Annual Report 2015

HKU-Pasteur Research Pole

7/F Jockey Club Building for Interdisciplinary Research

5, Sassoon Road, Hong Kong SAR

Malik Peiris, Director

Roberto Bruzzone, Co-Director

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*Refers to work done while a Post-doctoral Fellow in Dr. Leo Poon's group

4. Scientific Output 2014

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1. Summary

Mission. The University of Hong Kong (HKU) and Institut Pasteur (IP) have established the HKU-Pasteur Research Pole (HKU-PRP), a laboratory integrated into the School of Public Health of HKU, with a signing ceremony on December 17, 2013. HKU-PRP will further develop the successful partnership between HKU and IP through the recently established Pasteur Center for Global Health Research and Education (CGH), which aims to improve the health of populations worldwide, by expanding its current strength on respiratory and arboviral diseases to tackle basic cell biology and immunology processes that are relevant to the advancement of our understanding of pathogenetic mechanisms and rational vaccinedesign. HKU-PRP has been designated as one of the hubs of the CGH in recognition of its achievements and of the strategic partnership with the Center of Influenza Research of HKU, which has developed a multi-disciplinary research program of international excellence through the integration of basic, clinical and epidemiological research spanning the animal-human interface, thereby also enhancing capacity to confront other emerging viral pathogens.

Research. Our vision is to generate biological knowledge to better understand and treat infectious diseases by bringing together scientists with an interdisciplinary mind. We have organized our activity around four Group Leaders who have secured several independent extramural grants to continue research on respiratory infections and the mosquito borne disease of dengue. With respect to influenza research, we have extended our exploration of virus-host interaction and host response to viral infection by combining clinical studies and basic research investigations to gain insight into the mechanistic aspects of disease pathogenesis and adaptive immunity for improved protection. Collaboration with the First Affiliated Hospital of Guangzhou Medical University has led to very significant publications on avian influenza and the newly identified Middle East respiratory syndrome coronavirus (MERS-CoV), and laboratory space has been made available to HKU-PRP to further develop clinical research projects. We are continuing our large-scale seroepidemiology studies with international collaborators on MERS-CoV and have expanded the scope of these investigations to include Ebola virus disease with the Institut Pasteur-Dakar. With respect to dengue research, we are now extending our investigations on the characterization of host factors that are exploited in extensive remodeling of the endoplasmic reticulum to facilitate virus replication, trafficking, assembly and egress. The scientific output of HKU-PRP has been of the highest quality, with 28 papers published since January 2015.

Teaching. Our program of courses for postgraduate students and young scientists is drawing an increasing number of highly qualified applications from around the world, establishing a worldwide network of trainees. As the hub of the CGH, HKU-PRP will provide students, scientists and health professionals with consistent knowledge and interdisciplinary training through its international education program. In 2015 we have co-organized with the School of Biological Sciences at HKU the first Summer Course on Advanced Imaging, sponsored by the Croucher Foundation. In collaboration with the Australian National University, the 2015 edition of our annual international course on epidemiology and public health at the Pasteur Institute of Ho Chi Minh City (Vietnam) focused on Outbreak Investigation. The three events organized by HKU-PRP on this topic (2010 in Singapore; 2013 and 2015 in Vietnam) have been the first courses on Outbreak Investigation organized in the Pasteur Network.

Perspectives. We have developed a strong identity to promote the health and educational agenda of HKU, IP and the Pasteur Network, through research, teaching and public health activities. We have expanded our critical mass with the recruitment of a new Group Leader that will reinforce research on basic and applied immunology. The interdisciplinary nature of our research and teaching will facilitate synergies with the new School of Biomedical Sciences established by HKU to integrate basic science departments. In recognition of our achievements, HKU and IP are planning a 10-year extension of their collaborative agreement between HKU and IP. In summary, the results obtained in 2014 are clearly in line with our strategic objectives to confront major global health challenges through an innovative model for research collaboration and position HKU-PRP as a cluster of excellence within the School of Public Health.

2. Overview of the Programs

2.1 Research

The scientific activity of HKU-PRP has been significantly re-organized around three core projects to become an important component of the School of Public Health at HKU. We have successfully completed the search for a new Group Leader who is investigating immune correlates of protection to influenza for rational vaccine design.

Our research projects address key biological questions to gain insight into how viruses function and interact with their hosts in the cellular environment that constitutes their battleground. Three questions are of particular interest to us:

1. *How do viruses invade, replicate and escape infected cells?* This question encompasses both the virus point of view of the infectious process – by studying molecules and machinery of the host cells that are hijacked during the viral life cycle, as well as the cellular view – to investigate counterstrategies employed by the host in order to prevent virus infection at various steps, including replication, assembly and release.
2. *What makes a microbe pathogenic?* This question interrogates the the behavior and pathogenicity of emerging viruses by combining clinical studies that span serology, epidemiology and pathogenicity to delineate genetic determinants of virulence and the acquisition of traits that favor crossing of species barriers by zoonotic viruses.
3. *How do pathogens withstand the host immune response?* This question zooms in, on the one hand, on innate responses and the complex strategies devised by viruses to foil them and, on the other hand, on adaptive lines of defense of the host and how they could be harnessed and optimized by vaccination to improve protection.

Research in the Suki Lee lab focuses on the mechanisms of host innate immune response to viral infection and explores the potential of novel therapeutic targets for the treatment of diseases caused by these viruses. A major effort is devoted to dissect the signal transduction pathway of TLR10, an orphan receptor, which was shown to function as a new innate immune sensor during influenza virus infection. Suki Lee was the recipient of the Most Promising Young Researcher Award 2015 from the Research Office of the Food and Health Bureau of Hong Kong SAR.

The lab of Chris Mok uses a combination of in vitro and in vivo models to understand the behavior and pathogenicity of emerging viruses. Through the "Guangdong-Hong Kong Joint Research Centre for Clinical and Preventive Medicine against Emerging Infectious Diseases", laboratory space has been made available to HKU-PRP to develop collaborative research projects that focus on influenza, MERS and dengue viruses, which have recently caused outbreaks, albeit of different scale, in Guangzhou (PR China). Additional efforts are ongoing to understand the interplay between viral and host factors that influence the replication of influenza A/B viruses.

The main objectives of the Sumana Sanyal lab are to combine methods of cell biology and immunology to address aspects of host-pathogen interactions. Using influenza and dengue as model systems, the lab aims to determine the identity and function of specific host factors that are exploited by these viruses to complete their intracellular life cycle. A related research area is the investigation of counterstrategies employed by the host – either through upregulation of immune signaling pathways or expression of virus restriction factors – in order to prevent virus infection at various steps, including replication, assembly and release.

The newly established group of Sophie Valkenburg is studying the role of protective heterologous T and B cell immunity in mouse and human systems, by testing novel vaccines strategies and investigating immune correlates of protection for influenza. The primary focus is to study adaptive immunity, and how this could be harnessed by vaccination to improve protection from infection of diverse influenza viruses.

The research activity of HKU-PRP includes work by Jimmy Lai (joint appointment in the Department of Pathology), who combines chemical, biochemical and cell biological methods to study interactions between viruses with host receptors, in order to have a better understanding on viral host adaptation and cellular/tissue tropism. Work on MERS-CoV and Ebola virus is coordinated by Malik Peiris.

2.2 Teaching and Education

HKU-PRP pledges to extend the impact of research into the production and diffusion of knowledge by developing an advanced teaching program in life sciences that will train a highly selected group of students who will be at the forefront of biomedical research in their countries. This program is extremely competitive, and significantly contributes to solidifying the reputation of HKU-PRP as the premier regional hub for education. The Virology course has been held for the 12th consecutive year and the Immunology course has reached the 8th edition. In 2015 we have innovated by partnering with School of Biomedical Sciences and the Faculty Core Facility to organize the first Summer Course on Advanced Imaging, sponsored by the Croucher Foundation. The course will be repeated in 2017 and 2019. We have also expanded the number of subscriptions to the HKU-Pasteur Courses Series Newsletter, which now totals 516, and our groups in social media have grown, reaching 338 and 294 members for LinkedIn and Facebook, respectively.

We have continued the L'Oreal Research Scholarship scheme, which provides outstanding postgraduate students and postdoctoral fellows who are permanent residents of Hong Kong or Macau with a unique opportunity of exposure to the research environment in France through participation in research work at Institut Pasteur. One of the 2015 fellows has been hired as postdoctoral fellow at the Institut Pasteur and another recipient of the scholarship is applying for grants to join a lab at the Institut Pasteur, a very significant outcome in line with the stated goals of the scheme.

We have started in 2012, in collaboration with the Pasteur Institute of Ho Chi Minh City, a new series of international workshops for epidemiologists and public health personnel involved in surveillance activities, with the support of the Institut Pasteur International Network and the French Regional Scientific Cooperation. The 2015 workshop focused on Outbreak Investigation, a topical theme as the recent outbreaks of Ebola virus and MERS-CoV have illustrated, and we received more than 100 applications from all over the world.

All Group Leaders are actively engaged in the Teaching and Training program. Two postgraduate students (one PhD and one MPhil) have successfully defended their thesis in 2015. We hosted a Master student from the Netherlands and three undergraduate summer students from University College London and Imperial College London.

2.3 International Activity

We retain leadership roles in a number of global projects. Roberto Bruzzone is a member of the Executive Committee and Vice-Chair of the **International Severe Acute Respiratory and Emerging Infection Consortium** (www.isaric.tghn.org), a networkof networks aiming to ensure that clinical researchers have the open access protocols and data-sharing processes needed to facilitate a rapid response to emerging diseases.

Institut Pasteur and the Institut Pasteur International Network have established the Pasteur Center for Global Health Research and Education (CGH) that aims to improve the health of populations worldwide. HKU-PRP has been designated as the Asian the hub of the CGH and Roberto Bruzzone has been appointed Director of the International Teaching and Training Progam of Institut Pasteur.

Malik Peiris is the Coordinator of the Theme-based Research Scheme (TRS): **"Viral, host and environmental determinants of influenza virus transmission and pathogenesis"**, which has been awarded a HK$75 million grant. This large-scal multidisciplinary project builds on the success of the Area of Excellence scheme on **"Control of Pandemic and Inter-pandemic Influenza"**, which was also initiated and coordinated by Malik Peiris. The newly funded TRS aims at enhancing global public health by identifying the viral and host determinants of influenza virus transmission and pathogenesis leading to evidence-based interventions.

Malik Peiris continues to serve on a number of WHO working groups in relation to both avian and swine origin influenza virus and is the Co-Director of the WHO H5 Reference Laboratory at HKU.

3. Progress Report

3.1 SUKI LEE LAB

Main Objectives and Strategy

Acute respiratory viral infections remain a major cause of morbidity worldwide and of mortality in the developing world. Emerging respiratory viruses such as MERS, SARS, avian influenza H5N1 and pandemic H1N1 negatively impacted on societies and economies in many countries. The innate immune system is the first line of host defense and is central to a patient's effort to combat such emerging infections as well as common respiratory virus diseases. My lab focuses on virus-host interaction and host innate immune response to viral infection, with a major objective to investigate the underlining mechanisms of innate immunity relevant to viral pathogenesis, and to explore the potential of novel therapeutic targets for the treatment of diseases caused by these viruses.

Novel role of TLR10 as a nucleotide sensing receptor

Our lab recently identified a novel role of an orphan receptor, TLR10 in viral pathogenesis. We showed that influenza virus infection increased TLR10 expression and demonstrated the involvement of TLR10 in innate immune sensing of viral infection and the production of cytokine and interferon (IFN), suggesting a role of TLR10 as an innate immune sensor. In addition, we screened the possible ligand for TLR10 sensing and revealed that double stranded (ds) RNA could be ligand that triggered TLR10 mediated IFN response.

Achievements and Ongoing Research

During the past year, we further addressed key questions regarding this receptor. We found that TLR10 physically bound dsRNA in vitro, whilst such binding was favorable at a pH condition similar to that within endosomes. Myeloid differentiation primary response gene 88 (MyD88) was recruited to activate TLR10 for signal transduction which modulated interferon regulatory transcription factor (IRF)-7 dependent IFN expression. Moreover, immunoblotting analysis revealed previously unidentified proteolytic processing by cathepsins of TLR10 that governed TLR10 activity. These recent data further demonstrate that TLR10 is a novel nucleotide sensing receptor which could sense dsRNA within endosomes and subsequently recruit MyD88 to regulate IFN expression via IRF7.

Novel role of TLR10 as a nucleotide sensing receptor [Funding: RGC/GRF, AoE/M-12/16]

Our work is uncovering a novel pattern recognition receptor and providing new conceptual and important information to the understanding of innate immune sensing and signaling.

TLR10 binds dsRNA under acidic pH condition in vitro

To demonstrate that TLR10 could bind dsRNA, in vitro binding assays were performed. Previous studies have demonstrated that activation of nucleic acid sensing TLRs depends on pH environment. Thus, high affinity of nucleic acid to TLRs could only be observed at acidic pH such as within the endosomes. In the present study, binding assay was performed at pH 5.5. TLR10 was readily pulled down in vitro using biotinylated poly(I:C) at pH 5.5. Addition of competitive unlabeled poly(I:C) markedly decreased the amount of TLR10 being pull-down, suggesting that TLR10 specifically bound to poly(I:C). In contrast to pH 5.5, lack of detectable TLR10 was found in assay performed at pH 7.4. We have chosen this pH because it is similar to the physiological pH of which cells were cultured and, hence, to the condition at cell surface. The absence of detectable TLR10 in this condition suggests that binding of dsRNA to TLR10 does not occur at a pH mimicking the environment at the cell surface. Taken together, the data here demonstrate that TLR10 could physically interact

with a dsRNA analog in an acidic environment (pH 5.5), suggesting an intracellular compartment as the site, possibly the endosomes, for dsRNA binding to TLR10.

MyD88 is recruited to TLR10 upon dsRNA stimulation

Binding of Toll/interleukin-1 receptor (TIR)-domain containing adaptor proteins to the TIR domains of TLRs could activate TLR signalling. The highly conserved BB-loop within the TIR domain of TLRs was shown to interact with the TIR domain of the adaptor proteins. Previous study showed that in TLRs, one alanine/proline residue in the BB-loop is required to confer adaptor binding specificity. All known human TLRs have a proline residue at the BB-loop, except TLR3 which contains alanine at that position and, interestingly, all human TLR members, except TLR3, bind to Myeloid differentiation primary response gene 88 (MyD88) except TLR3, which binds TIR-domain-containing adapter-inducing IFN-β (TRIF). A change of alanine to proline in the BB-loop is sufficient to switch TLR3 signaling adaptor from TRIF to MyD88. Sequence analysis of TLR10 revealed the presence of proline residue in the BB loop. This suggests that MyD88 is a possible candidate as adaptor protein for TLR10 signaling. To confirm this, recruitment of MyD88 to TLR10 upon poly(I:C) stimulation was investigated by co-localization studies. Unstimulated cells displayed little or no co-localization of MyD88 to TLR10. When cells were challenged by transfected poly(I:C), recruitment of MyD88 to TLR10 was observed as early as 5 min, peaked at 10 min post ligand stimulation and was followed by a time-dependent decrease. Recruitment of MyD88 by TLR10 upon stimulation was further confirmed by immunoprecipitation. In accordance to these results, without poly(I:C) stimulation, MyD88 was barely detectable in samples immune-precipitated with anti-TLR10 antibody suggesting that there was only a very trace amount of MyD88 being recruited to TLR10 in un-stimulated cells. In contrast, there was a strong interaction between MyD88 and TLR10 detected after poly(I:C) stimulation. Similar to the co-localization study of MyD88 with TLR10, the strongest interaction was detected at 10 mins post stimulation and gradually decreased afterwards. As TRIF is another important adapter protein that is recruited by TLR3 upon stimulation for its signaling, we tested whether it interacted with TLR10. However, TRIF was not pulled-down by TLR10. Taken together, MyD88 was actively recruited to TLR10 upon poly(I:C) stimulation, which suggests it as an adaptor protein for TLR10 signaling.

TLR10 mediated IFN expression is regulated via IRF7

IRF3 and IRF7 play essential role in regulating the target genes of TLR signaling pathways. The activation of IRF3 and IRF7 is characterized by the phosphorylation of their C terminus by the IKK-related kinases TBK-1 and IKKε, followed by IRF dimerization and nuclear translocation. To determine whether TLR10-mediated signaling affects the phosphorylation of IRFs, subsequently leading to modulate type I IFN production, WT and cells overexpressing TLR10 (OE) were challenged by poly(I:C) transfection and the phosphorylation of IRF3 and IRF7 was examined. Our data indicate that there was no difference in IRF3 phosphorylation at all time points after poly(I:C) stimulation between WT and OE cells, whereas phosphorylation of IRF7 was found to be markedly reduced in TLR10 OE, with the greatest difference observed at 30 mins after stimulation. These boservations could not be accounted for by the slight difference in IRF7 phosphorylation that was observed in unstimulated (US) conditions between WT and OE cells.

Effect on IFN signaling in TLR10 knockdown cells upon poly(I:C) stimulation

The correlation of type I IFN response and TLR10 expression was further analyzed upon stimulation with poly(I:C) using an inducible reporter assay. The expression of luciferase reporter THP-1 based cell line was under the control of an IRF-inducible promoter, which is comprised of five IFN-stimulated response elements (ISRE) and an ISG54 minimal

promoter. Thus quantification of the luciferase activity reflects the induction of type I IFN signaling responses in the reporter cells. To examine the role of TLR10 in type I IFN signaling, siRNA against TLR10 was introduced to the reporter cells and their luciferase activities in response to poly(I:C) stimulation were compared to non-targeting control siRNA-treated cells. Upon poly(I:C) challenge, a signification increase in luciferase activity was observed in TLR10 knockdown cells, indicating that the type I IFN response would be augmented in TLR10-deficient environment. This finding further suggested a suppressive role of TLR10 in type I IFN signaling upon dsRNA stimulation. Overall, these data demonstrated a novel regulatory role of TLR10 in suppressing IFN signaling mediated through the IRF7 but not IRF3.

Proteolytic-cleavage of TLR10 is a multi-step event and is essential for signal activation

Cathepsins have been documented to mediate essential proteolytic cleavage of TLR3 and TLR9 for signal activation. In the present study, TLR10 has been shown to recognize dsRNA and trigger innate immune responses. The multiple bands pattern of TLR10 observed in WT cells suggested that TLR10 is likely to undergo constitutive proteolytic cleavage. To test this hypothesis, THP-1 cells were treated with cathepsin inhibitor, z-FA-FMK and whole cell lysates were blotted against TLR10. Similar to what observed, spontaneous proteolytic cleavage of TLR10 could be observed in resting WT cells. Upon cathepsin inhibition, cleavage of TLR10 was partially abrogated, as illustrated by the accumulation of a band at around 90 kDa and decreased abundances of TLR10 of smaller electrophoretic mobility. In contrast, cleavage of full-length TLR10 remained unaffected, suggesting that other proteases may contribute to this initial step. The effect of ligand stimulation on TLR10 cleavage was also investigated. Data demonstrated that such cleavage upon ligand stimulation was not readily observable within the initial 30 min, whereas it was detectable at later time points. As expected, ligand induced TLR10 cleavage was efficiently hindered by z-FA-FMK. In order to investigate the function and mode of activation of TLR10, parallel experiments were conducted in TLR10 O/E THP-1 cells for comparison. The pattern and dependence on cathepsin of TLR10 cleavage was not altered in in THP-1 cells over-expressing TLR10. TLR10 could decrease IFN-β induction after dsRNA stimulation in cells pre-treated with DMSO or medium control. In contrast, pre-treatment with z-FA-FMK significantly abolished the inhibitory effect of TLR10 mediated IFN-β expression in DMSO- ($p=0.0062$) and medium- ($p=0.0098$) treated cells, suggesting that cathepsin-mediated cleavage of TLR10 is a necessary event for its activation and downstream signaling.

Together, these data demonstrate for the first time that dsRNA as a ligand for TLR10 signaling. TLR10 physically binds dsRNA in vitro at a pH similar to that encountered within endosomes. Recruitment of MyD88 by TLR10 activated downstream signaling that which led to a suppressive function on interferon regulatory transcription factor (IRF)-7 dependent IFN production. Moreover, immunoblotting analysis revealed previously unidentified proteolytic modifications of TLR10by cathepsins that govern its activity. Our results identify TLR10 as a novel nucleotide sensing receptor which could bind dsRNA within endosomes and subsequently recruit MyD88 to trigger a signaling cascade that regulates IFN expression mediated via IRF7.

Publications

1. Yan S, Lee SM (2016) TLR10 modulates poly(I:C) induced pro-inflammatory response. In revision.
2. Yan S, Yip TF, Li ST, Ip K, Li PH, Peiris JS, Lee SM (2016) Toll like receptor (TLR)-10 is a novel nucleotide sensing receptor. In preparation.
3. Lee SM, Ng YP, Peiris JS, Ip NY (2016) Innate immune responses in human astrocytic and neuronal cells infected with avian H7N9 and pandemic H1N1 influenza A viruses. In preparation.

Presentations at Meetings

1. SMY Lee, KH Kok, M Jaume, DY Jin, JS Peiris (2015) Role of TLR10 as an innate immune sensor for influenza virus infection. *TOLL 2015: Targeting Innate Immunity,* Marbella, Spain (Poster).
2. S Yan, TF Yip, ST Li, Kelvin Ip, PH Li, Suki MY Lee (2015) TLR10 plays a regulatory role in type I interferon responses upon poly (I:C) activation. *TOLL 2015: Targeting Innate Immunity,* Marbella, Spain (Poster).
3. SMY Lee, KH Kwok, DY Jin, JS Malik Peiris (2015) Novel role of TLR10 as an innate immune sensor for respiratory virus infection. *Institut Pasteur International Network International Scientific Symposium,* Paris, France (Poster).
4. TF Yip, S Yan, JSM Peiris, SMY Lee (2015) Sub-cellular localization and ligand identification of Toll-like receptor (TLR)-10. *Institut Pasteur International Network International Scientific Symposium,* Paris, France (Poster).

Teaching

1. Suki Lee (2015) PBL teaching of Haematology/Immunology System and Endocrine System for year III MBBS students
2. Suki Lee (2015) Tutor in the 8th HKU-Pasteur Immunology Course, Hong Kong, Hong Kong SAR

Collaborations

1. **RT Guo** (Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, PR China): Determination of the crystal structure of TLR10.
2. **John Hiscott** (Istituto Pasteur-Fondazione Cenci Bolognetti, Italy): Effect of RIG-I agonists on TLR10 mediated signaling.

Funding

1. Determining the ligand and function of TLR10: a novel innate immune sensor in viral infection (**Principal Investigator**; Research Grants Council, General Research Fund – Ends: 09/2016).
2. Determining the ligand and function of an orphan receptor: Toll like receptor 10 (**Co-Investigator**; Area of Excellence "Control of Pandemic and Inter-pandemic Influenza" – Ends: 12/2016).
3. Pathogenesis and disease severity: Role of TLR10 as an innate immune sensor (**Co-Investigator**; Theme-based Research Scheme Viral, host and environmental determinants of influenza virus transmission and pathogenesis – Ends: 12/2019).
4. A potential new therapeutic option in treatment of influenza disease (**Principal Investigator**: Health and Medical Research Fund – Ends: 11/2015).
5. Association between basal leukocyte transcriptome profile and symptom development & disease severity after influenza virus infection in humans (**Co-Investigator**; Health and Medical Research Fund – Ends: 03/2017).

Personnel

Name	Position
Suki Man Yan LEE	Research Assistant Professor
Selena Sheung YAN	Research Associate
Tsz-Fung YIP	MPhil student (until 31 August 2015) Research Assistant (started 01 April 2016)
Ping-Hung LI	Research Technician
Shu-ting LI	Research Assistant
Kelvin Ka Kay IP	Research Assistant
Sharon TSE	Student Intern

3.2 CHRIS KA PUN MOK LAB

Main Objectives and Strategy

One of the main objectives of our group is to understand the behavior and pathogenicity of emerging viruses by combining clinical studies that span the areas of serology, epidemiology and pathogenicity. In this context, we have been the driving force in setting up with HKU and the First Affiliated Hospital of Guangzhou Medical University a research platform, the "Guangdong-Hong Kong Joint Research Centre for Clinical and Preventive Medicine against Emerging Infectious Diseases". This initiative will cover a wide range of research interests related to new emerging viruses identified in Guangzhou and laboratory space will be made available to HKU-PRP to develop collaborative research projects. We currently focus on three different pathogens: influenza, MERS and dengue viruses, which have recently caused outbreaks, albeit of different scale, in Guangzhou.

The second objective is to understand the interplay between the viral and host factors that influence the replication of influenza viruses. We are investigating the role of the human importin superfamily of nuclear transporters, which recognize nuclear localization signals to mediate the movement of proteins between the cytoplasm and the nucleus, on influenza A/B replication and pathogenicity. Human importins have been shown to be involved in the regulation of the mammalian adaptation of influenza A virus mediated by the interaction with viral polymerase basic protein 2 (PB2) and nucleoprotein (NP). Our team has been focusing on the genetic adaptation of influenza virus and has set up collaboration with the laboratory of Gülsah Gabriel (Heinrich-Pette Institute, Hamburg, Germany), who first discovered the interplay between importin and influenza proteins, and PC Shaw (Chinese University of Hong Kong, Hong Kong SAR), who is a structural biologist working on protein-protein interaction. We also aim to understand the functions of the unconjugated form of an antiviral protein, ISG15 upon influenza infection. ISG15 is known as an antiviral protein induced by interferon (IFN) α/β. Although its intracellular functions via protein ISGylation have been intensively investigated, the role of free, unconjugated ISG15 remains understood. Our group, in collaboration with Dr. Sumana Sanyal, another PI at HKU-Pasteur, will investigate the secretion pathways of free unconjugated ISG15 and further explore its functions.

Achievements and Ongoing Research

During 2015 we have made progress in characterizing the pathogenicity of emerging viruses responsible for outbreaks in the region. We have pursued a combined clinical and laboratory approach in collaboration with researchers at the First Affiliated Hospital of Guangzhou Medical University, which has culminated in establishing the "Guangdong-Hong Kong Joint Research Centre for Clinical and Preventive Medicine against Emerging Infectious Diseases". Several articles describing the clinical features, serology, epidemiology and pathogenicity of emerging viruses have resulted from this collaboration.

Identification and characterization of the novel H5N6 infection in humans [Funding: RGC Seed Funding for Basic Research]

Since the first detection in 1996, H5 subtype viruses have continued to reassort and evolve giving rise to multiple virus clades and gene constellations. Recently, clade 2.3.4.4 viruses have exhibited a predilection for genetic reassortment giving rise to H5N2, H5N5, H5N6 and H5N8 virus subtypes, which have become globally widespread, causing infections in wild birds or poultry in Asia, Europe and North America. In particular, H5N6 viruses have circulated in China and numbers of human infections have been reported. In collaboration

with the First Affiliated Hospital of Guangzhou Medical University, we have presented the clinical, virological and immunological features of human infection caused by A(H5N6) virus in a 59-year-old patient who developed Acute Respiratory Distress Syndrome (ARDS) and and provided a molecular characterization of the virus. This is one of the first reports in the world to describe the human infection of this new identified virus. The full genome sequence of the virus has been obtained and found that it is a reassorted virus with H5N1. Our molecular characterization showed that two lineages of H5N6 viruses with distant genetic background were co-circulating in China. Key mutations which were suspected to contribute to the virulence were identified. We are currently further characterizing the properties of the virus isolate by infection experiments using ex vivo lung cultures (collaboration with Dr. Michael Chan at HKU) and the ferret animal model (collaboration with Dr. Ron Fouchier at the Erasmus Medical Center, The Netherlands) to understand the pathogenicity or transmissibility of this novel virus.

Transcriptomic and serology studies of re-emergent avian-origin human H7N9 influenza disease in Guangdong province [Funding: Areas of Excellence Scheme on Control of Pandemic and Inter-pandemic Influenza]

Patients infected with A/H7N9 viruses exhibit a rapidly progressive pneumonia leading to respiratory failure and acute respiratory distress syndrome (ARDS) reminiscent of the disease caused by the highly pathogenic H5N1 avian virus. Since the identification in early 2013 of severe disease caused by influenza A(H7N9) virus infection, there have been few attempts to characterize the full severity profile of human infections. Our objective was to estimate the number and severity of H7N9 infections in Guangzhou, using a serological study. We tested 5340 residual sera from the First Affiliated Hospital of Guangzhou Medical University by hemagglutination inhibition (HI) and virus neutralization assays, and found two specimens that tested positive for H7N9 antibody at an HI titer $\geq$40 and a neutralization titer $\geq$40. We used a statistical model in a Bayesian framework to interpret this information, estimating that 51000 (95% credibility interval: 1000, 180000) human infections with influenza A(H7N9) virus occurred in Guangzhou in early 2014, with an infection-fatality risk of 5 deaths (95% credibility interval: 0.10, 16) per 1,000 infections. Through this project, our team has established a diagnostic HI test based on the use of lentiviral particles pseudotyped with H7N9 envelope proteins. This assay is safer as it does not rely on the use of live H7N9 virus and can be performed in a BSL-2 containment laboratory. HI positive samples will be further investigated by micro-neutralization assay, which is the gold-standard assay recommended by the WHO, using live H7N9 virus.

In response to virus infection, the host mounts innate immune and inflammatory responses that can results in reprogramming of the transcriptome of the immune cells in the peripheral blood. We have extracted total RNA from the peripheral blood cells of patients infected with H7N9 viruses are currently analyzing their gene expression profiles using gene microarray. This work will provide insight into the complexity of regulatory changes after H7N9 infections and a better understanding of the molecular mechanisms involved in related diseases.

Virological and immunological features of a patient with Middle East Respiratory Syndrome, China, 2015 [Funding: National Key Project of Clinical Faculty and Facility Construction on Infectious Diseases (2013-2014) and National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services]

One returning traveler initiated an outbreak of Middle East Respiratory Syndrome coronavirus (MERS-CoV) in South Korea resulting in 186 cases, which led to >35 deaths. We reported a 43-year-old male infected in South Korea who travelled to, and was diagnosed in China. We have provided serial data on clinical progression, viral load in different clinical specimens, plasma cytokine levels, serum antibody responses comparing multiple serological assays and T-cell responses. Among other things, this case report highlights importance of sputum collection that impacts on diagnostic yield and viral load, comparative serological data using conventional neutralization, pseudo-particle neutralization, plaque reduction neutralization and ELISA assays, and potent T cell responses to the spike protein of MERS-CoV. Thus, although it is one patient, it is one of the most intensively investigated patients with MERS so far.

Investigation of the role of human importin protein family on influenza replication and pathogenicity [Preliminary study]

The characterization of virus and host factors that are involved in the adaptation of avian influenza virus to mammalian cells (and mammalian transmission) remains one of the most critical questions in the field of influenza research. Mutations in any segment of the virus genome are an important mechanism of its adaptation to the mammalian host and the corresponding amino acid changes also contribute to pathogenesis. The group of Guelsah Gabriel (Heinrich-Pette Institute, Hamburg, Germany) has previously shown that avian influenza viruses undergo a switch in importin-alpha dependency upon avian–mammalian adaptation. Importin-alpha is a constituent of the classical nuclear import pathway. It acts as an adaptor protein that recognizes the nuclear localization signal (NLS) of the cargo protein, which is then transported as a ternary complex with the importin-beta receptor into the nucleus. It has been shown that importin protein can interact with PB2 and NP proteins of influenza A virus. For example, the human-like PB2 627K virus, but not avian-like PB2 627E, displayed reduced pathogenicity and replication in importin-alpha 7 knockout mice. However, the interplay between the mammalian importins and the adaptation of avian influenza virus in mammal has not been fully mapped out. In collaboration with the Gabriel's lab and Shaw's group, we aim to address several questions to delineate in mechanistic terms the interplay between influenza proteins and importins. Specifically, we will investigate the structural basis of the binding between importin and the influenza PB2/NP proteins and the interaction of the new identified PB2-Q591K mammalian adaptation and importins. Moreover, we will begin exploring the role of importins in the replication of influenza B virus, an endemic strain which only circulates in human population but not poultry.

Extracellular secretion of free ISG15 in primary human cells with influenza virus infection [Preliminary study]

Influenza is recognized as a major global public health threat because of the frequent, large-scale outbreaks of human disease lead to significant economic loss and deaths. It is estimated that about 5-15% of the total world population is infected every year. Despite the availability of a vaccine and the use of the antiviral drug oseltamivir for the clinical treatment to severe ill patients, infections of this virus still cause over 300,000 deaths per year during seasonal epidemics. The immune response of patients is a critical determinant of the disease outcome. Either suppression or hyperactivation of the immune response may harm the host. Thus, influenza viruses have evolved mechanisms to counteract the innate immune response, whereas mounting an uncontrolled immune response may be detrimental to the host. An egregious example is that of the avian influenza H5N1 virus, which causes a hyperinduction of the cytokine response during human infection, resulting

in immunopathological condition. Recent data have suggested that extracellular free ISG15, an ubiquitin-like molecule that is believed to play a role in in innate immunity, may function as an immuno-modulator. Because of its putative novel role, tight regulation of synthesis and secretion may help to control the disease severity during infection. However, there is very limited information on the function and regulation of free ISG15 in health and disease. We have begun to investigate the interplay between the host and viral factors on free ISG15 secretion during influenza virus infection. This project is its early phase.

Publications

1. Da Guan W*, **Mok CK***, Chen ZL, Feng LQ, Li ZT, Huang JC, Ke CW, Deng X, Ling Y, Wu SG, Niu XF, Perera RA, Da Xu Y, Zhao J, Zhang LQ, Li YM, Chen RC, Peiris M, Chen L, Zhong NS (2015) Characteristics of Traveler with Middle East Respiratory Syndrome, China, 2015. *Emerg Infect Dis* **21**:2278-2280 (*equal contribution).
2. Guan WD*, Gong XY*, **Mok CK***, Chen TT, Wu SG, Pan SH, Cowling BJ, Yang ZF, Chen DH (2015) Surveillance for seasonal influenza virus prevalence in hospitalized children with lower respiratory tract infection in Guangzhou, China during the post-pandemic era. *PloS One* **10**:e0117846 (*equal contribution).
3. **Mok CK**, Guan WD, Liu XQ, Lamers MM, Li XB, Wang M, Zhang TJS, Zhang QL, Li ZT, Huang JC, Lin JY, Zhang YH, Zhao P, Lee HHY, Chen L, Li YM, Peiris JSM, Chen RC, Zhong NS, Yang ZF. (2015) Genetic characterization of a highly pathogenic avian influenza A H5N6 virus isolated from a human patient in Guangdong, China. *Emerg Infect Dis* **21**:2268-2271.
4. Yang ZF*, **Mok CK***, Liu XQ*, Li XB, He JF, Guan WD, Xu YH, Pan WQ, Chen LY, Lin YP, Wu SG, Pan SH, Huang JC, Ding GY, Zheng K, Ke CW, Lin JY, Zhang YH, Lee HH, Liu WK, Yang CG, Zhou R, Peiris JS, Li YM, Chen RC, Chen L, Zhong NS (2015) Clinical, virological and immunological features from patients infected with re-emergent avian-origin human H7N9 influenza disease of varying severity in Guangdong province. *PLoS One* **10**:e0117846 (*equal contribution).
5. Yang ZF, **Mok CK**, Peiris JSM, Zhong NS (2015) Human infection with a novel avian influenza A(H5N6) virus. *N Engl J Med* **373**:487-489.
6. Blanc F, Furio L, Moisy D, Yen HL, Chignard M, Letavernier E, Naffakh N, **Mok CK**, Si-Tahar M. (2016) Targeting host calpain proteases decreases influenza A virus infection. *Am J Physiol Lung Cell Mol Physiol* **310**:L689-L699.
7. Fan Y, **Mok CK**, Zhang Y, Nal B, Kien F, Bruzzone R, Sanyal S (2016) Cell cycle independent role of cyclin D3 in host restriction of Influenza virus infection. *Submitted*.
8. Lin YP, Luo Y, Chen Y, Lamers MM, Zhou Q, Yang XH, Sanyal S, **Mok CK**[*], Liu ZM[*] (2016) Clinical and epidemiological features of the 2014 large-scale dengue outbreak in Guangzhou city, China. *BMC Infect Dis* **16**:102 (*co-corresponding authors).
9. Lin YP, Yang ZF, Liang Y, Li ZT, Bond HS, Luo YS, Chen Y, Chen TT, Guan WD, Lai JCC, Siu YL, Pan SH, Peiris JSM, Cowling BJ*, **Mok CK*** (2016) Population seroprevalence of antibody to influenza A(H7N9), Guangzhou, China. S*ubmitted*.

Seminars, Invited Lectures and Oral Presentations

1. Chris Mok (2105) Institut Pasteur-Korea, Pangyo, Republic of Korea.
2. Chris Mok (2015) Global Forum on Research and Innovation for Health 2015, Manila, The Philippines.
3. Chris Mok (2015) Erasmus Medical Center, Rotterdam, The Netherlands.

Presentations at Meetings

1. **CKP Mok**, Z Yang, NZ Zhong, JSM Peiris (2015) Human Disease Caused By Novel Reassortant Highly Pathogenic Avian Influenza A (H5N6) Virus. *Scientific Symposium of the Institut Pasteur International Network*, Paris, France (Poster).
2. **CKP Mok**, Z Yang, NZ Zhong, JSM Peiris (2015) Human Disease Caused By Novel Reassortant Highly Pathogenic Avian Influenza A (H5N6) Virus. *International Meeting on Respiratory Pathogens*, Singapore (Poster).
3. **CKP Mok**, HH Lee, ZF Yang, JS Peiris (2014) Host and the viral factors that contribute to the pathogenicity of the novel A/H7N9 influenza virus. *The 15th IUBMB-24th FAOBMB-TSBMB Conference*, Taipei, Taiwan (Poster).

Teaching

1. Chris Mok (2015) Tutor in the 9th HKU-Pasteur Virology Course, Hong Kong, Hong Kong SAR
2. Chris Mok (2015) Introduction to the Art and Science of Medicine Block – Problem Based Learning (MBBS Year 1 students), The University of Hong Kong, Hong Kong SAR.

Collaborations

1. **Gülsah Gabriel** (Heinrich-Pette Institute, Hamburg, Germany) and **PC Shaw** (The Chinese University of Hong Kong, Hong Kong SAR): Investigation of the role of human importin protein family on influenza replication and pathogenicity.
2. **Nan-Shan Zhong**, **Ling Chen**, **Zi-Feng Yang** (State Key Laboratory of Respiratory Disease, Guangzhou, PR China): Clinical and laboratory studies on emerging infectious diseases in Guangzhou.
3. **Michael Chan** (HKU) and **Ron Fouchier** (Erasmus Medical Center): Pathogenicity and transmissibility of H5N6 virus.

Funding

1. Characterization of the new identified human pathogenic avian-origin influenza (H5N6) virus (**Principal Investigator**; RGC Seed Funding for Basic Research – Ends: 2016).
2. Transcriptomic and serology studies of re-emergent avian-origin human H7N9 influenza disease in Guangdong province (**Principal Investigator**; Area of Excellence "Control of Pandemic and Inter-pandemic Influenza" – Ends: 2016).
3. Virological and immunological features of a patient with Middle East Respiratory Syndrome, China, 2015; (**Co-Investigator**; National Key Project of Clinical Faculty and Facility Construction on Infectious Dieases (2013-2014) and National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services).
4. The pathogenic role of the adaptation in the polymerase basic 2 protein of the new identified duck isolated H7N9 lineage in mammalian hosts (**Principal Investigator**; Health and Medical Research Fund – Ends 2017).
5. Study on the interaction of influenza A virus nucleoprotein and polymerase PB2 N-terminal region (**Co-Investigator**; Research Grants Council/General Research Fund – Ends: 2017).
6. The role of influenza PA-X protein on the virus replication and cytokine induction in human in vitro models (**Co-Investigator**; Theme-based Research Scheme Viral, host and environmental determinants of influenza virus transmission and pathogenesis – Ends: 12/2019).

7. Infection and immunopathogenesis of avian influenza H9N2 virus in tree shrew model (**Co-Investigator**; Theme-based Research Scheme Viral, host and environmental determinants of influenza virus transmission and pathogenesis – Ends: 12/2019).

Personnel

Name	Position
Chris Ka Pun MOK	Research Assistant Professor
Horace Hok Yeung LEE	PhD student
Fionn Nok Lam MA	Research Assistant-II MPhil Student (starting 01/Sep/2016)
Gannon MAK	PhD student (part-time)
Jane Kong San TSE	Research Technician
Xiao Kai CHEN	Research Assistant (in Guanzhou)
Si Hua PAN	Research Assistant (in Guanzhou)
Garrick Ka Wai YIP	Student Intern (IVE)

3.3 SUMANA SANYAL LAB

Main Objectives and Strategy

The main objectives of the lab are to combine methods of molecular biology and immunology to address aspects of host-pathogen interactions. Using influenza and dengue as model systems, we aim to determine the identity and function of specific host factors that are exploited by these viruses to complete their intracellular life cycle. We also investigate counterstrategies employed by the host – either through upregulation of immune signaling pathways or expression of virus restriction factors – in order to prevent virus infection at various steps, including replication, assembly and release. Among other factors, we are particularly interested in ubiquitin-like post-translational modifiers of protein function, such as ISG15 that play a significant role in modulating different pathways, most of which are innate signaling pathways such as RIG-I, TLR7 and inflammasome activation. Our major research projects are listed below.

Characterization of host factors involved in virus infections:

A molecular understanding of host cellular factors involved in virus infections is crucial not only to provide novel insights into pathways hijacked by them, but also for development of effective antimicrobials against such pathogens. Identification of host factors that can be targeted for developing novel anti-viral compounds has the additional benefit of avoiding potential resistance acquired in viruses by mutation and selection.

(a) *Role of Aup1 in dengue infection:* The complexity of the assembly and release of dengue virus provides a potentially rich source of host targets for interference. Propagation of dengue virus (DENV), West Nile (WNV) and other members of the family appears to involve extensive membrane and lipid remodeling to facilitate virus replication, trafficking, assembly and egress. However, we have been severely limited in our understanding of the role of fundamental biological pathways typically hijacked by flaviviruses. We recently discovered that Aup1 – a lipid droplet associated protein – is heavily expressed upon dengue infection. Preliminary results suggest that overexpression of Aup1 alone is sufficient to cause increased secretion of dengue virus like particles. The goal of this project is to investigate interactions between host factors such as Aup1 and dengue virus in order to understand their functional relevance.

(b) *Role of Tsg101 in influenza virus infection:* A major response of mammalian cells to viral infections is through upregulation of the interferon type I and II pathways. Viruses in turn implement counter strategies through either the inhibition of IFN response or by activation of proteins that inhibit the function of interferon-stimulated genes (ISGs). The primary antagonist of the host immune response for influenza is NS1. A key feature documented for NS1 is the dynamics of interaction with the interferon-stimulated gene 15 (ISG15). Upon type-I interferon treatment or virus infection, ISG15 is one of the immediate responders and is expressed in abundance. Based on limited proteomic analysis, the targets of ISGylation have been found to be of the order of a hundred or more genes. We have identified Tsg101 as one of the targets of ISG15 modification. We are currently exploring the functional relevance of this modification during influenza infection and how NS1 counteracts it. We find a strong correlation between the pathogenicity of the virus and the effectiveness of NS1 in preventing ISG15 mediated inhibition of Tsg101 function.

Targeting deubiquitylases as therapeutic strategies against viral infections

Influenza virus is responsible not only for annual epidemics, but also for frequent outbreaks of pathogenic avian flu strains that have become a serious public health issue worldwide. The ubiquitylation machinery is frequently exploited by a number of pathogens either to masquerade as host proteins or to inhibit immune signaling cascades. We have employed a chemoenzymatic strategy to identify deubiquitylating enzymes (DUBs) that are specifically expressed upon influenza infection and are currently investigating the role of these DUBs. Our ongoing studies involve characterization and pharmacological intervention of these DUBs in order to attenuate influenza infection. Preliminary data in macrophages and dendritic cells support the hypothesis that influenza takes advantage of DUBs to suppress signaling pathways such as RIG-I and inflammasome activation that require ubiquitin modification for recruitment of downstream effectors. We also propose to test small molecules that target these DUBs both in vitro and in vivo.

Regulation of immune signaling by deubiquitylases

Signaling cascades require tight control over activation and suppression to maintain downstream activities for appropriate durations. Such regulation is often executed by post-translational modifications such as phosphorylation and ubiquitylation. We are interested in deciphering the role of deubiquitylases (DUB) in the context of a number of innate and adaptive immune responses. We have identified DUBs that are either specifically recruited or inactivated in the T-cell receptor-signaling cascade, presumably to optimize the length and magnitude of downstream activities. Usp12, which resides in the nucleus, is redistributed to the cytosol in a TCR stimulus specific manner. In the absence of Usp12 surface expression of the TCR is drastically reduced. This phenotype is recapitulated upon inhibition of Usp12 translocation from the nucleus to the cytosol. Using proximity based labeling we identified LAT and Trat1 to be substrates of Usp12. Apart from Usp12, we are pursuing a set of DUBs identified through functional screening in mouse T-lymphocytes that participate in TCR signaling.

Achievements and Ongoing Research

Since joining HKU-PRP in November 2013, we have expanded on projects that were initiated at the Whitehead Institute/MIT while creating new directions at the current setting. Preliminary data generated were used to submit grant applications to RGC/GRF, HMRF and Area of Excellence for Control of Pandemic and Inter-pandemic Influenza as well as Transversal research grants available within the international network of Institute Pasteur. Results obtained in the TCR signaling project were recently accepted for publication in PNAS.

Characterization of host factors involved in virus infections:

(a) *Role of Aup1 in dengue infection* [Funding: RGC/GRF]

For pathogens that regulate cellular lipid metabolism, as many flaviviruses do, the role of host lipid biosynthetic enzymes that modify properties of organelles is an aspect of viral pathogenesis that has received scant attention. A functional screening strategy designed to identify proteins that are differentially modified by ubiquitin upon dengue infection revealed a set of lipid droplet and autophagy associated host proteins in which Aup1 was scored as a strong candidate. Aup1 is a lipid droplet/ER associated protein whose expression is induced in dengue-infected cells in a time-dependent manner. Aup1-deficient HepG2 cells become resistant to dengue infection. Interestingly, we find that the abundance of lipid droplets in dengue-infected cells is significantly lesser compared to

control cells. This phenomenon is inhibited in Aup1-deficient cells.

We have begun to dissect the mechanism by which Aup1 is exploited during DENV assembly. Our current hypothesis entails that induction of autophagy is a critical aspect to sustain dengue replication. Consumption of lipid droplets (lipophagy) generates the necessary fatty acids utilized by dengue and Aup1 is necessary for this step. Aup1$^{-/-}$ cells are unable to induce autophagy and LDs in turn are not consumed to facilitate virus replication. We have generated Aup1-GFP and Aup1-cherry fusion proteins stably expressed in hepatoma cells in culture in parallel with knock-out cells through the CRISPR mediated genome editing strategy. When transduced with DENV NS1 or exposed to live dengue virus, the expression level of Aup1 is significantly upregulated followed by induction of autophagy, which we can observe by confocal microscopy using the reporter constructs of Aup1. We have engineered a number of constructs for Aup1 with either deletions or mutations in specific domains of the protein to dissect the functional relevance of Aup1 interaction with NS1 during dengue pathogenesis. A number of reagents for dengue research, such as HeLa cells stably secreting dengue virus like particles (VLP), was established as part of Dr. Peigang Wang's research at HKU-PRP. Overexpression of Aup1 in these cells results in a substantial increase in VLP secretion, whereas a knock-out attenuates it. Our research builds on promising preliminary data that we generated, not only to understand the role of host factors involved in dengue biogenesis in mechanistic detail, but also to explore new aspects of the immunobiology involved in the host response against this pathogen.

(b) *Role of Tsg101 in influenza infection* [Funding: RGC/GRF]:

We have shown that Tsg101, a component of the ESCRT-I complex, is required for release of influenza A/WSN/33. Tsg101 is essential for transport of hemagglutinin (HA) from the Golgi to the plasma membrane prior to release of intact virus particles from the cell surface. We aim to extend these observations and explore whether this phenomenon holds true as a general host factor necessary for influenza virus biogenesis. We are testing different influenza strains in Tsg101 deficient A549 cells we have generated by the recently developed CRISPR knock out strategy. These A549 cells will be infected with the human H1N1 and H3N2 strains available in house as well as the avian influenza strains of H5N1 and H7N9. Differences may well exist between utilization of host proteins between the human versus the avian influenza strains. Thus, some influenza strains may be better equipped at counteracting the inhibitory effect of Tsg101 deficiency through other mechanisms. We will test viral titers from supernatants of infected cells (wild-type and Tsg101 deficient A549) using plaque assays. In addition, intracellular transport of HA will be measured using FACS to quantitate HA exposure at the cell surface. We have generated epitope-tagged Tsg101 (Tsg101-FLAG) for wild-type and a mutant that does not associate with the ESCRT complexes. These constructs will be expressed in Tsg101$^{-/-}$ cells to test whether they rescue influenza transport and release when infected. We anticipate that virus release will be restored to control levels with the wild-type construct. Expression of the Tsg101 variant that does not associate with the ESCRTs will indicate whether the role of Tsg101 in flu trafficking occurs independently of the ESCRT machinery. These will also be used in combination with confocal microscopy to investigate the localization and intracellular trafficking characteristics upon influenza infection. In addition, we have generated BirA-ligase fusion protein constructs of Tsg101, which are ideal in identifying transient interactors through proximity based labeling strategies. Since the subcellular localization of Tsg101 undergoes drastic changes upon either virus infection or interferon treatment, these will be used to determine the interactors under different physiological conditions. We are also addressing the mechanisms of Tsg101 regulation by post-

translational modification during virus infection. Many cellular antiviral mechanisms are initiated by induction of interferon (IFN). Interferon-stimulated gene 15 (ISG15) encodes an ubiquitin dimer-like protein that is associated with defense against a number of viral pathogens. Of note, post-Golgi transport of influenza HA to the plasma membrane, which requires the protein Tsg101 as described above, is blocked upon IFN treatment and Tsg101 itself is ISG15 modified upon IFN-I induction.

Our preliminary data indicate that differential post-translational modification of Tsg101 appears to regulate its function. Our results suggest that, during a productive virus infection, Tsg101 is phosphorylated at the Y390 residue, whereas upon IFN-I induction it is ISGylated. Upon infection with ΔNS1, which fails to suppress the IFN-I response, we can isolate ISGylated but not phosphorylated Tsg101. These data suggest that phosphorylation and ISGylation are mutually exclusive modifications regulating the function of Tsg101. Phosphorylated Tsg101 facilitates virus release, whereas ISG15 modified Tsg101 blocks viral protein transport from the Golgi to the PM. Similar analyses performed for VSV infection show hyper phosphorylation of Tsg101 at Y390 (unpublished). We will generate the corresponding phosphorylation and ISGylation mutants of Tsg101 for expression in A549 cells deficient in Tsg101. These cells will be infected with different strains of influenza to test how virus trafficking and release are affected. In addition subcellular localization and interacting partners of Tsg101 undergoes a drastic shift depending on the intracellular environment. Using proximity based labeling assay, we have now charted the distinct subcellular interactome of Tsg101 under conditions of mock treatment, influenza infection and interferon treatment, which further sheds light on the differential function and tight regulation imposed on Tsg101.

Targeting deubiquitylases as therapeutic strategies against viral infections [Funding: HMRF, Institut Pasteur]

One of our research goals is to determine how ubiquitin and ubiquitin like modifiers are utilized by the host innate immune system and their modulation during viral infections. Within this context, we are investigating the role of deubiquitylating enzymes (DUB) that are expressed during influenza infection and their effect on the innate immune response. Our preliminary data indicate that upon influenza A infection in human lung epithelial cells (A549) as well as bone marrow derived macrophages in culture, the extent of ubiquitylated material recovered from whole cell lysates is dramatically reduced. We hypothesize that this is a consequence of either one or both of the following reasons: (i) upregulation of deubiquitylating enzymes (DUBs) by influenza virus that de-ubiquitylates host factors to enable a productive virus infection; (ii) degradation of antiviral host restriction factors through the ubiquitin proteasome pathway to enable a productive influenza virus infection. In support of the first hypothesis we have identified DUBs, expressed during influenza infection, which function to block the inflammasome signaling pathway as well as the RIG-I pathway and, hence, compromise the host innate immune response. We anticipate that by targeting this pathway using a combination of chemoenzymatic and pharmacological methods, we will be able to restore a more robust innate immune response and restrict virus replication. Targeting specific enzymes of the ubiquitylation machinery is an emerging therapeutic strategy. We propose to test two specific small molecule inhibitors to these DUBs that are available and have shown considerable promise as drugs in phase I and II clinical trials. Questions that we are specifically focused on addressing are as follows: Are certain DUBs specifically induced during influenza infection? Our preliminary results support the conclusion that specific DUBs are induced upon H1N1 infection. Using a version of ubiquitin (Ub) modified at its C-terminus with vinyl methyl ester (Ub-VME) and a

combination of large-scale immunoprecipitation and proteomic analyses by mass spectroscopy, we have successfully identified DUBs that are expressed during H1N1 infection. What is the purpose of DUBs expressed during viral infections? Do they provide an advantage to virus replication? Are there differences between DUBs expressed by different strains of influenza virus, specifically between the seasonal human influenza H1N1 and the pathogenic avian influenza H5N1 and H7N9? We hypothesize that the DUB expression profiles for seasonal H1N1 and the pathogenic avian viruses would be different and their identity would provide insights into what host factors allow avian flu to become transmissible in humans. Given our success with the seasonal influenza, we aim to use the same strategy to identify and compare DUBs expression profiles with avian influenza.

Regulation of immune signaling by deubiquitylases [Funding: AoE/M-12/16]

Amongst the myriad post-translational modifications the ubiquitin conjugation system appears to play a crucial role in regulating immune signaling cascades. When a naïve T cell encounters a foreign antigen, it undergoes clonal expansion. The strength of the interaction between the antigen and the T cell receptor is a critical determinant for activating a signal cascade and mounting an immune response. Therefore, understanding the molecular mechanism of proximal TCR signaling events following receptor engagement is critical for the purpose of modulating it.

Although some of the E3 ligases participating in the TCR signaling pathway have been identified, the ubiquitin specific proteases that carry out deubiquitylation have been less well studied. Ubiquitin is attached to a substrate by the concerted activities of a series of E1, E2 and E3 enzymes and removed by the presence of deubiquitylating enzymes (DUBs). A balance between the forward and the backward reactions determine the outcome of the strength and duration of the signal and subsequent phenotypic effects. TCR signaling to NFκB requires assembly of large multi-protein complexes consisting of several kinases, scaffold proteins, ubiquitin ligases and deubiquitylating enzymes. The TCR forms a multisubunit complex with CD3 consisting of cytoplasmic immunoreceptor tyrosine based activation motifs (ITAMs). A series of phosphorylation steps that involves PI3K and PDK1 culminates in PKC phosphorylation. The NEMO/IkappaB (IKK) complex integrates signals from upstream stimuli and results in NFκB activation. Several studies have identified key signal mediators involved in the pathway such as Zap70, SLP-76, PLC γ, SAP, Fyn, LCK, PKCθ, Vav1, Bcl10, Malt1 and Carma1. Biochemical characterization of these effectors have suggested a putative sequence of events where PKCθ activity is followed by nucleation of the multiprotein Carma-Bcl10-Malt (CBM) complex within lipid microdomains to recruit the inhibitor of NFκB kinase (IKK). The signal initiated by these core events is then disseminated through adaptor proteins such as LAT and SLP-76 and ultimately induces global changes in gene transcription and acquisition of effector functions.

Reports on the dynamics of TCR surface expression suggest that in accordance with other receptors, the TCR is internalized and recycled rapidly with a rate constant of ~0.01min^{-1}. Engagement by an antigen-presenting cell (APC) causes an intracellular retention, although the kinetics of internalization remains unaffected. Although scant evidence exists for the mechanism of surface dynamics of TCR, available data on well-characterized receptors such as the transferrin receptor (TfR) or epidermal growth factor receptor (EGFR) indicate that internalization and recycling occurs via a dynamic interplay between monoubiquitylation and deubiquitylation process. Several enzymes of the ubiquitylation machinery have been identified that appear to play a crucial role in orchestrating maturation, differentiation and

function of T cells. Amongst the well documented are TRAF6, GRAIL and the SOCS proteins of the E3 ligase family and CYLD, USP9X from the deubiquitylase (DUB) family. Adding another layer of complexity is the diverse array of ubiquitin-chain linkages that dictate the outcome of such modifications in the context of cellular responses such as localization, degradation and signaling.

We have employed a ubiquitin specific activity-based probe to target functional DUBs in the TCR signaling pathway. We used a C-terminally modified ubiquitin (Ub) with vinyl methyl ester (vme) to capture DUBs that are recruited upon TCR activation in both mouse T-lymphocytes and Jurkat cells. This approach has been successfully used previously to identify DUBs that are either cellular or expressed upon infection by chlamydia. Upon stimulation with anti-CD3 antibodies followed by large scale immunoprecipitation we identified a set of cytosolic DUBs, including CYLD and Usp9X, which have been described previously in the context of TCR signaling. We focused on those that were differentially recovered between control and stimulated cells and identified Usp12 and Usp46 that displayed enhanced recovery in the TCR stimulated cells compared to resting cells. Usp12 localizes primarily to the nucleus, but becomes enriched in the cytosol upon stimulation. Usp12$^{-/-}$ Jurkat cells generated through Cas9/CRISPR-mediated genome editing were defective in several downstream activities including NFκB, NFAT and Erk1/2 phosphorylation. In addition surface expression of TCR was severely attenuated upon stimulation in Usp12$^{-/-}$ cells. Through proximity based labeling with a promiscuous BirA-ligase fused to Usp12 (BirA*-Usp12) we identified several adaptor proteins of the TCR signaling pathway including LAT, Trat1 and SLP76. Expression of LAT and Trat1 was attenuated in Usp12$^{-/-}$ cells suggesting that Usp12 acts directly on these proteins to stabilize the TCR complex at the cell surface. We are determining the mechanism of TCR stabilization through the activity of Usp12 on these TCR adaptors.

Publications

1. Jahan AS, Lestra M, Swee LK, Fan Y, Lamers MM, Tafesse FG, Theile CS, Spooner E, Bruzzone R, Ploegh HL, **Sanyal S** (2016) Usp12 stabilizes the T cell receptor complex at the cell surface during signaling. *Proc Natl Acad Sci USA* **113**:E705-E714.
2. Lin YP, Luo Y, Chen Y, Lamers MM, Zhou Q, Yang XH, **Sanyal S**, Mok CKP*, Liu ZM* (2016) Clinical and epidemiological features of the 2014 large-scale dengue outbreak in Guangzhou city, China. *BMC Infect Dis* **16**:102 (*co-corresponding authors).
3. Fan Y, Mok CK, Zhang Y, Nal B, Kien F, Bruzzone R, **Sanyal S** (2016) Cell cycle independent role of cyclin D3 in host restriction of Influenza virus infection. *In revision*.

Seminars, Invited Lectures and Oral Presentations

1. Sumana Sanyal (2015) The University of Cambridge, UK.
2. Sumana Sanyal (2015) University College London, UK.
3. Sumana Sanyal (2015) The Whitehead Institute for Biomedical Research, MIT, Cambridge, MA, USA.
4. Sumana Sanyal (2015) Cambridge Institute of Medical Research, Cambridge, UK
5. Sumana Sanyal (2015) Department of Cell Biology and Infection; Institut Pasteur, Paris.
6. Sumana Sanyal (2015) Department of Cell Biology & Infection of the Institut Pasteur, Annual Retreat, Morzine, France
7. Sumana Sanyal (2015) DKFZ, Heidelberg, Germany.

8. Sumana Sanyal (2015) EMBL, Heidelberg, Germany.
9. Sumana Sanyal (2015) Keystone Symposium "The Human Proteome"; Stockholm, Sweden.

Presentations at Meetings

1. AS Jahan*, M Lestra*, LK Swee*, S Sanyal (2015) *Keystone Symposium on the Human Proteome*, Stockholm, Sweden (Oral).
2. J Zhang, S Sanyal (2015) Role of Aup1 in the assembly and egress of dengue virus. *Scientific Symposium of the Institut Pasteur International Network*, Paris, France (Poster).
3. AS Jahan, S Sanyal (2015) Role of deubiquitylating enzymes in influenza virus infection. *International Meeting on Respiratory Pathogens*, Singapore (Poster).
4. MM Lamers, S Sanyal (2015) Differential regulation of Tsg101 identified through proximity dependent labeling. *International Meeting on Respiratory Pathogens*, Singapore (Poster).
5. Fan Y, Mok CK, Zhang Y, Nal B, Kien F, Bruzzone R, Sanyal S (2016) Cell Cycle Independent Role of Cyclin D3 in Host Restriction of Influenza A Virus Infection. *International Meeting on Respiratory Pathogens*, Singapore (Oral).

Teaching

1. Sumana Sanyal (2015) Lecture in the Molecular Biology of the Cell Course, Institut Pasteur, Paris, France.
2. Sumana Sanyal (2015) Introduction to the art and science of medicine for MBBS Block – Problem Based Learning (MMBS Year 1), The University of Hong Kong, Hong Kong SAR.
3. Sumana Sanyal (2016) Lecturer and Tutor in the 7th HKU-Pasteur Cell Biology Course, Hong Kong, Hong Kong SAR.

Collaborations

1. **Caroline Demeret** (Institut Pasteur, Paris): Role of deubiquitylases in influenza virus infections.
2. **Lee Kim Swee** (BiomedX Innovation Center, Heidelberg, Germany): Ubiquitin-mediated regulation of the T-cell receptor-signaling pathway.
3. **Joseph Ashour** (Mount Sinai School of Medicine, New York, NY, USA): Manipulation of host factors in influenza and dengue infections.
4. **Adolfo Garcia-Sastre** (Mount Sinai School of Medicine, New York, NY, USA): Studying the function of Isg15 and its mode of restricting influenza virus trafficking, specifically, the efficacy of influenza NS1 in preventing ISG15 activity.
5. **Hidde Ploegh** (The Whitehead Institute for Biomedical Research, MIT, Cambridge, MA, USA): Studying host factors and their mechanism of function during influenza virus biogenesis centered on Tsg101.

Funding

1. Host factors involved in dengue infection (**Principal Investigator**; Research Grants Council / General Research Fund – Ends: 11/2016).
2. Role of Tsg101 in influenza virus infection (**Principal Investigator**; Research Grants Council / General Research Fund – Ends: 10/2017).
3. Development of therapeutic strategies against viral infections by targeting the ubiquitylation

machinery and its modulation of the host innate immune response (**Principal Investigator**; Health and Medical Research Fund – Ends: 06/2017).

4. Deciphering influenza viral polymerase interplay with host ubiquitin proteasome system in correlation with pathogenesis (**Co-principal Investigator**; Institut Pasteur – Programme Transversaux de Recherche – Ends: 06/2017)
5. Elucidating the role of Tsg101 in influenza virus assembly and release (**Principal Investigator**; Area of Excellence Control of Pandemic and Inter-pandemic Influenza – Ends: 12/2016).
6. Mechanism of Influenza NS1 mediated inhibition of interferon type-I response: effect on Isg15 (**Principal Investigator**; RGC Seed Funding for Basic Research – Ends: 06/2016).
7. Regulation of host factors in influenza virus infections through ubiquitin and ubiquitin like modifiers (**Co-Investigator**; Theme-based Research Scheme Viral, host and environmental determinants of influenza virus transmission and pathogenesis – Ends: 12/2019).

Personnel

Name	Position
Sumana SANYAL	Research Assistant Professor
Tami Jingzhu ZHANG	Technical Officer/Postdoctoral associate
Yun LAN	Technical Officer
Mingyuan LI	Technical Officer
Ivonna Ying FAN	PhD student Technical Officer (starting 01/Jul/2015)
Akhee Sabiha JAHAN	PhD Student
Lewis Yu Lam SIU	Research Technician
Joao POMBO	Research Assistant-II Mphil Student (starting 01/Sep/2016)
Mart LAMERS	Student Intern (Master student, Erasmus Medical Centre)
Dora ANGYAL	Student Intern (Medical student, Erasmus Medical Centre)
Agathe LE QUANG	Student Intern (Master student, University of Montpellier)

3.4 SOPHIE VALKENBURG Lab

NB: Dr. Sophie Valkenburg is on maternity leave from September 2015 until April 2016. The work outlined in this progress report was conducted in 2015 as a post-doctoral fellow of the Centre for Influenza Research in the laboratory of Dr. Leo Poon.

Main Objectives and Strategy

The main objectives of the lab are determining the role of protective heterologous T and B cell immunity in mouse and human systems, by investigating novel vaccines and immune correlates of protection for influenza. Our primary focus is to study adaptive immunity to influenza, and how this could be harnessed and optimized by vaccination to improve protection from diverse influenza virus infection. HA-specific antibodies can block influenza infection, whilst T cells recognize influenza-infected cells. A vaccine which ultimately combines antibody and T cell based immunity for influenza will provide a full-proof immunological barrier to influenza infection, which our studies will ultimately help develop. Our major research projects, which aim to elucidate how cross-reactive T and B cell responses to influenza provide broad immunity, are listed below.

Broadly reactive influenza vaccines in mouse models

A vaccine that is broadly protective against different strains and subtypes of influenza is needed in lieu of the current seasonal vaccine which is requires yearly update and is not protective against pandemic or outbreak strains.

(a) *A T cell based universal vaccine*: In collaboration with Liyange Perera and Thomas Waldmann at NIH, our group is determining the mechanism of protection of a Vaccinia Wyeth vaccine vector encoding 5 influenza proteins, HA, NA, NP and Matrix 1 and 2 proteins, with a molecular IL-15 adjuvant to enhance vaccine memory responses, termed Wyeth/5Flu/IL-15. The vaccine has been highly effective in mice providing protection against avian, pandemic and seasonal strains of influenza. The vaccine elicits effective influenza-specific T cell memory responses that establish early local T cell responses upon influenza challenge, significantly reducing viral lung titers and thus survival. Importantly, depletion of T cell subsets showed that memory CD4 T cell responses were necessary for vaccine mediated protection, an under appreciated role of helper subset. The vaccine is currently being investigated for use in a sequential combination regime with inactivated influenza viruses by Scarlett Yan and Olive Li within the Poon laboratory at HKU. Furthermore, the induction of influenza-specific T follicular helper cells is being determined.

(b) *An HA-stem based vaccine*: The HA protein contains a stem region with conserved conformational epitopes that are relatively conserved between different influenza strains, leading to the induction of broadly neutralizing antibodies that recognize influenza viruses of different subtypes, in some cases groups (group 1 or 2) or even both influenza A and B viruses. Using a protein minimization technique, the Varadarajan lab at the Indian Institute of Science developed a HA-mini stem recombinant protein vaccine that mimics the pre-fusion native form of the HA protein by utilizing a trimerization motif, Foldon. Both an H5 and H1 form of the vaccine, H5-Foldon and H1-Foldon, have been assessed in the mouse influenza challenge model, and found to induce broadly HA binding antibodies for different subtypes and groups. Vaccine antibodies were able to mediate protection from heterologous H5N1 challenge. The mechanism of action of HA-stem specific antibodies is being investigated and will be assessed in the context of antibody dependent cellular cytotoxicity (ADCC) and its effect on the virus life cycle.

Human correlates of protection from influenza

(a) *Protective pre-existing T cell responses*: Whilst T cell responses have been shown to be highly effective in mediating protection in mouse models, corresponding data in human influenza infection is not as robust. All adults have established influenza-specific memory T cell responses; however we have repeated infection during our lifetime that can range from mild to life threatening infection. The half-life of T cell memory and cross reactivity may explain the variability in protection from repeated infection. In collaboration with Benjamin Cowling at HKU, we aim to determine the correlation between higher baseline early effector T cell memory responses and protection from influenza infection or reduced symptom severity and viral shedding in a household transmission setting. Blood samples are obtained from infected index cases, and uninfected household contacts that are monitored for influenza transmission, at day 0 and day 28. The aim of the study is to find if there is an immunological difference between contacts positive for influenza transmission during sampling and contacts negative for influenza transmission. Sample collection has been ongoing since June 2013 due to the limited and specific nature of cases and intensity of monitoring households.

(b) *ADCC avian cross-reactivity*: H5N1-specific ADCC antibodies have been found from the blood of healthy unexposed adults, and therefore ADCC antibodies must target conserved epitopes of the HA protein. In collaboration with Joe Wu at HKU, the level of H1 and H7-specific ADCC antibodies is being probed in a large community cohort study, using archived serum from Red Cross blood collection. This study will also determine the effect of the 2009 pandemic on the level of HA-stem antibodies and their relationship with ADCC responses, as well as the pandemics relationship with increasing breadth of responses against potential outbreak strains like H7N9.

(c) *H3N2 vaccine in elderly cohort*: The 2015 Northern hemisphere winter influenza season had excess mortality in over 65 year olds due to vaccine mismatch between the H3N2 circulating strain and vaccine strain (A/Texas/50/2012). The updated vaccine containing the A/Switzerland/9715293/2013 virus became available after the peak of the influenza season. In collaboration with Yat Hang Tam at HKU, the benefit of re-vaccinating the elderly in establishing H3N2-Switzerland specific T and B cell and ADCC responses is being assessed between subjects who received one dose of the updated vaccine versus two doses of vaccine.

Achievements and Ongoing Research

Broadly reactive influenza vaccines in mouse models [Funding HMRF]

(a) *A T cell based universal vaccine*

In collaboration with Scarlett Yan (Poon lab at HKU) we have established a sequential vaccine regime, whereby mice are vaccinated with Wyeth/5flu/IL-15 in combination with inactivated influenza virus to establish both effective T cell immunity and potentially HA-stem specific antibody responses. Preliminary data from H3N2 and H1N1 infection has shown successful protection in mice, whilst the induction of broadly reactive antibodies is currently being determined from stored serum.

(b) *An HA-stem based vaccine*

The H1-Foldon and H5-Foldon vaccine were assessed in the BalbC mouse model. HA-Stem vaccine antibodies provided heterologous protection against avian, seasonal and pandemic influenza viruses, and also were protective in an adoptive serum transfer experiment. Vaccine antibodies bound a broad array of recombinant HA proteins. Whilst the HA-mini stem vaccine was clearly protective in mice with significant gains in survival, the vaccine did

not reduce viral loads. This has lead us to further studies on the mechanism of action of the HA-stem antibodies and whether they provide ADCC action is currently being determined using alpha-test kit from Promega and further in vitro experiments.

Human correlates of protection from influenza [Funding HMRF]

(a) *Protective pre-existing T cell responses*

Patient recruitment is ongoing for the household study for the winter and summer influenza seasons in Hong Kong. A 13-colour flow cytometry assay to measure influenza-specific IFNg producing early effector T cell responses is under development.

(b) *ADCC avian cross-reactivity*

A databank of age-stratified serum from the Red Cross was used to probe ADCC activity in the Hong Kong community. The level of ADCC responses towards the Avian H7N9 HA protein before and after the H1N1 2009 pandemic showed an age related increase in ADCC activity, whilst ADCC activity towards the relatively conserved internal NP protein remained unchanged.

(c) *H3N2 vaccine in elderly cohort*

In collaboration with Yat Hang Tam (HKU) and his network of field nurses, we recruited over 300 donors at three time-points (d0, d7 and d30 post-vaccination), over two vaccine seasons (May-August and October-December 2015). Peripheral blood mononuclear cells and serum were cryobanked for future T cell and ADCC assays. An NK cell line was obtained from Conkwest and Fox Chase Cancer Centre to improve the ADCC assay. The NK cell line significantly increases the number of serum samples and proteins that can be assessed in parallel whilst also streamlining the assay initially developed by Jegaskanda and Kent (J Immunol, 2013). Initial results by Isabella Chan in the Poon lab at HKU show a high level of H3-specific ADCC responses before and after vaccination with no effect of vaccination on ADCC responses. The impact of repeated vaccination in the elderly upon T cell immunity remains to be determined.

Publications

1. Fan RL, **Valkenburg SA**, Wong CK, Li OT, Nicholls JM, Rabadan R, Peiris JS, Poon LL (2015) Generation of live attenuated influenza virus by using codon usage bias. *J Virol* **89**:10762-10773.
2. **Valkenburg SA**, Mallajosyula VV, Li OT, Chin AW, Carnell G, Temperton N, Varadarajan R, Poon LL (2016) Stalking influenza by vaccination with pre-fusion headless HA mini-stem. *Sci Rep* **6**:22666.
3. Chan MC, Kuok DI, Leung CY, Hui KP, **Valkenburg SA**, Lau EH, Nicholls JM, Fang X, Guan Y, Lee JW, Chan RW, Webster RG, Matthay MA, Peiris JS (2016) Human mesenchymal stromal cells reduce influenza A H5N1-associated acute lung injury in vitro and in vivo. *Proc Natl Acad Sci USA* **113**:3621-3626.
4. **Valkenburg SA**, Josephs TM, Clemens EB, Grant EJ, Nguyen TH, Wang GC, Price DA, Miller A, Tong SY, Thomas PG, Doherty PC, Rossjohn J, Gras S, Kedzierska K (2016) Molecular basis for universal HLA-A*0201-restricted CD8+ T-cell immunity against influenza viruses. *Proc Natl Acad Sci USA*, in press.

Presentations at Meetings

1. **Valkenburg SA**, Li OTW, Peiris JSM, Perera LP, Poon LLM (2015) Universal influenza vaccine utilizes T cells for mechanism of protection as killers and coordinators. Keystone Symposia on Viral Immunity (A2), Breckenridge, USA (Poster).
2. Valkenburg SA, Mallajosyula VVA, Li OTW, Chin AWH, Carnell G, Temperton N, Varadarajan R, Poon LLM (2016) Targeting influenza by vaccination with prefusion headless HA ministem. Victorian Infection and Immunity, Lorne, Australia (Oral).

Teaching

1. Sophie Valkenburg (2015) Tutor in the 8th HKU-Pasteur Immunology Course, Hong Kong, Hong Kong SAR.

Collaborations

1. **Leo LM Poon** (The University of Hong Kong): Primary supervisor as a post doctoral fellow. Key collaborator and contributor to ongoing projects.
2. **Ragahavan Varadarajan** (Indian Institute of Science, Bangalore, India): Characterization of a headless-trimeric pre-fusion conformation HA recombinant protein vaccine in a mouse model to generate broadly reactive HA-stem antibodies.
3. **Benjamin Cowling** (The University of Hong Kong): Determining the correlation between baseline T cell responses and protection from transmission in a household transmission setting.
4. **Joseph Wu** (The University of Hong Kong): Probing ADCC antibody responses towards avian influenza viruses in the community.
5. **Yat Hang Tam** (The University of Hong Kong): Two-dose vaccine immune effect in Elderly for the H3N2-mismatch.
6. **Liyange Perera and Thomas Waldmann** (NIH, NAID, USA): Vaccinia vector H5N1 vaccine for broad T cell responses, with an emphasis on CD4 mediated heterologous protection.
7. **Katherine Kedzierska** (The University of Melbourne, Australia): Mutation rates in T cell epitopes during infection and human T cell responses towards influenza.
8. **Arthur Young** (Innvax, USA): Characterization of a lipopeptide T cell epitope vaccine in MHC-humanized mouse model.

Funding

1. Understanding alternate immune correlates of protection in household transmission of influenza (Principal Investigator; Health and Medical Research Fund – Ends: 06/2017)
2. Vaccination scheme development to stimulate both B and T cell dependent heterosubtypic protection against Influenza A viruses in mice (Co-Investigator; Health and Medical Research Fund – Ends: 06/2017)
3. Probing community susceptibility to influenza infection by measuring alternate antibodies (**Principal Investigator**; Seed Funding for basic research – Ends: 09/2016)
4. Human lung local immunity to influenza virus - role of lung antibody and resident memory T cells (**Co-Investigator**; Research Grants Council/General Research Fund – Ends: 12/2015)
5. Understanding vaccine induced T cell protection from influenza viruses (**Principal Investigator**; Health and Medical Research Fund – Ends: 06/2016)

Personnel

Name	Position
Sophie VALKENBURG DOAK	Research Assistant Professor (from 01/April 2016)
Máiréid BULL	MPhil Student (starting September 2016)

3.5 JIMMY CHUN CHEONG LAI GROUP

Main Objectives and Strategy

Our group aim to study the interactions between viruses with the host receptors, in order to have a better understanding on viral host adaptation and cell/tissue tropism. Main projects include the study of influenza virus-cell receptor interactions at the atomic level by combination of chemical, biochemical and cell biological methods; and the investigation of the interplay between different influenza surface proteins during viral infection. We are also interested in the effect of human B4GALNT2 gene expression on influenza virus infection; and the role of dipeptidyl peptidase-4 (DPP4) as the host-receptor for Middle East respiratory syndrome coronavirus (MERS-CoV). In addition, in collaboration with the department of clinical oncology, we are performing a clinical study that investigates the potential usefulness of immunotherapy as treatment of nasopharyngeal carcinoma (NPC), which is caused by a combination of environmental, genetic and viral factors, being often linked to Epstein–Barr virus (EBV) infection.

Study of influenza virus-host receptor

The objective of the study is to obtain a better understanding of the influenza viral tropism. Sialic acids are known to be the receptor molecules of influenza viruses, but the diversity of sialylated glycans is not equivalent in different animal species and organs. Therefore, it is likely that the interactions between influenza viral proteins and different sialylated glycans are involved in the viral adaptation to the host and one possible mechanism underlying species jump, e.g. from avian to human. In our study, we have produced influenza virions or virus-like particles (VLP) of different influenza subtypes and/or different viral origin. The interactions between hemagglutinin (HA), neuraminidase (NA) and a variety of sialylated glycans are being investigated using chemical methods. Functional study of the virions are also carried out on cell/tissue cultures.

Interplays between influenza surface proteins in cell receptor interactions

Influenza HA and NA are two major glycoproteins both interacting with sialic acids receptor on cell surface. It has been long recognized that a balance between HA receptor-binding and NA receptor-destroying functions is important for the influenza virulence and transmission. However, interplays between the two viral proteins were not clearly studied. In this project we aim to investigate the role of HA-receptor binding properties on the NA functions. The effect of HA inactivation on NA enzymatic activity will be tested in native virions. VLPs containing NA with or without corresponding HA will also be produced for the comparison of their NA activities.

Effect of human B4GALNT2 gene expression on influenza virus infection

B4GALNT2 is involved in the biosynthesis of human Sd^a blood group antigen by adding a terminal GalNAc side chain. Published data suggested that Sd^a glycotope is expressed in both N- and O-linked glycans and the present of Sd^a in animal tissue is a potential protective mechanism in against avian influenza viruses. Our objective is to over-express B4GALNT2 gene in different cell lines and test for their susceptibility against avian and human influenza viruses.

Immunotherapy against nasopharyngeal carcinoma

The aim of the project is to develop an effective immunotherapy treatment against Epstein-Barr virus (EBV)-associated nasopharyngeal carcinoma (NPC), which, differently from Western countries, is endemic in southern China, including Hong Kong. EBV is present in

virtually all poorly differentiated and undifferentiated nonkeratinizing NPC (type II and III, according to the WHO classification), making the viral antigens expressed by tumor cells attractive targets for immunotherapy. Our strategy is to generate LMP/EBNA1-specific T cells from PBMC isolated from NPC patients using an adenoviral vector. The safety and efficacy of expanded T cells can be assessed upon adoptive CTL infusion as immunotherapy.

Achievements and Ongoing Research

In 2015, we have continued our exploration of the basis of influenza receptor specificity and the role O-linked sialylated glycans in influenza viral infection. We also developed a methodology for the inactivation of HA in influenza viruses, which led to our new finding that influenza NA activity is affected by the functionality of HA.

Study of influenza virus receptor [Funding: HMRF, RGC, URC]

(a) *The role of O-linked sialylated glycans in influenza viral infection*

Cell surface sialosides contain both N-linked and O-linked glycans. N-linked sialosides were found to be important in the influenza viral infection whereas the role of O-glycans as influenza receptor remains unclear. In our work, histochemical analysis using lectins and antibodies indicating the presence of sialylated-O-glycans in MDCK and A549 cell-lines, as well as human lung and bronchial tissues.

Efficient influenza infections were observed in GnT1-deficient cells in which maturation of N-glycans is blocked, while reduced infection were observed in cells pretreated with GalNAc-O-Bn that inhibit the O-glycan synthesis. These data indicate the biologically relevant role of sialylated O-linked glycans in influenza virus infections. NMR spectroscopy study on influenza HA interacting with O-glycans analogues also reflected significant binding of HA to these glycans. However, we observed a variation in binding affinity to the O-glycans when different influenza subtypes were included in the study. To investigate the role of sialyl-O-glycans during influenza virus cell attachment and infection, virus neutralization assays were performed in the presence of sialyl-N- and O-glycans at various concentrations. Our data reveal a significant difference between H1N1 and H3N2 influenza virus subtypes in their IC50 values against sialylated O-glycans. A manuscript describing these finding is in preparation.

(b) *Molecular determinants of HA and NA affecting H9N2 virus tropism*

H9N2 infection is one of the most widespread influenza viruses in poultry in Asia and has transmitted occasionally to swine and human. We hypothesize that poor transmission between humans by H9N2 viruses may depend on NA not being well adapted for human airway. Our role in this project is to investigate the binding and cleavage activities of N2 neuraminidase using enzymatic methods coupled with NMR spectroscopy, using the above described approach. Virus-like particles containing N2 have been engineered but the yield was unsatisfactory therefore native virions were used instead. In order to eliminated the HA binding to sialic acids during the study of NA, a novel methodology using acidic pH to inactivated HA receptor-binding function has been developed. At low pH, HA undergo irreversible conformational change and no longer binds to sialic acids. Different sialylated glycans were included in the study especially those contain the two different sialic acid species, Neu5Ac and Neu5Gc, and those with the two different sialic acid linkage, alpha2-3 and alpha2-6. We found that NA from H9N2 has weak binding and enzymatic activity against Neu5Gc, indicates a poor adaptation for the mammalian system. These NA also display lower sialidase activity against alpha2-6 linked sialylated glycans with compared to human seasonal influenza viruses, reflected the H9N2 virus remains avian-like influenza.

However, sialic acid binding affinity to the hemadsorption site (which is conserved in avian influenza viruses) of the tested H9N2 is lower than other avian influenza. In summary, our data suggest that the NA of H9N2 viruses is not well adapted for mammalian system, and the HA inactivation methodology developed during this study could become a useful tool in influenza research.

(c) *Study of the emerging H7N9 influenza*

Zoonotic H7N9 disease is a lurking threat to public health but the major question is whether this virus poses a risk of acquiring transmission potential in humans (pandemic potential). We would like to perform a risk assessment of the H7N9 virus. *In vitro* and *ex vivo* viral tropism, and the ability of viral replication in mammalian cells by H7N9 viral polymerase complex were investigated by Michael Chan's group at HKU. Pseudotyped viruses containing H7 hemagglutinin were successfully constructed for the study of virus-receptor interactions. Potential gain-of-function or loss-of-function amino acid mutations were introduced into the H7 hemagglutinin to investigate the risk posed by such mutation in the process of viral adaptation to humans. To overcome safety concerns, pseudotyped viruses were utilized instead of replicative virions, therefore eliminating any ethical consideration linked to performing experiments that could fall into the category of Dual Use of Research Concern. Studies using *in vitro* pseudotyped viral infections suggest an increased infectivity upon the potential gain-of-function single amino acid mutations. These wild-type and mutant pseudotyped viruses were tested for infections in *ex vivo* human cultures but the signal produced were below the detection limited and. Therefore, alternative methodology using fluorescence VLP is being tested. NMR spectroscopy showed that wild-type H7N9 HA binds similarly to both avian and human sialic acid receptor analog, however further funding is required for similar study on HA with potential gain-of-function and loss-of-function mutations.

(d) *Comparison of native influenza virus and virus-like-particles in their receptor-binding properties*

Our group has previously developed non-infectious influenza virus-like-particle (VLP) containing influenza HA or NA as tools for influenza study. Together with the NMR technology, these VLPs have been applied to address HA/NA functions, resulting in a series of publications. However, the similarity between native virions and VLPs in virus-receptor interaction has not been thoroughly investigated. In order to validate previous and prospective data obtained using VLP, a systematic study comparing VLP and native virions on their receptor interactions was performed and no significant difference were observed in receptor specificity using HAs from both human and avian influenza isolates. In addition, we tested for the possibility of using inactivated virus for the receptor study as the data obtained will be closely comparable to natural infections. Native H9N2 and H1N1 virions were inactivated by UV treatment or aldehyde fixation. We found that both HA and NA functions were significantly reduced by UV irradiation or 4% paraformaldehyde treatment and HA-receptor binding were abolished upon glutaraldehyde fixation. However, treatment of viruses in 0.2% paraformaldehyde overnight had minimum effect on both HA and NA, an important finding that can be exploited to use inactivated virus for future receptor studies of influenza.

Interplays between influenza surface proteins in cell receptor interactions [Funding: HMRF, RGC, URC]

In a preliminary study of neuraminidase inhibition antibodies in patient sera, we observed a difference in NA activity level between NA proteins on VLP surface and native virons. Using fetuin as a substrate, NA on VLP surface displayed a lower enzymatic activity when

compared to the same amount of NA on the whole virus. However, this phenomenon was not observed when using methylumbelliferone N-acetylneuraminic acid (MUNANA) as the substrate. We hypothesize that HA binding to complexes sialylated glycans (e.g. fetuin) promotes the NA-substrate recognition, thereby enhancing its enzymatic activity. H1N1pdm viruses were treated in acidic pH to remove the HA function and their NA activity were compared with viruses treated in neutral pH. NA activity against fetuin was largely reduced after HA inactivation but only minor reduction was detected using MUNANA as the substrate, which supports our hypothesis. VLPs containing NA with or without corresponding HA were also produced to compare their NA activities and we found that the presence of HA significantly enhanced NA cleavage of fetuin. We will further investigate the effect of HA avidity and specificity on the NA activities if extra funding will be available. A manuscript describing these finding is in preparation.

Effect of B4GALNT2 gene expression on influenza virus infection (Preliminary study)

B4GALNT2 catalyzes the last step in the biosynthesis of the human Sd^a blood group antigen through the addition of an N-acetylgalactosamine residue via a beta-1,4 linkage to a subterminal galactose residue substituted with an alpha-2,3-linked sialic acid. In order to test our hypothesis that high expression of B4GALNT2 acts a protective mechanism against avian influenza virus infection, MDCK cells stably expressing human B4GALNT2 gene were derived and our preliminary data show a decrease of viral titers at 24 hours after avian virus infection, when compared to parental MDCK cells. Details of the protective effect upon B4GALNT2 expression will be further investigated by Wong Ho Him as part of his MPhil project.

Immunotherapy against nasopharyngeal carcinoma [Funding: Ester Lee and Chew Pik Foundation, Croucher Foundation]

NPC is endemic in China and Southeast Asia where it is tightly associated with infections by EBV. The role of tumor-associated viral antigens in NPC renders makes them promising candidates for cellular immunotherapy. In earlier preclinical studies, a novel adenoviral vector–based vaccine termed AdE1-LMPpoly has been generated; it encodes EBV nuclear antigen-1 (EBNA1) fused to multiple CD8+ T-cell epitopes from the EBV latent membrane proteins, LMP1 and LMP2. Our group has previously reported data of an early phase I clinical trial using AdE1-LMPpoly as an immunotherapeutic tool for EBV-associated NPC (*Cancer Res* 72:1116–1125). Twenty-four NPC patients were selected and EBV-specific T cells were successfully expanded from sixteen patients. Transient increase in the frequencies of LMP1&2- and EBNA1-specific T-cell responses was observed after adoptive CTL transfer and the median overall survival compared with patients who did not receive T-cell therapy increased from 220 to 523 days. We finished the phase I clinical trial in2015 with a total of fifty-two NPC patients involved in which the CTL infusion is shown to be safe and well tolerated. Phase II clinical trial will be performed upon approval by the review board. At present there is no facility present in Hong Kong that allows the expansion of T-cells for adoptive therapy in a GMP accredited standard. As a preparation for the phase II clinical trial, a physical facility will be established to initiate the process of expansion of T-cells here in Hong Kong and I will be trained in the laboratory of Rajiv Khanna (Queensland Institute of Medical Research, Australia) to (*i*) establish criteria to identify a location where T-cells can be expanded in a safe environment; (*ii*) provide training in compliance with Australian/Hong Kong regulations regarding cell based therapy; (*iii*) transfer know-how and protocols to expand donor T-cells and test for CTL efficiency.

Publications

1. Mayr J, Lau K, **Lai JC**, Gagarinov I, Chan RW, von Itzstein M, Nicholls JN, Haselhorst T (2016) Unraveling the role of O-glycans in influenza virus infections. *In preparation*.
2. **Lai JC**, Herath MT, Peiris JS, Nicholls JN (2016). Influenza virus hemagglutinin binding to sialic acid receptor enhance the virus neuraminidase activity in complexes sialylated glycans. *In preparation*.

Seminars, Invited Lectures and Oral Presentations

1. Jimmy Lai (2015) The role of O-glycans in influenza infection. Department of Pathology, The University of Hong Kong, Hong Kong SAR.

Collaborations

1. **Xuechen Li** (Department of Chemistry, The University of Hong Kong): Molecular determinants of influenza virus tropism and binding; expertise in glycan synthesis, to produce glycans of interest as influenza receptor analogues.
2. **Guang Zhu** (Division of Life Science, The University of Hong Kong Science and Technology): Access to equipment and technical support regarding NMR spectroscopy.
3. **Mark von Itzstein and Thomas Haselh**orst (Institute for Glycomics, Griffith University, Australia): Study of O-linked sialylated glycans and synthesis of O-glycans analogue.
4. **Michael Chan** (School of Public Health, The University of Hong Kong): Comparison of native influenza virus and virus-like-particles in their receptor-binding properties using the *ex vivo* human culture model.
5. **Dora Kwong** (Department of Clinical Oncology, The University of Hong Kong ong): Clinical trials of immunotherapy against EBV-associated NPC.
6. **Rajiv Khanna** (Department of Immunology, Queensland Institute of Medical Research, Australia): Immunotherapy against EBV and technology transfer to develop methods of T cells expansion.

Funding

1. Molecular determinants of H9N2 virus hemagglutinin and neuraminidase affecting virus tropism for the human and swine respiratory tract (**Co-Investigator**; Health and Medical Research Fund – Ends: 10/2015).
2. Risk assessing human transmission potential of H7N9 viruses using ex vivo cultures of the human respiratory tract (**Co-Investigator**; Commissioned Studies on Emerging Influenza A Viruses with Epidemic Potential RRG-03 – Ends: 11/2015).
3. Comparison of native influenza virus and virus-like-particles in their receptor-binding properties (**Principal Investigator**; Small Project Funding – University Research Committee – Ends: 10/2015).
4. Immunotherapy against nasopharyngeal carcinoma (**Co-Investigator**; Ester Lee and Chew Pik Foundation, Croucher Foundation and other donors – Ends: open).

Personnel

Name	Position
Jimmy Chun Cheong LAI	Postdoctoral Fellow (Joint Appointment with the Department of Pathology in the Nicholls Lab)
Thusitha Kumara K. HERATH M.	PhD student
Ho Him WONG	Research Assistant MPhil student (starting 01/Jan/2016)

3.6 ROBERTO BRUZZONE and MALIK PEIRIS GROUPS

This section summarizes the main results of research work done by PhD students under the supervision of Roberto Bruzzone and by HKU-PRP staff on MERS coronavirus under the supervision of Malik Peiris.

Main Objectives and Achievements

We focus here on current projects investigating the dynamics of host-pathogen interactions with dengue and influenza viruses. Work on MERS coronavirus in camels and humans has led to the development of a safe diagnostic test for sero-epidemiological studies and has resulted in the publications of ten manuscripts in 2015.

Role of Cyclin D3-influenza A Virus M2 Ion Channel Interaction in Influenza Virus Infection and Pathogenesis [Funding: Research Fund for the Control of Infectious Diseases and AoE/M-12/06]

To identify new host factors that modulate the replication of influenza A virus, we had previously performed a genome-wide yeast two-hybrid screen using as a bait the cytoplasmic tail (CT) of viral matrix protein 2 (M2), which has the longest CT among the three virus envelope proteins. The screen revealed a high-score interaction of M2with cyclin D3, a key regulator of cell cycle G1/S transition. M2–cyclin D3 interaction was validated through GST pull-down and recapitulated in influenza A/WSN/33-infected cells. Knockdown of *Ccnd3* by small interfering RNA significantly enhanced virus progeny titers in cell culture supernatants. Being a key regulator of cell cycle progression, *Ccnd3* knockdown by default leads to cell cycle G0/G1 phase arrest, providing a favorable environment for influenza A virus replication. However, we have obtained several lines of evidence to support that the increase in virus production was due to cyclin D3 deficiency *per se*, and not merely to deregulation of the cell cycle. Thus, a combined knockdown of *Ccnd3* and *Rb1*, which rescued cell cycle progression into the S phase, failed to normalize virus production. By separating cyclin D3 from its cell cycle activity, we determined that increase in virus production in its absence was not commensurate with a mere inhibition of cell cycle progression. Our study describes the identification and characterization of cyclin D3 as a novel interactor of influenza A virus M2 protein and a restriction factor of influenza A virus infection. The role of cyclin D3 in the context of influenza infection has not been described previously. More interestingly, our results suggest a novel function of cyclin D3 that is beyond its classical function in cell cycle regulation.

We speculate that the inhibitory effect of cyclin D3 on influenza A virus infection is due to its interaction with viral M2 protein, as measured by competitive co-immunoprecipitation assays. It is possible that cyclin D3-M2 interaction either masks the domain on M2 mediating binding to M1, or has a higher affinity than that between M1-M2. An alternative explanation is that cyclin D3-M2 interaction sequesters the amount of available M2. Consequently, limited-M1–M2 binding during the budding process of influenza A virus infection results in less progeny virions being efficiently packaged and released from host cells. Co-evolution and adaptation of virus-host interactions is best described as a zero-sum biological arms race. However, owing to high mutation rates, viruses such as influenza are well equipped to rapidly evolve strategies to evade host restriction factors. Consequently, in case of influenza A virus infection, the effect of cyclin D3 is successfully antagonized by M2, which redirects cyclin D3 from the nucleus to the cytosol followed by proteasomal degradation, thereby granting an advantage to virus production. Our study

provides important insights into the mechanism through which cyclin D3 restricts influenza A virus infection.

This work has been carried out by Ivonna Ying Fan, who successfully defended her PhD thesis in 2015. Ivonna has been offered a 3-year postdoctoral fellowship from MRC to join the lab of Dario Alessi (University of Dundee, UK), one of the world's leading laboratory in cellular signaling. A manuscript describing the results of this project is in under revision.

Invovlement of C-type lectin receptor CLEC5A in influenza virus pathogenesis

In conclusion, we have identified CLEC5A as a novel host factor implicated in the modulation of IAV pathogenesis. This project was part of the PhD work of Ooiean Teng, who was jointly supervised by Roberto Bruzzone and Hui-Ling Yen, Assistant Professor in the School of Public Health, and successfully defended her thesis in October 2014. A manuscript has been submitted.

Characterization of MERS coronavirus in camels and humans [Funding: National Institute of Allergy and Infectious Diseases, National Institutes of Health; European Community Seventh Framework Program]

Middle East respiratory syndrome (MERS) is an emerging respiratory disease of global public health concern, which has been widely disseminated by travelers from the Middle East to North America, North Africa, Europe and Asia. To date, 26 countries have reported cases of MERS-CoV and WHO has been notified of 1,684 laboratory-confirmed cases of infection with MERS-CoV having caused more than 600 deaths related since September 2012. Saudi Arabia is the most affected country, but South Korea has also known a severe outbreak in 2015.

The current challenge is to contain the epidemic by preventive actions and get research efforts under way to better understand virus pathogenesis, fill the gaps in understanding transmission in the animal reservoir and from animals to humans. Seroepidemiology is a precious tool to investigate the sources of zoonotic transmission. HKU-PRP and their partners have conducted several studies to investigate the MERS-CoV seroprevalence in dromedary (one hump on the the back) camels in order to have a better understanding of the geographical distribution of MERS-CoV in its suspected animal reservoir using a novel pseudoparticle neutralization assay that doest not require Biosafety Level 3 containment and, therefore, is well adapted for large-scale seroepidemiology studies. We have now systematically investigated potential cross-reactions that may confound the use of this and of the standard microneutralization assay in seroepidemiological studies in animals. Our previous data demonstrate that these two serological assays are free of cross-reaction with Bovine coronavirus and other coronavirusesand, therefore, can be used with confidence in seroepidemiological studies to identify animal species that may serve as reservoirs or vectors of MERSCoV.

We have found high seroprevalence of MERS-CoV in dromedary camels in Egypt, Saudi Arabia and Nigeria. Studies in Mongolia showed that the Bactrian camels (two humps) appear to be seronegative. We also tested archived dromedary sera in Saudi Arabia and Australia. While dromedaries were seronegative in Australia, our study revealed that MERS-CoV has been circulating in camels in the Arabian Peninsula for at least several decades and it is not a newly emerged virus from camels.

Apart from seroepidemiological investigations, wetook part in a clinical study in collaboration with the First Affiliated Hospital of Guangzhou Medical University and

reported the case of the MERS patient from South Korea, infected during the outbreak, who traveled to Hong Kong and China where he was hospitalized. This study has provided a detailed and serial data on clinical progression, viral load in different clinical specimens, plasma cytokine levels and serum antibody responses, comparing multiple serological assays and T-cell responses. This case report is one of the most intensively investigated patients with MERS thus far; it highlights the importance of sputum collection for diagnostic accuracy, and further confirms that sensitibity and specificity of the pseudotype neutralization assay, which does not require a Biosafety Level 3 containment.

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Seminars, Invited Lectures and Oral Presentations

1. Roberto Bruzzone (2015) International Conference on Scientific Insight and Response of Ebola Virus Disease; Beijing, PR China.
2. Roberto Bruzzone (2015) Oxford University/Mahidol Oxford Tropical Medicine Research Unit; Bangkok, Thailand.
3. Roberto Bruzzone (2015) Global Health Risk Framework: A Workshop on Research and Development of Medical Products; Hong Kong, Hong Kong SAR.
4. Roberto Bruzzone (2015) City University of Hong Kong; Hong Kong, Hong Kong SAR.
5. Malik Peiris (2015) Courage Fund Infectious Disease Conference; Singapore.
6. Malik Peiris (2015) 3rd International One Health Congress; Amsterdam, The Netherlands.
7. Malik Peiris (2015) One Health Master Class - SEA-EU-NET 2015; Bangkok, Thailand.
8. Malik Peiris (2015) 1st International Meeting on Respiratory Pathogens; Singapore.
9. Malik Peiris (2015) 10th Asia-Pacific Congress of Medical Virology; Taipei, Taiwan.
10. Malik Peiris (2015) National University of Singapore; Singapore.
11. Malik Peiris (2015) XVII International Symposium on Respiratory Viral Infections; Vancouver, Canada.

Presentations at Meetings

1. M Li, K Kwok, L Siu, JS Zhang, R Bruzzone, PG Wang (2015) Molecular Dissection of Dengue Virus Egress: Involvement of KDEL Receptors. *Gordon Research Conference "Infections of the Nervous System"*, Hong Kong, Hong Kong SAR (Poster).
2. Fan Y, Mok CK, Zhang Y, Nal B, Kien F, Bruzzone R, Sanyal S (2016) Regulation of host factors in influenza virus infections through ubiquitin and ubiquitin like modifiers. *International Meeting on Respiratory Pathogens*, Singapore (Oral).

Teaching

1. Roberto Bruzzone (2015) Introduction to the Art and Science of Medicine Block – Problem Based Learning (MBBS Year 1), The University of Hong Kong, Hong Kong SAR.
2. Roberto Bruzzone (2015) CMED6227 – Biological Basis of Disease (Master of Public Health students), The University of Hong Kong, Hong Kong SAR.
3. Roberto Bruzzone (2015) Course Director, Molecular Biology of the Cell Course (Institut Pasteur, Paris, France).
4. Roberto Bruzzone (2015) Course Director, 1st Croucher Summer Course in Advanced Imaging: From Systems Biology to Single Cell & Single Molecule Analysis.
5. Roberto Bruzzone (2015) Course Director, 8th HKU-Pasteur Immunology Course.
6. Roberto Bruzzone and Malik Peiris (2015) Course Directors, 12th HKU-Pasteur Virology Course.
7. Malik Peiris (2015) CMED6104 – Emerging infectious diseases: the "One Health" concept (Master of Public Health students), The University of Hong Kong, Hong Kong SAR.

Collaborations

1. **Ziad A Memish** (College of Medicine, Alfaisal University, Riyadh, KSA), **Gazi Kayali** (St. Jude Children's Research Hospital, Memphis, USA) and **Maged G Hemida** (King Faisal University, Al Hofuf, KSA): MERS coronavirus in animals and humans.

Funding

1. Role of Cyclin D3-influenza A Virus M2 Ion Channel Interaction in Influenza Virus Infection and Pathogenesis (AoE/M-12/06).
2. Characterization of MERS coronavirus in camels and humans (NIAID, National Institutes of Health and FLUPIG, 7th Framework Program of the European Union).

Personnel

Name	Position
Roberto BRUZZONE	Professor
Malik PEIRIS	Professor
Ranawaka A.P.M. PERERA	Postdoctoral Fellow (Peiris Lab)
Mingyuan LI	Technical Officer (Sanyal Lab)
Ooiean TENG	Research Associate (Yen lab)
Ivonna Ying FAN	PhD student (Sanyal Lab)
Akhee Sabiha JAHAN	PhD Student (Sanyal Lab)
Horace Hok Yeung LEE	PhD student (Chris Lab)
Gabriel BENET	Scientific Officer (International Volunteer of the French Ministry of Foreign Affairs)
Lewis Yu Lam SIU	Research Technician (Sanyal Lab)

3.7 BARBARA GAYRAUD-MOREL (Visting Scientist from the Institut Pasteur)

Barbara Gayraud-Morel a senior stem cell biologist from the Institut Pasteur working in the laboratory of Shahragim Tajbakhsh *(Department of Developmental Biology and Stem Cells*) has been seconded to HKU-PRP and has been appointed as Honorary Research Associate under the School of Public Health until February 2019. Stem cell biology will be one of the two priority research areas of the newly formed School of Biomedical Sciences. The presence of Barbara Gayraud-Morel at HKU-PRP gives us an opportunity to further develop our synergies with HKU. In collaboration with the Stem Cell and Regenerative Medicine Consortium and the Dr Li Dak-Sum Research Centre on Regenerative Medicine, HKU-PRP will begin to work towards developing a human lung epithelium model from inducible Pluripotent Stem Cells (iPSC). Barbara Gayraud-Morel is interested in developing tissue engineering with stem cells. Her extensive experience in stem cell transplantations will allow her to perform functional tests of engineered lung epithelium followed by transplantation into the mouse. We present below a brief introduction on stem cell biology and outline the background for the proposed project.

Stem cells

Stem cell research has generated a broad interest in the scientific community since stem cells were found to represent the cellular identity allowing growth and repair of most tissues during normal homeostasis or after injury. It has also attracted the general public attention since stem cells are presented as the key for regenerative medicine that should impact our health and wellness. Stem cells research commonly distinguishes two broad types of stem cells, the embryonic stem cells and the adult stem cells. Recently, the emergence of iPSCs (inducible Pluripotent Stem Cells) brought a third category allowing the generation of cells with embryonic stem cells properties from differentiated adult cells.

Embryonic stem cells (ESC) are undifferentiated cells derived from pre-implantation stage embryos. In the embryo, they develop into cells and tissues of the three primary germ layers. Embryonic stem cells can be derived in vitro from the inner cell mass of the embryo and cultured for a prolonged period of time without differentiation under defined culture conditions. *In vitro*, they can differentiate stochastically or be directed to a specific differentiated cell type. Identification of molecular factors and the time window of their action on embryonic stem cells to induce a homogeneous differentiation toward a specific cell type has a fundamental importance since it is the key of their potential use in regenerative medicine.

Adult stem cells have been identified in many tissues. They are characterized by their capacity to self-renew and to differentiate in a functional cell type of the tissue of origin. They represent a small population within a tissue and the difficulty to identify them relies often on the characterization of specific transcription factor expression or cell surface markers. The function of adult stem cells is to sustain the replacement of cells during normal tissue homeostasis or cell loss after an injury. The contribution of stem cells in a given tissue is highly variable. The demand can be high like in the small intestine or the skin where the epithelium is entirely replaced within 3-5 days and 28 days respectively. In contrary, some tissues that have a low turn over, like the skeletal muscle or the lung, which require less stem cell contribution to maintain homeostasis. Finally, some organs like the heart or the brain contain some stem cells but they have a very limited ability to regenerate the tissue after damages. Stem cells reside in a microenvironment, called the niche, which maintain their stemness and influence their properties. The niche can be defined by several factors like for example, cell-cell or cell-extracellular matrix interactions. The destruction

of the niche by an injury triggers stem cell activation and initiate the regeneration. The identification and characterization of the niche for many stem cells is still poorly defined and need further investigations.

iPSCs are adult cells which have been reprogrammed by the forced expression of "stemness" factors which give them property-like embryonic stem cells. iPSCs are pluripotent and therefore able to differentiate into all cell types of the body, except extra-embryonic tissue. Like ESC they can be maintained undifferentiated in culture and expanded in large scale. iPSCs have been generated from mouse and human adult cells. The discovery of iPSCs has open even wider the scope of application of stem cells in regenerative medicine. Indeed, iPSCs derived from the patient to treat circumvent the risk of immune rejection associated with cell therapy, representing a critical advantage compare to ESC. Although, recent results from the first human iPSCs clinical trial performed in Japan for retinal repair is challenging this aspect of autogenic cell therapy. Nevertheless, iPSCs or ESCs rise several challenges which need to be addressed to generalize their use, tumorigenicity being one of them. Aside from potential use for therapeutical applications, iPSCs can be derived from patients with any type of pathology and can provide a new source of in vitro disease modeling for academic research and pharmaceutical industries to develop new therapeutic agents and perform high-throughput drug screening.

Satellite cells: the adult skeletal muscle stem cells

Satellite cells are the principal cell type assuring postnatal skeletal muscle growth and regeneration and are therefore identified as the stem cell of the skeletal muscle (Relaix and Zammit, 2012). Satellite cells reside between the plasmalemma of the myofibre and the surrounding basement membrane. The elements composing their niche are still poorly defined. In a resting adult muscle, satellite cells are quiescent and express the transcription factor Pax7. After injury, satellite cells get activated, re-enter cell cycle, proliferate and differentiate before to fuse into new myofibres. During the myogenic differentiation they will express sequentially the four Myogenic Regulating Factors (MRFs), *Myf5*, *Myod*, *Myogenin* and *Mrf4*.

Achievement and Ongoing Research

Research projects developed in Shahragim Tajbakhsh's laboratory aim to characterize stem cells and their progenitors in the skeletal muscle during embryonic, fetal and post-natal development, to understand muscle tissue growth, regeneration and ageing.

My research interests are focused on factors and regulating adult muscle stem cells, the satellite cells. I have previously studied the function of the myogenic determination gene *Myf5* during homeostasis and regeneration. We have shown that the Myf5 protein is expressed at different level within the quiescent satellite cells and participates to the population heterogeneity and that regeneration is perturbed in *Myf5* null mice after an acute muscle injury (Gayraud-Morel et al., 2007). Using mouse models modified at the *Myf5* locus, we surprisingly revealed by transcriptomic analysis that *Myf5* heterozygous cells were more primed for myogenic commitment. Paradoxically, transplantation of these cells in a regenerating muscle resulted in normal myofibre regeneration but also in a higher self-renewal capacity compared to wild-type stem cells (Gayraud-Morel et al., 2012). Overall, these results show that Myf5 is participating to satellite cell heterogeneity and that transplantation experiments unveiled cell fate properties of *Myf5* haploinsufficient cells.

To further investigate satellite cell heterogeneity I performed large and challenging serial transplantation assays of satellite cell sub-populations. Taking advantage of the *TgPax7nGFP* mouse model, Pax7Hi cells, 10% of satellite cells expressing the higher level

of *Pax7*, and Pax7Lo cells, 10% of cells expressing the lower level of *Pax7*, have been isolated and characterized. Pax7Hi quiescent satellite cells are transcriptionnally less primed for commitment, have a lower metabolic status and perform asymmetric DNA segregation (Rocheteau et al., 2012). Still, both populations were able to sustain 6 rounds of serial transplantation. Interestingly, Pax7Hi cells tended to yield Pax7Hi cells whereas pax7Lo cells gave less frequently Pax7Hi cells. Likewise, using transplantation assays, we were able to support the stemness character of the satellite cells (Sambasivan et al., 2011) or to investigate in vivo the myogenic potential of mESC differentiated in vitro under defined culture conditions (Chal et al., 2015).

More recently, in collaboration with the Michel Cohen-Tannoudji's laboratory (Institut Pasteur, Paris), I have investigated the role of *Notchless* (*Nle*), a gene involved in the formation of the large ribosomal sub-unit, during myogenesis. Interestingly, the understanding of ribosome functions has recently evolved and ribosomes are no longer considered as constitutive translational machinery but rather as complexes which provides a new level of regulation of gene expression. Previous works have demonstrated the importance of *Nle* in the maintenance of hematopoietic (Le Bouteiller et al., 2013) and intestinale (Stedman et al., 2015) stem cells. Given these findings, we were interested in determining whether *Nle* plays a role during myogenesis where distinct cell states including quiescence, proliferation, differentiation and self-renewal can be analysed. To address this question we used a conditional knock-out mice where the *Nle* deletion can be achieved ubiquitously with the $R26^{CreERT2}$ mice or specifically in muscle stem cells using the $Tg{:}Pax7\text{-}CreER^{T2}$ mice. We used the constitutive HSA-Cre (Human Smooth muscle Actin) recombinase to delete *Nle* in differentiated myofibres. The results show that in absence of *Nle*, consecutively to an injury, ribogenesis is impaired and the proliferation of muscle stem cells is compromised on a p53 dependent pathway. However, *Nle* seems dispensable for quiescent stem cells and differentiated myofibres, which support the hypothesis that different procedures are required to assemble functional ribosomes depending on the cell state. These results are currently analyzed to be submitted for publication.

Perspectives/futures directions at HKU-PRP

As a stem cell biologist, I bring my expertise to HKU-PRP where several groups work on different infectious diseases. My goal is explore new interactions that can emerge between stem cell biology, tissue regeneration, and infection. Indeed following a physical trauma or a chronic/acute infection, an inflammatory response results to restore the tissues to homeostasis. In both situations, failure to recover from the insult results can lead to chronic pathologies. These domains of research have been traditionally studied in isolation from one another, yet common elements define the host response. While settling in Hong Kong, I have had an opportunity to discuss with the research groups at HKU-PRP. I plan to explore possible links, given also the strong initiatives in HKU to establish a stem cell core in the context of the clinic. One possible avenue to investigate involves the use of human stem cells or iPSCs to develop a tissue model for viral infection studies.

In parallel, I will take advantages of my visiting scientist position in Hong Kong to establish collaborations with leading labs involved in stem cell research where whole transcriptome single cell studies are being performed as well as tissue engineering is well established
The laboratory of Tom H Cheung at the Hong Kong University of Science and Technology. Tom Cheung works on muscle stem cells, with a major focus on pathways controlling of satellite cell quiescence and stem-cell mediated tissue repair;
The laboratory of Ron Li inthe Stem Cell and Regenerative Medicine Consortium at the University of Hong Kong University. Ron Li's lab (located in the same building as HKU-PRP) is dedicated to cardiac stem cell research. He has developed a mini-heart model from hES

and iPSCs cells which mimics heart physiology. I expect to benefit from this expertise to engineer an artificial contractile skeletal muscle tissue made from mouse or human muscle stem cells.

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3.8 Teaching and Education

HKU-Pasteur Courses

The main objective of our educational pillar is to further develop an advanced teaching program in life sciences that will train a highly selected group of students who will be at the forefront of biomedical research in their countries. Our courses are extremely competitive and comparable in quality to that of established benchmarks, such as EMBO and Cold Spring Harbor courses and, therefore, are solidifying the reputation of HKU-PRP and Hong Kong as the premier regional hub for education. Some figures help to illustrate the overall impact of the program in Hong Kong. In 2015 we received more than 170 applications from over 25 countries; 69 students with global geographic representation were selected for participation. Each course includes a full-time senior faculty member of HKU, as well as of Institut Pasteur in the organizing committee, thereby strengthening the ties between the two institutions. In 2015 we have assembled an outstanding faculty, including members of the National Academy of Sciences USA and/or EMBO, HHMI Investigators, Professors at College de France and Fellows of the Royal Society. Many lectures are open to the scientific community of HKU and HK. More than 20 lectures were given as open seminars in 2015, and an open symposium (over 150 registered participants) featuring seven overseas speakers was organized at the end of the Croucher Summer Course (**see Annex 5.7 for a complete list of the 2015 speakers**).

HKU-PRP teaching approach is unique in Hong Kong and in the region as we bring together students from all over the world, coming from countries with markedly different resources to train their students. The Virology course has been held for the 12th consecutive year (**see Annex 5.3**) and the Immunology course has reached the 8th edition (**see Annex 5.5**). In 2015 we have innovated by partnering with School of Biomedical Sciences and the Faculty Core Facility to organize the first Summer Course on Advanced Imaging, sponsored by the Croucher Foundation (**see Annex 5.4**). As systems biology, combined with high throughput approaches, has deeply influenced our understanding of cell biology, a strong interest has emerged in the ability to assess and interrogate cellular functions at the level of the single cell and the single molecule. This course and symposium in advanced imaging have provided ample opportunities for close interaction between the oversea experts and local investigators to foster research collaboration and development of single-molecule and single-cell imaging in Hong Kong. The course will be repeated in 2017 and 2019. Full reports of all events have been separately provided to the Advisory Committee.

HKU-Pasteur courses are supported with external grants that are received, on a competitive basis, from Institut Pasteur International Network, the Li Ka Shing Faculty of Medicine at HKU, the Croucher Foundation, the French Consulate and other private donations. Our funds cover advertising costs, travel and accommodation for all lecturers (except from industry). Selected students are expected to pay for their travel costs. Registration fees (HKD 1,000) include tuition, all course materials, accommodation (on sharing twin basis for overseas participants) and food (lunch and coffee breaks).

Additional teaching and training

Directors and Group Leaders at HKU-PRP are teaching in the Problem-Based Learning modules for MBBS students and are all actively involved in the HKU-Pasteur course series. Robero Bruzzone started a new course for MPH students, entitled "Biological basis of diseases" (CMED6227). Malik Peiris teaches "Emerging infectious diseases: the One Health

concept" (CMED6104). Two postgraduate students (Ivonna Fan YING, PhD and Tsz-Fung YIP, MPhil) have successfully defended their thesis in 2015. HKU-PRP regularly hosts undergraduate/postgraduate students from overseas institutions for internships. In 2015 we welcomed Mart Lamers from Erasmus Medical Center (Rotterdam, The Netherlands) as a final year Master student for his research project. His outstanding contributions have resulted in two publications and the establishment of collaboration between the Chris Mok lab and one of the top institutions in virus research. In addition, we had three undergraduate summer students from University College London (Elaina Sylvie Cayrouse and Elizabeth Catherine Vacher) and Imperial College London (Megan Hei Tung Chan). For the past three years Chris Mok has supervised each summer, from June to August, one internship students from the Institute of Vocational Education (IVE).

We have continued the L'Oreal Research Scholarship scheme, which we have established in 2013, to support short-term scholarships at the Institut Pasteur (http://loreal-scholarship.com/). The Scholarship is concretely helping to create a very solid bridge between Hong Kong and France by providing selected candidates having demonstrated a strong scientific potential with a unique opportunity to be exposed to the rich cultural environment in Paris through research work at the Institut Pasteur. Two of the awardees, Sarah Wong and Candice Cheng, have been hired as postdoctoral fellows at the Institut Pasteur, a very significant outcome in line with the stated goals of the scheme.

We have also expanded the number of subscriptions to the HKU-Pasteur Courses Series Newsletter, which now totals 516, and our groups in social media have grown, reaching 338 and 294 members for LinkedIn and Facebook, respectively. The average open rate of the newsletter is 78% and the average click-through rate is 24.4%. The use of "HKU-Pasteur Research Pole" Facebook page (created in March 2013) as a means of communication has been strengthened. We now reaching an average of 588 people on a weekly base with our posts (November 2015 – April 2016). The average engagement rate is 6.3% (November 2015 – April 2016). The total number of "likes" on the Facebook page has increased by 30% (November 2015 – April 2016) and reached 178.

Public Health Workshop Series at Pasteur Institute of Ho Chi Minh City (Vietnam): Outbreak Investigation

To take into account the increasing convergence in health threats and interdependency of responses, we have established in 2012 at the International Teaching and Training Center of the Pasteur Institute of Ho Chi Minh City a world-class training program for epidemiologists, researchers and public health officials. The 2015 edition of the course, organized in close partnership with the Pasteur Institute of Ho Chi Minh City, the International Network of Institut Pasteur, the Australian National University and the Pasteur Center for Global Health, focused on "Outbreak Investigation", a key aspect for the public health sector to mount countermeasures in the control of infectious diseases. The course was based on introductory lectures completed by directed, computer-based tutorials with international and local experts, and a field study in a local setting. It allowed participants to acquire the skills to choose the appropriate analytical study design during an outbreak investigation, develop questionnaires and draw conclusions that guide public health actions, communicate findings of an outbreak investigation for various audiences and conduct similar training in their own institutions (**see Annex 5.6**). The reputation and impact of this course series, which has been generously supported since its inception by the Regional Health Cooperation Office of the French Ministry of Foreign Affairs, have drawn a huge response from the region with more than 100 applications from all ASEAN countries and beyond received in 2015 (for 24 slots).

3.8 International Activity

HKU-PRP exerts a leadership role in a number of research and educational programs of global scope.

International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC)

Roberto Bruzzone has been the Vice-Chair and on the Executive Committee of ISARIC since its official launching in 2012. ISARIC - is a global initiative aiming to ensure that clinical researchers have the open access protocols and data-sharing processes needed to facilitate a rapid response to emerging diseases that may turn into epidemics or pandemics. ISARIC has become a Consortium of over 70 networks and individuals involved in health research. In 2014 ISARIC has laid down the foundations for more challenging co-ordinated studies, including clinical trials of pathogen-specific therapies with pragmatic endpoints.

ISARIC has playing a major role in the Ebola virus crisis and has urged the deployment of alternative trial designs to fast-track the evaluation of new Ebola treatments. It has been involved in the coordination of two clinical trials in West Africa. ISARIC is now assisting with the deployment of research on Zika virus and has set up a web site for shared resourced and information.

Theme-based Research Scheme "Viral, host and environmental determinants of influenza virus transmission and pathogenesis"

Malik Peiris is the Coordinator of the Theme-based Research Scheme (TRS): "Viral, host and environmental determinants of influenza virus transmission and pathogenesis", which has been awarded a HK$75 million grant. The program addresses two outstanding "grand-challenge" research questions in influenza: i) the biological determinants of influenza virus transmission from animals-to-humans and from human-to-humans; and ii) the pathogenesis of severe influenza disease. The specific goals of the TRS are to:

- Understand the viral, host and environmental determinants of influenza virus transmission between humans, and from animals to humans;
- Understand the viral and host determinants of pathogenesis of severe influenza;
- Develop evidence based interventions to reduce transmission and novel therapeutic strategies targeting the host.

The TRS will promote the implementation of the "One Health" concept to manage influenza risks, with a strong educational component embedded in the program.

The Pasteur Center for Global Health Research & Education

Institut Pasteur and the Institut Pasteur International Network are developing a Center for Global Health Research and Education (CGH) that aims to improve the health of populations worldwide. The CGH will develop interdisciplinary international research projects to improve knowledge on diseases and promote transformative discoveries in the very countries where the research is being conducted and where populations are the most affected. Besides strengthening synergy between research and education by establishing a comprehensive training program, the CGH will reinforce scientific knowledge and technical skills in the five continents in order to train and equip the next generation of researchers and global health leaders.

HKU-PRP has been designated as the Asian the hub of the CGH to promote the One-Health approach of infectious diseases, thereby providing the opportunity to further develop our teaching mission by aligning our educational program with the overarching objective to improve human, animal and environmental health through research and public health. Roberto Bruzzone has been appointed Director of the International Teaching and Training Progam of Institut Pasteur. He has co-organized in this capacity an Educational Forum at Institut Pasteur (June 2015) to discuss and confront the scope and design of the Global Teaching Program with other major institutions involved in global education. The first meeting of the Educational Advisory Committee has taken place at the Institut Pasteur in December 2015.

The WHO Collaborative Center Avian Influenza

The School of Public Health, Li Ka Shing Faculty of Medicine of The University of Hong Kong (HKU) hosted special ceremonies last Thursday (August 13, 2015) to unveil the plaques of respectively the WHO Collaborating Centre for Infectious Disease Epidemiology and Control, as well as a WHO H5 Reference Laboratory. The ceremonies were officiated by Dr Margaret Chan, Director-General of the World Health Organization. Malik Peiris has been appointed as Co-Director of the WHO H5 Reference Laboratory at HKU. Its mandate is to provide international reference laboratory services and training on H5 and other animal influenza viruses with zoonotic or pandemic potential, to provide WHO with data and risk assessment relevant to such threats and advice on selection for relevant viruses for pre-pandemic vaccine development.

4. Scientific Output

4.1 Publications cited in PubMed – 2015 to present

1. Al Hammadi ZM, Chu KW, Eltahir YM, Al Hosani F, Al Mulla M, Tarnini W, Hall AJ, Perera RA, Abdelkhalek MM, Peiris JSM., Al Muhairi SS, Poon LLM (2015) Asymptomatic MERS-CoV infection in humans possibly linked to infected dromedaries imported from Oman to United Arab Emirates, May 2015. *Emerg Infect Dis* **21**:2197-2200.
2. Bruzzone R (2015) The double life of connexin channels: single is a treat. J *Invest Dermatol*, **135**:940-943.
3. Chan SM, Damdinjav B, Perera RA, Chu DK, Khishgee B, Enkhbold B, Poon LL, Peiris M (2015) Absence of MERS-Coronavirus in Bactrian Camels, Southern Mongolia, November 2014. *Emerg Infect Dis* **21**:1269-1271.
4. Chin AW, Perera RA, Guan Y, Halfmann P, Kawaoka Y, Peiris M, Poon LL (2015) Pseudoparticle neutralization assay for detecting Ebola neutralizing antibodies in biosafety level 2 settings. *Clin Chem* **61**: 885-886.
5. Chu DK, Oladipo JO, Perera RA, Kuranga SA, Chan SM, Poon LL, Peiris M (2015) Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels in Nigeria, 2015. *Euro Surveill* **20**(49).
6. Da Guan W*, Mok CK*, Chen ZL, Feng LQ, Li ZT, Huang JC, Ke CW, Deng X, Ling Y, Wu SG, Niu XF, Perera RA, Da Xu Y, Zhao J, Zhang LQ, Li YM, Chen RC, Peiris M, Chen L, Zhong NS (2015) Characteristics of Traveler with Middle East Respiratory Syndrome, China, 2015. *Emerg Infect Dis* **21**:2278-2280 (*equal contribution).
7. Dutry I, Li J, Li PH, Bruzzone R, Peiris JS, Jaume M (2015) The effects of macrophage polarity on influenza virus replication and innate immune responses. *J Clin Cell Immunol* **6**:297.
8. Fan RL, Valkenburg SA, Wong CK, Li OT, Nicholls JM, Rabadan R, Peiris JS, Poon LL (2015) Generation of live attenuated influenza virus by using codon usage bias. *J Virol* **89**:10762-10773.
9. Guan WD*, Gong XY*, Mok CK*, Chen TT, Wu SG, Pan SH, Cowling BJ, Yang ZF, Chen DH (2015) Surveillance for seasonal influenza virus prevalence in hospitalized children with lower respiratory tract infection in Guangzhou, China during the post-pandemic era. *PloS One*, **10**:e0117846 (*equal contribution).
10. Hemida MG, Al-Naeem A, Perera RA, Chin AW, Poon LL, Peiris M (2015) Lack of Middle East respiratory syndrome coronavirus transmission from infected camels. *Emerg Infect Dis* **21**:699-701.
11. Hemida MG, Elmoslemany A, Al-Hizab F, Alnaeem A, Almathen F, Faye B, Chu DK, Perera RA, Peiris M (2015) Dromedary Camels and the transmission of Middle East Respiratory Syndrome Coronavirus (MERS-CoV). *Transbound Emerg Dis* **doi**:10.1111/tbed.12401.
12. Li MY, Grandadam M, Kwok K, Lagache T, Siu YL, Zhang JS, Sayteng K, Kudelko M, Qin CF, Olivo-Marin JC, Bruzzone R, Wang PG (2015) KDEL receptors assist dengue virus exit from the endoplasmic reticulum. *Cell Rep* **10**:1496-1507.
13. Li MY, Bruzzone R, Wang PG (2015) New tricks for KDEL receptors. *Oncotarget* **6**:30425-30426.
14. Memish ZA, Alsahly A, Masri MA, Heil GL, Anderson BD, Peiris M, Khan SU, Gray GC (2015) Sparse evidence of MERS-CoV infection among animal workers living in Southern Saudi Arabia during 2012. *Influenza Other Respir Viruses* **9**:**64-67**.
15. Mok CK, Da Guan W, Liu XQ, Lamers MM, Li XB, Wang M, Zhang TJ, Zhang QL, Li ZT, Huang JC, Lin JY, Zhang YH, Zhao P, Lee HH, Chen L, Li YM, Peiris JS, Chen RC, Zhong NS, Yang ZF (2015) Genetic characterization of highly pathogenic avian influenza A(H5N6) virus, Guangdong, China. *Emerg Infect Dis* **21**:2268-2271.

16. Park SW, Perera RA, Choe PG, Lau EH, Choi SJ, Chun JY, Oh HS, Song K, Bang JH, Kim ES, Kim HB, Park WB, Kim NJ, Poon LL, Peiris JS, Oh MD (2015) Comparison of serological assays in human Middle East respiratory syndrome (MERS)-coronavirus infection. *Euro Surveill* **20**(41).

17. Park WB, Perera RA, Choe PG, Lau EH, Choi SJ, Chun JY, Oh HS, Song KH, Bang JH, Kim ES, Kim HB, Park SW, Kim NJ, Man Poon LL, Peiris M, Oh MD (2015) Kinetics of Serologic Responses to MERS Coronavirus Infection in Humans, South Korea. *Emerg Infect Dis* **21**:2186-2189.

18. Taylor A, Foo SS, Bruzzone R, Vu LD, King NJ, Mahalingam S (2015) Fc receptors in antibody-dependent enhancement of viral infection. *Immunol Rev* **268**:340-364.

19. Yang ZF*, Mok CK*, Liu XQ*, Li XB, He JF, Guan WD, Xu YH, Pan WQ, Chen LY, Lin YP, Wu SG, Pan SH, Huang JC, Ding GY, Zheng K, Ke CW, Lin JY, Zhang YH, Lee HHY, Liu WK, Yang CG, Zhou R, Peiris JS, Li YM, Chen RC, Chen L, Zhong NS (2015) Clinical, virological and immunological features from patients infected with re-emergent avian-origin human H7N9 influenza disease of varying severity in Guangdong province. *PLoS One* 10:e0117846 (*equal contribution).

20. Yang ZF, Mok CK, Peiris JSM, Zhong NS (2015) Human infection with a novel avian influenza A(H5N6) virus. *N Engl J Med* **373**:487-489.

21. Zhao J, Perera RA, Kayali G, Meyerholz D, Perlman S, Peiris M (2015) Passive immunotherapy with dromedary immune serum in an experimental animal model for MERS coronavirus infection. *J Virol* **89**:6117-6120.

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23. Jahan AS, Lestra M, Swee LK, Fan Y, Lamers MM, Tafesse FG, Theile CS, Spooner E, Bruzzone R, Ploegh HL, Sanyal S (2016) Usp12 stabilizes the T cell receptor complex at the cell surface during signaling. *Proc Natl Acad Sci USA* **113**:E705-714.

24. Fan Y, Mok CK, Zhang Y, Nal B, Kien F, Bruzzone R, Sanyal S (2016) Cell cycle independent role of cyclin D3 in host restriction of influenza virus infection. *Submitted*.

25. Lin YP, Luo Y, Chen Y, Lamers MM, Zhou Q, Yang XH, Sanyal S, Mok CKP*, Liu ZM* (2016) Clinical and epidemiological features of the 2014 large-scale dengue outbreak in Guangzhou city, China. *BMC Infect Dis* **16**:10 (*co-corresponding authors).

26. Lin YP, Yang ZF, Liang Y, Li ZT, Bond HS, Luo YS, Chen Y, Chen TT, Guan WD, Lai JCC, Siu YL, Pan SH, Peiris JSM, Cowling BJ*, Mok CKP* (2016) Population seroprevalence of antibody to influenza A(H7N9), Guangzhou, China. *Submitted* (*co-corresponding authors).

27. Miguel E, Perera RA, Baubekova A, Chevalier V, Faye B, Akhmetsadykov N, Ng CY, Roger F, Peiris M (2016) Absence of Middle East Respiratory Syndrome Coronavirus in Camelids, Kazakhstan, 2015. *Emerg Infect Dis* **22**:doi:10.3201.

28. Valkenburg SA, Mallajosyula VV, Li OT, Chin AW, Carnell G, Temperton N, Varadarajan R, Poon LL (2016) Stalking influenza by vaccination with pre-fusion headless HA mini-stem. *Sci Rep* **6**:22666.

4.2 Presentations at Meetings – 2015 (Oral/Posters)

- ***Y Fan***, CK Mok, Y Zhang, B Nal, F Kien, Bruzzone R, S Sanyal (2016) Regulation of host factors in influenza virus infections through ubiquitin and ubiquitin like modifiers. *International Meeting on Respiratory Pathogens*, Singapore (Oral).
- ***AS Jahan***, S Sanyal (2015) Role of deubiquitylating enzymes in influenza virus infection. *International Meeting on Respiratory Pathogens*, Singapore (Poster).
- ***MM Lamers***, S Sanyal (2015) Differential regulation of Tsg101 identified through proximity dependent labeling. *International Meeting on Respiratory Pathogens*, Singapore.
- ***SMY Lee***, KH Kok, M Jaume, DY Jin, JS Peiris (2015) Role of TLR10 as an innate immune sensor for influenza virus infection. *TOLL 2015: Targeting Innate Immunity,* Marbella, Spain (Poster).
- ***SMY Lee***, KH Kwok, DY Jin, JS Malik Peiris (2015) Novel role of TLR10 as an innate immune sensor for respiratory virus infection. *Institut Pasteur International Network International Scientific Symposium,* Paris, France (Poster).
- ***M Li,*** K Kwok, L Siu, JS Zhang, R Bruzzone, PG Wang (2015) Molecular Dissection of Dengue Virus Egress: Involvement of KDEL Receptors. *Gordon Research Conference on Infections of the Nervous System*, Hong Kong, Hong Kong SAR (Poster).
- ***CKP Mok***, HH Lee, ZF Yang, JS Peiris (2014) Host and the viral factors that contribute to the pathogenicity of the novel A/H7N9 influenza virus. *The 15th IUBMB-24th FAOBMB-TSBMB Conference*, Taipei, Taiwan (Poster).
- ***CKP Mok***, Z Yang, NZ Zhong, JSM Peiris (2015) Human Disease Caused By Novel Reassortant Highly Pathogenic Avian Influenza A (H5N6) Virus. *Scientific Symposium of the Institut Pasteur International Network*, Paris, France (Poster).
- ***CKP Mok***, Z Yang, NZ Zhong, JSM Peiris (2015) Human Disease Caused By Novel Reassortant Highly Pathogenic Avian Influenza A (H5N6) Virus. *International Meeting on Respiratory Pathogens*, Singapore (Poster).
- ***SA Valkenburg***, OTW Li, JSM Peiris, LP Perera, LLM Poon (2015) Universal influenza vaccine utilizes T cells for mechanism of protection as killers and coordinators. *Keystone Symposia on Viral Immunity (A2)*, Breckenridge, USA (Poster).
- ***SA Valkenburg***, VVA Mallajosyula, OTW Li, AWH Chin, G Carnell, N Temperton, R Varadarajan, LLM Poon (2016) *Targeting influenza by vaccination with prefusion headless HA ministem. Victorian Infection and Immunity*, Lorne, Australia (Oral).
- ***S Yan***, TF Yip, ST Li, Kelvin Ip, PH Li, SMY Lee (2015) TLR10 plays a regulatory role in type I interferon responses upon poly (I:C) activation. *TOLL 2015: Targeting Innate Immunity,* Marbella, Spain (Poster).
- ***TF Yip***, S Yan, JSM Peiris, SMY Lee (2015) Sub-cellular localization and ligand identification of Toll-like receptor (TLR)-10. *Institut Pasteur International Network International Scientific Symposium,* Paris, France (Poster).
- ***TJ Zhang***, S Sanyal (2015) Role of Aup1 in the assembly and egress of dengue virus. *Gordon Research Conference on Infections of the Nervous System*, Hong Kong, Hong Kong SAR (Oral).

4.3 Seminars, Invited Lectures – 2015

1. ***Roberto Bruzzone*** (2015) International Conference on Scientific Insight and Response of Ebola Virus Disease; Beijing, PR China.
2. ***Roberto Bruzzone*** (2015) Oxford University/Mahidol Oxford Tropical Medicine Research Unit; Bangkok, Thailand.
3. ***Roberto Bruzzone*** (2015) Global Health Risk Framework: A Workshop on Research and Development of Medical Products; Hong Kong, Hong Kong SAR.
4. ***Roberto Bruzzone*** (2015) City University of Hong Kong; Hong Kong, Hong Kong SAR.
5. ***Jimmy Lai*** (2015) Department of Pathology, The University of Hong Kong, Hong Kong SAR.
6. ***Chris Mok*** (2105) Institut Pasteur-Korea, Pangyo, Republic of Korea.
7. ***Chris Mok*** (2015) Global Forum on Research and Innovation for Health 2015, Manila, The Philippines.
8. ***Chris Mok*** (2015) Erasmus Medical Center, Rotterdam, The Netherlands.
9. ***Malik Peiris*** (2015) Courage Fund Infectious Disease Conference; Singapore.
10. ***Malik Peiris*** (2015) 3rd International One Health Congress; Amsterdam, The Netherlands.
11. ***Malik Peiris*** (2015) One Health Master Class - SEA-EU-NET 2015; Bangkok, Thailand.
12. ***Malik Peiris*** (2015) 1st International Meeting on Respiratory Pathogens; Singapore.
13. ***Malik Peiris*** (2015) 10th Asia-Pacific Congress of Medical Virology; Taipei, Taiwan.
14. ***Malik Peiris*** (2015) National University of Singapore; Singapore.
15. ***Malik Peiris*** (2015) XVII International Symposium on Respiratory Viral Infections; Vancouver, Canada.
16. ***Sumana Sanyal*** (2015) The University of Cambridge, UK.
17. ***Sumana Sanyal*** (2015) Department of Cell Biology and Infection; Institut Pasteur, Paris, France.
18. ***Sumana Sanyal*** (2015) Keystone Symposium "The Human Proteome"; Stockholm, Sweden.
19. ***Sumana Sanyal*** (2015) University College London, UK.
20. ***Sumana Sanyal*** (2015) The Whitehead Institute for Biomedical Research, MIT, Cambridge, MA, USA.
21. ***Sumana Sanyal*** (2015) Cambridge Institute of Medical Research, Cambridge, UK.
22. ***Sumana Sanyal*** (2015) Department of Cell Biology & Infection of the Institut Pasteur, Annual Retreat, Morzine, France.
23. ***Sumana Sanyal*** (2015) DKFZ, Heidelberg, Germany.
24. ***Sumana Sanyal*** (2015) EMBL, Heidelberg, Germany.

4.4 Active Grants – 2015 to present

Area of Excellence, Control of Pandemic and Inter-pandemic Influenza

Principal Investigator:	Dr Suki Lee
Amount:	HK$250,000.00
Period:	01/May/2015 to 31/Dec/2016

Area of Excellence, Control of Pandemic and Inter-pandemic Influenza

Principal Investigator:	Dr Chris Mok
Amount:	HK$250,000.00
Period:	01/May/2015 to 31/Dec/2016

Area of Excellence, Control of Pandemic and Inter-pandemic Influenza

Principal Investigator:	Dr Sumana Sanyal
Amount:	HK$250,000.00
Period:	01/May/2015 to 31/Dec/2016

BNP-Paribas

Principal Investigator:	Dr Roberto Bruzzone
Amount:	HK$200,000.00
Period:	01/Jan/2015 to 31/Dec/2015

Health and Medical Research Fund (HMRF)

Principal Investigator:	Dr Suki Lee
Amount:	HK$998,544.00
Period:	01/Jan/2013 to 30/Nov/2015

Health and Medical Research Fund (HMRF)

Principal Investigator:	Dr Ben Cowling
Co-Investigator:	Dr Suki Lee
Amount:	HK$796,778.00
Period:	01/Apr/2015 to 30/Mar/2017

Health and Medical Research Fund (HMRF)

Principal Investigator:	Dr Michael Chan
Co-Investigator:	Dr Chris Mok
Amount:	HK$978,704.00
Period:	01/Mar/2013 to 28/Feb/2015

Health and Medical Research Fund (HMRF)

Principal Investigator:	Dr John Nicholls
Co-Investigator:	Dr Chris Mok
Amount:	HK$794,000.00
Period:	01/Nov/2013 to 31/Oct/2015

Health and Medical Research Fund (HMRF)

Principal Investigator:	Dr Chris Mok
Amount:	HK$638,340.00
Period:	01/Jul/2015 to 30/Jun/2017

Health and Medical Research Fund (HMRF)

Principal Investigator:	Dr Sumana Sanyal
Amount:	HK$981,120.00
Period:	01/Jul/2015 to 30/Jun/2017

IPP-PTR

Principal Investigator: Dr Sumana Sanyal
Amount: EUR143,000.00
Period: 01/Jul/2015 to 30/Jun/2017

Research Grants Council

Principal Investigator: Dr Suki Lee
Amount: HK$632,266.00
Period: 01/Oct/2014 to 30/Sep/2016

Research Grants Council

Principal Investigator: Dr Sumana Sanyal
Amount: HK$654,557.00
Period: 01/Dec/2014 to 30/Nov/2016

Research Grants Council

Principal Investigator: Dr Sumana Sanyal
Amount: HK$769,020.00
Period: 01/Nov/2015 to 31/Oct/2017

Seed Fund, University of Hong Kong

Principal Investigator: Dr Chris Mok
Amount: HK$120,000.00
Period: 01/Apr/2014 to 31/Mar/2016

Seed Fund, University of Hong Kong

Principal Investigator: Dr Sumana Sanyal
Amount: HK$120,000.00
Period: 01/Jul/2014 to 30/Jun/2016

UGC-Matching Fund Scheme (6th Phase)

Principal Investigator: Dr Roberto Bruzzone
Amount: HK$723,027.00
Period: 01/Apr/2014 to 31/Mar/2016

Viral, Host and Environmental Determinants of Influenza Virus Transmission and Pathogenesis

Principal Investigator: Dr Suki Lee
Amount: HK$800,000.00
Period: 01/Jan/2015 to 31/Dec/2019

Viral, Host and Environmental Determinants of Influenza Virus Transmission and Pathogenesis

Principal Investigator: Dr Chris Mok
Amount: HK$300,000.00
Period: 01/Jan/2015 to 31/Dec/2019

Viral, Host and Environmental Determinants of Influenza Virus Transmission and Pathogenesis

Principal Investigator: Dr Sumana Sanyal
Amount: HK$450,000.00
Period: 01/Jan/2015 to 31/Dec/2019

4.5 Pending Grant Applications

BNP-Paribas

Principal Investigator: Dr Roberto Bruzzone
Amount: HK$100,000.00
Period: 01/Jan/2016 to 31/Dec/2016

Health and Medical Research Fund (HMRF)

Principal Investigator: Dr Roberto Bruzzone/Dr Mingyuan Li
Amount: HK$1,170,000.00

Health and Medical Research Fund (HMRF)

Principal Investigator: Dr Sophie Doak
Amount: HK$1,151,000.00

Health and Medical Research Fund (HMRF)

Principal Investigator: Dr Jimmy Lai
Amount: HK$900,900.00

Health and Medical Research Fund (HMRF)

Principal Investigator: Dr Suki Lee
Amount: HK$1,093,000.00

Health and Medical Research Fund (HMRF)

Principal Investigator: Dr Chris Mok
Amount: HK$988,300.00

Health and Medical Research Fund (HMRF)

Principal Investigator: Dr Sumana Sanyal
Amount: HK$1,200,000.00

Health and Medical Research Fund (HMRF)

Principal Investigator: Dr Selena Yan/Dr Suki Li
Amount: HK$1,155,800.00

Research Grants Council

Principal Investigator: Dr Sophie Doak
Amount: HK$1,490,000.00

Research Grants Council

Principal Investigator: Dr Suki Lee
Amount: HK$1,143,000.00

Research Grants Council

Principal Investigator: Dr Chris Mok
Amount: HK$993,000.00

Research Grants Council

Principal Investigator: Dr Sumana Sanyal
Amount: HK$1,170,000.00

5. Annexes

5.1 List of Staff

Name	***Position***	
PEIRIS, Malik	Director	
BRUZZONE, Roberto	Co-Director	
DOAK, Sophie	Research Assistant Professor	(starts 01/Apr/2016)
LEE, Man Yan Suki	Research Assistant Professor	
MOK, Ka Pun Chris	Research Assistant Professor	
SANYAL, Sumana	Research Assistant Professor	
LAI, Chun Cheong Jimmy	Post-Doctoral Fellow	(Joint Appointment with Department of Pathology in the Nicholls Lab)
ZHANG, Jingshu Tami	Technical Officer Post-Doctoral Fellow	(ended 31/Dec/2015) (effective 01/Jan/2016)
LAN, Yun	Technical Officer	
LI, Mingyuan	Technical Officer	
YAN, Sheng Selena	Research Associate	
BENET, Gabriel	Scientific Officer	(International Volunteer of the French Ministry of Foreiogn Affairs)
TREMOLET, Sebastien	Scientific Officer	(International Volunteer of the French Ministry of Foreiogn Affairs; ended Jun/2015)
LI, Ping Hung	Research Technician	
SIU, Yu Lam Lewis	Research Technician	
TSE, Kong San Jane	Research Technician	
CHEN, Xiao Kai	Research Assistant	(ended 31/Mar/2016)
IP, Ka Kay Kelvin	Research Assistant	
JAHAN, Akhee	Research Assistant PhD Student	(ended 31/Jul/2015) (started 01/Aug/2015)
LI, Shuting	Research Assistant	
MA, Nok Lam Fion	Research Assistant	
PAN, Sihua	Research Assistant	(ended 29/Feb/2016)
POMBO, Joao	Research Assistant	
WONG, Ho Him	Research Assistant	
FAN, Ying	PhD Student Technical Officer	(ended 30/Jun/2015) (ended 31/Mar/2016)
LEE, Hok Yeung Horace	PhD Student	
HERATH M Thusitha Kumara K	PhD Student	

MAK, Ganon	PhD Student (Part-time)	
YIP, Tsz Fung	MPhil Student	(ended 31/Aug/2015)
	Research Assistant	(started 01/Apr/2016)
ANGYAL, Dora	Student Intern (Erasmus MC)	
LAMERS, Mart	Student Intern (Erasmus MC)	
LE QUANG Agathe	Student Intern (University of Montpellier)	
TSE, Sharon	Student Intern	
YIP, Ka Wai Garrick	Student Intern (IVE)	
LI, Suk Yin Anne	Administrative Assistant	
CHAU, Man Hao	Laboratory Attendant	(ended 26/Nov/2015)
CHEUNG, Wai Sze	Laboratory Attendant	

5.2 Income & Expenses for the year ending 30 June 2016 (forecast)

INCOME:

Central Fund				
		$1,721,214.00		
COLA adjustment		$111,464.31		
Notional Rental Recovery		$223,608.00	$2,056,286.31	
Endowment Fund	c/f	$2,151,069.96		
		$3,825,808.00	$5,976,877.96	
Institut Pasteur	c/f	$1,487,711.25		
		$2,312,215.00	$3,799,926.25	
External Grants*	c/f	$839,650.39		
2015-2016 (see next page)		$3,540,193.92	$4,379,844.31	
Other Donations	c/f	$1,008,000.00		
		$300,000.00	$1,308,000.00	
Teaching				
Virology		$320,017.05		
Immunology		$244,600.21		
Cell Biology		$251,969.73	$816,586.99	
				$18,337,521.82

EXPENSES:

Staff Cost			$6,850,299.01	
Research				
Reagents, etc.		$2,585,304.23		
Conferences/Meetings		$463,382.21	$3,048,686.44	
Equipment/Maintenance			$411,928.70	
Administration			$112,883.34	
Teaching & Training			$898,037.17	
				$11,321,834.66

BALANCE: **$7,015,687.16**

*External Grant 2015-2016:

	c/f	New Grants
Matching Fund	$255,112.03	--
AOE – Suki	--	$170,000.00
AOE – Chris	$(4,409.01)	$320,000.00
AOE – Sumana	$115,294.58	$170,000.00
BNP	$121,414.07	$150,000.00
Donation – Chris	$2,436.35	$31,000.00
HMRF – Chris	--	$319,170.00
HMRF – Sumana	--	$490,560.00
International Research Grant – Suki	--	$11,496.67
International Research Grant – Sumana	--	$3,352.92
IPP – PTR	--	$381,650.00
RGC – Suki	$77,208.14	$321,133.00
RGC – Sumana 1	$43,980.10	$327,278.50
RGC – Sumana 2	--	$256,006.67
RFCID – Suki	$228,614.13	$313,546.17
Seed Fund – Chris	--	$60,000.00
Seed Fund – Sumana	--	$60,000.00
TRS – Suki	--	$80,000.00
TRS – Chris	--	$50,000.00
TRS – Sumana	--	$25,000.00
	$839,650.39	$3,540,193.92

TOTAL: **$4,379,844.31**

5.3 HKU-Pasteur Virology Course 2015

12th HKU-PASTEUR VIROLOGY COURSE

FOR RESEARCH POSTGRADUATE STUDENTS

12 - 24 July 2015

HKU-Pasteur Research Pole, Hong Kong

Institut Pasteur

香港大學 · 巴斯德研究中心
HKU-Pasteur Research Pole

THE UNIVERSITY OF HONG KONG
LI KA SHING FACULTY OF MEDICINE
香港大學李嘉誠醫學院

Outbreaks

DIRECTORS:
Roberto Bruzzone (HKU-PRP) Malik Peiris (HKU-PRP) Noel Tordo (Institut Pasteur)

TUTORS:
Vincent Deubel (Institut Pasteur) Chris Mok (HKU-PRP)

LECTURERS:

Roberto Bruzzone (Hong Kong)	Ali Mirazimi (Sweden)	Amadou Sall (Senegal)
Heinz Feldman (USA)	Lisa Ng (Singapore)	Pei-Yong Shi (Singapore)
Didier Fontenille (Cambodia)	Cheik Ibrahima Niang (Senegal)	Noel Tordo (France)
Sandra Junglen (Germany)	Hiroshi Nishiura (Japan)	Marco Vignuzzi (France)
Suresh Mahalingam (Australia)	Malik Peiris (Hong Kong)	Victor Volchkov (France)
		Winfried Weissenhorn (France)

The 12th HKU-Pasteur Virology course will focus on "**Outbreaks**" with particular emphasis on hemorrhagic fevers.

REGISTRATION - DEADLINE FOR APPLICATIONS: 30 April 2015
Please return the completed form, including two letters of recommendation to hku-pasteur@hku-hk
Registration fees (HKD 1,000) include accommodation (on sharing twin basis) and food (breakfast, lunch, coffee breaks and two dinners)
The course (MMPH6171) is included in the coursework curriculum for research postgraduate studies of the University of Hong Kong

For more information, please contact
Anne LI at +852 2831 5516 or hku-pasteur@hku-hk
check www.hkupasteur.hku.hk for program updates

Posters for Public Lectures

HKU-PASTEUR VIROLOGY COURSE

FOR RESEARCH POSTGRADUATE STUDENTS

Institut Pasteur

香港大學 · 巴斯德研究中心
HKU-Pasteur Research Pole

THE UNIVERSITY OF HONG KONG
LI KA SHING FACULTY OF MEDICINE
香港大學李嘉誠醫學院

12th HKU – PASTEUR VIROLOGY COURSE

OPEN LECTURES

"Molecular biology of filovirus"

by

Dr Viktor Volchkov
INSERM, France

Date: **Wednesday, 15th July 2015**

Time: **9:00 to 12:00**

Venue: **Seminar Room 7, 7th Floor**
HKJC Building for Interdisciplinary Research
5 Sassoon Road, Pokfulam, Hong Kong

ALL ARE WELCOME

HKU-PASTEUR VIROLOGY COURSE

FOR RESEARCH POSTGRADUATE STUDENTS

Institut Pasteur

香港大學 · 巴斯德研究中心
HKU-Pasteur Research Pole

THE UNIVERSITY OF HONG KONG
LI KA SHING FACULTY OF MEDICINE
香港大學李嘉誠醫學院

12th HKU – PASTEUR VIROLOGY COURSE

OPEN LECTURES

09:00 – 11:00 "Integrated structural biology approaches to understand filovirus replication and pathogenicity"
by Dr Winfried Weissenhorn
Institut De Biologie Structurale, France

11:30 – 12:30 Part 1
14:30 – 15:30 Part 2 "The New Institut Pasteur – Guinea"
by Dr Noel Tordo
Institut Pasteur, France

Date: Thursday, 16th July 2015

Venue: Seminar Room 7, 7th Floor
HKJC Building for Interdisciplinary Research
5 Sassoon Road, Pokfulam, Hong Kong

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Direction des Affaires Internationales

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CONSULAT GÉNÉRAL DE FRANCE À HONG KONG ET À MACAO

L'ORÉAL
RESEARCH SCHOLARSHIP

SARS-CoV virions budding from infected cells (Departments of Pathology and Microbiology, HKU).
Françoise Barré-Sinoussi (b. 1947) co-recipient of the 2008 Nobel Prize in Medicine for the "discovery of human immunodeficiency virus"

HKU-PASTEUR VIROLOGY COURSE

FOR RESEARCH POSTGRADUATE STUDENTS

Institut Pasteur

香港大學 · 巴斯德研究中心
HKU-Pasteur Research Pole

THE UNIVERSITY OF HONG KONG
LI KA SHING FACULTY OF MEDICINE
香港大學李嘉誠醫學院

12th HKU – PASTEUR VIROLOGY COURSE

OPEN LECTURES

09:00 – 12:00 "Vaccines against filoviruses"
by Dr Andrea Marzi
National Institute of Allergy & Infectious Diseases
USA

13:30 – 16:00 "Crimean Congo hemorrhagic fever virus; pathogenesis, epidemiology and prevention"
by Dr Ali Mirazimi
Karolinska Institute
Sweden

Date: Thursday, 21st July 2015

Venue: Seminar Room 2, Ground Floor
HKJC Building for Interdisciplinary Research
5 Sassoon Road, Pokfulam, Hong Kong

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HKU-PASTEUR VIROLOGY COURSE

FOR RESEARCH POSTGRADUATE STUDENTS

Institut Pasteur

香港大學 · 巴斯德研究中心
HKU-Pasteur Research Pole

THE UNIVERSITY OF HONG KONG
LI KA SHING FACULTY OF MEDICINE
香港大學李嘉誠醫學院

12th HKU – PASTEUR VIROLOGY COURSE

OPEN LECTURES

09:00 – 11:00	"Molecular Evolution of Chikungunya Virus" by Dr Marco Vignuzzi Institut Pasteur, France
11:30 – 12:30 Part 1 14:00 – 15:00 Part 2	"Immune response to CHKV" by Dr Lisa Ng Singapore Immunology Network, Singapore
15:30 – 17:30	"Animal models of arboviruses to screen for anti-inflammatory and antivirals drugs" by Dr Suresh Mahalingam Grifith University, Australia

Date: Wednesday, 22nd July 2015

Venue: Seminar Room 2, Ground Floor
HKJC Building for Interdisciplinary Research
5 Sassoon Road, Pokfulam, Hong Kong

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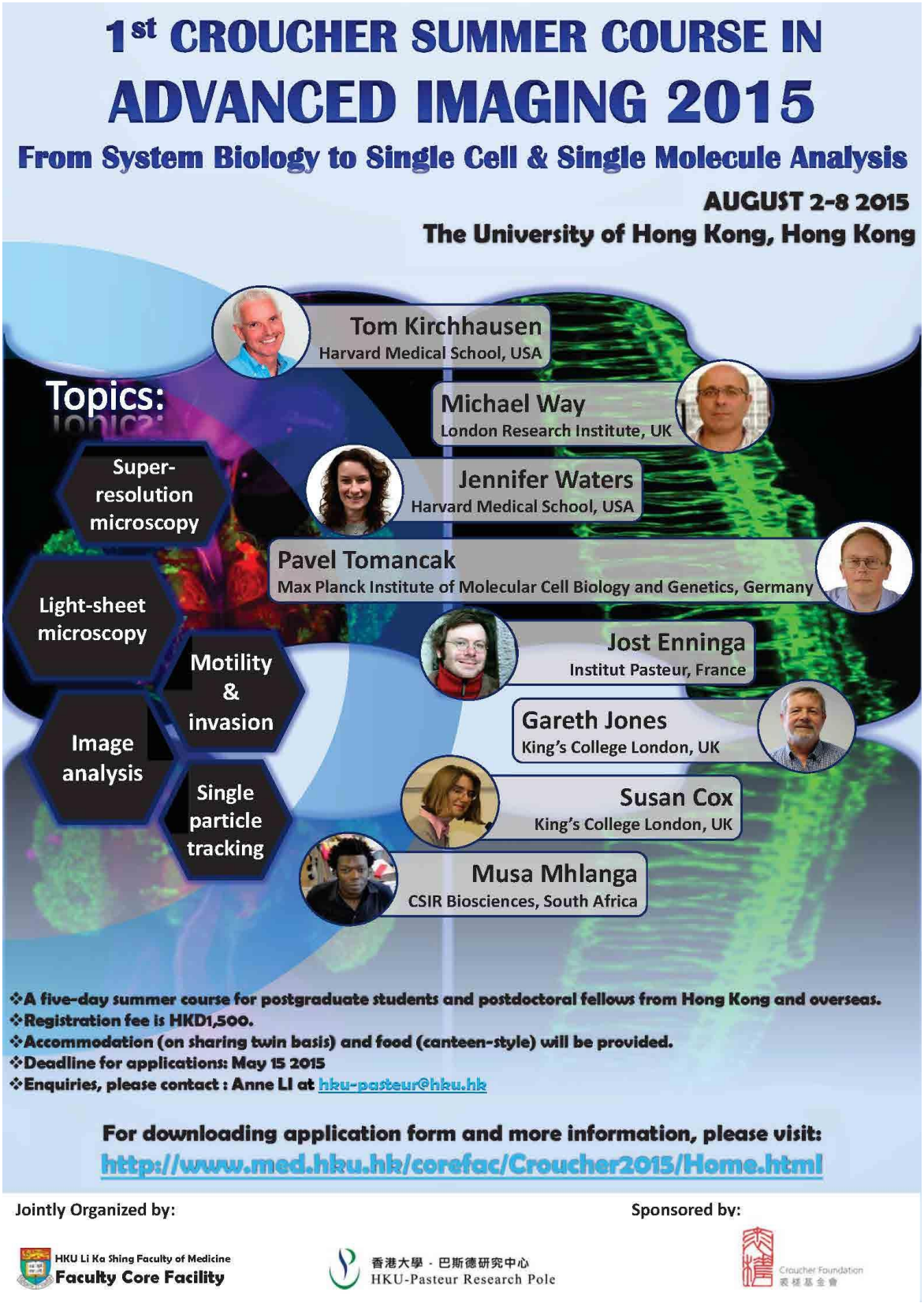
L'ORÉAL
RESEARCH SCHOLARSHIP

SARS-CoV virions budding from infected cells (Departments of Pathology and Microbiology, HKU)
Françoise Barré-Sinoussi (b. 1947) co-recipient of the 2008 Nobel Prize in Medicine for the "discovery of human immunodeficiency virus"

5.4 1st Croucher Summer Course in Advanced Imaging: From Systems Biology to Single Cell & Single Molecule Analysis




CROUCHER SYMPOSIUM IN ADVANCED IMAGING
From Systems Biology to Single Cell & Single Molecule Analysis
AUGUST 7 2015 (Friday) 8:30am – 5pm
Venue
Cheung Kung Hai Lecture Theatre 4
William MW Mong Building, Li Ka Shing Faculty of Medicine, The University of Hong Kong
Invited Speakers
Tomas Kirchhausen
Harvard Medical School, USA
Michael Way
London Research Institute, UK
Michael Loy
Hong Kong University of Science & Technology, HK
Peter Gabriel Pitrone
Max Planck Institute of Molecular Cell Biology and Genetics, Germany
Jost Enninga
Institut Pasteur, France
Cheng-han Yu
The University of Hong Kong, HK
Gareth Jones
King's College London, UK
Jade Shi
Hong Kong Baptist University, HK
Susan Cox
King's College London, UK
Musa Mhlanga
University of Cape Town, South Africa
Registration
Registration is free.
Sandwich lunch will be provided to registered participants.
For registration, please go to:
https://hkuems1.hku.hk/hkuems/ec_hdetail.aspx?guest=Y&ueid=37317
Enquiries, please contact
Faculty Core Facility
Li Ka Shing Faculty of Medicine
The University of Hong Kong
corefac@hku.hk
Jointly Organized by:
HKU Li Ka Shing Faculty of Medicine
Faculty Core Facility
香港大學 · 巴斯德研究中心
HKU-Pasteur Research Pole
Sponsored by:

Croucher Symposium in Advanced Imaging:
From Systems Biology to Single Cell & Single Molecule Analysis

- **Date: 7 August 2015**
- **Venue: Cheung Kung Hai Lecture Theatre 4**

Program	
8:30 – 8:45	**Registration**
8:45 – 9:00	**Welcome address:** **Prof. Peter Mathieson (President), The University of Hong Kong** **Prof. Suet Yi Leung (Associate Dean), LKS Faculty of Medicine, The University of Hong Kong** **Photo session: (Invited guests, Speakers, Organizing Committee)**
	Chairman : Randy Poon & Musa Mhlanga
9:00 – 09:30	**L1: Microscopes, movies and cells** **Tomas Kirchhausen** ***Harvard Medical School, USA***
9:30 – 9:55	**L2: Optical Microscopy for 21st Century Life Scientists** **Michael Loy** ***Hong Kong University of Science & Technology, HK***
9:55 – 10:25	**L3: Using Vaccinia virus to understand Arp2/3 driven actin polymerization** **Michael Way** ***The Francis Crick Institute, UK***
10:25 – 11:00	**Coffee Break**
	Chairman : Shuk Han Cheng & Yun Wah Lam
11:00 – 11:30	**L4: Vacuolar rupture caused by invasive bacterial pathogens- causes and consequences** **Jost Enninga** ***Institut Pasteur, France***
11:30 – 12:00	**L5: Applications of light sheet microscopy in developmental biology** **Peter Gabriel Pitrone** ***Max Planck Institute of Molecular Cell Biology and Genetics, Germany***
12:00 – 12:45	**Lunch Break**
	Chairman: George Tsao & Roberto Bruzzone & Musa Mhlanga
12:45 – 14:00	**Advanced Imaging Platform presentation (Sandwich & drinks provided)** **Sponsored by Carl Zeiss, NBI, PerkinElmer and Coherent** *(Please see page 10 for titles of lunch presentation)*
	Chairman: Chenghan Yu & Jade Shi
14:00 – 14:30	**L6: Mechanisms of cell invasion** **Gareth Jones** ***King's College London, UK***
14:30 – 14:55	**L7: Adhesion transformation, integrin signaling, and endocytosis in the absent of matrix force** **Chenghan Yu** ***The University of Hong Kong, HK***
14:55 – 15:25	**L8: Bayesian analysis of localization microscopy reveals nanoscale podosome dynamics** **Susan Cox** ***King's College London, UK***
15:25 – 16:00	**Coffee Break**
	Chairman: George Tsao & Roberto Bruzzone
16:00 – 16:25	**L9: Cytotoxic dynamics of Natural Killer cells at the single cell level** **Jade Shi** ***Hong Kong Baptist University, HK***
16:25 – 16:55	**L10: A high content single cell imaging method for the denovo identification of subcellular localizations of mRNAs and proteins** **Musa Mhlanga** ***University of Cape Town, South Africa & Institute for Molecular Medicine, Portugal***

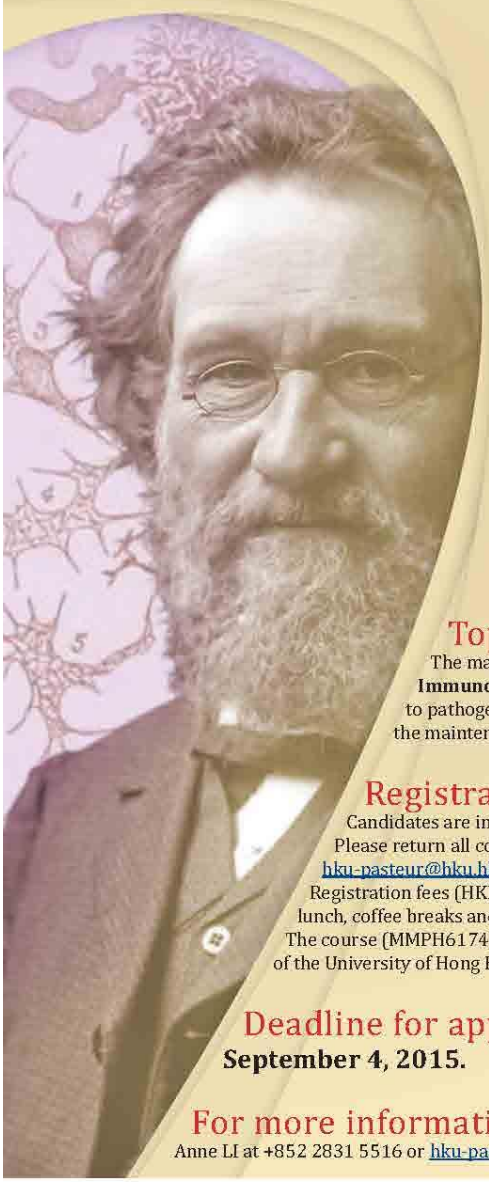
5.5 HKU-Pasteur Immunology Course 2015

8th HKU-PASTEUR IMMUNOLOGY COURSE

for Research Postgraduate Students

22 November - 4 December 2015

HKU-Pasteur Research Pole, Hong Kong



Directors:

Roberto BRUZZONE, HKU-Pasteur Research Pole
Liwei LU, The University of Hong Kong
Daniel SCOTT-ALGARA, Institut Pasteur

Lecturers:

Oreste ACUTO	United Kingdom
Antonio BERTOLETTI	Singapore
Roberto BRUZZONE	Hong Kong
Katja FINK	Singapore
Thomas GEBHARDT	Australia
Florent GINHOUX	Singapore
Jeroen GUIKEMA	The Netherlands
Hiroshi KAWAMOTO	Japan
Katherine KEDZIERSKA	Australia
Bin LI	P R China
Liwei LU	Hong Kong
Daniel SCOTT-ALGARA	France
Wenwei TU	Hong Kong
Stephen TURNER	Australia
Jose VILLADANGOS	Australia

Topics:

The major theme of the 8th HKU – Pasteur Immunology Course will be **Immunological Memory**. The course will explore how the immune system responds to pathogens that have been encountered previously and the mechanisms underlying the maintenance of antigen-specific lymphocytes in health and chronic diseases.

Registration:

Candidates are invited to download course application form at www.hkupasteur.hku.hk. Please return all completed forms, including two letters of recommendation to hku-pasteur@hku.hk.

Registration fees (HKD 1,000) include accommodation (on sharing twin basis) and food (breakfast, lunch, coffee breaks and two dinners).

The course (MMPH6174) is included in the coursework curriculum for research postgraduate studies of the University of Hong Kong.

Deadline for applications:

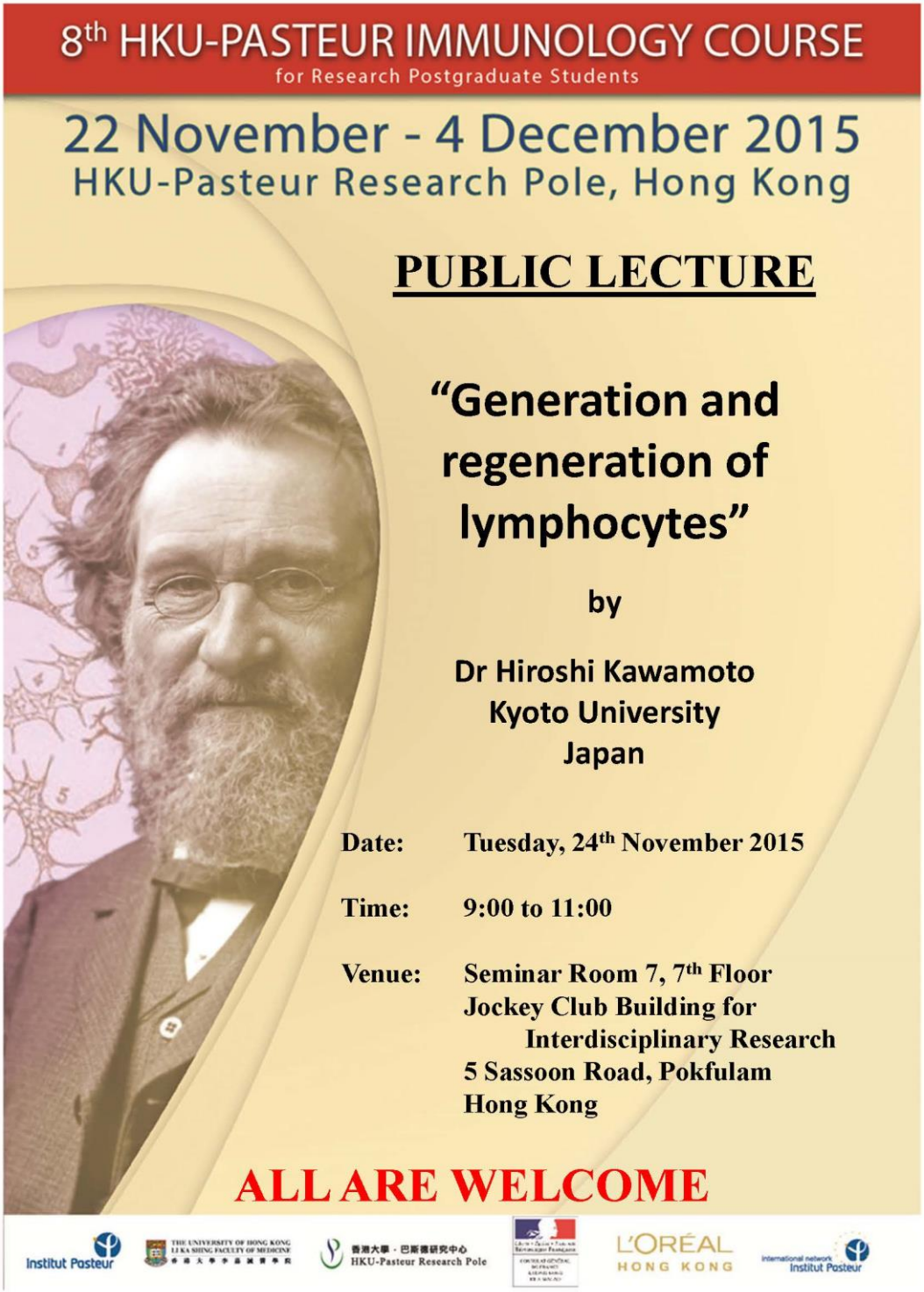
September 4, 2015.

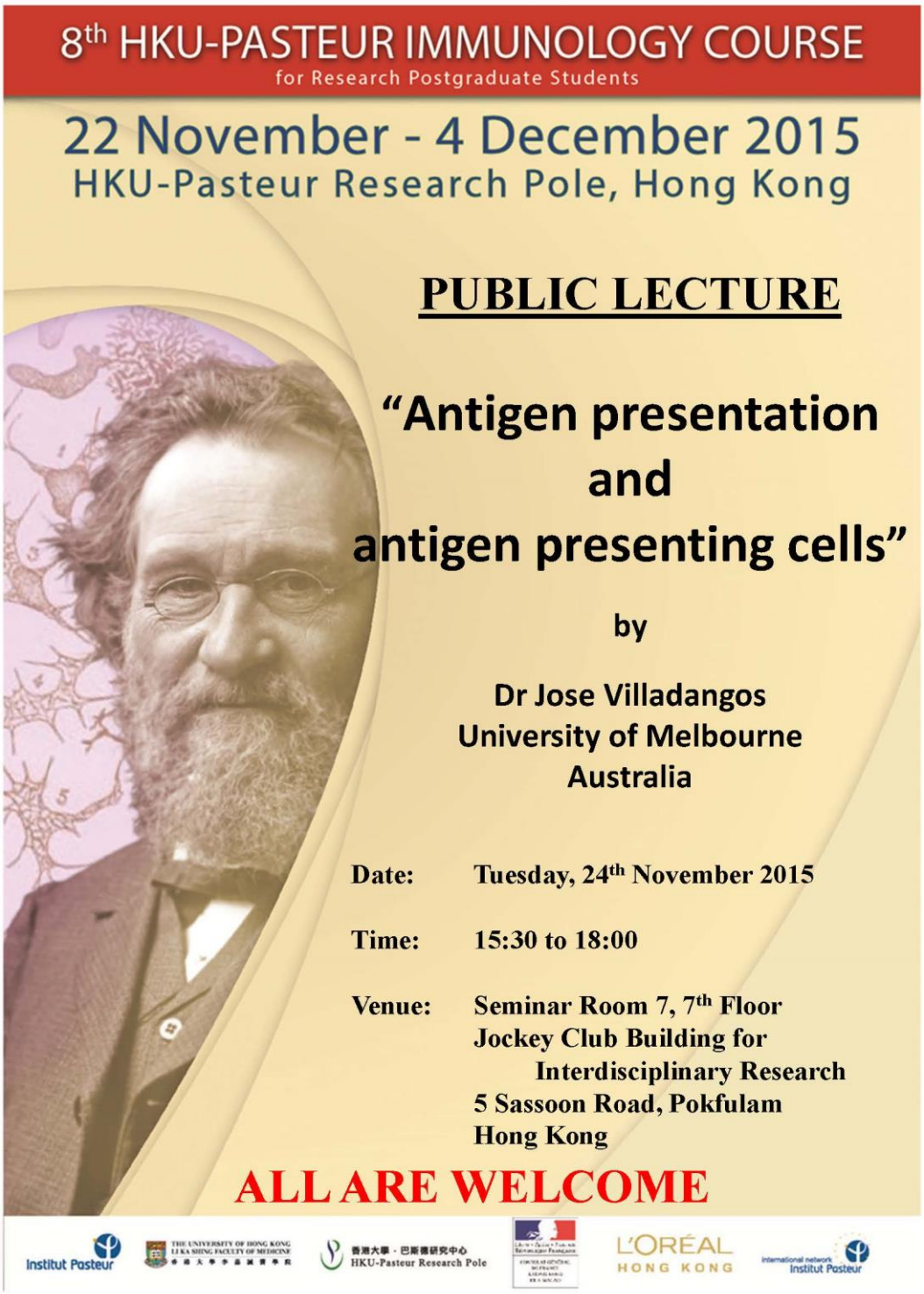
For more information, please contact:

Anne LI at +852 2831 5516 or hku-pasteur@hku.hk. Check www.hkupasteur.hku.hk for programme updates.

Institut Pasteur | The University of Hong Kong Li Ka Shing Faculty of Medicine | 香港大學・巴斯德研究中心 HKU-Pasteur Research Pole | L'ORÉAL HONG KONG | International network Institut Pasteur

Posters for Public Lectures




8th HKU-PASTEUR IMMUNOLOGY COURSE
for Research Postgraduate Students
22 November - 4 December 2015
HKU-Pasteur Research Pole, Hong Kong
PUBLIC LECTURE
"Antigen presentation and antigen presenting cells"
by
Dr Jose Villadangos
University of Melbourne
Australia
Date: Tuesday, 24th November 2015
Time: 15:30 to 18:00
Venue: Seminar Room 7, 7th Floor
Jockey Club Building for Interdisciplinary Research
5 Sassoon Road, Pokfulam
Hong Kong
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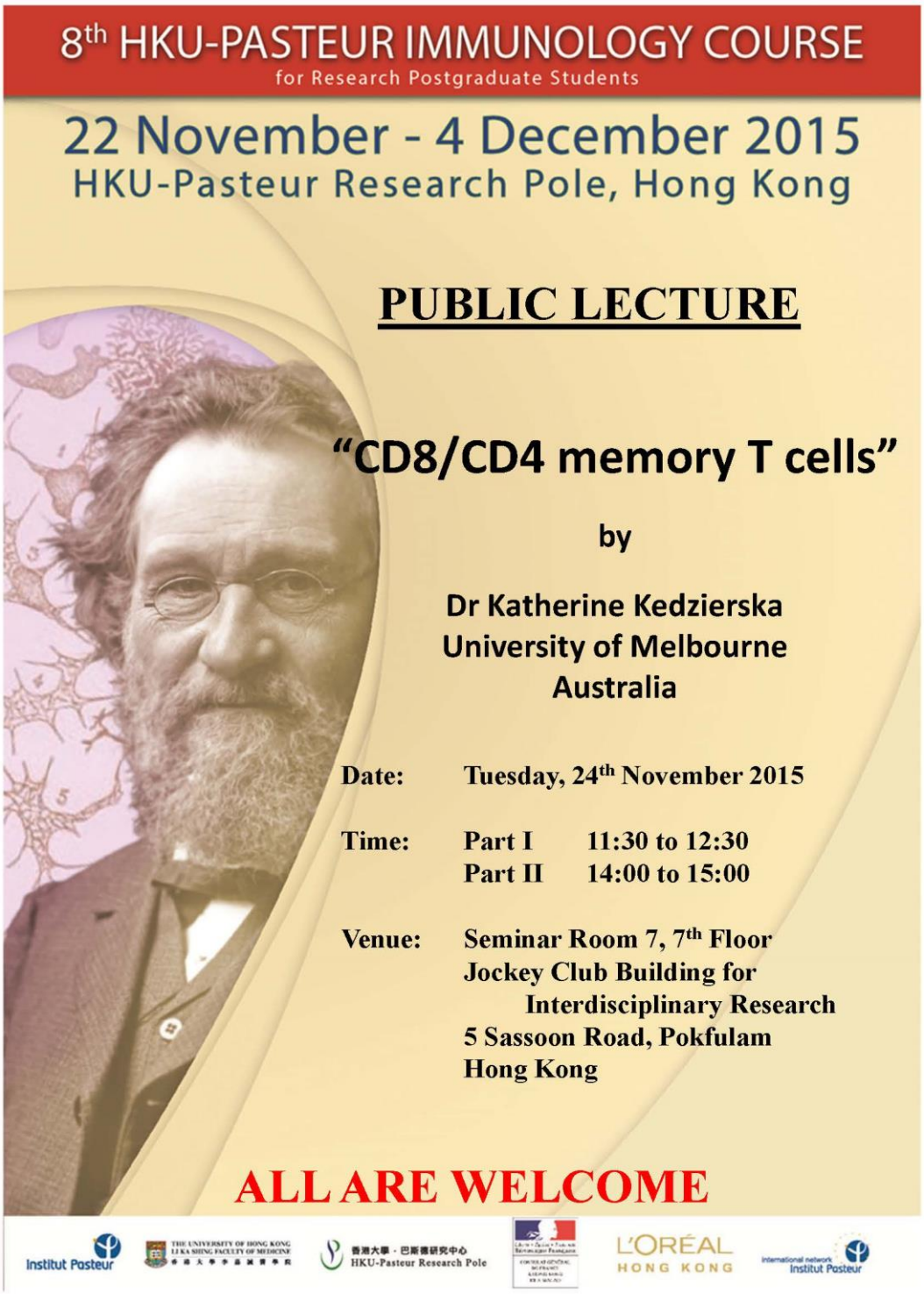
8th HKU-PASTEUR IMMUNOLOGY COURSE
for Research Postgraduate Students
22 November - 4 December 2015
HKU-Pasteur Research Pole, Hong Kong
PUBLIC LECTURE
"CD8/CD4 memory T cells"
by
Dr Katherine Kedzierska
University of Melbourne
Australia
Date: Tuesday, 24th November 2015
Time: Part I 11:30 to 12:30
Part II 14:00 to 15:00
Venue: Seminar Room 7, 7th Floor
Jockey Club Building for
Interdisciplinary Research
5 Sassoon Road, Pokfulam
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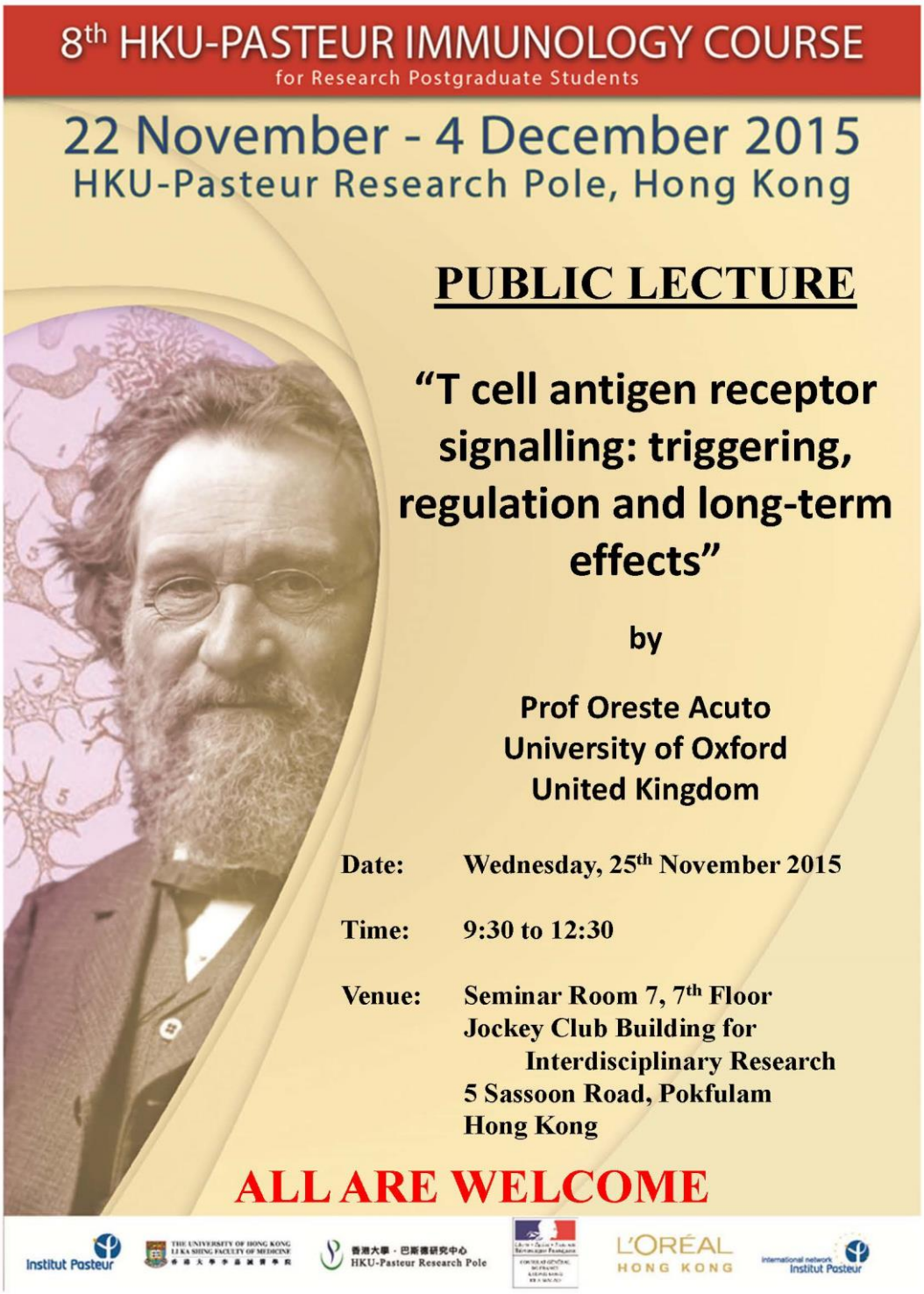
8th HKU-PASTEUR IMMUNOLOGY COURSE
for Research Postgraduate Students
22 November - 4 December 2015
HKU-Pasteur Research Pole, Hong Kong
PUBLIC LECTURE
"T cell antigen receptor signalling: triggering, regulation and long-term effects"
by
Prof Oreste Acuto
University of Oxford
United Kingdom
Date: Wednesday, 25th November 2015
Time: 9:30 to 12:30
Venue: Seminar Room 7, 7th Floor
Jockey Club Building for Interdisciplinary Research
5 Sassoon Road, Pokfulam
Hong Kong
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5.6 International Course: Outbreak Investigation 2015

香港大學 - 巴斯德研究中心
HKU-Pasteur Research Pole

Pasteur Institute - Ho Chi Minh City
Training Center

International Course

Outbreak Investigation

2-6 November 2015, Ho Chi Minh City, Vietnam

Course Directors & Faculty

S DAVIS, D HARLEY (Australian National University, Australia)

R BRUZZONE (HKU-Pasteur Research Pole, The University of Hong Kong)

VU TQH, HOANG QC (Pasteur Institute - Ho Chi Minh City, Vietnam)

T MOUNTS (US-CDC, Vietnam); **M VAN KERKHOVE** (Institut Pasteur, France)

Objective: The recent outbreaks of Ebola virus and MERS-CoV have illustrated that effective control and prevention of disease *outbreaks* depend on early detection, rapid on-site *investigation* and timely and appropriate interventions. This workshop will allow participants to acquire the skills to choose the best analytical study design during an outbreak investigation, develop questionnaires and draw conclusions that guide public health actions. Participants will also gain skills in conducting risk assessments of public health events and learn how to effectively communicate findings of an outbreak investigation for various audiences.

Participants' Profile: The course is specifically designed for health personnel from countries in South East Asia involved in surveillance activities and outbreak investigation. The course will be based on introductory lectures completed by directed, computer-based tutorial with experts and local tutors, and a field study in a local setting. Participants are strongly encouraged to pass on the skills they have learnt at this course through conducting similar trainings in their own institutions or countries.
Taught in English. Limited to 24 participants.

Applications: Candidates are invited to download application forms at www.hkupasteur.hku.hk. Please return the completed form, including 1-2 letters of recommendation, to hku-pasteur@hku.hk. The course teaching committee will review applications and select participants.

No registration fees – Free accommodation will be provided

Deadline for applications: September 4, 2015

international network Institut Pasteur

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5.7 List of Public Lectures organized by HKU-PRP

04/02/2015
"Innate Lymphoid Cell Development and Diversity" by Dr James Di Santo, Institut Pasteur, France

03/03/2015
"Frontline Experience in Fighting Ebola virus disease in West Africa" by Dr Amadou Sall, Institut Pasteur de Dakar, Senegal

04/03/2015
"A vulnerable site of the envelope protein of dengue virus suggest new strategies for vaccine design" by Dr Felix Rey, Institut Pasteur, France

27/03/2015
"Scrub typhus: studying the infection cycle of the intracellular bacterium Orientia tsutsugamushi" by Dr Jeanne Salje, University of Oxford, Mahidol Oxford Tropical Medicine Research Unit, Thailand

13/07/2015
"Hemorrhagic fevers in Africa" by Dr Amadou Sall, Institut Pasteur, Dakar

13/07/2015
"Epidemiological studies of Ebola: Effectiveness of case isolation and travel ban" by Dr Hiroshi Nishiura, University of Tokyo, Japan

13/07/2015
"Anthropology of Ebola Virus Outbreak" by Dr Cheikh Ibrahima Niang, University Cheikh Anta Diop, Dakar

15/07/2015
"Molecular biology of filovirus" by Dr Viktor Volchkov, INSERM, France

16/07/2015
"The New Institut Pasteur – Guinea" by Dr Noel Tordo, Institut Pasteur, Paris, France

21/07/2015
"Vaccines against filoviruses" by Dr Andrea Marzi, National Institute of Allergy and Infectious Diseases, USA

21/07/2015
"Crimean Congo hemorrhagic fever virus; pathogenesis, epidemiology and prevention" by Dr Ali Mirazimi, Karolinska Institute, Sweden

22/07/2015
"Molecular Evolution of Chikungunya Virus" by Dr Marco Vignuzzi, Institut Pasteur, Paris, France

22/07/2015

"Immune response to CHKV" by Dr Lisa Ng, Singapore Immunology Network, Singapore

22/07/2015

"Animal models of arboviruses to screen for anti-inflammatory and antivirals drugs" by Dr Suresh Mahalingam, Griffith University, Australia

07/08/2015

"Microscopes, movies and cells" by Dr Tomas Kirchhausen, Harvard Medical School, USA

07/08/2015

"Optical Microscopy for 21st Century Life Scientists" by Dr Michael Loy, Hong Kong University of Science & Technology, Hong Kong

07/08/2015

"Using Vaccinia virus to understand Arp2/3 driven actin polymerization" by Dr Michael Way, The Francis Crick Institute, United Kingdom

07/08/2015

"Vacuolar rupture caused by invasive bacterial pathogens- causes and consequences" by Dr Jost Enninga, Institut Pasteur, France

07/08/2015

"Applications of light sheet microscopy in biology" by Dr Peter Gabriel Pitrone, Max Planck Institute of Molecular Cell Biology and Genetics, Germany

07/08/2015

"Advanced 3D Fluorescence Imaging Systems from ZEISS" by Dr Daniel Koch, Carl Zeiss, Hong Kong

07/08/2015

"Advancing Cancer Immunotherapy; How is fluorescence microscopy helping to understand tumour microenvironments?" by Mr Chris Johnson, PerkinElmer, Hong Kong

07/08/2015

"NBI Product and Service: A User-Friendly 3D Two-Color Super-Resolution Localization Microscope" by Dr Du Shengwang, Nanobioimaging Ltd, Hong Kong

07/08/2015

"Mechanisms of cell invasion" by Dr Gareth Jones, King's College London, United Kingdom

07/08/2015

"Adhesion transformation, integrin signaling, and endocytosis in the absent of matrix force" by Dr Chenghan Yu, The University of Hong Kong, Hong Kong

07/08/2015

"Bayesian analysis of localization microscopy reveals nanoscale podosome dynamics" by Dr Susan Cox, King's College London, United Kingdom

07/08/2015

"Cytotoxic dynamics of Natural Killer cells at the single cell level" by Dr Jade Shi, Hong Kong Baptist University, Hong Kong

07/08/2015

"A high content single cell imaging method for the denovo identification of subcellular localizations of mRNAs and proteins" by Dr Musa Mhlanga, CSIR Biosciences, South Africa

05/10/2015

"Modeling and Computation for a new era of life science" by Dr Magnus Fontes, Institut Pasteur, France

27/10/2015

"The gut microbiome: from metagenomics to 'experimentomics'" by Dr Philippe Sansonetti, Institut Pasteur, France

29/10/2015

"Molecular and cellular pathogenesis of Shigella: finding a second breath" by Dr Philippe Sansonetti, Institut Pasteur, France

09/11/2015

"Skeletal muscle stem cells in development and regeneration" by Dr Shahragim Tajbakhsh, Institut Pasteur, France

24/11/2015

"Generation and regeneration of lymphocytes" by Dr Hiroshi Kawamoto, Kyoto University, Japan

24/11/2015

"CD8/CD4 memory T cells" by Dr Katherine Kedzierska, University of Melbourne, Australia

24/11/2015

"Antigen presentation and antigen presenting cells" by Dr Jose Villadangos, University of Melbourne, Australia

25/11/2015

"T cell antigen receptor signalling: triggering, regulation and long-term effects" by Dr Oreste Acuto, University of Oxford, United Kingdom