Oxford-MRC DTP Symposium

21 June 2018

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DOMINVS ILLVMINATIO MEA

Programme

09:00 **Registration + Tea and Coffee**

09:30 **Opening remarks – Professor Marella de Bruijn**, Director of Oxford-MRC DTP

09:40 Student Talks – Session 1 (Chair – Paolo Spingardi)

Margot Overman – FMRIB, Nuffield Department of Clinical Neuroscience
"Modulating reward learning in healthy adults with transcranial direct current stimulation: Applications for the treatment of depression"
Hannah Farley – Mammalian Genomics Unit, MRC Harwell Institute
"PIERCE1 mediates left-right patterning through ciliary motility"
Egon Jacobus – Department of Oncology
"Hypoxia: a double-edged sword for the activity of cancer-killing (oncolytic) adenoviruses"

10:40 **Coffee Break**

11:10 Student Talks – Session 2 (Chair – Deniz Kaya)

Dominic Owens – MRC Molecular Haematology Unit, WIMM
"Runx1 Regulation by Ctcf during developmental haematopoiesis"
Shahana Sengupta – OCDEM, Radcliffe Department of Medicine
"Investigating a candidate causal allele in Type 2 diabetes (T2D) susceptibility gene PAM as a cause of neonatal diabetes mellitus (NDM)"
Isabel Wassing – Sir William Dunn School of Pathology
"RAD51 phosphorylation promotes mitotic DNA synthesis"

12:10 Keynote Lecture
Professor Dame Kay Davies - DPAG
"*Genetic approaches to therapy of muscular dystrophy*"

13:00 **Lunch Break and Poster Presentations**

14:00 **Rachel Bray (Careers Services)**
"Career Planning for DPhil Students"

14:20 MRC DTP Oxford Alumni Presentations

Cornelia Meisenberg – Postdoctoral Research Fellow at The Institute of Cancer Research
Oliver Sampson – Senior Life Sciences Recruitment Consultant at SRG
Phillip Tait – Senior Knowledge Exchange Facilitator at University of Oxford
Ricarda Gaentzsch – Director (Scientific Operations) at Seven Bridges Genomics

15:40 **Coffee Break**

16:10 **Jonathan Silk** – Head of Cell Research at Adaptimmune
"Life in a Biotech company"

16:30 **John Pollard** – Principal Research Fellow (VP), Head Biological Sciences at Vertex Pharmaceuticals
"How understanding causal human disease biology can impact drug discovery"

16:50 **Catarina Vicente** – MRC WIMM, Radcliffe Department of Medicine
"My career in science communication and public engagement (so far)"

17:10 **Prize Ceremony and Drinks Reception**

Student Talk Abstracts

Session 1

Modulating reward learning in healthy adults with transcranial direct current stimulation: Applications for the treatment of depression

Margot Overman, J. O'Shea, M. Browning

FMRIB, Nuffield Department of Clinical Neuroscience

Rationale: Depression is characterised by a pervasive negative attention bias, which may be caused by a belief that negative events have a higher information content than positive events. Here, I investigated whether transcranial direct current stimulation (tDCS) could modulate information content estimates for rewarding and adverse outcomes of healthy adults.

Methodology: Forty healthy volunteers participated in one sham and one active tDCS session. In each session, participants completed an information bias learning task (IBLT) while receiving tDCS targeting bilateral dorsolateral prefrontal cortex (DLPFC) for 20 minutes at 2 mA. In the IBLT, the information content of positive and negative outcomes was manipulated by varying the volatility of stimulus-outcome associations. Information content estimates were inferred from participants' learning rates, which were derived from the data with computational modelling techniques.

Results: Active bifrontal tDCS was associated with an increase in learning rates for positive outcomes in stable but not volatile environments. These behavioural changes were maintained following cessation of the stimulation. Paradoxically, the increase in learning rates was associated with reduced winnings in the task.

Conclusions: The findings indicate that tDCS enhanced people's estimates of the information content for positive but not negative outcomes, particularly when positive events were uninformative. While this behavioural change was associated with reduced performance of the task, augmenting learning from positive events may ameliorate negative attention biases in a broader context. Additional research is needed to investigate whether tDCS targeting reward learning may present a novel intervention strategy for depressive disorders.

PIERCE1 mediates left-right patterning through ciliary motility

Hannah Farley, J. Keynton, J. Thompson, D. Asante, H. Hilton, C. Esapa, P. Lackie, J. Lucas, D. Norris

Mammalian Genomics Unit, MRC Harwell Institute

Motile cilia in the embryonic node drive a leftwards fluid flow that serves to establish normal left-right asymmetry. *Pierce1* mutants exhibit randomised situs and heart defects which are indicative of faulty left-right patterning. Live imaging of node cilia demonstrates reduced ciliary beat frequency and altered beat pattern; particle image velocimetry has confirmed that while motile, these cilia no longer drive leftwards nodal flow. Motile cilia lining the adult trachea function to clear mucus from the lungs. Analysis of *Pierce1*$^{-/-}$ tracheal cilia reveals abnormal beating, making PIERCE1 a candidate primary ciliary dyskinesia (PCD) gene. This is supported by transmission electron microscopy work showing inner and outer dynein arm deficiencies in the tracheal cilia of homozygous animals. Current work focuses on further characterisation of ciliary motility defects in this mouse line, as well as functional characterisation of the PIERCE1 protein in cell culture.

Hypoxia: a double-edged sword for the activity of cancer-killing (oncolytic) adenoviruses

Egon Jacobus, Iris, K. Fisher and L. Seymour

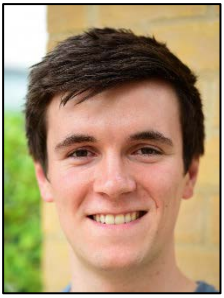
Department of Oncology

Oncolytic viruses can specifically infect and lyse tumour cells as they spread from cell to cell ultimately provoking an anti-cancer immune response. Great progress has been made in the field with the approval of Imlygic®, a treatment for melanoma using Herpes Simplex Virus Type I. However, little is known about how oncolytic viruses behave in regions of hypoxia found in solid carcinomas. Here we set out to define the influence of hypoxia on the activity of oncolytic adenoviruses, ultimately aiming to design a virus or a combination therapy that obviates any oxygen dependence.

We show that hypoxic cells successfully support a primary adenovirus infection cycle. Transcription and translation of early and late viral genes in 1% hypoxia showed increased levels of notably E1A, but also E2B, Fibre and encoded therapeutic transgenes, leading to higher levels of viral genome synthesis and a 5-fold increase in infectious virus production. Nonetheless, in studies involving cell to cell viral spread, hypoxic cells produced up to 12-fold fewer infectious particles. Hypoxic inhibition of virus spread was confirmed by assessing plaque size of a virus with a novel oxygen-independent fluorescent reporter. We hypothesized that hypoxic cells either elicit or respond stronger to antiviral signals. Using re-oxygenation studies we showed that hypoxic and normoxic cells have similar abilities to respond to adenovirus infection and that hypoxic cells appear more susceptible to paracellular antiviral signalling.

We analysed the localisation of infection foci in DLD1 xenografts relative to areas of hypoxia. Immunohistochemical analysis showed that the dissemination of adenoviruses correlated negatively with hypoxia. We conclude that hypoxia boosts the oncolytic activity of adenoviruses, but it can inhibit viral spread.

Session 2



Runx1 Regulation by Ctcf during developmental haematopoiesis

Dominic Owens, V. Frontera, D. Downes, J. Hughes, M. de Bruijn

MRC Molecular Haematology Unit, MRC WIMM, Radcliffe Department of Medicine

Runx1 is a key master transcription factor that is required for the generation of haematopoietic stem cells (HSCs). HSCs are generated during embryonic development by a process of endothelial-to-haematopoietic transition (EHT) that is critically dependent on *Runx1*. Runx1 exhibits complex transcriptional regulation and is dynamically regulated during EHT. The *Runx1* gene spans 224kb on mouse chromosome 16, has two alternative promoters and multiple *cis*-regulatory elements. Ctcf/cohesin is involved in the regulation of tissue-specific gene expression by complex mechanisms that are poorly understood. Many studies into basic mechanisms of transcriptional regulation by Ctcf/cohesin have been performed on smaller, structural genes that are not highly dynamically regulated during development. We have performed NG Capture-C in haematopoietic cell lines and shown that multiple Ctcf sites in the *Runx1* locus interact specifically and dynamically with *Runx1* promoters when the gene is transcribed. We have performed site-specific CRISPR/Cas9-mediated deletions of combinations of Ctcf sites in the *Runx1* locus in mESCs to elucidate their function. We are utilising *in vitro* EHT differentiation protocols to determine the role that specific Ctcf binding sites play in the regulation of *Runx1* during developmental haematopoiesis. Studying the complex transcriptional regulatory mechanisms of *Runx1* during developmental haematopoiesis could shed new light into basic mechanisms of transcriptional regulation by Ctcf/cohesin. Furthermore, a deeper understanding of *Runx1* regulation during developmental haematopoiesis could inform future protocols for the *de novo* generation of HSCs from pluripotent stem cells *in vitro*.

Investigating a candidate causal allele in Type 2 diabetes (T2D) susceptibility gene PAM as a cause of neonatal diabetes mellitus (NDM)

Shahana Sengupta, L. Bonnycastle, M. Umapathysivam, B. Hastoy, A. Raimondo, A. Swift, A. Clark, H. Huopio, F. Collins, M. Laakso, A. L. Gloyn

OCDEM, Radcliffe Department of Medicine

Genome wide association studies (GWAS) have been integral to identifying more than 450 risk loci for type 2 diabetes (T2D) of which the majority influence beta-cell function. Sentinel T2D risk alleles in *PAM*, D563G and S539W, which influence beta-cell function were identified through exome array and sequencing and mediate risk through alterations to PAM protein function [1-4]. We hypothesised that rare fully penetrant *PAM* alleles could cause severe beta-cell dysfunction presenting as monogenic diabetes and performed whole exome sequencing in probands with neonatal diabetes and their unaffected parents.

We identified a novel de novo nonsynonymous coding variant (R36S) in *PAM* in one proband. We set out to investigate the impact of the variant allele on amidating activity, protein stability and cellular localisation as a measure of PAM protein function. The luminal R36S-PAM isoform demonstrated reduced protein expression and secretion in HEK293 cells stably transfected with recombinant PAM, which could be rescued by inhibiting the proteosomal pathway with MG132; whilst the membrane-integral isoform displayed increased expression in the human beta-cell model EndoC-βh1. Despite this, R36S-PAM retains normal catalytic activity both in in vitro assays of recombinant R36S-PAM and in serum from the subject with the R36S-PAM allele and shows no significant differences in subcellular localisation with immunofluorescence in EndoC-βh1 cells. Residue R36 is highly conserved and maps to a region outside the catalytic domains which may play a role in the secretion of soluble proteins. Efforts are now being focused on exploring the impact of R36S-PAM on the dynamics of insulin vesicle trafficking and secretion in human beta-cells.

We have identified a novel coding variant in a gene involved in beta-cell dysfunction in T2D. Understanding how this variant influences PAM protein function will shed light on the non-catalytic function of PAM in insulin secretion.

RAD51 phosphorylation promotes mitotic DNA synthesis

Isabel Wassing, L. Rampazzo, A. Bassett, F. Esashi

Sir William Dunn School of Pathology

DNA replication is a challenging process which, when perturbed, poses a fundamental threat to genomic stability and human health. Emerging evidence demonstrates that certain 'difficult-to-replicate' regions of the genome remain unreplicated into mitosis. Such regions are proposed to undergo a process termed mitotic DNA synthesis (MiDAS) which involves active induction of replication fork breakage, followed by break-induced DNA synthesis. This process is important to ensure complete genome replication prior to chromosome segregation. However, the mechanism mediating MiDAS is not fully understood.

Faithful repair of DNA breaks is achieved via homologous recombination, which is catalyzed by RAD51 recombinase. RAD51 is canonically recruited to DNA breaks via the BRCA1-PALB2-BRCA2 complex, but this pathway is inhibited in mitosis. Our group previously identified an alternative pathway for RAD51 recruitment in which PLK1-dependent phosphorylation of RAD51 mediates its recruitment to broken DNA. Interestingly, this phosphorylation peaks during mitosis. However, the role of RAD51 phosphorylation during mitosis remains unknown.

In this study, we show that RAD51 inhibition in mitosis decreases the mitotic incorporation of thymidine analogue EdU, demonstrating that MiDAS is a RAD51-dependent process. Analyses of mitotic EdU incorporation in CRISPR-generated RAD51 phospho-mutants further show that PLK1-dependent RAD51 recruitment promotes MiDAS. Finally, spindle assembly checkpoint (SAC) inhibition significantly reduces cell survival of the RAD51 phospho-null mutant, demonstrating the importance of the SAC for the completion of replication prior to anaphase. Taken together, these observations demonstrate, for the first time, the role of RAD51 in mitosis and its potential impact on chemotherapeutic treatments targeting the spindle checkpoint.

Keynote Lecture

Genetic approaches to therapy of muscular dystrophy

Professor Dame Kay Davies

Department of Anatomy, Physiology and Genetics at the University of Oxford

Professor Dame Kay Davies is the Dr Lee's Professor of Anatomy and the co-Director MDUK Neuromuscular Disease Centre in the Department of Anatomy, Physiology and Genetics at the University of Oxford. She is also Associate Head of the Medical Sciences Division and a Fellow of Hertford College. She is a founding fellow of the Academy of Medical Sciences and was elected a Fellow of the Royal Society in 2003. Her research interest is in the genetic background of neuromuscular and neurological disorders and her research group focuses on the development of therapy for Duchenne muscular dystrophy. She is also co-founder of Summit Therapeutics and Oxstem.

MRC DTP Oxford Alumni

Cornelia Meisenberg – Postdoctoral Research Fellow at The Institute of Cancer Research

Cornelia did her DPhil from 2008 to 2012 where she focused on determining the role of ubiquitylation in the regulation of the highly important DNA repair factor APE1. Following this she secured 2 consecutive postdoctoral positions at the University of Sussex next to a tutoring job. She is currently a postdoctoral Research Fellow at the Institute of Cancer Research in London studying chromatin remodellers in cancer biology.

Oliver Sampson – Senior Life Sciences Recruitment Consultant at SRG

After working as a Molecular Biologist and Analytical Chemist at RAFT Medical Research and GlaxoSmithKline respectively, Oliver pursued a DPhil in Radiation Biology where he studied the role of AKT in tumour cell survival post irradiation. He finished in 2010 and adopted a management position in an animal feed company. Oliver currently holds a position as Life Sciences Recruitment Consultant at SRG in addition to independently consulting on career development and post graduate career coaching at a number of leading institutions across the UK.

Phillip Tait - Senior Knowledge Exchange Facilitator at University of Oxford

After finishing his DPhil in the Department of Oncology in 2009, Phillip focussed his career on Innovation. He held positions as Innovation Manager at both the BBSRC and at the Science & Technology Facilities Council (STFC), where he worked nationally to foster academic-business collaborations. Phillip is now a Senior Knowledge Exchange Facilitator at the Department of Physics, working to increase economic and societal impact from fundamental physics research and he also holds an STFC Innovation Fellowship.

Ricarda Gaentzsch - Director (Scientific Operations) at Seven Bridges Genomics

In her MRC funded DPhil, Ricarda focused on comparing the DNA methylation pattern in the genomes of human and transgenic mice. After finishing in 2013, she secured an MRC Early Career postdoc before moving to the molecular diagnostics company GeneFirst. She first joined her current company Seven Bridsges Genomics in 2016 as a Scientific Project Manager and has since progressed to become Director of Scientific Operations.

Industry Speakers

Life in a Biotech company

Jonathan Silk

Adaptimmune

Dr Jonathan Silk is the Head of Cell Research at Adaptimmune, a Biotechnology company focussing on T-cell therapy, based in Abingdon. He previously worked at the Weatherall Institute of Molecular Medicine as a post-doctoral scientist with Professor Vincenzo Cerundolo and will be giving a career talk about the transition of scientists from Academia to Industry.

How understanding causal human disease biology can impact drug discovery

John Pollard

Vertex Pharmaceuticals

John is Vice President, Principal Research Fellow and Head of Biological Sciences at Vertex Pharmaceuticals research site in Oxfordshire where he has responsibility for all aspects of biological research including protein sciences, structural biology and in vitro and in vivo pharmacology. John joined Vertex in 1999 following a PhD at Southampton University and postdoctoral positions at St.Andrews and Birmingham Universities in bioorganic chemistry. During his tenure at Vertex, John has led a series of research and clinical development projects across multiple disease areas including cancer, kidney and liver disease; and has served as the global research lead for oncology as well as leading numerous collaborations with academic groups and pharma companies.

Science Communication and Outreach

My career in science communication and public engagement (so far)

Catarina Vincente

MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine

Catarina is responsible for internal and external communications at the MRC Weatherall Institute of Molecular Medicine, particularly their online presence, and coordinate the Institute's Public Engagement activities.

She has a background in genetics and cell biology. She did her undergraduate degree in Genetics at the University of York, with a year-in-industry placement at the National Heart and Lung Institute at Imperial College. She graduated from her DPhil in the lab of Prof Jordan Raff at the Sir William Dunn School of Pathology, University of Oxford, in 2013. Her thesis project focused on centrioles and cell division in the Drosophila embryo.

Following her DPhil she worked for three years at the Company of Biologists, a not-for-profit publishing company in Cambridge, where she was the community manager for the Node, a community website for developmental biologists. She was also the online editor and press contact for the journal Development.

After a short stint as Digital Communications Officer at Lady Margaret Hall College, She joined the MRC WIMM in September 2017.

Posters

1. **Sema3A affects T cell migration and killing in tumours** Mike B Barnkob, Violaine André, Uzi Gileadi, Salvatore Valvo, Margarida Rei, JiLi Chen, Corinna Kulicke, Lena Cords, Huw Colin-York, Yale Michaels, Andreas V Hadjinicolaou, Youxin Kong, Vitul Jain, Viveka Mayya, Philip Macklin, Lars R Olsen, Tudor A Fulga, Marco Fritzsche, E Yvonne Jones, Michael L Dustin, Vincenzo Cerundolo

2. **Hypermutagenic Phenotype and DNA Replication Defects in Cancer Polε Variants.** Sibyl Bertrand, Ignacio Soriano-Moruno, Catherine Green, Stephen Kearsey

3. **Behavioural interventions to reduce the consumption of meat: evidence from two systematic reviews of intervention studies.** Filippo Bianchi, Emma Garnett, Claudia Dorsel, Paul Aveyard, Susan A Jebb

4. **Prevalent Methodology and Measurement Gains of Computer Adaptive Test Use in Patient Reported Outcomes – A Scoping Review.** Diasmer Panna Bloe

5. **Characterising changes in brain activity during retinopathy of prematurity screening and treatment.** M Buckle, C Hartley, A Hoskin, G Schmidt Mellado, CK Patel and R Slater

6. **The impact of glycation on dendritic cell responses to proteins.** S. L. Harris, A. E. Moghaddam and Q. J. Sattentau

7. **From Mobile Mes to connected Community – Investigating Social Networks in Transition.** Mary Kempnich

8. **The IFITM3 SNP rs12252 Differentially Affects Gene Expression and Viral Susceptibility in Influenza Patients.** Henry Laurenson-Schafer, Lin Qing,Yan Zhao, Shokouh Makvandi-Nejad, Dannielle Wellington, Philip Hublitz , Jan Rehwinkel, Nin Li, Yonghong Zhang, Tao Dong

9. **Investigating the role of FGF signalling in the mouse left-right organizer.** Rosie Little, Jennifer Keynton, Rebecca Walker, Dominic Norris

10. **Adiposity and Ischaemic Heart Disease in the UK Biobank: A prospective cohort study of 500 000 adults.** Debbie E Malden

11. **Maximising transduction in ATII cells: a comparison of different rAAV serotypes.** Helena Meyer-Berg, Steve Hyde and Deborah Gill

12. **Learning the payoffs and costs of actions.** Moritz Moeller and Rafal Bogacz

13. **Normative values for hippocampal volume in 12,245 healthy participants of the UK Biobank.** Lisa Nobis, Sanjay Manohar, Stephen M. Smith, Fidel Alfaro-Almagro Mark Jenkinson, Clare E Mackay, Masud Husain

14. **Phosphatase exclusion-based triggering and stoichiometry of the B cell receptor.** Caitlin O'Brien-Ball, Martin Wilcock, Anna Lippert, James McColl, Aleks Ponjavic, Mafalda Santos, Richard Cornall, David Klenerman and Simon Davis

15. **Runx1 Regulation by Ctcf during developmental haematopoiesis.** Dominic Owens, Vincent Frontera, Damien Downes, Jim Hughes, Marella de Bruijn

16. **Tracking RNA: Xist under the Magnifying Glass.** Lisa Rodermund, Heather Coker, Roel Oldenkamp, Tatyana Nesterova, Lothar Schermelleh, Neil Brockdorff

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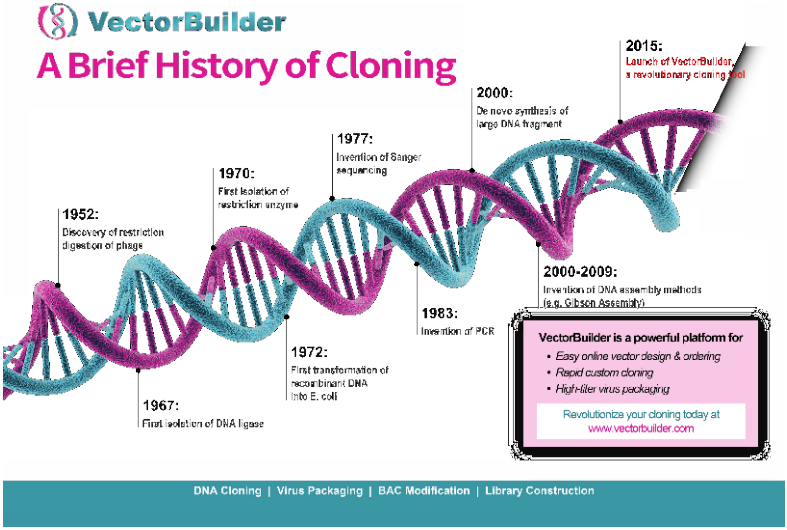

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