cellular properties determine the frequency of occurrence of certain alterations.
Cancer genomes and epigenomes show a strong degree of tissue-specificity: many alterations (such as activating point mutations in the oncogene KRAS) are frequent in some types of cancer but rare in others. In my presentation I will show how properties of the tissue of origin and its environment shape the tissue-specific alteration profiles of human tumors.

Biography

2008 Diploma in Bioinformatics, University Tübingen
2008 – 2013 PhD candidate at the MDC (with M. Andrade), Berlin
2013 – 2017 PostDoc at the CRG (with L. Serrano), Barcelona
2017 – 2018 Staff scientist at the CRG (with L. Serrano), Barcelona
Since 2018 Group leader at IEO, Milan

9. Christoph Bock, CeMM

Single-cell Sequencing of Epigenetic Landscapes and Induced Perturbations

1 CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria
2 Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria
3 Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases, Vienna, Austria

Most diseases develop through the complex interplay of genetic and environmental influences, involving signaling pathways, metabolic changes, and diverse cellular phenotypes. Our research is based on the hypothesis that the "epigenetic landscape" constitutes a highly informative intermediate layer of information processing that allows cells to maintain their regulatory state and cellular identity over time, while retaining the flexibility to respond swiftly to a broad range of perturbations. In our definition, the "epigenetic landscape" is not restricted to epigenetic marks such as DNA meth-ylation and histone modifications. Rather, it reflects the full spectrum of transcription regulation by which cells translate various inputs into sustainable changes in their cell state. Notably, the epigenet-ic landscape not only reflects a cell's current state, but also its developmental history (e.g., cell-of-origin in cancer) and its potential for future adaptation (e.g., plasticity in response to drug treatment). International consortia have mapped the epigenetic landscape in hundreds of cells types (ENCODE, IHEC, BLUEPRINT, etc.) and thousands of individuals (GTEx, GoDMC etc.). Yet, fundamental ques-tions about the role and regulation of the epigenetic landscape in health and disease remain unre-solved. In our research, we develop and apply high-throughput assays as well as computational methods for addressing two central challenges: Heterogeneity and Causality. First, I will focus on heterogeneity and present our ongoing work within and beyond the Human Cell Atlas, dissecting epigenetic landscapes in hematopoietic differentiation, cancer patients, and organ-oids. Second, I will outline different ways in which we seek to obtain causal, mechanistic insights in high throughput, including CRISPR single-cell sequencing (CROP), time series of drug response in patients, and deep learning algorithms for the functional dissection of gene-regulatory networks.

Funding: C.B. is supported by an ERC Starting Grant (n° 679146) of the European Union and by a New Frontiers Group award of the Austrian Academy of Sciences.

Biography

Christoph Bock is a principal investigator at the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences. He pursues interdisciplinary, highly collaborative research aimed at understanding disease biology and advancing precision medicine. His research group combines experimental biology (high-throughput sequencing, epigenetics, CRISPR screening, synthetic biology) with computer science (bioinformatics, machine learning, artificial intelligence). He is also a guest professor at the Medical University of Vienna, scientific coordinator of the Biomedical Sequencing Facility at CeMM, and group leader at the Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases. He coordinates an upcoming EU Horizon 2020 project on the single-cell analysis of human organoids, which constitutes part of the European contribution to the Human Cell Atlas, and he co-founded Genom Austria, a citizen science project that is the Austrian partner in the International Network of Personal Genome Projects. Christoph Bock is an elected member of the Young Academy of the Austrian Academy of Sciences and has received several major research awards, including the Max Planck Society's Otto Hahn Medal (2009), an ERC Starting Grant (2016-2021), and the Overton Prize of the International Society of Computational Biology (2017). He co-founded Aelian Biotechnology, a Vienna-based startup company that develops and applies single-cell methods for high-throughput biology and drug discovery.

10. Dario Lupiáñez, MDC

Structural variation in the 3D genomic era: Implications for disease and evolution

3D spatial organization is an inherent property of the vertebrate genome to accommodate the roughly 2m of DNA in the nucleus of a cell. On a larger scale, chromosomes display a nonrandom nuclear organization highly influenced by their gene density and transcriptional status. On a subchromosomal scale, the 3D organization of chromatin brings genomic sites that lie far apart along the linear genome into proximity. Within such organization, topologically associating domains (TADs) emerge as a fundamental structural unit that guides regulatory elements to their cognate promoters to induce transcription.

Structural and quantitative chromosomal rearrangements, collectively referred to as structural variation (SV), contribute to a large extent to the genetic diversity of the human genome and thus are of high relevance for cancer genetics, rare diseases and evolutionary genetics. Recent studies have shown that SVs can not only affect gene dosage but also modulate basic mechanisms of gene regulation. SVs can alter the copy number of regulatory elements or modify the 3D genome by disrupting higher-order chromatin organization such as TADs. As a result of these position effects, SVs can influence the expression of genes distant from the SV breakpoints, thereby causing the appearance of certain pathogenic phenotypes or evolutionary traits. Therefore, the impact of SVs on the 3D genome and on gene expression regulation has to be considered when interpreting the phenotypical consequences of these variant types.

In this talk, I will show examples at different genomic loci, highlighting the potential of SVs to induce developmental disease by distinct pathomechanisms. Furthermore, I will discuss about the iberian mole Talpa occidentalis, a unique case of true XX mammalian hermaphroditism, and a prominent example of how SVs can also be a force of evolutionary innovation.

Biography

Darío G. Lupiáñez is a group leader at the Berlin Institute for Medical Systems Biology (BIMSB), Max Delbrück Center for Molecular Medicine (MDC). He received his master´s degree and PhD from the University of Granada, working under the supervision of Prof. Rafael Jiménez and Prof. Miguel Burgos. Then he worked as a postdoctoral scientist at the Max Planck Institute for Molecular Genetics in Berlin, within the group of Prof. Stefan Mundlos. Since 2017, he leads the "Epigenetics and Sex Development" group at the BIMSB.
His research focuses on the study of gene regulation in development, disease and evolution, with a special emphasis on 3D chromatin organization.

11. Tineke Lenstra, NKI

A single-molecule understanding of transcriptional bursting

Transcriptional in single cells is a stochastic process, which arises from the random collision of molecules. This stochastic behaviour results in variability in gene activity between cells, and as well as within a cell over time. In our lab, we use single-molecule imaging techniques to visualize the dynamics of transcription in living cells. We and others have shown that genes are often transcribed in bursts, with periods of activity followed by periods of inactivity. Transcriptional bursting is observed from bacteria to yeast to human cells, but the origin and regulators of bursting are still largely unknown. In this talk, I will focus on how transcriptional bursting in yeast is regulated by transcriptional activators and nucleosomes.

Biography

Tineke Lenstra received her Bachelor's and Master's degree in biomedical sciences from Utrecht University. As a PhD student in the laboratory of Frank Holstege at University Medical Centre Utrecht, she used genome-wide expression analysis to study transcription regulatory complexes, for which she was awarded a cum laude PhD in 2012. During her postdoc in laboratory of Daniel Larson at the National Cancer Institute in Bethesda, USA, she studied transcription dynamics in single cells with cutting-edge single-molecule techniques. In 2016, she established an independent group at the Netherlands Cancer Institute (NKI) in Amsterdam, and in 2019, she joined Oncode Institute. Her lab focuses on the regulatory mechanisms of stochastic transcription in eukaryotic cells. For her work, Tineke has received a number of awards, including the NVBMB prize, the NCI Director's Innovation Award, and the ERC starting grant.

12. Claudia Ribeiro de Almeida, BI

Modulation of G-quadruplexes by RNA helicase DDX1 is required for antibody gene rearrangements in B lymphocytes

Laboratory of Lymphocyte Signalling and Development, The Babraham Institute, Cambridge, United Kingdom.

B cells undergo immunoglobulin heavy-chain (IgH) class switch recombination (CSR), which replaces the exon encoding for IgH constant region (CH), thus modifying the isotype and effector properties of antibodies. CSR is linked with the transcription of long non-coding RNAs (lncRNAs) initiated upstream highly repetitive "switch"-regions (called S-regions), located 5' to each CH exon. Due to their G-rich nature, S-region transcription leads to the formation of RNA:DNA hybrid structures between nascent RNA and the template DNA strand called R-loops. It is thought that R-loops occur co-transcriptionally and thereby expose the non-template DNA to the activity of the CSR-catalyzing enzyme, activation-induced cytidine deaminase (AID). More recently, G-quadruplex (G4) structures within the switch transcripts themselves have been implicated in targeting AID to S-regions. However, the molecular players required for modulation of switch RNA secondary structures are largely unknown.

We now show that the DEAD-box RNA helicase 1 (DDX1) is required for CSR. In vivo deletion of DDX1 in activated B cells results in a general decrease in the steady-state levels of switched IgH isotypes. Upon immunization, DDX1 conditional knock-out mice show increased levels of antigen-specific IgM antibodies and a severely reduced IgG response. The mechanism of action of DDX1 is to bind to G4 structures present in switch lncRNAs and convert them into S-region R-loops, thus promoting AID targeting to IgH. Notably R-loop levels over S-regions are diminished by chemical stabilization of G4 RNA or by the expression of a DDX1 ATPase deficient mutant that acts as a dominant-negative protein to reduce CSR efficiency.

These studies highlight the importance of lncRNA-mediated mechanisms in CSR and provide direct evidence for RNA helicase activity in converting G4 into R-loop structures at the IgH locus. While B cells induced to undergo CSR provide a clear example of G4 RNA regulation by DDX1 as a specific mechanism to target AID to the IgH locus, these findings may well extrapolate to other molecular functions of G4 RNA.

Biography

Claudia Ribeiro de Almeida is a Group Leader at the Babraham Institute's Lymphocyte Signalling and Development programme. She studied Molecular Biology & Genetics at the University of Lisbon before moving to the Netherlands in 2005 to obtain her PhD at the Erasmus MC, Rotterdam. During her PhD she made significant contributions to the field of lymphocyte development by characterizing the function of the CTCF transcription factor, including its role in immunoglobulin κ locus V(D)J recombination. As a Postdoctoral Researcher, she initially worked at the MRC-Clinical Sciences Centre (now MRC-LIMS) before joining the Sir William Dunn School of Pathology in Oxford as an EMBO fellow in 2012. While in the lab of Prof. Nick Proudfoot, Claudia has found that RNA-unwinding proteins called helicases are required to modulate RNA secondary structures during Class Switch Recombination (CSR). She moved to the Babraham Institute in November 2018 to start her research group, which focus on understanding how RNA molecules participate in the mechanisms of immunoglobulin gene rearrangements thereby allowing B cells to combat infections by producing different types of antibodies.

13. Eva Novoa, CRG

Charting the epitranscriptome in native RNA molecules

Huanle Liu, Oguzhan Begik, Morghan C Lucas, David Wiener, Schraga Schwartz, Christopher E Mason, John S Mattick, Martin A Smith, Eva Maria Novoa

RNA modifications are chemical entities present in both coding and non-coding RNAs, which can play pivotal roles in cellular function, including cell fate, sex determination and cellular differentiation, among others. There are currently more than 170 different RNA modifications described to date, and from these, more than 70 have been associated to human diseases, including neurological disorders and cancer. Unfortunately, the limited availability of antibodies and chemicals selective to RNA modifications has so far limited our transcriptome-wide view to only a handful of RNA modifications. Consequently, the abundance, location, and function of the majority of RNA modifications remains unknown.

Here we show that by using direct RNA sequencing from Oxford Nanopore Technologies, which allows direct sequencing of native RNA molecules, we can accurately detect and quantify RNA modifications in full-length native RNA transcripts. Using this technology, we show that we can detect N6-methyladenosine (m6A) RNA modifications both in vitro and in vivo with an overall accuracy of 87-90%. We are now developing novel methods to detect and quantify other RNA modifications, as well as to train algorithms that will distinguish between modifications that current next-generation sequencing-based methods cannot distinguish, such as 5-methylcytosine (m5C) from 5-hydroxymethylcytosine (hm5C). Our results open new avenues to investigate the universe of RNA modifications in full-length transcripts, with single molecule resolution, allowing us to query the epitranscriptome in ways that, until now, had not been possible.

Biography

Eva Maria obtained her BSc in Biochemistry in 2007 and MSc in Bioinformatics in 2009. She then conducted research in three different continents, including the Institute for Research in Biomedicine (Barcelona, Spain), the Massachusetts Institute of Technology and the Broad Institute (Cambridge, USA), and the Garvan Institute of Medical Research (Sydney, Australia). During those years, she generated a substantial research profile in the field of protein translation, codon usage evolution and post-transcriptional regulation. In 2018, she joined the Center for Genomic Regulation (Barcelona, Spain) as Group Leader, leading the 'Epitranscriptomics and RNA Dynamics" laboratory (https://www.crg.eu/en/programmes-groups/novoa-lab). Her lab is focused on dissecting the language of RNA modifications, and how its orchestration can regulate our cells in a space--, time- and signal-dependent manner. Using a combination of experimental and computational techniques, including the use of state-of-the-art sequencing technologies such as Oxford Nanopore Technologies, her lab aims to decipher the secrets of three post-transcriptional regulatory layers: the epitranscriptome, the RNA structurome and ribosome specialization. Eva has received fellowships from "LaCaixa", EMBO, HFSP, and the ARC, and her work has been awarded with the Fisher Scientific Prize for Young Researchers (2013) given by the Spanish Society of Molecular Biology and Biochemistry, and the Young Researcher Award (2016) given by the Catalan Society of Biology.

14. Eric Miska, Gurdon Institute, University of Cambridge

New biology and therapeutics from RNA structure and modification

Since August Weismann (1834-1914) formulated the distinction between innate and acquired characteristics at the end of the 19th century, the debate relating to the inheritance of acquired traits has raised many controversies in the scientific community. Following convincing arguments against (e.g. William Bateson) this debate was then set aside by the majority of the scientific community. However, a number of epigenetic phenomena involving RNA, histone modification or DNA methylation in many organisms have renewed interest in this area. Transgenerational effects likely have wide-ranging implications for human health, biological adaptation and evolution, however their mechanism and biology remain poorly understood. We recently demonstrated that a germline nuclear small RNA/chromatin pathway can maintain epi-allelic inheritance for many generations in C. elegans. We will discuss evidence for such inheritance in an emerging vertebrate model, the cichlid fish of Malawi.

Biography

As a postdoc, Eric studied the then newly-discovered class of miRNA genes in the nematode C. elegans as a major functional genomics project. He also developed the first miRNA microarray which led to the first map of miRNA expression in human cancer.

Since establishing his own research group in 2005 at the Gurdon Institute he has continued to investigate gene regulation by non- coding RNA and other epigenetic mechanisms. Highlights include assigning biological function to some of the first animal miRNAs. Next the Miska Lab discovered the piRNA pathway in C. elegans, demonstrating that the piRNA pathway of C. elegans functions upstream of a nuclear RNAi pathway. Also showing that piRNAs and nuclear RNAi lead to a mutigenerational RNA memory in C. elegans. Together with collaborators the first virus to naturally infect C. elegans was discovered. Thus having a new host-pathogen paradigm in hand the lab was able to demonstrate that RNA interference provides immunity to natural viral infection in animals.

He is also leading a major collaborative effort to "re-invent" African cichlids as a vertebrate model for the study of non-DNA based inheritance. Currently the Herchel Smith Professor of Molecular Genetics and Deputy Director of the Gurdon Institute, he is also the 2013 recipient of the Hooke Medal awarded by the British Society of Cell Biology. A full member of EMBO since 2012 his work on RNA-modifying enzymes has led to the Cambridge University spin-out company, STORM Therapeutics Limited, which aims to deliver new medicines by targeting RNA in cancer.

Selected Short Talks Abstracts

1. Johanna Grinat, MDC

Johanna Grinat[1,], Julian Heuberger[1,*], Frauke Kosel[1], Ramon Oliveira Vidal[2], Annika Wulf-Goldenberg[3], Diana Behrens[3], Bálint Melcher[4], Sascha Sauer[2], Michael Vieth[4], Francis Stewart[5] and Walter Birchmeier[1]*
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* These authors contributed equally.

Epigenetic control of Lgr5$^+$ intestinal cancer stem cells by the histone methyltransferase Mll1

Wnt/β-catenin-dependent genes have been shown to be crucial for intestinal carcinogenesis and the formation and maintenance of cancer stem cells. Here we identified the histone methyltransferase Mll1 as an epigenetic regulator of human and mouse intestinal stem cells and tumors. Mll1 is highly expressed in Lgr5$^+$ stem cells and human colon carcinomas with nuclear β-catenin. It is a prerequisite for the β-catenin-mediated expansion of stem cells in organoids and in intestinal tumors. Knockdown of Mll1 decreases the self-renewal and proliferation of colon cancer sphere cultures and halts tumor growth in xenografts. Mll1 knockdown cells reduce the expression of intestinal Lgr5$^+$ stem cell genes. Mll1 directly controls the expression of distinct stem cell genes including Wnt-dependent *Lgr5* via H3K4 tri-methylation. The knockdown of Mll1 increases repressive H3K27me3 promoter marks at the expense of activating H3K4me3 marks. Our results demonstrate that Mll1 is essential for Wnt/β-catenin-induced tumorigenesis and cancer stemness.

2. Tobias Hoffmann, CRG

Functional impact of frequent cancer mutations in the splicing regulatory protein RBM10 and discovery of cancer relevant targets

Alterations in alternative splicing contribute to every cancer hallmark and tumours can become addicted to particular splice variants. Frequent mutation of Splicing Factors (SF) in cancer contribute to these alterations. The SF RBM10 is frequently mutated in lung adeno- and bladder carcinomas. We analysed RNA sequencing data from dozens of bladder tumours from the UROMOL project and identified alternative splicing events associated with the presence of RBM10 mutations. These can potentially explain the contributions of RBM10 alterations to bladder cancer progression. Work in different bladder cancer cell lines confirmed the regulation of these events by RBM10.

Cancer-associated alterations in RBM10 include both truncating as well as missense mutations. Molecular modelling, NMR and biochemical studies enable us to predict potential effects of missense mutations, including loss of binding to protein partners or RNA. For example, our data suggest that mutation R343G rather affects RNA binding and the frequent mutation S781L might affect the folding of the second Zinc-finger domain. The effects of these and other mutations are being studied in cell culture as well as in mouse- and human-derived bladder organoids.

3. Charlène Estrada, Curie

Charlene Estrada[1], Damarys Loew[2], Florent Dingli[2], Corine Bertolotto[3], Celio Pouponnot[1], Sabine Druillennec[1] and Alain Eychène[1]

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Two key players in melanoma, MITF and RAF proteins, form cytosolic complexes

NRAS and *BRAF* are frequently mutated in cutaneous melanoma. Targeted BRAF inhibitors are currently used in clinics but can be prescribed only for BRAF-mutated patients since they paradoxically overactivate the ERK pathway in NRAS mutant cells. We have developed mouse models enabling to study the role of RAF kinases in NRAS-mutated melanoma. Our results show that NRAS-driven melanoma can escape simultaneous loss of BRAF and CRAF via ARAF, which is sufficient to sustain ERK activation and cell survival.

Because the role of ARAF in NRAS-driven cutaneous melanoma was so far unknown, we sought new partners of ARAF using proteomics. After selecting putative hits by a functional siRNA-based screen, we focused on the transcription factor MITF, which is required for proliferation of NRAS-mutant cells. We confirmed the formation of ARAF/MITF complexes in the cytosol. MITF plays a key role in melanoma since it is correlated with tumor cells phenotype, either stem-like or proliferative, depending on its level of expression or activity. Although it is known that ERK directly phosphorylates MITF on S73, no interaction between a RAF kinase and MITF has been described so far.

Our current project aims at better characterizing the interaction between ARAF and MITF and studying its functional consequences on the activity of both partners. Using PLA and coIP, we showed that MITF also interacts with BRAF and CRAF. At the functional level, two hypotheses – not mutually exclusive – are possible: 1/ RAF function is modulated by MITF; 2/ RAF/MITF interaction impacts on MITF activity. We are assessing the transcriptional activity of MITF and its localization upon modulation of RAF expression.

Altogether, this work highlights a new level of interaction between two major actors in melanoma and provides new insights into the functional relevance of this interaction. Characterizing MITF/RAF protein interaction could offer new paths for melanoma treatment, especially for NRAS-mutated patients.

4. Johannes Bigenzahn, CeMM

Johannes Bigenzahn[1], Giovanna M. Collu[2], Felix Kartnig[1], Melanie Pieraks[1], Gregory I. Vladimer[1], Leonhard X. Heinz[1], Vitaly Sedlyarov[1], Fiorella Schischlik[1], Astrid Fauster[1,3], Manuele Rebsamen[1], Katja Parapatics[1], Vincent A. Blomen[3], André C. Müller[1], Georg E. Winter[1], Robert Kralovics[1], Thijn R. Brummelkamp[1,3], Marek Mlodzik[2], Giulio Superti-Furga[1]

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Genetic drug resistance screens identify LZTR1 as regulator of RAS ubiquitination and signaling

Small molecule tyrosine kinase inhibitor (TKI)-based treatment of chronic myeloid leukemia (CML), characterized by the presence of the Philadelphia chromosome (Ph+) encoded BCR-ABL tyrosine kinase, is a paradigm of targeted cancer therapy. However, the development of TKI resistance limits the clinical long-term success of these therapeutics. We used a genetic screening approach in the near-haploid CML cell line KBM-7 to identify six genes whose individual loss-of-function led to TKI drug resistance. We investigated in detail the role of the leucine zipper like transcription regulator 1 (LZTR1) gene, as it was mechanistically enigmatic despite its involvement in many human developmental and oncological diseases including Noonan syndrome, Schwannomatosis and glioblastoma. We found that LZTR1 inactivation led to enhanced MAPK pathway activity and reduced TKI sensitivity of CML cells in a RAS-dependent way. LZTR1 missense mutations identified in human diseases failed to revert the loss-of-function phenotype. Knockdown of the LZTR1 orthologue CG3711 in Drosophila led to an increase in ectopic wing vein formation that could be rescued by impairment of RAS gene function, confirming the epistatic relationship and suggesting an evolutionary conserved role for LZTR1 in RAS regulation. Endogenous LZTR1 protein associated with the four main RAS isoforms KRAS4A, KRAS4B, NRAS and HRAS in proximity biotinylation proteomic and immunoprecipitation experiments in a manner that for KRAS required farnesylation and palmitoylation. Ectopic expression of LZTR1 along with all four RAS proteins led to their ubiquitination, compatible with the proposed role of LZTR1 as a cullin 3 (CUL3) E3 ligase adaptor. Loss of LZTR1 led to reduced ubiquitination of endogenous KRAS and its increased abundance as well as enhanced localization at the plasma membrane. In summary, LZTR1 acts as a conserved regulator of RAS GTPase ubiquitination and MAPK pathway activation, providing a mechanistic explanation to its genetic involvement across a variety of human pathologies.

5. Jonathon Coates & Sarah Ross, BI

Dissecting the complex response of cytotoxic T cells to hypoxia

The function of immune cells is defined by environmental cues. Cytotoxic T lymphocytes (CTLs) destroy diseased or damaged cells and are a critical component of the adaptive immune system. The sites of infection or disease where CTLs function are often oxygen deficient (hypoxic). Our aim is to determine how oxygen levels regulate cellular programs to shape effector T cell functions. Using quantitative mass spectrometry, we have uncovered that hypoxia reconfigures the proteome of primary cultures of CTLs, modulating the abundance of molecules that regulate CTL function. Approximately 8% of the mass of a CTL was comprised of glycolytic enzymes and we found that hypoxia can boost this to around 12%. Glycolytic metabolism is linked to promoting the effector function of CTL; correlating with the increase in glycolytic enzymes, hypoxia increased the expression of molecules, such as perforin and granzymes, that CTL use to kill diseased cells. However, the increase in these pro-inflammatory molecules was accompanied by an increase in the abundance of the inhibitory checkpoint molecules PD-1, CTLA-4 and Tim-3, and increased production of the anti-inflammatory cytokine, IL-10, along with a decreased ability of the CTL to produce IFN-γ and TNF-α in response to TCR-stimulation. This data establishes a role for hypoxia in reconfiguring the CTL proteome to promote cytotoxic functions whilst restraining wider inflammation and immune cell activation. Interestingly, not all of the changes in protein abundance we observed in CTL in response to hypoxia depended on the expression of the hypoxia inducible transcription factor, HIF1α. This highlights that multiple factors are involved in reconfiguring gene expression in response to oxygen levels. Currently, we are determining how these pro- and anti-

inflammatory responses are co-ordinated by hypoxia, with a focus on understanding how the transcriptional and epigenetic landscape of CTLs are modulated by acute and chronic hypoxia.

Commercial Research Session Abstracts

1. Chris Torrance, CEO PhoreMost Ltd

PhoreMost: Drugging the Undruggable

PhoreMost has developed a next-generation phenotypic screening platform called SITESEEKER® that can discern the best new targets for future therapy and crucially, how to drug them, which has the potential to significantly increase the diversity and affordability of novel therapeutics for cancer and other unmet diseases. Based on the Company's core proprietary 'Protein Interference' technology, SITESEEKER® systematically unmasks cryptic druggable sites across the entire human genome and directly links them to useful therapeutic functions in a live-cell context. Using this platform, PhoreMost is building a pipeline of novel drug discovery programmes aimed at addressing a range of unmet diseases.

2. Katherine Ewings, Group Leader at CRUK-TDL

Translating cutting edge science into innovative new therapies for cancer patients

Cancer Research UK's Therapeutic Laboratories (CRUK-TDL) is the in-house Cancer Research UK drug discovery unit with a principal focus on establishing and prosecuting biologically-themed multi-project alliances with industry. Our expertise spans cancer target validation and disease positioning, immunology, assay development and HTS, protein crystallography, medicinal chemistry, PK-PD and cancer models. Through our alliance approach, we align these key skills with internationally competitive academic research and the development capabilities of industry partners to maximise cancer patient benefit.

3. Richard Sainson, Senior Director, Translational Medicine, Kymab

Kymab: a brief overview of our platforms and late stage pipeline

Based on the Babraham site in Cambridge, Kymab is a clinical-stage biopharmaceutical company that is developing novel human antibody-based therapies for different diseases. We have several unique platforms that allow us to generate repertoires of fully-human, species cross-reactive and affinity-matured antibodies against challenging targets. We have developed biologics designed to modulate the immune system in order to generate a powerful immune response in cancer and reinstate homeostasis in autoimmune diseases. Here we will present a brief overview of our platform, our pipeline with a focus on the lead assets, KY1005 (anti-OX04L), KY1044 (anti-ICOS) and KY1043 (anti-PD-L1 immunocytokine) that are already in clinical trials in atopic dermatitis and advanced malignancies or close to enter Phase I.

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Poster_Abstracts

1. Julieta Ramírez, CRG

Julieta Ramírez Cuéllar, Guillermo P. Vicent, Miguel Beato

Genome wide analysis performed in 3D breast cancer organoids reveal new pathways involved in hormone-dependent gene regulation

Breast cancer is a highly heterogeneous disease, dictated mainly by the spatial and temporal relation between the tumour and the microenvironment. The microenvironment of a cell is an organized combination of extracellular matrix, neighbouring cells, and interstitial fluid that influence cellular function (Warrick, 2008).

However, how these external signals integrate and impact on gene regulation is far from understood, as most studies have been carried out in cells cultivated as monolayers on plastic. Organoids aim at recapitulating normal and pathological tissue conformation, structure and function, and are increasingly used.

We aim to elucidate how the signalling network mediating hormone response differs in an organoid system from what has been found in conventional 2D culture, focusing on coordination of chromatin remodelling and differential gene expression in the control of cell proliferation and cell fate decisions.

We have established the culture conditions for growing organoids from breast cancer cells. Initial work is performed with T47D cells exhibit hormone-dependent cell proliferation. The expression of hormone receptors and the hormone- dependent activation of Src/Ras/Erk kinase signalling pathway (Vicent et al., 2006) are comparable in organoids and 2D culture conditions, indicating an initial similar response to the hormonal stimulus.

However, RNA-seq in the presence and absence of progestin R5020 shows 2,000 new differentially regulated genes in 3D. Surprisingly, ChIP-seq of PR in 3D shows a highly-reduced number of PR binding sites down to 16 % from the original described in 2D (Ballaré, 2013). ATAC-seq detected an overall reduced chromatin accessibility in 3D organoids, limiting PR binding to regions that are occupied at very low hormone concentration.

Monitoring the genome architecture using Hi-C will help track the reorganization of the 3D nucleus resembling the physiological situation. This will allow us to identify new physiologically significant signalling pathways controlled by the microenvironment.

2. Anastasiia Lozovska, IGC

Anastasiia Lozovska, Patricía Duarte and Moisés Mallo
Instituto Gulbenkian de Ciência, Oeiras, Portugal

Gene regulation during trunk-to-tail transition in the vertebrate embryo

During embryogenesis the vertebrate embryo extends posteriorly and forms the trunk and tail regions by sequentially adding new tissue differentiated from the progenitors located in a caudal growth zone, first in the epiblast and at later stages in the tail bud. How the same pool of progenitors gives rise to the different body compartments and how the transition between body regions is orchestrated is not fully understood. According to the previous findings of our lab, Gdf11, a signalling molecule of the TGF-b family, and the transcription factor Oct4 play opposite roles in the trunk-to-tail transition. While Gdf11 is important to initiate formation of tail structures, Oct4 activity is associated with trunk development. By combining transcriptomics and genomics data we aim to elucidate how these major regulators control formation of the trunk and tail regions and what kind of interaction is behind the trunk-to-tail transition.

3. Gert-Jan Kuijntjes, NKI

Linking Transcriptional Bursting to Chromatin Structure at Single Cell, Single Nucleosome Level

Transcriptional bursting is defined as the rapid initiation of clusters of polymerases that cause bursts in mRNA production during a period of gene activity. Bursting is conserved from bacteria to humans and the majority of genes show this behaviour. Even though this phenomenon has been studied for over two decades, the causes and functions of transcriptional bursting remain largely unknown.
Previous studies have identified several factors that influence either the burst duration or the frequency of burst initiation. One of the identified factors is the local organization of chromatin, which has been linked to transcriptional bursting mainly on the most basic compaction level of nucleosomes. This linking relies heavily on population-based nucleosome mapping assays, such as the MNase assay. As cell populations show a high degree of heterogeneity, the results of these assays represent the population average, which makes it rather well impossible to pinpoint the exact factors contributing to transcriptional bursting.
In this study, we use a novel technique in order to map nucleosomes of native chromatin of a single locus using magnetic tweezers, and link these data to the bursting dynamics of this locus via single-live-cell imaging. First, we isolate the single-copy Galactose locus from *Saccharomyces cerevisiae* in its native chromatin context in different transcriptional states. Secondly, we analyse the purified chromatin using magnetic tweezers in order to map the exact location of nucleosomes and to get insight in the nucleosome stacking of single chromatin fibres. Subsequently we analyse the transcriptional behaviour of the galactose genes using the PP7-system, and correlate these data to our magnetic tweezer analysis, bridging the gap between cellular and *in vitro* studies of chromatin and transcription analysis.

4. Ana Neves-Costa, IGC

Disease protection by DNA damage responses induced by Anthracyclines

We identified the anthracycline family of chemotherapeutic drugs (epirubicin, doxorubicin and daunorubicin) as potent antagonists of inflammatory cytokine production that are able to confer strong protection in a mouse model of severe sepsis. We also showed that Ataxia Telangiectasia Mutated (ATM)-dependent events are required for the protection, thus implicating the DNA damage response (DDR). The anti cancer activity of anthracyclines is not fully understood, but it is known that these drugs inhibit type II topoisomerases and induce oxidative damage in the DNA as a consequence of the reactive oxygen species generated by their intracellular metabolism.
Here we test the capacity of different anthracyclines to generate DNA damage in immune-responsive cells both directly, as assayed by comet assays, and by quantifying histone H2AX phosphorylation levels. We conclude that cytokine up-regulation by anthracyclines is independent of DNA damage, as well as the regulation of a series of critical pro-inflammatory mediators. Importantly, the anthracycline-mediated effects on the expression of the inflammatory program are also observed in ATM-/-mice.

5. Sara Lundgren, FIMM

Sara Lundgren[1], Sara Kuitunen[1*], Kirsi Pietiläinen[2 3], Jaakko Kaprio[1,4], Miina Ollikainen[1]*
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4Department of Public Health, University of Helsinki, Helsinki, Finland
**Equal contribution*

Epigenetic aging in BMI-discordant monozygotic twin pairs

Background: Obesity is a heritable complex phenotype which itself increases the risk of many age-related diseases and outcomes, including cancers, type 2 diabetes, cardiovascular disease, and death. One way of estimating biological aging is by inferring age using DNA methylation data. Some previous work suggests that individuals with elevated weight also display accelerated aging, but the results have been mixed. A new epigenetic clock which has not yet been evaluated in relation with obesity, GrimAge, is estimated by first predicting levels of seven plasma proteins from DNA methylation data, which are then used along with smoking history to predict age and in turn age acceleration (Lu et al, 2019). We aim to assess the relation between excess weight and age acceleration.
Methods: Subjects are participants selected from the population-based FinnTwin12 and FinnTwin16 cohorts (n=1540), including monozygotic (MZ) pairs discordant for BMI, defined as a difference in measured BMI > 3 kg/m2 (n=38 MZ twin pairs). Usage of MZ twins allows assessment of epigenetic differences independent of genetic and shared environmental effects. DNA methylation was measured using the Illumina HumanMethylation450 BeadChip, and GrimAge will be calculated using

the method developed by the Horvath group. First, association between BMI and GrimAge will be assessed, and differences in GrimAge and age acceleration between cotwins from BMI-discordant pairs will be tested. Additionally, we will assess the relation between clinical measures including body fat percentage, subcutaneous and intra-abdominal fat, and liver fat with GrimAge and age acceleration.

Results/Conclusions: We hypothesize that GrimAge is higher in heavier co-twins, and that age acceleration is positively related with BMI and fat accumulation in different depots.

6. Lluís Riera, BRIC

Identification and characterisation of *IFNAR1*^{C291*}, a rare mutation in familial Parkinsonian disorders

Parkinsonian disorders, including Parkinson's disease (PD), are the second most common class of neurodegenerative disorders. PD produces a progressive decline in motor and cognitive functions with no cure. Despite numerous efforts, PD is largely sporadic with unknown aetiology. Nevertheless, studies of rare familial forms have identified genetic mutations– shedding light on the underlying pathophysiology. Here, we report the identification of a novel, nonsense, disease-linked mutation of the interferon α/β receptor 1 (*IFNAR1*) gene, p.(C291*), in a family affected by parkinsonian disorders. We found that the p.(C291*) mutation confers loss-of-function and defective neuronal IFNβ-IFNAR-signaling, as it encodes for a truncated version of the receptor, with partial deletion of the extracellular domains, transmembrane domain and cytoplasmic tail. Heterozygous *IFNAR1*^{C291*} expression in primary neurons had a dominant-negative effect, causing defective neuronal morphology and the pathological hallmarks of PD: accumulation of senesced mitochondria, increased oxidative stress, lowered ATP production, and intracellular inclusions containing phosphorylated α-synuclein, Tau and ubiquitin. Our data reveal a new link between innate immunity and Parkinsonian disorders, highlighting new pathogenic mechanisms involved in the aetiology of the disease.

7. Juan Castillo-Fernandez, BI

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Effects of ageing on the transcriptome and methylome of the oocyte assessed at the single-cell level

Early embryo development prior to zygotic genome activation is heavily supported by maternal transcripts and epigenetic states produced in the oocyte. Given that age is a major factor affecting fertility, oocyte quality for proper embryo development might be compromised by advanced maternal age, which is common in modern society. Characterising the molecular effects of ageing

on the oocyte can identify such factors affecting its quality. Here we profiled the transcriptome and methylome of germinal vesicle oocytes from mice at 12 weeks old (young group) and 44-54 weeks old (aged group) using parallel single-cell RNA sequencing and whole-genome bisulfite sequencing. Global exploration of the transcriptome datasets revealed a lower complexity of expressed genes in the aged group (Wilcoxon test, p = 3.6x10-6) and also a weak correlation ($p < 0.05$) and a strong correlation ($p < 1x10-5$) of principal components 1 and 2, respectively, with age. Furthermore, we found evidence of differential expression at 314 genes (false discovery rate < 0.05) between young and aged oocytes. Based on the expression levels of these differentially expressed genes we identified a subgroup of aged oocytes that expressed a subset of genes (n=56) at levels similar to those observed in the young group, which we refer to as the young-like group. Concordant with this finding, a dispersion analysis revealed more variability in the gene expression levels of aged oocytes compared to young oocytes (3,641 vs 39 genes). When looking at the methylome we identified 806 differentially methylated regions at domains known to be either hypermethylated or hypomethylated in the oocyte that were able to cluster into two separate groups young and aged oocytes. Lastly, we were able to identify coordinate changes in gene expression and gene body DNA methylation at 15 genes. The impact of such coordinated, age-related changes in gene expression and methylation on the fate of the early embryo will require further investigation.

8. Sarada Achyutuni, CeMM

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Identification of vulnerabilities of CALR mutant driven neoplasms

Myeloproliferative neoplasms (MPNs) are characterized by an abnormal expansion of myeloid clonal lineages, wherein hematopoietic stem cells (HSCs) or progenitors with somatic mutations drive the oncogenic program of dysregulated hematopoiesis. CALR, JAK2 and MPL are known major drivers. Mutations in CALR are insertions and/or deletions that lead to a 'frameshift' to a very specific alternative reading frame. Our lab has previously shown that mutant proteins specifically induce the activation of thrombopoietin receptor (MPL) and to further clarify the mechanism of CALR mutants, we have also developed a conditional knock-in mouse model with CALR mutant (del52).

Genome-wide genetic perturbation screens divulge information about potential gene-gene dependencies that would be either detrimental for mutant cell survival or would exacerbate the disease progression. In this study, we have performed a genome-wide in vitro CRISPR screen to identify the group of differentially regulated genes in Wild-type, Heterozygous & Homozygous mutant calreticulin mouse cell lines. Currently, we are validating the significant hits and will further assess their relevance in CALR-del52 driven MPN in vivo. This project will be crucial in identifying the most relevant signaling pathways/molecules in CALR mutant driven MPN. This would not only augment our existing knowledge on the mechanism and biology of the disease, but also facilitate the development of novel therapeutic intervention.

9. Alex Whale, BI

Alex Whale, Ryan Hull and Jon Houseley
Babraham Institute

Investigating mechanisms of environmentally-stimulated Copy Number Variation

Copy number variation (CNV) is widespread in eukaryotes and is implicated in a variety of human disorders, in particular cancer whereby CNV promotes both carcinogenesis and chemotherapy resistance. Our group recently demonstrated that copper resistance arises in yeast through stimulated CNV: exposure to copper drives CNV at the copper resistance gene, CUP1, and leads to the emergence of novel alleles containing CUP1 amplifications, allowing cells to adapt rapidly to copper-rich environments. Here we begin to characterise the mechanism of environmentally-stimulated CNV.

Through screening candidate mutants using a yeast deletion collection, we have identified a number of factors that are either critical for stimulated CNV to occur, or that function to suppress CNV. The results of this screen have identified proteins involved in various aspects of the DNA damage response, transcriptional initiation / elongation and histone post-translational modification as being essential for stimulated CNV, while factors involved in protecting replication fork stability have a significant role in supressing CNV.

We propose a mechanism for stimulated CNV by which localised transcription destabilises replication forks during DNA synthesis and increases the frequency of error-prone DNA repair. We have demonstrated that the Structure Specific Endonucleases (SSEs) Mus81 and Yen1, which target stalled replication forks promote fork collapse and initiate the process of CNV. Furthermore, we showed that these collapsed replication forks are repaired through Break-Induced Replication (BIR), a homologous recombination-dependant process which re-establishes DNA synthesis. Stability of BIR along chromatin is negatively impacted by histone 3 K56 acetylation (H3K56ac) and through genetically or pharmacologically increasing levels of H3K56ac we were able to reduce BIR efficiency, causing error-prone BIR repair that led to significant levels of CNV.

Our research suggests that stimulated CNV may be a widespread and controllable mechanism allowing cells to adapt towards more hostile environments. Although stimulated CNV could have a significant impact on driving both tumourgenesis and drug resistance, here we speculate it may also be a process that could be therapeutically inhibited.

10. Vicky Chung Wai Yee, MDC

Mammalian Sex Determination in Single Cell Resolution

In mammals, embryonic gonad is the sole bipotent organ, which is capable to differentiate into either testis in male or ovary in female upon primary sex determination. Despite knowing this fate commitment step in gonadal cells are gene-directed, the systemic gene expression profile and coordination remain elusive.

The research goal here is to investigate the regulatory network in primary sex determination step in single cell resolution, and via expression perturbation of sex determining factors, in models of

interest. High throughput single cell omics (scRNA- & scATAC-seq) are employed to profile the transcriptomes and chromatin accessibility maps of gonads right after sex determination. Our results advances the understanding on the regulatory network underlying gonadal sex fate commitment.

11. Anna Schrempf, CeMM

Novel insights into error-prone repair pathways of CRISPR-Cas9 induced DNA breaks

During CRISPR-Cas9 editing, the resulting DNA double-strand break is resolved by either error-free repair or one of two error-prone pathways: canonical non-homologous end joining (c-NHEJ) or polymerase theta-mediated end joining (TMEJ). Surprisingly, we observed residual error-prone repair of Cas9-breaks in HAP1 cells harboring loss-of-function mutations in repair genes rendering them incompetent in both c-NHEJ and TMEJ (LIG4 POLQ KO). Importantly, we could confirm the presence of residual error-prone repair, distinct from c-NHEJ and TMEJ, using a flow cytometry-based assay. However, accurate prediction of Cas9 editing outcomes demands a complete understanding of the involved double-strand break repair machinery. To identify genes involved in this novel error-prone repair pathway, we are setting up a DNA-repair focused CRISPR knock-out screen in wildtype and LIG4 POLQ KO HAP1 cells. By targeting Cas9 to a fluorophore and monitoring fluorescence upon CRISPR-Cas9 editing, we can distinguish cells which underwent error-prone repair by their loss of fluorescence. By further validation of candidate genes, we aim to identify an additional DNA repair mechanism that deals with Cas9-generated lesions which will be highly relevant for understanding and predicting the editing outcome of CRISPR-Cas9.

12. Christopher Fell, CeMM

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Molecular Neuropathology of HACE1-Deficiency

HACE1, encoding the HECT Domain and Ankyrin Repeat Containing E3 Ubiquitin Protein Ligase 1, has been of interest to biomedical research groups for its role in cancer, heart function and inflammation. Surprisingly, mutations in HACE1 have recently been shown to cause a rare autosomal recessive neurodevelopmental syndrome called Spastic Paraplegia and Psychomotor Retardation with or without Seizures (SPPRS; OMIM #616756). SPPRS is marked by global delay of developmental milestones, most prominent of which are intellectual disability (ID), hypotonia and ataxia. Magnetic resonance imaging (MRI) findings were variable among patients, but included enlarged ventricles, hypoplastic corpus callosum and atrophy of the cerebrum and brain stem. Guided by patient clinical descriptions, our group performed detailed phenotypic analyses of Hace1-deficient mice and SPPRS patient fibroblasts and uncovered surprising new roles for HACE1 in both human and mouse brain development. The present study builds on this previous work by working towards and understanding the molecular underpinnings of HACE1-deficiency using cellular models.

13. Dimitrios Tsallos , FIMM

Dimitrios Tsallos[1], Komal K. Javarappa[1], Joseph Saad[1], Bulat Zagidullin[1], Alina Matulina[1], Celine Pallaud[2], Pedro Maerques Ramos[2], Satu Mustjoki[3], Jing Tang[1], Caroline Heckman[1]

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Eltrombopag promotes megakaryocyte cell survival and signaling in presence of specific cytotoxic agents

Thrombocytopenia is a frequent cause of hemorrhage related morbidity and mortality in patients receiving cancer treatment. Approximately two thirds of patients with hematologic cancers and one fourth of patients with solid tumors experience treatment-related thrombocytopenia. The development of supportive strategies for the treatment of chemotherapy-induced thrombocytopenia has the potential to improve both treatment outcome and quality of life during treatment. We try to identify novel drug combinations with eltrombopag (EP), a thrombopoietin receptor agonist, to protect megakaryocytes (MKs) during treatment. Here we describe off target signaling activation of EP and which treatments support MK survival in presence of EP. To produce MKs, we expanded CD34+ cells from healthy donors and treated cells with 14 common cancer treatments alone or in combination with a stable concentration of EP. The drug response of MKs is measured using high-throughput flow cytometry. MK signal activation of pSTAT3, pSTAT5, pAKT, pERK by TPO, EP, selected treatments alone or in combination with EP was normalized to serum-free media. MKs were distinguished at different levels of maturation (CD41a, CD42b) and expression of TPO receptor (CD110), the target of EP. The greatest net effect was observed when EP was combined with BCL-2 inhibitors (venetoclax and navitoclax), while other common cancer treatments, including platin based treatments, topoisomerase inhibitors and nucleoside analogs, showed a moderate combinatorial benefit. Signaling patterns from the controls revealed a distinct pathway activation between TPO and EP that is not correlated with the expression of TPO receptor. Most treatments in combination with EP achieved signaling activation similar to that of TPO alone, while only ruxolitinib and azacitidine revealed antagonistic effects upon combination with EP. We identify EP's ability to activate signaling pathways in presence of cytotoxic treatments, validating the observed MK viability in the drug screening assay. Identifying therapies that can be successfully combined with EP could enhance anti-tumor effects of cancer treatments and predict outcome of clinical trial. Protecting MKs directly could prevent drug induced thrombocytopenia and minimize the use of platelet transfusion in cancer patients.

14. Mathilde Meyenberg, CeMM

Mutational Profiles in Obesity Induced Colon Cancer

Mutational signatures provide a new way of investigating the evolution of cancer. However, many etiologies of known signatures remain unidentified, while endogenous factors involved in DNA damage and mutagenesis remain understudied. This project aims to study how genome integrity is impacted in the obese condition by identifying and characterizing mutational signatures associated with high fat diet induced sterile inflammation and the concomitant metabolic dysregulation. To do so, male wild type C57BL/6J mice are placed on a normal or high fat diet and mutational signatures are tracked over time by sequencing clonally derived intestinal organoids. Analysis of the observed signatures reveals which pathways were dysregulated and which mutational outcome resulted. In the second part of the project, we will validate the dysregulation of predicted pathways and generate CRISPR-Cas9 mediated knockout organoids, targeting genes in candidate pathways. In this in vitro system we can investigate functional consequences of the disturbed pathways on DNA damage repair and mutagenesis. Mutational signature analysis of knockout organoids will confirm the mechanistic connection between mutational process and resulting mutational profile. By studying the DNA damage response in the obese condition, we expect to bring new understanding to the molecular basis of obesity induced colorectal cancer and identify new markers and targets for treatment of pre-cancerous lesions.

15. Lennart Enders, CeMM

Functional characterization of beta-cell dedifferentiation using an endogenous reporter cell-line for compound and genetic screening

The metabolic disease type II diabetes is characterized by insulin resistance and pancreatic beta-cell failure. This involves increased rates of beta-cell death. However, there is a growing body of evidence that beta-cell failure also includes a loss of cell identity and dedifferentiation to a progenitor or stem cell-like state. This process involves the loss of Foxo1 transcription factor activity and can be modeled pharmacologically. We aim to establish a screen to comprehensively identify all genes involved in beta-cell dedifferentiation and compounds that can reverse it.
As a read-out, insulin and glucagon expression will be analyzed using automated microscopy and FACS-based readouts. Therefore, the insulin and glucagon gene loci in beta-cell lines were edited using the PITCh (Precise Integration into Target Chromosome) system to couple expression of the respective gene to the expression of fluorescent proteins via IRES elements.
We successfully generated endogenous reporter cell-lines for the expression of insulin and glucagon and showed that the fluorescent signal correlates with the expression of insulin in these edited cells. After establishing the reporter cell-lines, we started testing the PLACEBO compound library for compounds affecting the expression of insulin or glucagon in a pharmacological model of dedifferentiation. First hit compounds could be validated and were tested in human primary pancreatic islets from cadaveric donors. A further edited beta-cell line expressing Cas9 is used for a genome-wide screen to identify modulators of insulin and glucagon expression and beta-cell differentiation. Promising genes can then be further analyzed mechanistically.

16. Piotr Jung, BI

What is the Plekhs1 function in PI3K Class IA signalling?

Phosphatidylinositol-3-kinase (PI3K) signalling is one of the most important intracellular pathways, which can be considered as a master regulator for cancer. Upon activation, the catalytic subunit of class I PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2] to phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P3], leading to subsequent recruitment of a variety of pleckstrin homology domain-containing proteins such as AKT to the cell membrane . This process is negatively regulated by PTEN and INPP4B. Loss of function of the PTEN tumour suppressor, resulting in dysregulated activation of the PI3K signalling network, is recognized as one of the most common driving events in prostate cancer development although upstream regulators of PI3K Class IA in this processes are still poorly understood.

To address the question what are the potential interactors of PI3K Class IA in 'healthy' and 'cancerous' prostate tissue, Tamara Chessa from Stephens and Hawkins lab has constructed specific PI3K 'Avitag' mouse lines, which allow us to efficiently pulldown subunits of the kinases from isolated tissues or cells along with their interactors. Based on the preliminary data, poorly characterised protein called Plekhs1 was found to interact with PI3K, although the interaction was much stronger, when PTEN was absent.

To further investigate the function of Plekhs1 we generated two genetically-modified mouse strains: a Plekhs1-KO and a Pekhs1-Avitag. 'Cancerous' prostates isolated from Plekhs1-KO mice show decrease in size (preliminary data) and lower AKT phosphorylation comparing to the 'cancerous' prostate with undisrupted Plekhs1 expression. Using Pelkhs1-Avitag mice we were able to perform a very efficient pulldown. We observed enhanced phosphorylation of Plekhs1, in the prostate where PTEN is absent as well as increased interaction with PI3K Class IA, what is consistent with previously achieved results in our laboratory.

17. Polly Machin, BI

Characterisation of Tiam1-dependent neutrophil adhesion

Neutrophil chemotaxis and extravasation into the bloodstream towards inflammatory stimuli is adhesion dependent, and involves the engagement of β-integrins with extracellular ligands. Rac-GEFs activate Rac by removing GDP so free intracellular GTP can bind, and Rac-GAPs turn off the Rac signal through enhancing the intrinsic GTP hydrolysis action of Rac. Multiple Rac-GEFs have been shown to be expressed in the neutrophil and already some Rac-GEFs have been assigned Rac-dependent roles, however, this is not complete. The Rac-GEF Tiam1 is being characterised in neutrophils, as knockout of Tiam1 (Tiam1$^{-/-}$) in the mouse has previously shown Tiam1 to affect neutrophil recruitment in an adhesion-integrin dependent manner. Here, we show that Tiam1$^{-/-}$ mouse neutrophils adhered to pRGD, a fibronectin-like protein polymer which engages with β1 and β2 integrins, leads to an increase in the number of polarised neutrophils, and to a dysregulation in F-actin structure in polarised neutrophils. We also show that there is an increase in the number of peripheral focal adhesion complexes of Tiam1$^{-/-}$ neutrophils on pRGD compared to wild type (WT) neutrophils. This could explain the increased random migration of Tiam1$^{-/-}$ neutrophils seen on pRGD. Comparatively, on ICAM-1/Fc where adhesion of neutrophils is solely β2-integrin dependent,

Tiam1$^{-/-}$ neutrophils tend to be less polarised than WT neutrophils, with no dysregulation in polymerised F-actin structure. This could explain the increased adhesion of Tiam1$^{-/-}$ neutrophils on ICAM-1/Fc. The phenotype of the Tiam1$^{-/-}$ mouse neutrophil may be explained through future Rac activity assays involving the Raichu Rac-FRET activity reporter, where preliminary data indicates an increase in adhesion-dependent global Rac activity in Tiam1$^{-/-}$ neutrophils.

18. Elizabeth Hampson, BI

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Exploring GPCR Trafficking Control by P-Rex Rac GEFs

P-Rex family proteins are guanine nucleotide exchange factors (GEFs) that activate the Rho GTPase Rac. They mediate cellular processes such as cell proliferation, migration and membrane protrusions that commonly involve actin reorganisation. P-Rex proteins are important for tumour growth and metastasis in several types of cancer including prostate, breast and melanoma.
Previous unpublished work by the Welch group identified a novel role for P-Rex1 in the trafficking of G-protein-coupled receptors (GPCRs). Overexpression of P-Rex1 inhibited internalisation of the GFP-tagged GPCR Sphingosine-1-Phosphate Receptor 1 (S1PR1) which usually occurs upon stimulation with S1P, through clathrin-mediated endocytosis. This did not require GEF activity. Instead, large portions of the P-Rex1 protein were required, showing that GPCR trafficking control is an adaptor function of P-Rex1. Importantly, overexpression of P-Rex1 inhibited the S1P-induced phosphorylation of the S1PR1, the first step required for receptor endocytosis.
My aim is to characterise the role of endogenous P-Rex1 in GPCR trafficking, to elucidate the mechanism and the consequences for cellular functions.
I used CRISPR targeting to delete endogenous P-Rex1 from neuronal PC12 cells stably expressing S1PR1-GFP. Immunofluorescence imaging demonstrated that GPCR trafficking control is a function of endogenous P-Rex1, as deletion of P-Rex1 increased the S1P-stimulated internalisation of S1PR1-GFP. These data corroborate previous results from overexpression of P-Rex1. I have begun to characterise these P-Rex1 KO PC12 cells further. Deletion of P-Rex1 reduces cell viability and proliferation and inhibits the formation of neurite protrusions. P-Rex1 KO reduced the S1P-induced activation of Rac1 and the constitutively high level of Rac3 activity, without obviously altering other GPCR signalling pathways, including Akt, p38 MAPK and Erk1/2.
Future work will involve investigating mechanistic details of the novel function of P-Rex1 in GPCR trafficking, elucidating receptor specificity and effects on cellular responses. I will investigate correlations between expression levels of P-Rex1 with selected GCPRs on the surface of cancer cell lines, aiming to use these GPCRs to target P-Rex1 in cancer progression and metastasis.

19. Joseph Saad, FIMM

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FLT3 Activating Mutations Confer Significant Sensitivity to BET Inhibition in AML

BACKGROUND: Despite recent advances, the management of patients with acute myeloid leukemia (AML) remains challenging due to the lack of effective treatments. Preclinical studies of AML have demonstrated promising therapeutic activity of bromodomain and extra terminal (BET) family protein inhibitors. To identify patients that may respond to BET inhibition, we sought to discover and evaluate predictive molecular biomarkers of sensitivity, focusing on the BET inhibitor JQ1.
METHODS: Following written informed consent and approved protocols in accordance with the Declaration of Helsinki, fresh bone marrow and peripheral blood samples were obtained from AML patients (n=139) and analyzed for their whole exome profiles and ex vivo responses to JQ1 exposure. Associations between somatic mutations and drug responses were then investigated using logistic regression models. Predictive biomarkers emerging from our patient cohort were carried forward for validation using publicly available AML cell line and patient datasets.
RESULTS: Enabling a genome-wide search for predictive biomarkers for BET inhibitor response in AML, our bioinformatics pipeline revealed that FLT3-mutated samples in the cohort were significantly more sensitive to JQ1 compared to FLT3 wild-type samples, irrespective of their NPM1 mutation status. The superior response was maintained independently of whether the mutation was an internal tandem duplication or a point mutation in the tyrosine kinase domain of the gene.
CONCLUSION: The study suggests that FLT3-mutated AML patients may benefit from BET inhibition. Moreover, this observation proposes drug partners worth investigating in combination with BET inhibitors in this patient subpopulation, namely FLT3 and HDAC inhibitors. Although our finding requires further validation in a larger cohort and ultimately by clinical investigations, this study provides evidence of molecular indicators that could help stratify AML patients for emerging targeted therapies.

20. Lauren Maggs, BI

Determing the Interactome of Autotaxin

Autotaxin (ATX) is an extracellular lysophospholipase D which generates lysophosphatidic acid (LPA) by catalysing the hydrolysis of lysophosphatidylcholine (LPC). There are two types of LPA receptor. The EDG and non-EDG receptors. The non-EDG receptors receive LPA from the plane of the cell membrane, whereas the EDG receptors receive LPA directly from the extracellular space. Activation of these receptors leads to a signalling cascade in the cell that results in a variety of physiological responses, including cell survival and migration. ATX has been implicated in tumourgenesis and metastasis.

A population of secreted ATX binds to the surface of exosomes. Exosomal ATX is not as active as free ATX but does have some activity and the level of LPA present in the exosomes increases with levels of ATX. Along with generating LPA, ATX is believed to transport LPA to the LPA receptors on

the cell surface. This project hypothesises that the role of exosomal ATX is to deliver LPA directly to the ligand binding pockets of the EDG-type receptors, whereas free ATX provides LPA for the non-EDG receptors. This hypothesis will be investigated by determining the proteins exosomal and free ATX are bound to.

Two methods will be used to investigate the interactome of exosomal and free ATX. First, an affinity capture method, utilises the bacterial biotin ligase BirA. BirA specifically biotinylates a 15 amino acid tag, known as the 'AviTag'. Through transient overexpression in Huh7 cells it has been confirmed that Avi-GFP-ATX is secreted. Co-transfection of Avi-GFP-ATX and BirA has confirmed Avi-GFP-ATX is also biotinylated.

The second method, BioID, is a form of proximity dependant labelling. It utilises BirA*, a promiscuous biotin ligase that will biotinylate any nearby lysine residue. Transient overexpression of BirA*-ATX showed this protein is also secreted, however it did not result in biotinylated proteins. ATX tagged to a more efficient biotin ligase, MiniTurbo, was then trialled. MiniTurbo-ATX is also secreted and biotinylates proteins in the media to a level detectable by western blot.

21. Louise Webb, BI

Louise M.C. Webb, Alyssa Silva-Cayetano, Alexandre Bignon, Sigrid Fra-Bido, Noudjoud Attaf-Bouabdallah, Alice Denton, Edward Carr, and Michelle Linterman.

Intrinsic Notch signalling biases T follicular helper subset differentiation in older persons

T follicular helper (TFH) cells provide help to B cells enabling high affinity antibody production. TFH differentiation must be regulated to ensure optimal help and remains an attractive target for therapeutic intervention. Using an in vitro model of human TFH cell differentiation we have asked how age effects this process since the ability to mount appropriate immune response declines with age.

Using a model where human naïve CD4+ T cells are stimulated in vitro, we show that cells from older donors differentiate into TFH cells by default. This occurs in the absence of the polarising cytokines normally required to drive differentiation into TFH cells. We have confirmed this finding in vivo following immunisation of young and old mice. Despite increased proportions of TFH cells, older mice (like humans) show reduced antibody production and affinity maturation. It is already understood that an increase in the ratio of TFH:B cells can be detrimental for the evolution of the antibody response that occurs during the germinal center reaction. Using RNA-Seq, we found increased expression of the transcription factor RBPJ in TFH cells generated from older donors. RBPJ is a transcription factor and acts as an effector of the Notch signalling pathway. We show that RBPJ drives CXCR5 expression on T cells and we are able to block age-driven TFH differentiation by manipulating the Notch pathway.

This work suggests that age causes changes in the way T cells perceive signals, resulting in Notch signalling which drives increased differentiation into TFH cells. It not only provides an explanation for the decline in acquired immunity in the elderly population but also offers a pathway that can be manipulated to circumvent this.

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22. Celine Sin/Jörg Menche, CeMM

ROADdt: Regulatory network remodeling along disease development trajectories

The human body is comprised of over 200 different cell types varying in size, shape, and function. The differentiation and subsequent maintenance of these different phenotypic states are governed by complex gene regulatory networks that dynamically orchestrate the activation and deactivation of genes. Abnormalities in these networks may lead to dysfunctional expression programs, e.g. uncontrolled cell proliferation. In order to understand the conditions resulting in disease, we must understand the underlying gene regulatory networks governing the gene expression program.
As cells move through the differentiation space, the networks that govern gene regulation are remodeled in order to achieve the appropriate gene expression program. While statistical physics and network theory have demonstrated numerous relationships between the structure of networks and the dynamic processes that act on them, few studies link these mathematically rigorous principles to gene regulatory networks, none at the level of cell-trajectory-states. The overall goal of this project is to understand the fundamental architecture of gene regulatory networks associated with cell differentiation processes in disease. We hypothesize that the gene regulatory networks of different cell-trajectory-states along the differentiation trajectory – e.g. transitory, branching, or terminal states – are each characterized by distinct structural features. I will present our first steps in this direction, starting from single-cell RNA seq profiles of tumors. Ultimately, we expect that detailed characterization of the gene regulatory networks in these disease processes will reveal basic principles applicable to other diseases and cell developmental processes.

23. Sarah Whiteside, BI

Unique transcriptional profiles of tumour-associated Treg cells contain candidates for targeted immunotherapy

Immunotherapy has revolutionized cancer treatment and improved clinical outcomes for patients with metastatic disease. However, existing immunotherapies fail to induce durable clinical response in a majority of patients treated, necessitating a broader understanding of how tumors evade immune attack. $CD4^+$ regulatory T (Treg) cells are a powerful suppressive subset of T cells that are required to limit deleterious autoimmune and allergic inflammation. However, Treg cells can also serve a detrimental function, suppressing anti-tumor immunity and preventing immune rejection of disease. Proposed strategies to target the immunosuppressive function of Treg cells in cancer are poorly specific and lead to loss of systemic Treg suppressive activity and unwanted inflammation. By comparing the global transcriptional profiles of Treg cells in distinct tissues, we identified specific features of tumour-associated Treg cells that distinguish them from activated $CD4^+$ and $CD8^+$ conventional T (Tconv) cells. Future work aims elucidate the transcriptional relationships between

effector and suppressor immune cell types in the tumour, with the ultimate goal of identifying novel targets for tumour-specific Treg cell depletion.

24. Prasanna Channathodiyil, BI

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Role of gene copy number variation in the acquisition of resistance to selumetinib in colorectal cancer

Copy number amplification of BRAFV600E or KRASG13D drives selumetinib resistance in colorectal cancer cells by reactivating the ERK1/2 signalling pathway. Here, we investigate the underlying mechanisms of selumetinib resistance through gene copy number variation (CNV). By generating a panel of 24 independent selumetinib resistant cell lines from the BRAFV600E mutant colorectal cancer cell line COLO205 we demonstrated that BRAF amplification associated with selumetinib resistance is highly reproducible. Determination of BRAF breakpoints in 7 resistant cell lines generated from the parental COLO205 revealed unique breakpoints in 4 cases but identical breakpoints in 3 cases. Identical breakpoints indicate the presence of pre-existing mutants in the parental COLO205 population. We then derived 3 clones from single COLO205 cells and generated 7 resistant lines, all of which showed unique BRAF amplifications. Remarkably, there was no difference in the average time to resistance between the single cell derivatives and the parental line. CNVs commonly emerge from replication defects when transcriptional changes affect replication fork stability. Based on Edu incorporation and cyclin B1 staining, we detected the presence of a replicating sub-population of cells in selumetinib treated COLO205. We developed a method for mRNA sequencing following intracellular staining with Cyclin B1 and flow sorting, and analysed the transcriptomic profile of these cells. This revealed that replicating cells have decreased mRNA levels of essential replication factors. We predict that DNA replication under such conditions could cause genomic instability leading to CNV. We are currently characterising these cells by single cell DNA sequencing methods.

25. Andrea Lopez, BI

Andrea F. Lopez-Clavijo[1], Simon A. Rudge[1], Gregory West [1], Steven Wingett, Michael J O Wakelam[1]

Hypoxia-induced lipidomics changes point to an important role for stearoyl CoA desaturase

AIMS/INTRODUCTION: In cell membranes, double-bond formation (desaturation) of the acyl chain of phospholipids can decrease membrane thickness and modify membrane fluidity, thereby affecting physiological function. Oxygen-dependent SCD1 catalyses the formation of oleic acid (18:1) and palmitoleic acid (16:1) by the incorporation of a single double bond upon the free fatty acids stearic acid (18:0) and palmitic acid (16:0). SCD1 knockout was shown to inhibit hepatitis C,

denge and zika virus entry into cultured human liver cells. A number of cancers depend on SCD activity to survive, moreover the core of a tumour is hypoxic and lipid desaturation has been shown to be essential for such cancer cells to survive. In this work we have examined lipidomics changes in cells cultured under low nutrient conditions under an oxygen concentration of 21 % or 1 %. Changes in lipids are correlated to biosynthetic, metabolic and signalling pathways.

The synthesis of each lipid class examined was decreased in SCD1KO cells under both normoxia and hypoxia, but with a significantly greater decrease under hypoxic conditions compared to 21% oxygen. Additionally, there was an increase in the prevalence of saturated lipid species, in particular there were higher levels of saturated PA molecular species (34:0, 36:0 38:0, and 40:0). The amount of Cer and dhCer molecular species in SCD1KO cells was elevated compared to the wild type, suggesting increased de novo synthesis of sphingosine and its conversion to ceramide, which is correlated with the activation of SMPD2 and 3 enzymes.

Hypoxic incubation of both wild type (WT) and SCD1 KO cells caused increased synthesis of phospholipids, which correlates with an increase activation of CEPT1 enzyme (involved in the production of PC species from DG) and CHPT1 enzyme (DG→PE). Increased phospholipid synthesis might suggest changes in cell membrane fluidity and lipogenesis. Interestingly, hypoxia in wild type and SCD1 KO cells induces a similar phospholipids profile, confirming the role of SCD as an oxygen-dependent enzyme. However, there are at least 4 additional lipid metabolising enzymes activated in SCD negative cells compared to WT (CDS2, PTPMT1, PTDSS1 and PISD) highlighting additional pathways regulated directly, or indirectly by changes in oxygen concentration.

It has been reported that under hypoxic conditions cells either synthesise or take up FA from their surroundings. Thus, we used serum-free (i.e. fatty acid free) culture conditions (OPTIMEM) and observed accumulation of neutral lipids, CE and TG, present in lipid droplets, in both hypoxic WT and SCD KO cells suggesting decreased lipolysis. Since we determined accumulation of CE and TG alongside increased phospholipid biosynthesis it would suggest that the cells have responded to hypoxia by increasing de novo fatty acid synthesis.

Conclusions:

- The amount of each lipid class measured increases by ~20 % during hypoxia, indicating that elevated lipid synthesis is required for cell survival. This may bring about morphological changes in cell size/cell membrane structure and in lipid droplet content to adapt for the hypoxic environment.
- In the absence of SCD1 the results also show elevated lipid synthesis in hypoxic cells with a reduction of around 3-fold during hypoxia in SCD1 KO cells.
- The results have identified lipid enzyme changes/responses to SCD1 KO during hypoxia and normoxia.
- Future work will focus on elucidating how SCD impacts the activities of the enzymes that drive de-novo lipid synthesis during hypoxia and normoxia to identify the cellular signalling pathways, cellular process, and how the enzymes are regulated.

26. Joana Ferreira da Silva, CeMM

Joana Ferreira da Silva, Sejla Salic, Marc Wiedner, Paul Datlinger, Patrick Essletzbichler, Alexander Hanzl, Giulio Superti-Furga, Christoph Bock, Georg Winter, Joanna I. Loizou

Genome-scale CRISPR screens are efficient in non-homologous end-joining deficient cells

The mutagenic repair of Cas9 generated breaks is thought to predominantly rely on non-homologous end-joining (NHEJ), leading to insertions and deletions within DNA that culminate in gene knock-out (KO). In this study, by taking focused as well as genome-wide approaches, we show that this pathway is dispensable for the repair of such lesions. Genetic ablation of NHEJ is fully compensated for by alternative end joining (alt-EJ), in a POLQ-dependent manner, resulting in a distinct repair signature with larger deletions that can be exploited for large-scale genome editing. Moreover, we show that cells deficient for both NHEJ and alt-EJ were still able to repair CRISPR-mediated double-strand breaks, highlighting how little is yet known about the mechanisms of CRISPR-based genome editing.

27. Amandine Moretton, CeMM

Amandine Moretton and Joanna Loizou

Crosstalk between metabolism and DNA repair

Compelling evidence has underscored the importance of cellular metabolism in DNA damage and repair, yet a systematic analysis aimed at identifying such interactions remains unreported. The overall goal of this project is to functionally explore genetic interactions between metabolism and DNA repair, taking a global and unbiased approach based on a metabolic CRISPR-Cas9 library.
Here, we utilized a pooled CRISPR-Cas9 sgRNA library targeting some 3,000 metabolic genes, followed by the induction of DNA double-strand breaks by exposure to etoposide, an inhibitor of topoisomerase 2. After a minimal recovery time such that wild-type cells were able to clear the DNA damage, cells were stained for γH2AX, a marker of DNA double-strand breaks. Next, cells that retained high levels of γH2AX were FACS sorted, and genomic DNA was subjected to next generation sequencing to identify enriched sgRNAs representing metabolic genes that are required for DNA repair. In addition, treated and untreated cells were harvested at an extended time point, to identify metabolic genes that are synthetic lethal with etoposide treatment. Through data analysis, candidate metabolic genes that impact the clearance of DNA damage have been identified and will be validated across different cell lines and using different DNA double-strand break inducing agents. Next, we will explore whether the identified genes affect apoptosis, cell cycle or proliferation. Finally, a limited number of metabolic genes will be mechanistically investigated to understand their roles in DNA repair.

This project will shed light on how the fundamental cellular process of DNA damage maintenance is affected by alterations in cellular metabolism.

28. Junil Kim, BRIC

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Gene network reconstruction using single cell transcriptomic data reveals key factors for autophagic process

"What are the regulators?" is one of the frequently asked questions in biology to understand cellular processes. To answer this, experimental approaches including gain- and loss-of-function have been used. However, these approaches can catch indirect targets as well. Genome-wide analysis using chromatin immunoprecipitation followed by sequencing (ChIP-seq) is useful but limited by the availability of the antibody. Single cell RNA-seq (scRNA-seq) technology sheds light on tracing cellular trajectories in various biological systems. However, inferring upstream regulators from expression profiles is still challenging.

To understand regulator-target causal relationships, we introduce an approach called TENET, a new algorithm to reconstruct gene regulatory networks (GRNs) from scRNA-seq data. Based on transfer entropy, which measures the amount of directed transfer of information between two random processes, TENET identifies causal relationships between genes using the transcriptional profile aligned along the pseudo-time. Applied to differentiation from mouse embryonic stem cells to neural precursor cells, TENET reconstructs a GRN which includes Yamanaka factors Pou5f1, Nanog, and Sox2 as hub regulators. Robust validation using ChIP-seq and knockdown/knock-in experiment found that the causal relationships predicted by TENET far better performed than other algorithms such as SCODE. Applying TENET to the scRNA-seq data obtained during autophagic process prioritized key transcription factors that potentially controls majority of autophagy related genes. Strikingly, knocking down of the top prioritized factor X followed by green flurescent protein (GFP)-tagged LC3-positive autophagosome can block the autophagic process. Our results show that TENET identifies master regulators from single cell transcriptomic data.

Here, we proposed a novel approach for reconstructing GRN based on transfer entropy between gene expression profiles along cellular dynamics. GRNs inferred by TENET can reveal not only reliable causal relationships between genes but also critical regulators for cellular processes. Furthermore, TENET can be integrated with spatial single-cell data and may provide a new perspective of single cells' behavior linked to their niches.

29. Jana Block, CeMM

Jana Block[1,2,3], Rico Chandra Ardy[1,2,3], Paula Sanchez Moreno[4], Laia Alsina[5,6,7], Julien Viaud[8], Marianne Guisset[9], Rainiero Ávila Polo[10] Rocío Cabrera-Pérez[10], Cecilia Domínguez Conde[1,2], Elisabeth Salzer[1,2,3,11], Laurène Pfajfer[1,9], Raul Jimenez Heredia[1,2,3], Michael Caldera[2], Julia Pazmandi[1,2,3], Estíbaliz Iglesias Jiménez[6,12], Angela Deyà-Martinez[6,7], Marisol Camacho Lovillo[4], Birgit Hoeger[1,2,3], Jörg Menche[2], Jordi Anton Lopez[6,7,12], Joan Calzada-Hernández[6,12], Olaf Neth[4,*], Loïc Dupré[1,9,*], Kaan Boztug[1,2,3,11,13,*]

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Novel inborn error of immunity linking aberrant cytoskeletal dynamics and severe immune dysregulation

Disorders of immune regulation comprise a poorly characterized group of inborn errors of immunity with predominant autoimmunity and/or autoinflammation. Recently, study of patients with autoinflammatory syndromes carrying mutations in actin regulators have suggested that actin cytoskeleton integrity is key to prevent the onset of autoinflammation. However, discovery of additional molecules linking actin cytoskeleton with specific inflammatory pathways will be necessary to decipher the complex network controlling immune homeostasis in humans.

We discovered a novel gene defect affecting actin and microtubule cytoskeleton dynamics in a patient with impaired immune regulation. Clinically, the patient suffered from recurrent fever, hepatosplenomegaly, infections with unknown etiology and severe amyloid A amyloidosis very early in life. Using whole exome sequencing, we identified a hemizygous missense mutation in DOCK11. We were able to show that this mutation leads to impaired downstream signaling indicating a loss-of-function mutation. Moreover, patient primary cells showed morphological abnormalities, including reduced polarization and decreased formation of actin-rich protrusions. Further assessment of patient CD8 T cells revealed impaired immune synapse formation and reduced cytotoxic capacity. Genetic knockdown of DOCK11 in Jurkat T cells phenocopied the aberrant morphology, reversible upon overexpression of wild-type but not mutant DOCK11. Our study identifies human DOCK11, a hitherto poorly studied protein, as a key regulator of actin and microtubule cytoskeleton dynamics. Naturally occurring variants in actin-related genes will allow us to further dissect the molecular mechanisms by which the highly controlled cytoskeleton meshwork interacts with the inflammatory machinery.

30. **Cristina Mayor-Ruiz**, CeMM

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Plasticity of the cullin-RING ligase repertoire shapes sensitivity to ligand-induced protein degradation

Inducing protein degradation via small-molecules is a transformative therapeutic paradigm. While structural requirements of target degradation are emerging, mechanisms determining the cellular response to small-molecule degraders remain poorly understood. To systematically delineate effectors required for targeted protein degradation, we applied genome-scale CRISPR/Cas9 screens for five drugs that hijack different substrate receptors (SRs) of cullin RING ligases (CRLs) to induce target proteolysis. We found that sensitivity to small-molecule degraders is dictated by shared and drug-specific modulator networks including the COP9 signalosome and the SR exchange factor CAND1. Genetic or pharmacologic perturbation of these effectors impairs CRL plasticity and arrests a wide array of ligases in a constitutively active state. Resulting defects in CRL decommissioning prompt widespread CRL auto-degradation that confers resistance to multiple degraders. Collectively, our study informs on regulation and architecture of CRLs amenable for targeted protein degradation, and outlines biomarkers and putative resistance mechanisms for upcoming clinical investigation.

31. Mahesh Tambe, FIMM

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Paradox-breaker pan-RAF Inhibitors Induce an AML-specific Cytotoxic Response and Synergize with Venetoclax to Display Superior Antileukemic Activity

Paradox breaker pan-RAF inhibitors have proved effective against solid cancer models harboring mutations in NRAS, KRAS and BRAF oncogenes. However, the efficacy of pan-RAF inhibitors in acute myeloid leukemia (AML) remains to be investigated. In AML, the RAS-RAF-MEK-ERK (MAPK) pathway is deregulated due to activating mutations of the NRAS or KRAS oncogenes. However, targeting the MAPK pathway by MEK inhibitors, the best explored class of MAPK inhibitors, have not proven effective in the clinical setting. Here we show that inhibition of pan-RAF, MEK and ERK effectively stopped AML cell proliferation but only pan-RAF inhibition induced apoptosis in a subset of primary AML patient blasts ex vivo and AML cell lines. Importantly, pan- RAF inhibition was not cytotoxic to mononuclear bone marrow cells obtained from healthy individuals. Moreover, pan-RAF inhibition had durable long-term anti-leukemic effect as compared to MEK inhibition in vitro. Interestingly, pan-RAF inhibition while not the MEK inhibition downregulated protein synthesis of prosurvival myeloid cell leukemia-1 (Mcl-1) and induced apoptosis in cells dependent on Mcl-1 for their survival. Furthermore, switching the dependency of cells from Mcl-1 caused resistant to cytotoxic effect of pan-RAF inhibitors. Importantly, cells resistant to pan-RAF and/or Bcl-2 inhibition were sensitized by combined inhibition of pan-RAF and Bcl-2. In summary, our results show that paradox breaker pan-RAF inhibitors are effective as single agents to induce cell death in AML blasts, likely through a combined MAPK pathway and Mcl-1 dependent mechanism. Moreover, combined inhibition of RAF

kinases and Bcl-2 can overcome resistance of AML cells to either drug alone and lead to superior anti-leukemic activity.

32. Montserrat Estruch-Alrich, BRIC

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PI3K inhibition combined with standard chemotherapy effectively inhibits kit mutant acute myeloid leukemia (AML)

INTRODUCTION: AML patients currently demonstrate a dismal overall survival rate (5y OS 25%) in response to standard chemotherapy regimens. The c-KIT receptor (KIT) is recurrently mutated in AML. Mutations in KIT frequently cooccur in AML patients with inv(16) and t(8;21) driver aberrations where they confer inferior prognosis. The KIT mutation KIT(D816Y) in exon 17 confer conformational changes leading to constitutive activation of PI3K and its downstream signalling pathways, which ultimately promotes cell growth, survival and chemotherapy resistance.

AIM: We applied the inv(16)/KIT(D816Y) mouse AML model to investigate whether therapeutic targeting of PI3K in combination with standard chemotherapy would inhibit growth and survival of AML synergistically in a preclinical AML trial program.

RESULTS: Colony forming-cell assays were applied to assess the inhibitory effect of a range of PI3K inhibitors and standard chemotherapeutics on clonogenic growth of inv(16)/KIT(D816Y) AML cells vs wild-type bone marrow (BM) cells. Among all tested drugs the pan-PI3K inhibitor BKM120, Doxorubicin and Ara-C were selected and subjected to drug combination tests, which demonstrated high synergy scores for BKM120+Doxorubicin and for BKM120+Ara-C. We subsequently conducted in vivo pre-clinical AML trials using varying doses of BKM120 alone and in combination with varying doses of a Doxorubicin/Ara-C 3+5 chemotherapy regimen. Treatment with BKM120 per oral gavage demonstrated in vivo inhibition of the PI3K pathway, decreased AML burden in BM, spleen, and peripheral blood, and reduced splenomegaly in a dose dependent manner. Combinatorial treatment with BKM120 and Doxorubin/Ara-C 3+5, increased survival of mice with inv(16)/KIT(D816Y) AML significantly compared to untreated mice and mice subjected to single treatment with either BKM120 or chemotherapy.

PERSPECTIVES: Our findings provide a rationale for development of precision medicine strategies that target cancer-specific vulnerabilities, such as oncogenic KIT/PI3K signaling axis in individual AML

patients, in order to enhance their sensitivity toward conventional chemotherapy, leading to improved clinical outcome.

33. Courtney Hanna, BI

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Endogenous retroviral insertions drive non-canonical imprinting in extra-embryonic tissues

Genomic imprinting is an epigenetic phenomenon that allows a subset of genes to be expressed mono-allelically based on parent-of-origin, and is typically regulated by differential DNA methylation inherited from gametes. Imprinting is pervasive in murine extra-embryonic lineages and, uniquely, the imprinting of several genes has been found to be conferred non-canonically through maternally-inherited repressive histone modification H3K27me3. However, the underlying regulatory mechanisms of non-canonical imprinting in post-implantation development remain unexplored.
We identified imprinted regions in post-implantation epiblast and extra-embryonic ectoderm (ExE) by assaying allelic histone modifications (H3K4me3, H3K36me3, H3K27me3), gene expression and DNA methylation in reciprocal C57BL/6 and CAST hybrid embryos. We distinguished loci with DNA methylation- dependent (canonical) and independent (non-canonical) imprinting by assaying hybrid embryos with ablated maternally-inherited DNA methylation. We find that non-canonical imprints are localized to endogenous retrovirus-K (ERVK) long terminal repeats (LTRs), which act as imprinted promoters specifically in extra-embryonic lineages. Transcribed ERVK LTRs are CpG-rich and located in close proximity to gene promoters, and imprinting status is determined by their epigenetic patterning in the oocyte. Finally, we show that oocyte-derived H3K27me3 associated with non-canonical imprints is not maintained beyond pre-implantation development, and is replaced by secondary imprinted DNA methylation on the maternal allele in post-implantation ExE, while being completely silenced by bi-allelic DNA methylation in epiblast. Our study reveals distinct epigenetic mechanisms regulating non-canonical imprinted gene expression between embryonic and extra-embryonic development, and identifies an integral role for ERVK LTR repetitive element.

34. Hana Imrichova, CeMM

Deciphering dynamic transcriptional and regulatory portraits upon KRAS degradation

Regulation of gene expression is essential for any human cell. Importantly, expression is often dysregulated in most cancer types. Although gene control has been intensively studied, many questions remain. One major challenge is to infer high-throughput causal relations in gene regulatory networks and accurately interpret them. Causality is encoded by temporal transition states within the biological system. State transitions are associated with processes such as the

differentiation of embryonic stem cells, or population dynamics in cancer that often underpin drug resistance or metastasis, highlighting the need to model the dynamic dysregulation in cancer. Our ability to comprehend these processes depends on tools to properly interpret dynamic changes in an unbiased and holistic manner.
Novel targeted therapies are being developed to block the function of key regulatory proteins via pharmacologic inhibition or, increasingly, via induced protein degradation. Therefore, development of bioinformatics methods to identify and prioritize therapeutic targets is a key challenge in the field of "transcriptional therapeutics".
To infer causalities and regulatory cascades that have the strongest effects on the repertoire of genes involved in cancer-related processes, thereby identify targets for therapeutic interference, we are developing a computational approach for automated integrative analysis of high-resolution transcriptome and epigenome time-series data generated upon TF perturbations.
Using this methodology together with the innovative approach of targeted protein degradation, we study the consequences of acute mutant KRAS degradation on chromatin remodeling and transcription in (i) mouse embryonic fibroblasts and (ii) in human pancreatic cancer cells. This allows us to reveal causalities in transcriptional control elicited by oncogenic KRAS and might lead to identification of relevant targets for the treatment of RAS-driven tumors.

35. Ed Horton, BRIC

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Targeting breast cancer metastasis through analysis of cancer cell responses to extracellular matrix

Metastatic disease accounts for approximately 90% of cancer-related deaths, and understanding this process is of significant therapeutic interest. The tumour microenvironment, composed of stromal and cancer cells surrounded by extracellular matrix (ECM), plays a crucial role in the metastatic process. The ECM scaffold that a cancer cell encounters when it leaves the primary tumour is different in composition depending on the organ to which it travels, and will therefore induce different intracellular signalling when compared to the primary tumour site. Here, we aim to understand the role of metastatic organ ECM on cancer cell signalling to elucidate how these cancer cell-ECM interactions could be targeted to reduce cancer cell invasion and metastatic growth. We have a mouse breast cancer model along with fibroblasts from primary (mammary) and metastatic (lung) sites for in vitro investigations. Lung or mammary fibroblasts were cultured to allow the deposition of ECM, fibroblasts were removed and cancer cells were seeded onto the tissue-specific fibroblast cell-derived matrices. Kinase profiling identified several kinases with higher signalling in cancer cells on lung fibroblast-derived ECM, and inhibitors against these kinases also reduced proliferation of cancer cells in lung ECM, as seen in a drug screen using a library of 250 kinase inhibitors. In addition, we performed kinase profiling and drug screening to examine proliferation and invasion, instead using human breast cancer cells seeded onto multiple individual ECM components. We identified kinases that are activated upon attachment to specific ECM molecules and we have identified small molecules that inhibit cancer cell proliferation and/or invasion. Selected kinase inhibitors are now being tested in vivo for efficacy against lung metastases. Our aim

is to use our approach to identify how to target ECM-dependent signalling and metastases for individual cancer patients.

36. Kirsty Hooper, BI

Kirsty Hooper, Katherine Fletcher, Elise Jacquin, Oliver Florey

Molecular mechanisms regulating LC3–associated phagocytosis

LC3-associated phagocytosis (LAP) is a non-canonical autophagy pathway that regulates immune function, inflammation and aging. Following the engulfment of pathogens, or apoptotic cell debris, LAP can be activated to modulate lysosomal degradation of phagosome contents. A defining feature of LAP is the lipidation of ATG8 proteins, such as LC3, to the phagosome membrane. This pathway shares the core ATG8 lipidation machinery (e.g ATG3, 4, 5, 7, 10, 12, 16L1) with canonical autophagy, but is independent of upstream autophagy regulators (e.g. mTOR). While LAP is known to play an important role in a range of immune cell responses, such as pathogen killing, cytokine secretion and antigen presentation, the molecular mechanisms regulating this pathway are poorly understood. The activation of NADPH oxidase and generation of reactive oxygen species (ROS) have been implicated in LAP, although how this is achieved remains unknown. Recently we discovered an essential role for the C-terminal WD40 domain of ATG16L1 in supporting LAP, which allows us to genetically dissect the canonical and non-canonical autophagy pathways and interrogate their specific molecular mechanisms. Here, we present our preliminary results using a newly developed ATG16L1 K490A knock-in mouse model. Further, we highlight an essential role for the V-ATPase proton pump during LAP, and other related non-canonical autophagy processes, and propose a model as to how this links with the known role of NADPH oxidase activity.

37. Emma Minihane, BI

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Targeting melanoma's MCL1 bias unleashes the apoptotic potential of BRAF and ERK1/2 pathway inhibitors

BRAF and MEK1/2 inhibitors are effective in melanoma but resistance inevitably leads to disease progression. Despite increasing the abundance of the pro-apoptotic BIM and BMF proteins, ERK1/2 pathway inhibition is predominantly cytostatic reflecting residual pro-survival BCL2 family activity. Here, we show that uniquely low BCL-XL expression in melanoma biases the pro-survival pool towards MCL1. Consequently, BRAF or MEK1/2 inhibitors are synthetic lethal with the novel MCL1 inhibitor AZD5991, driving tumour cell death and inhibiting tumour growth in vivo. Combination of ERK1/2 pathway inhibitors with BCL2/BCL-XL inhibitors is typically stronger in CRC, correlating with a low MCL1:BCL-XL ratio; indeed the MCL1:BCL-XL ratio is predictive of ERK1/2 pathway inhibitor synergy with MCL1 or BCL-XL/BCL2 inhibitors. Death induced by inhibition of ERK1/2-plus-MCL1 is BAK/BAX-dependent and requires BIM and BMF. Finally, AZD5991 delays acquired BRAFi resistance and enhances the efficacy of an ERK1/2 inhibitor in a model of acquired BRAFi-plus-MEKi resistance. Thus combining ERK1/2 pathway inhibitors with MCL1 antagonists in melanoma could improve therapeutic index and patient outcomes.

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