Department of Pathology
Annual Research Symposium
Li Ka Shing Centre (Cambridge Research Institute)
Monday 10 April 2017

08:30 - 09:00	Registration	
09:00 - 09:10	Nabeel A Affara	Welcome
09:10 - 09:20	Heike Laman	Gender Champions: Highlighting Achievements
Chair: Carole Sargent		
09:20 - 09:40	Heike Laman	Loss of FBXO7 results in a Parkinson's-like dopaminergic degeneration via an Rpl23-Mdm2-p53 pathway
09:40 - 10:00	Maja Wållberg	Glucagon Like Peptide-1 Receptor - a possible role for beta cell physiology in susceptibility to autoimmune diabetes
10:00 - 10:20	Paul Bergen	Ordered export of proteins to build a bacterial cell surface nanomachine
10:20 - 10:40	Laura Caller	Much Ado About Nothing: Using TMT proteomics to study host cell changes during human BK Polyomavirus infection
Tea & Coffee (10:40 - 11:10)		
Chair: Ben Skinner		
11:10 - 11:30	Max Blanck	Non-apoptotic, non-necroptotic cell death downstream of TNFR1?
11:30 - 11:50	Klaus Okkenhaug	Activated PI3Kδ Syndrome (APDS): a new primary immunodeficiency
11:50 - 12:10	Dora Pereira	Bench to Clinical Trials: the journey of a novel iron supplement for the treatment of anaemia in Sub-Saharan Africa
12:10 - 12:30	Rachel Ulferts	How cells patrol the health of their acid vesicles
12:30 - 12:50	2nd Year PhD Students	One-Minute Poster Introductions
Lunch (12:50 - 14:10)		
Chair: Ian Brierley		
14:10 - 14:20	A Brief presentation from Green Impact Team	
14:20 - 14:40	Sarah Moody	MALT lymphoma: a paradigm where antigenic stimulation meets genetic changes
14:40 - 15:00	Brian Ferguson	Inflammation and cell death during virus infection
15:00 - 15:20	Stephen Bentley	Evasion of vaccines and antibiotics in the pneumococcus
15:20 - 15:40	Aartjan te Velthuis	Subgenomic RNAs link influenza A virus virulence to RNA Polymerase activity
Tea & Coffee (15:40 - 16:10)		
Chair: Gillian Fraser		
16:10 - 16:30	Nick Coleman	The Oncostatin M Receptor as a Therapeutic Target in Squamous Cell Carcinomas
16:30 - 16:50	David Carpentier	Recruitment and modulation of the cellular kinesin-1 motor complex by vaccinia virus during egress from the host cell
16:50 - 17:10	Allister Crow	Antibiotic resistance and toxin secretion mediated by a novel ABC transporter
17:10 - 17:30	Naomi McGovern	Tissue resident macrophages – from human adult to embryo
Poster Prize & Prize for Best Research Talk (1730 - 1740)		
Drinks Reception (1740 – 2000)		

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Speaker	Heike Laman
Title	*Loss of FBXO7 results in a Parkinson's-like dopaminergic degeneration via an Rpl23-Mdm2-p53 pathway*
Contributors to the Research	Simon Stott, Suzanne J Randle, Paulina Rowicka, Jing Xia, Jeffrey W Dalley, Roger Barker & Heike Laman
Abstract	Parkinson's disease (PD) is the second most common neurodegenerative disease, and it has been estimated that approximately 1 in 500 people (~127,000) in the UK are currently afflicted. The majority of PD cases are idiopathic with a median age of onset of 68, although some predisposing environmental and genetic factors have been identified. PD is characterized by the loss of dopamine (DA)-producing neurons in the substantia nigra pars compacta, although pathology is not restricted to this site. Diagnosis depends on the presence of specific clinical symptoms and signs, including a resting tremor, rigidity, bradykinesia, and postural instability and is often accompanied by an array of non-motor features. Post-mortem, the disease is characterized histopathologically by the presence of intra-cytoplasmic protein aggregates known as Lewy bodies (LBs), which contain α-synuclein. However, PD is not exclusively a disease of old age. Approximately 1 in 20 of all UK sufferers is under the age of 40, and in this cohort there are many genes that have been linked to the development of their disease. One example of a rare mutation that is associated with a juvenile Parkinsonian-pyramidal disease (PPD) is PARK15, mostly known as FBXO7 (F-box only 7), which encodes an F-box protein (FBP), a substrate docking subunit within E3 ubiquitin ligases. Pathogenic mutations in FBXO7 were first found by high resolution SNP arrays of a consanguineous Iranian family with early-onset PD and later in Dutch, Italian, UK, Turkish, and Kurdish families. Mutations are inherited recessively, suggesting they produce a hypomorphic protein or cause a loss of function. My laboratory has extensively characterized the tissue-specific functions of Fbxo7, which is a remarkably multi-functional E3 ubiquitin ligase using mouse models. Because there continues to be a huge unmet need in the PD field for genetic models that recapitulate key clinical and neuropathological aspects of PD, we investigated whether mice lacking Fbxo7 would constitute a better model of progressive DA cell loss. We engineered a tissue-specific KO of *Fbxo7,* and I will present our findings on the analysis of this mouse.

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Speaker	Maja Wållberg
Title	*Glucagon Like Peptide-1 Receptor - a possible role for beta cell physiology in susceptibility to autoimmune diabetes*
Contributors to the Research	Asha Recino, Anja Schmidt-Christensen, Kerry Barkan, Nick Holmes, David Spring, Jacob Hecksher-Sorensen, Malin Flodström-Tullberg, Dan Holmberg, Anne Cooke, Graham Ladds and Maja Wållberg
Abstract	Type 1 diabetes is a disease that destroys the insulin producing cells of the pancreas. Once these cells are gone or incapacitated, many cells in the body can no longer take up glucose. Type 1 diabetes patients rely on insulin injections to survive, and even with access to this, it is difficult to achieve good glucose control. There are many health problems associated with type 1 diabetes. Around 400,000 people in the UK live with type 1 diabetes, and the number is increasing. Despite almost a century of intense research since the discovery of insulin, it is not known what causes the disease or how it can be prevented. There is no cure. There is strong evidence indicating that type 1 diabetes is an autoimmune disease, caused by immune mediated destruction of the beta cells. But it remains unknown what factors might trigger such an autoimmune response. We have found that type 1 diabetes prone, non obese diabetic (NOD) mice, are severely deficient in expression of the G-protein coupled receptor Glucagon Like Peptide (GLP)-1R. This receptor is important for insulin release, and also for maintenance, resistance to stress, and regeneration of beta cell mass. We believe that this novel finding may explain why NOD mouse beta cells are attacked in the first place, and why they cannot resist the attack and/or regenerate. We propose that defining the mechanisms responsible for inadequate expression of the receptor will lead to identifying novel drug targets for the treatment of both type 1 and type 2 diabetes.

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Speaker	Paul M Bergen
Title	*Ordered export of proteins to build a bacterial cell surface nanomachine*
Contributors to the Research	Owain J Bryant, Katie R Kemplen, Colin Hughes, Gillian M Fraser
Abstract	To enable swimming and population swarming motility, bacteria build long, complex rotary 'nanomachines', called flagella, on their cell surface. Their sequential assembly requires export of thousands of structural subunits across the cell membrane and this is achieved by dedicated type III export machinery located at each flagellum base. Subunits then transit up to 20 cell lengths through a narrow channel in the external flagellum to reach the assembly site at the tip of the nascent structure. Our lab has recently uncovered how subunit transit is achieved without a conventional energy source in the external channel, showing that the energy for transit is intrinsic to the subunits themselves as they link in a chain that is pulled to the flagellum tip1. Flagellum assembly is biphasic, with subunits for the long cell-surface filament only being exported once the cell-proximal rod and hook structures are complete. The order of subunit export is imposed by the autocleaved FlhB2 membrane-bound export gate and by the intermittently exported molecular ruler, FliK3, which monitors the length of the growing rod/hook and transmits this information back into the cell to the cytoplasmic domain of FlhB. This export machinery 'specificity switch' is a critical, yet unexplained, event in the sequential assembly of flagella. Here, we will describe molecular, biochemical and biophysical data that indicate how FliK triggers the export specificity switch by promoting a radical conformational change in the FlhB export gate. References: 1. L.D. Evans, S. Poulter, E.M. Terentjev, C. Hughes & G.M. Fraser, Nature, 2013, 504, 287 2. G.M. Fraser, T. Hirano, H.U. Ferris, L. Devgan, M. Kihara & R.M. Macnab, Mol Microbiol, 2003, 48, 1043 3. M. Erhardt, H.M. Singer, D.H. Wee, J.P. Keener & K.T. Hughes. EMBO J, 2011, 30, 2948

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Speaker	Laura Caller
Title	*Much Ado About Nothing: Using TMT proteomics to study host cell changes during human BK Polyomavirus infection*
Contributors to the Research	Mike Weekes Lab (CIMR), Mike Hollinshead (Dept. of Pathology)
Abstract	The potentially oncogenic human pathogen BK Polyomavirus (BKPyV) persistently infects up to 90% of adults. While infections are generally asymptomatic in the majority of people, in immunocompromised individuals BKPyV can cause severe and debilitating pathologies predominantly affecting the renourinary tract. For example, BKPyV associated nephropathy is a significant problem in renal transplant recipients and can lead to a high chance of graft rejection, and in haematopoietic stem cell transplant patients lytic BKPyV replication causes haemorrhagic cystitis. To investigate modulation of host cells by BKPyV infection, as well as potential host responses to infection, we have analysed global changes to the cellular proteome of two human primary cell lines of the renourinary tract that are caused by BKPyV infection. We have employed Tandem Mass Tagging (TMT)-mass spectrometry approaches, which enables comparison of up to 10 independent samples in parallel, to analyse the whole cell proteome over the complete replication cycle of BKPyV. Using these techniques, we have identified more than 7000 cellular proteins and demonstrated a range of virus-induced changes to cellular pathways. For example, BKPyV infection induces a specific set of cell cycle regulators to provide an optimal environment for viral genome replication and vision assembly, but it is what is lacking that has generated the most interest. These studies are expanding our understanding of virus-host interactions during polyomavirus replication.

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Speaker	Max Blanck
Title	*Non-apoptotic, non-necroptotic cell death downstream of TNFR1?*
Contributors to the Research	Senior Author: Christine Watson. Researched carried out by: Max Blanck
Abstract	Necroptosis has emerged as an important cell death mechanism in diverse pathological conditions. In vitro necroptosis may be artificially induced downstream of TNF-Receptor 1 (TNFR1) under conditions in which translation and caspases are inhibited. However, we show that in some cell lines TNF can induce a non-apoptotic, non-necroptotic from of cell death. In the presence of cycloheximide EpH4, MDA-MB-468 and HT-29 cells all have the capacity to undergo TNF induced apoptotic cell death or non-apoptotic cell death when caspases are inhibited with Z-VAD-FMK. Since non-apoptotic cell death, but not apoptosis, is blocked by the RIP1 kinase inhibitor necrostatin-1, we hypothesised that non-apoptotic cell death in MDA-MB-468 and EpH4 cells was necroptosis. Surprisingly, a different broad band caspase inhibitor, prevents non-apoptotic cell death in MDA-MB-468 and EpH4 cells but not in HT-29 cells. This suggests that necroptosis is not the prevailing non-apoptotic cell death mechanism in MDA-MB-468 and EpH4 cells. In a series of pharmacological and genetic ablation experiments we demonstrate that TNF induced non-apoptotic cell death in MDA-MB-468 and EpH4 cells follows a new, previously unknown, pathway. This new non-apoptotic pathway and necroptosis shared the features of RIP1 kinase dependence and negative regulation by caspase 8. Genetic ablation of caspase 8 using CRISPR/Cas9 technology results in induction of non-apoptotic cell death without caspase inhibition by ZVAD and proves that cell death is indeed non-apoptotic. It is demonstrated that small molecule inhibition or genetic deletion of RIP3 or MLKL inhibit necroptosis but not non-apoptotic cell death in MDA-MB-468 cells. In contrast to necroptosis, non-apoptotic cell death in MDA-MB-468 cells is dependent on FADD and on NEMO. In conclusion, our work demonstrates that MDA-MB-468 and EpH4 cells undergo a new form of non-apoptotic cell death in response to TNF treatment. This newly identified cell death pathway is negatively regulated by caspase 8 and is dependent on FADD and NEMO as well as on RIP1 kinase activity.

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Speaker	Klaus Okkenhaug
Title	*PI3Kδ hyper-activation results in increased susceptibility to S. pneumoniae airway infection*
Contributors to the Research	Anita Chandra[1,2], Anne-Katrien Stark[1], Rafeah Alam[1], Valentina Carbonaro[1], Krishnendu Chakraborty[1] and Klaus Okkenhaug[1] [1] Babraham Institute, Cambridge, CB22 3AT, UK [2] Department of Medicine, University of Cambridge, UK
Abstract	PI3Kδ signalling is critically required for immune cell development and function. Activated PI3K delta Syndrome (APDS) is a recently described cause of primary immunodeficiency, resulting from gain of function mutations affecting the p110δ catalytic- or p85α regulatory subunits. APDS leads to a combined immunodeficiency resulting in the clinical features of recurrent sino-pulmonary bacterial infections, herpesvirus infections, bronchiectasis, autoimmunity and lymphoproliferation. Respiratory tract infections secondary to Streptococcus pneumoniae are a frequent finding. We have created a conditional mouse model of APDS (p110δE1020K) to use a tool to further understand the pathophysiology of APDS. Consistent with APDS patients, p110δE1020Kmice show increased susceptibility to airway infection with S. pneumoniae. The time course of infection leading to pathology is extremely short, thereby lessening the likelihood of involvement of an antigen-specific immune response. Surprisingly, however, this susceptibility disappears if p110δE1020K expression was restricted to T cells or myeloid cells but is present in mice in which p110δE1020K expression is restricted to B cells. Therefore hyperactive p110δ B cells recapitulate the susceptibility to S. pneumoniae. Further experiments indicate that this susceptibility occurs via an antibody-independent mechanism. Following a detailed characterisation of splenic and lung B cells from S. pneumoniae infected mice we have identified a novel expanded population of B220-CD19+IL10+ B cells from the PI3KδE1020K mice. We postulate that this B cell subset has immunoregulatory properties and contributes to the susceptibility seen to S. pneumoniae in mice with hyperactive PI3Kδ signalling.

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Speaker	Dora Pereira
Title	*Bench to Clinical Trials: the journey of a novel iron supplement for the treatment of anaemia in Sub-Saharan Africa*
Contributors to the Research	Author: Dora Pereira Contributors: Professor Andrew A. Prentice, MRC Unit The Gambia Dr Jonathan Powell and Dr Nuno Faria, MRC Elsie Widdowson Laboratory, Cambridge Dr Mohamad Farshard Aslam and Dr Yemisi Latunde-Dada, King's College London Professor Greg Anderson and Dr David Frazer, QIMR, Brisbane, Australia
Abstract	Iron deficiency anaemia remains the largest nutritional deficiency disorder in the world, affecting nearly 1.2 billion people, the majority of whom are children and women from resource-poor countries. The World Health Organization recognises that "worldwide, at any given moment, more individuals have iron-deficiency anaemia than any other health problem" and that reducing the global burden of this condition remains one of the top global nutritional targets. Nevertheless, despite considerable worldwide efforts and investment, in the past 25 years we have been unable to reduce the global burden of this disease. Oral iron supplementation remains the treatment of choice for anaemia in developing countries because it is relatively easy and inexpensive. The current paradigm for oral iron delivery is that only soluble ionic iron can be efficiently absorbed through DMT1 present in the apical membrane of duodenal enterocytes. This paradigm has supported the development, and use of interventions that aim to deliver a large bolus of ionic iron to the enterocytes. This non-physiological approach leads to the presence of 'gut-reactive' soluble iron that circumvents the natural chaperone and absorption/exclusion mechanisms of the gut and ultimately causes frequent gastrointestinal side-effects leading to poor compliance and, in children, increased infection including bloody diarrhoea, and detrimental changes to the gut microbiome. Over the past 12 years, I have been part of the team developing an innovative nanoparticulate iron supplement (namely IHAT) which mimics natural food iron and we think will result in a safer form of iron supplementation, particularly in population groups at high risk for enteric infection. This technology won the first prize at the RSC Emerging Technologies Competition in 2014 and is now starting Phase II testing. I will present the many challenges in translating IHAT from bench to field clinical trials and how the possibility of being able to address a global challenge kept me going.

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Speaker	Rachel Ulferts
Title	*How cells patrol the health of their acid vesicles*
Contributors to the Research	Department of Pathology, University of Cambridge: Rachel Ulferts, Liam Lee, Talita Veith, Suzanne Turner and Rupert Beale The Babraham Institute: Katherine Fletcher and Oliver Florey
Abstract	Influenza A virus (IAV) infection causes accumulation of the autophagy protein LC3 at intracellular membranes. The proton channel activity of the viral M2 protein is necessary for this process. It has been proposed that M2 prevents fusion of autophagosomes to lysosomes during influenza infection. However, here we provide evidence that IAV induced LC3 positive intracellular vesicles do not represent double-membrane autophagosomes, but are instead the target of a non-canonical autophagy process that results in LC3 lipidation of endosomal single-membrane vesicles. IAV induced LC3-lipidation depends on ATG16L1, the component of the lipid transfer complex responsible for membrane targeting. The mechanism of recruitment of the complex to membranes differs from that of canonical autophagy. The ULK-1 complex and phosphoinositol-3-phosphate are essential for canonical macroautophagy, but dispensable for this process. Furthermore, the FIP200 binding domain of ATG16L1 is not required. Instead the C-terminal WD40 domain of ATG16L1, though dispensable for canonical autophagy, is critical for M2 proton channel induced LC3 lipidation. Electron micrographs of LC3 targeted vesicles superficially resemble autophagosomes, but we show they are single-membrane vesicles derived by endocytosis. The molecular mechanism closely resembles that observed for LC3 lipidation in response to compounds known to affect pH or ion distribution in intracellular vesicles. Many pathogens encode ion channels, and some of these have been shown to affect LC3 lipidation. We therefore propose that this phenomenon represents a novel cellular pathway detecting a ‘danger’ signal of abnormal pH or ion concentrations of intracellular vesicles.

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Speaker	Sarah Moody
Title	*MALT lymphoma: a paradigm where antigenic stimulation meets genetic changes*
Contributors to the Research	Leire Escudero-Ibarz, Ming Wang, Alexandra Clipson, Eguzkine Ochoa Ruiz, Deborah Dunn-Walters, Xuemin Xue, Naiyan Zeng, Alistair Robson, Shih-Sung Chuang, Sergio Cogliatti, Hongxiang Liu, John R Goodlad, Margaret Ashton-Key, Markus Raderer, Yingwen Bi and Ming-Qing Du
Abstract	Mucosa Associated Lymphoid Tissue (MALT) lymphomas occur in the context of chronic microbial infections or autoimmune disorders, with antigen stimulation thought to play a key role in the lymphoma development. In support of this, biased usage of certain IGHV gene segments has been previously reported in MALT lymphomas. Recurrent genetic abnormalities occur in the form of t(11;18)/*API2-MALT1*, t(1;14)/*BCL10-IGH*, t(14;18)/*IGH-MALT1* and inactivation of *TNFAIP3* via deletion or somatic mutation, which all promote NF-κB activation. However, neither antigenic stimulation nor the known genetic features are sufficient alone to drive malignant transformation. It is likely that both co-operate in lymphoma development, but which features co-occur and what functional impact this has is currently unknown. In addition, MALT lymphoma occurs at a diverse range of anatomic sites, with the majority of cases at some anatomic sites remaining devoid of known genetic abnormalities. Our initial studies have focused on identifying novel genetic abnormalities which may co-operate in lymphoma development. A panel of genes implicated in the development of other lymphomas which share the characteristic of constitutive NF-κB activation, primarily the activated B-cell subtype of diffuse large B-cell lymphoma (ABC-DLBCL) was screened using massive parallel Fluidigm Access Array PCR and Illumina MiSeq sequencing. To date we have screened 179 MALT lymphomas of various anat omic sites for somatic mutation in molecular pathways including BCR signalling (*CD79A, CD79B*, *CARD11*), NF-κB (*TNFRSF11A*, *TNFAIP3*, *TRAF3*), TLR (*MYD88*), plasma cell differentiation (*PRDM1*), histone modifiers (*CREBBP*, *EP300*, *EZH2*, *MLL2, MEF2B*, *KDM2B*), antigen presentation and recognition (*B2M*, *CD58*), and apoptosis (*TP53)*. With the exception of TNFAIP3 (19%), the remaining genes were infrequently mutated, indicating that MALT lymphomas have a distinct mutation profile compared to other B-cell lymphomas with constitutive NF-κB activation. In addition we have sequenced the rearranged immunoglobulin heavy chain sequences of MALT lymphomas to discover potential associations with genetic abnormalities. We identified biased usage of several IGHV genes including *IGHV4-34*, *IGHV1-69*, *IGHV3-7* and *IGHV7-4-1*. Biased usage of *IGHV1-69* was found specifically in MALT lymphomas of the salivary gland, but no association with genetic abnormalities was identified. Importantly, autoreactive *IGHV4-34* rearrangements were significantly associated with *TNFAIP3* inactivation, suggesting their oncogenic co-operation during lymphoma development. Currently, we are investigating the functional impact of *IGHV4-34* usage with *TNFAIP3* inactivation, in addition to identifying novel genetic abnormalities of MALT lymphoma using whole exome sequencing.

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Speaker	Brian Ferguson
Title	*Inflammation and cell death during virus infection*
Contributors to the Research	Julia Zinngrebe, Eva Rieser, Jenny Cao, Helena Teague, Kathi Lauer, Lucia Taraborrelli, Nieves Peltzer, Torsten Hartwig, Hongwei Ren, ldikó Kovács, Cornelia Endres, Peter Draber, Maurice Darding, Silvia von Karstedt, Johannes Lemke, Balazs Dome, Michael Bergmann, Henning Walczak, Brian Ferguson
Abstract	During virus infection, cells respond by activating both anti-viral gene transcription and programmed cell death pathways. Both of these features of innate immunity are essential for fighting infection but must be balanced appropriately to drive virus clearance whilst limiting pathology. Pattern recognition receptors (PRRs) contribute to these responses by directly sensing infection and triggering not only pro-inflammatory and interferon gene transcription but also cell death signalling. Understanding how PRR ligation can result in multiple distinct outputs from a single input will help to define the relative contributions of inflammation and cell death to host defence against viruses. In this context the linear ubiquitin chain assembly complex (LUBAC) co-ordinates these events by functioning downstream of PRRs to drive pro-inflammatory transcription over the activation of apoptosis or necroptosis. The importance of this is highlighted by the fact that patients with perturbed linear ubiquitination, caused by mutations in the Hoip or Hoil-1 genes, simultaneously suffer from immunodeficiency and autoinflammation. This talk will discuss the consequences of LUBAC mutations for host defence against virus infection with a specific focus on its impact on viral nucleic acid sensing.

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Speaker	Stephen Bentley
Title	*Evasion of vaccines and antibiotics in the pneumococcus*
Contributors to the Research	
Abstract	Somewhat paradoxically, Streptococcus pneumoniae (the pneumococcus) is one of the leading causes of death due to bacterial infection worldwide yet it is commonly found as a harmless resident of the human nasopharynx. The pneumococcus is the leading vaccine preventable cause of pneumonia, septicaemia and meningitis and is estimated to be responsible for about 15 million cases of disease and over 800,000 deaths in young children in the year 2000. Pneumococcal disease is both treatable, through administration of antibiotics, and preventable, by vaccination, but both of these strategies are compromised by the extraordinary adaptability of this genetically diverse pathogen. The frequency of detection of multidrug resistant pneumococci has steadily increased since the 1970's, but it is notable that resistant isolates tend to be members of a small number of genotypic lineages, some of which have been seen to spread around the globe. The recent deployment of conjugate pneumococcal vaccines (PCV), targeting the bacterial capsular polysaccharides of the lineages most associated with disease and antibiotic resistance, has led to reductions in rates of disease. However, the enormous existing pneumococcal strain diversity has allowed vaccine evasion through serotype replacement and capsular switching. This bacterium has been studied in detail for decades – it is the organism which Avery and colleagues used in the 1940's to confirm DNA as the "transforming principle" – and several genetic loci have been studied as candidate virulence determinants. In more recent times we have used population genomics to reveal details of the evolutionary processes required for evasion of vaccines and antibiotics and shown how current clinical interventions are, at least partly, driving the evolution of the pathogen.

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Speaker	Aartjan te Velthuis
Title	*Subgenomic RNAs link influenza A virus virulence to RNA polymerase activity*
Contributors to the Research	Josh Long, David Bauer, Marian Killip, Rick Randall, and Ervin Fodor
Abstract	Influenza A virus infections usually cause mild to moderately severe respiratory disease in humans and induce innate immune responses through the generation of RNAs that activate the RNA sensor Retinoic acid-inducible gene-I (RIG-I). However, infections with the 1918 pandemic virus or highly pathogenic avian influenza viruses (HPAIV) such as the H5N1 subtypes, can lead to viral pneumonia, systemic disease and death. A strong innate immune dysregulation, resulting in enhanced cytokine and chemokine production, known as 'cytokine storm', underlies this pathology. Interestingly, the influenza virus RNA polymerase can produce short subgenomic RNAs that potently activate RIG-I and induce the expression of the cytokine interferon (IFN). These subgenomic viral RNAs, termed mini viral RNAs (mvRNA), are derived by long internal deletions in genomic RNA segments and are shorter than 125 nucleotides in length. The polymerases of the 1918 pandemic and H5N1 HPAIV are particularly efficient at generating mvRNAs and inducing IFN expression, whereas polymerases with mutations that increase nucleotide incorporation fidelity or with human-adaptive mutations in the PB2 subunit produce fewer mvRNAs. Analysis by deep-sequencing shows that the majority of the mvRNAs are produced in a process that involves misincorporation and nascent strand re-alignment on the template RNA. Together, these results provide an advance in our understanding of the molecular basis of influenza virus virulence by i) defining specific viral RNAs produced during influenza virus genome replication as the prime agonists for RIG-I and the subsequent generation of pro-inflammatory cytokines, and ii) demonstrating that polymerases of avian influenza virus origin over-produce these RNAs in mammalian cells.

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Speaker	Nick Coleman
Title	*The Oncostatin M Receptor as a Therapeutic Target in Squamous Cell Carcinomas*
Contributors to the Research	Justyna Kucia, Valtteri Tulkki, Stephen Smith, Cinzia Scarpini, Marta Paez-Ribes, Maja Wallberg, Kate Hughes, Maria M Caffarel
Abstract	The oncostatin M receptor (OSMR) shows frequent gene copy-number gain and over-expression in cervical squamous cell carcinomas (SCCs), associated with adverse clinical outcomes. In SCC cells that overexpress OSMR, the major ligand OSM induces multiple pro-malignant effects, including invasion, secretion of angiogenic factors and metastasis. OSMR over-expression in SCC cells produces increased responsiveness to OSM and activates cell autonomous feed-forward signaling, via further expression of OSMR and OSM, with sustained STAT3 activation despite expression of the negative regulator SOCS3. The pro-malignant effects associated with OSMR overexpression are critically mediated by JAK/STAT3 activation, which is induced by exogenous OSM and also by autocrine OSM:OSMR interactions. Specific inhibition of OSM:OSMR interactions by neutralizing antibodies significantly inhibited STAT3 activation and feed-forward signaling, leading to reduced invasion, angiogenesis and metastasis. In 1,351 clinical SCC samples, OSMR levels correlated with multiple cognate genes, including OSM, STAT3 and downstream targets. OSMR is therefore a key driver of SCCs, in which it is an important novel therapeutic target.

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Speaker	David Carpentier
Title	*Recruitment and modulation of the cellular kinesin-1 motor complex by vaccinia virus during egress from the host cell*
Contributors to the Research	David C. J. Carpentier, William N.D. Gao, Helen A. Ewles, Stacey-Ann Lee, Edline W.X. Yee, Alexander Van Loggerenberg, Nele Dieckmann, Geoffrey L Smith
Abstract	Vaccinia virus is the prototype member of the Poxviridae and the agent used as a vaccine in the eradication of Smallpox. Vaccinia virus replication occurs in cytoplasmic structures known as viral factories. Here a series of assembly and maturation events leads to the formation infectious virions known as intracellular mature virus (IMV). These are released from the host cell upon cell lysis. A subpopulation of these IMVs migrate away from the viral factories and become wrapped by an additional double membrane of early endosomal or trans-Golgi origin. These fully formed intracellular enveloped virions (IEVs) migrate to the cell periphery where they are released onto the cell surface by fusion of their outer envelope. These released virions either remain attached to the host cell (cell associated enveloped virus or CEV) or are released into the extracellular matrix (extracellular enveloped virus or EEV). CEVs and EEVs play an important role in the spread of infection within a host as any mutations that affect IEV formation or IEV egress results in severe attenuation of the virus. IEV egress is mediated by kinesin-1, a microtubule associated motor protein complex consisting of two kinesin heavy chains (KHC) and two kinesin light chains (KLC) and involved in the intracellular trafficking and localisation of membrane bound organelles and ribonucleoprotein complexes. Both the cellular and viral mechanisms of kinesin-1 recruitment and activity modulation remain poorly characterised. Three viral proteins, A36, F12 and E2, have been shown to associate with the kinesin-1 complex and be important for efficient IEV egress. The interaction of the integral membrane protein A36 and of a complex made up of the cytoplasmic proteins F12 and E2 has been mapped to different regions of KLC. Interestingly the two interactions show strong preferences for different KLC isoforms. While A36 is the known protein directly linking IEVs to kinesin-1 disrupting its ability to interact with KLC does not completely abrogate IEV egress, although its efficiency and localisation in polarised cells is severely altered. On the other hand A36 mediated IEV egress is completely reliant on the activity of the F12/E2 complex. A36 binding of KLC in the absence of F12, while still possible, is reduced, suggesting the three proteins may cooperatively associate with KLC. Future work will involve identifying which KLC iso/spliceforms are involved in IEV egress. This work will contribute to our understanding of the diverse roles of different kinesin-1 isoforms and how viruses hijack them.

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Speaker	Allister Crow
Title	*Antibiotic resistance and toxin secretion mediated by a novel ABC transporter*
Contributors to the Research	Allister Crow, Nicholas Greene, Elise Kaplan, Vassilis Koronakis
Abstract	ABC transporters are integral membrane proteins that coordinate binding and hydrolysis of ATP with the passage of substrates from one side of a membrane to other. Here we present the structure of a bacterial ABC transporter with a completely novel protein fold. Functional characterisation of this ABC transporter reveals roles in antibiotic resistance and protein secretion, and a combination of site-directed mutagenesis and in vivo functional studies have been used to probe its mechanism.

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Research Symposium
Li Ka Shing Centre
(Cambridge Research Institute - Addenbrooke's Biomedical Campus)
Monday 10 April 2017

Speaker	Naomi McGovern
Title	*Tissue resident macrophages – from human adult to embryo*
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Abstract	Macrophages are found in all lymphoid as well as non-lymphoid tissues and recent studies have shown that tissue-resident macrophages are derived from two sources; circulating monocytes and embryonic derived macrophages that self-maintain. During gestation macrophages are the first immune cells to seed embryonic tissues. Through work with the placenta we are provided with the opportunity to study the earliest human extra-embryonic macrophages, called Hofbauer cells (HBC). HBC are located within the villious core of the placenta and appear before fetal blood circulation begins, allowing the study of embryonic macrophages without the risk of blood monocyte contamination. HBC are the only immune cell on the fetal side of the placenta barrier. However, today the role of HBC in placenta development and transplacental infection remains poorly understood. Using the skills I developed through my work with both fetal and adult tissue antigen presenting cells I have begun to perform an in-depth characterisation of HBC. Through my preliminary data, I have identified HBC subsets and demonstrate subset specific functionality in terms of their suspectibility to viral infection. Through my Sir Henry Dale Fellowship and work at the Dept. of Pathlogy I will continue to charactise HBC and their role in placental biology.