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

# Annual Report 2007

**HKU-Pasteur Research Center**

**Dexter HC Man Building**

**8, Sassoon Road**

**Hong Kong SAR, China**

**Roberto Bruzzone, CEO**

**Malik Peiris, Scientific Director**

Hong Kong, February 19, 2008

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# 1 EXECUTIVE SUMMARY

Following the departure of Dr Ralf Altemyer, who was Director between 2003-2006, the Board of Directors has appointed at the last meeting (Paris, September 2006) Dr Roberto Bruzzone and Prof Malik Peiris as Chief Executive Officer (CEO) and Scientific Director(SD), respectively. During the past year, **the new directorate team has carried out a strategic review of the role of HKU-Pasteur Research Centre** within Hong Kong University, within Hong Kong and the Asia Pacific Region.

In research, we concluded that the **HKU-PRC has a particular niche to fill in bringing together** the cutting edge developments in the **cell-biology of infections disease, which is the strength of Institut Pasteur**, with **the expertise in the clinical virology and immunology of infectious disease, which is the strength of HKU**. Through this synergy, **we aim to develop novel approaches to confront the challenges posed by viral infections in Hong Kong and the Asia-Pacific region in general by utilizing the RESPARI network** of the Pasteur Institutes**, as well as the regional networks** established by the HKU team.

**We have maintained our research focus on respiratory viral infections**, with special emphasis on influenza and SARS, **and on** the mosquito borne disease of **dengue**. With influenza and coronaviruses in particular, we have a local niche advantage of expertise and complementarities with ongoing projects at HKU. We have decided to **focus on exploiting expertise in key research tools established over the recent pas**t, including viral pseudoparticles, viral-like particles, fluorescent markers, siRNA screening and cell imaging to achieve our research goals at HKU-PRC.

**This research direction then naturally extends to training activities** in virology, immunity and cell biology of infectious disease. To the highly successful **virology training course** that is already ongoing on an annual basis, **we aim to add training courses in immunity and the cell biology of infection**. **We next aim at** consolidating and expanding these achievements by **combining strategic**

**investments in infrastructure** that will enable the long-term **development of highly competitive projects**.

*Research*

**The research program functions as three complementary teams:** viz**.** Virus-Host Interactions led by Dr Beatrice Nal-Rogier, Viral Infection & Immunity led by Dr Joanna Ho, and the Screening Platform led by Dr Jean-Michel Garcia.

**The Virus-Host Interactions team** lead by Dr Beatrice Nal-Rogier has expanded its studies on the **identification of novel cellular targets that interact with viral components, viz. interactomes**. We have performed, in collaboration with the Viral Genomics and Vaccination Laboratory (Institut Pasteur-Paris) a Yeast-Two-Hybrid (Y2H) screening to analyze the interactomes of the envelope proteins of 5 human coronaviruses that are of low and high pathogenicity in humans. **This work has led to the identification of new virus-host protein interactions paving the way to a comparative analysis of the differential interactomes that should provide new insights on the pathogenicity of these viruses**. Using a similar strategy we have identified a specific interaction with a protein involved in the establishment of cellular tight junctions at the apical side of epithelial cells. These data are very exciting in view of recent findings that implicate cell polarity as a crucial factor during early and late stages of coronavirus infection.

On the basis of these and other preliminary data **one grant has been submitted at the last call for proposals from the Research Grant Council (RGC) and three more to the Research Fund for the Control of Infectious Diseases (RFCID)**. One declaration of invention has been filed with the Patent Office of Institut Pasteur.

**The Viral Infection & Immunity team** led by Dr Joanna Ho **has been recently funded by RFCID** for a project entitled “Interaction of NK activatory receptors and hemagglutinin from avian flu – Potential implication of NKp44 in anti-flu therapy”. **Work carried out this year has led to the completion of a manuscript that was published in the Journal of Virology** (the top specialist journal in the field). The Viral Infection & Immunity group has been strengthened by the recruitment of

2 research assistants and one MPhil student. In addition, two PhD candidates are currently working as technicians pending the result of their application.

**A second RFCID-funded project addresses the role of antibody mediated enhancement (ADE) in the pathogenesis of SAR**S. *In vitro* studies have revealed that s**pecific antibodies induced by either SARS-CoV infection** (for example in convalescent sera from patients with SARS) **or SARS vaccines** (sera from animals immunized with putative SARS vaccines) **can facilitate the entry of SARS-CoV to human B cell lines** which are otherwise refractory to the virus (in the absence of such antibody) because these B cells lack the ACE2 receptor necessary for viral entry. **Such enhancing antibodies may potentially lead to lymphopenia**, which is a notable feature of SARS. This is also of major relevance as a possible adverse consequence of a potential SARS vaccine. ADE has been incriminated in immunopathology of the animal coronavirus disease feline infectious peritonitis where vaccination has led to enhanced disease and it is clearly important to investigate its role in SARS. We have compared humoral immune response elicited by different vaccine formulations and uncovered distinct patterns of antibody-mediated entry of SARS-CoV into hematopoietic cells despite similar abilities of the sera to neutralize receptor-mediated infection of ACE2-bearing cells. Interestingly, immunization of mice with whole inactivated SARS-CoV virion elicited neutralizing antibody response lacking ADE potency. **One additional proposal to look into the cellular mechanisms of ADE has been prepared for the annual RFCID call**. Continuing this line of investigation **we are initiating studies on the role of ADE in facilitating viral entry of avian viruses in human FcR bearing cells**.

**The HKU-PRC screening platform team**, led by Dr Jean-Michel Garcia, **remains actively implicated in drug discovery** projects but has more recently broadened its interests to **sero-epidemiology studies** for immuno-surveillance and diagnosis of highly pathogenic avian influenza infections. One important achievement has been **the publication of a manuscript describing the validation of the H5 hemagglutinin pseudotyped lentiviral particles (H5pp) for sero-diagnostic and epidemiological studies**. This line of investigation is being actively pursued with appropriate collaborations with industry as well as academic partners. The

more detailed validation of this method is ongoing using clinical sera from patients with H5N1 disease in Vietnam (in collaboration with Dr Menno De Jong, Hospital for Tropical Diseases, Ho Chi Minh City) and we are also testing H5N1 sera of vaccinated subjects from clinical trials (in **collaboration with the pharmaceutical organizations, Sanofi-Pasteur and Baxter**). **These companies have shown a keen interest in the potential use of our method** as an additional serological technique to detect vaccine-induced immune responses, have taken out rights to license this method and are exploring this possibility using this platform on a fee-for service basis. **We have been also been involved in the transfer of this technology** to the Institut Pasteur-Cambodia and the Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam. We have continued in parallel our efforts in the drug discovery program and **have successfully completed the first phase of the screening campaign for inhibitors of dengue replication, which has identified 20 hits**. One declaration of invention is in preparation.

**An extension of the application of viral pseudotypes** in serology has been to explore the possible role of lentiviral pseudotypes and virus like particles (VLPs) **to investigate virus receptor interactions of influenza**. We have used VLPs expressing the H5 virus haemagglutinin on the lentivirus backbone in experiments utilizing Saturation Transfer Difference Nuclear Magnetic Resonance (STD-NMR) in collaboration with Dr Mark von Itzstein at the Institute of Glycomics, Griffith University, Australia. **A paper reporting the feasibility of this approach for investigating viral haemagglutinin-glycan interactions has recently been published in Angewandte Chemie, the highly prestigious chemistry journal**. This technique now opens up the possibility of a systematic analysis of the viral haemagglutinin-glycan receptor interactions of the highly pathogenic H5N1 virus. It provides, on the one hand, a novel tool to re-assess the glycans that bind the avian H5-haemagglutinin and, on the other hand, an exploration of the impact of viral mutations on such binding.

**Three new grant proposals have been submitted: to the Pasteur network (ACIP), to the RFCID and to the Area of Excellence.**

*Teaching*

**The project to establish a Teaching & Training Center of Excellence in Biomedical Research has received a boost by the decision of the Li Ka Shing Faculty of Medicine to grant an interest-free loan** to equip part of the 3rd floor of the Dexter HC Man Building where lab space for practical course has been planned. **The annual Pasteur-Asia Virology Course has now been validated by HKU as part of its Research Postgraduate Curriculum**. The 4th Pasteur Asia Virology course, on the theme "INFLUENZA AND OTHER EMERGING RESPIRATORY VIRAL INFECTIONS" was held in July 2007 was very successful. Twenty nine students from 8 countries were selected and scientists from all over the world (15 different countries) came to share their expertise. **Two new courses, Immunology and Cell Biology are being planned as part of a gradual expansion of the teaching activity of the center**. In addition, HKU-PRC is actively collaborating with HKU Faculty to help strengthening the imaging facility on the campus. **A Cell Imaging workshop has been organized in February 2008 and two more international workshops have been planned in 2008 (applications submitted to the Croucher Foundation)**. HKU-PRC continues its efforts to promote scientific exchanges between Hong Kong and Institut Pasteur through the Pasteur-Croucher Exchange Program. Two students are now working on their PhD thesis in Paris and the 3rd seminar series, held in September 2007, attracted more quality candidates.

*Network projects*

HKU-PRC relies on a number of strategic collaborations and network projects. **Dr Roberto Bruzzone has become the Scientific Coordinator of the RESPARI program** that federates the 8 Institutes of the Pasteur-Asia network in a multi-center project that tackles several aspects of acute respiratory infections, through the implementation of clinical, epidemiological, fundamental and translational research projects. **Prof Malik Peiris is the Coordinator of the 8-year research program "Control of Pandemic and Inter-pandemic Influenza"** that has been awarded a HK$ 76 millions by the University Grants Committee in the fourth round of its **Areas of Excellence** (AoE) scheme. The HKU-PRC participated in this application and will contribute its expertise on the cell biology of viral infections in relation to pathogenesis, host response and innate-immunity.

*Financial situation*

The financial situation has been defined under the Consolidated Agreement stipulating that the **Centre will receive intramural funding from HKU** (HK$6.1 million per annum in cash plus in kind contributions) up to the end of the revised term (November 2011). Starting in 2005 **more intramural funds have been made available from Institut Pasteur** (€80K per annum in cash). The center still holds grants from the **French Ministry of Health** and the **Li Ka Shing Foundation**, and has successfully competed with projects submitted to Hong Kong funding agencies. However, the **draw-down of the special RAP/PDF fund** allocated to the center at its foundation may require re-adjusting the level of staff employed. In summary, **the financial position of the Centre is considered as healthy** with a total net asset of HK$5.73M and a balance of cash and cash equivalent of HK$7.38M stood at June 30, 2007

# 2 PROGRESS REPORT

## 2.1 PROGRAM 1: VIRUS-HOST INTERACTIONS

### 2.1.1 Objectives and strategy

The objective of the Virus-Host Interactions (VHI) Unit is the identification of novel cellular targets to understand in molecular terms the steps that regulate early (entry) and late (assembly and budding) stages of viral infections.

Our general hypothesis is that focusing on the virus-cell interactions will allow defining crucial steps that can lead to the development of new anti-viral therapies. Specifically, our efforts are concentrated on

1. The characterization of **cellular factors able to enhance or restrict** early (entry) and late (assembly and budding) stages of viral infections, using SARS-CoV and other human coronaviruses, avian influenza and dengue as experimental models.
2. The use of **large-scale genomic approaches** like yeast-2-hybrid (Y2H) and siRNA library screens to identify cellular pathways that regulate viral infections.

### 2.1.2 Achievements

**We have progressed towards the characterization of novel molecular mechanisms that regulate the SARS-CoV pathogenesis**. Among the 3 major virus-cellular factor interactions identified, 2 are being investigated in greater detail. First, we have **confirmed the restricting role of ezrin on SARS-CoV early stages of infection** and have made significant steps towards the characterization of the determinants of interaction. Second, we have **discovered the interaction of SARS-CoV with PALS1, a tight junction associated protein that plays a fundamental role in the maintenance of epithelial cell architecture**. Our hypothesis is that the virus deteriorates the integrity of the airway epithelium by hijacking PALS1. Third, we have broadened our studies on the SARS-CoV cellular interactome to include the four other human coronaviruses. We have shown that ezrin also interacts with the other human coronaviruses

OC43, NL63 and 229E but not HKU1. We are the first to describe the differential sets of cellular factors these 5 viruses bind to during their life cycle.

More recently, the team has begun the search for **cellular regulators of H5-influenza entry. We have identified six cellular factors that interact with three structural proteins of the highly pathogenic avian influenza virus**. The underlying mechanisms of interactions are being investigated.

To better understand the viral and cellular determinants of dengue virus infection, **we have developed a viral-like particle (VLP) production system** in mammalian cells and established a dengue VLP producing stable cell line (HeLa-prME) **as a safe and convenient tool for the study of egress of dengue virus**. Furthermore, we have set up a chemical luminescence dot-blot (CLDB) system to quantify VLP production by HeLa-prME cells, which makes it amenable to a screening approach.

**We have strengthened the imaging capability of the center**. In order to remain competitive, **HKU-PRC needs to invest in high performance imaging tools** to conduct fluorescence microscopy studies in situ. HKU-PRC is equipped with a fluorescence microscope (**Zeiss Axiovert 200**) that has been **upgraded with a Monochrome CCD camera and Axiovision software**. This equipment will allow the team to perform high quality analysis of labeled fixed cells. For precise sub-cellular localization experiments we have **acquired a fully motorized Zeiss Axio observer Z1 inverted microscope equipped with the ApoTome module**, which allows the very fast production of extremely high-quality optical sections through fluorescence-labeled biological specimens. We have received this new microscope in October and personnel have received specialized training.

**Local and international contacts** have been actively pursued in 2007, with particular attention to the following collaborations:

- Dr. F. Tangy and P.O. Vidalain, Institut Pasteur, with the objective to study interactions between viral proteins and host factors using state-of-the-art technology;

- Pr. George Tsao, Department of Anatomy at HKU, to further develop the HKU Core Imaging Facility in a BSL2 environment;
- Dr Michael Chan, Department of Microbiology at HKU, to develop cellular models of polarized lung epithelium to study infection by SARS-CoV and H5N1;
- Dr John Nicholls, Department of Pathology at HKU, for electron microscopy analysis of viral particles (production, subcellular localization);
- Dr. Xiaowei Zhuang, Harvard University, to set up life-cell imaging of viral entry using pseudotyped particles.
- Dr. Ulf Nehrbass and Dr. Regis Grailhe at Institut Pasteur Korea, with the aim to run genome-wide siRNA screenings with live viruses in a BSL-3 environment.

### 2.1.3 Current projects

**The work of the group is focusing on the characterization of cellular factors that interfere with viral infection with specific emphasis on SARS-CoV, avian influenza and dengue**. These projects are carried out in parallel taking advantage of both similar and complementary technical approaches (pseudoparticles, VLPs, fluorescent markers, siRNA screening) to maximize interactions and productivity.

The mechanism of restriction of viral infections by ERM proteins is the most advanced of these projects and we expect a **high impact publication** in the field of cell biology of viral infection. A **close collaboration** with Frederic Tangy and Pierre-Olivier Vidalain at Institut Pasteur aims to **characterize the particular motifs** on both SARS-CoV Spike and ERM proteins **involved in the interaction; identify inhibitors of interaction;** and **define the viral interactome** of ERM proteins (a library of structural viral proteins from other coronaviruses and other viruses will be constituted and interaction with ERM proteins will be determined). In addition using a large-scale yeast two-hybrid screening strategy, we **have found that the cytosolic tail of the E envelope protein of SARS-CoV specifically interacts with PALS1, a tight junction-associated protein**, and this E-PALS1 interaction has been confirmed in vitro by GST-pull down experiments.

We are currently studying the determinants of interaction between the SARS-CoV E envelope protein and the PALS1 and have planned to investigate the consequences of SARS-CoV E interaction with PALS1 on tight junction structure and polarity of infected airway epithelial cells. **Our hypothesis is that SARS-CoV E interaction with PALS1 contributes to the lung epithelium damages observed in SARS-CoV infected patients.**

We have begun the **investigation of enhancing and restricting cellular factors of the highly pathogenic H5N1** virus, with a particular emphasis on early and late stages of the replication cycle. Our hypothesis is that **through identification of the specific interactions between cellular and viral structural proteins**, we will contribute to the better understanding of the cell biology of H5N1 infection and **delineate new targets that may lead to the rational design of anti-viral strategies.** We have performed a Yeast-Two-Hybrid (Y2H) screening to identify cellular partners of the cytosolic domains of the hemagglutinin (HA), M2 proteins and the full-length M1 protein. Six proteins related to the cytoskeleton, vesicle trafficking, cell cycle regulation and DNA modification were identified with high confident scores. Their relevance for influenza life cycle and viral pathogenesis will be investigated. Intrinsic viral factors are also of great importance in determining the outcome of the infectious cycle. Thus, **we have started to investigate factors controlling the assembly and release of H5** pseudotyped lentiviral particles (H5pp). **Our hypothesis is that H5 hemagglutinins of different clades/strains may possess different properties in regard to their expression, cleavage, intracellular trafficking and receptor binding affinity**, all of which may contribute to influence the efficiency of H5pp production. This is based on our observation that the H5 haemagglutinins of clade 1 (A/Cambodia/408008/05), clade 2.1 (A/Indonesia/5/05) and clade 2.2 (A/Anhui/1/05) viruses have markedly different efficiencies in their producing H5 pseudotyped particles (H5pp).

To better understand the viral and cellular determinants of dengue virus egress, **we have developed a VLP production system** in mammalian cells. We have established a dengue VLP producing stable cell line (HeLa-prME) by using a codon-optimized type 1 dengue prME gene that highly increases expression level

of prME. VLPs can readily form in cells expressing the glycoproteins of dengue virus without any other viral element. Biochemical analysis of VLPs produced by HeLa-prME cells demonstrates that they have gone through the same secretion pathway and maturation process as wild type dengue viruses. Therefore, **the VLP producing HeLa-prME cell line constitutes a safe and convenient tool for the study of egress of dengue virus**. Furthermore, we have set up a chemical luminescence dot-blot (CLDB) system to quantify VLP production by HeLa-prME cells. Our preliminary data shows that **this system displays a wide lineage range, and is sensitive** enough to detect VLPs in small volumes of culture medium of HeLa-prME cells, **making it amenable for use in screening projects**.

### 2.1.4 Funding

These projects have been financed in part through the RESPARI (French Ministry of Health) and DENFRAME network programs. Five grants are currently under review.

1. Regulation of early steps of coronavirus infection by the ezrin ERM protein (submitted to the **Research Grants Council**).
2. Recruitment of the tight junction factor PALS1 by the SARS-CoV E envelope protein: consequences for integrity of the infected airway epithelium (Submitted to the **Research Fund for the Control of Infectious Diseases**).
3. Mechanistic study on the assembly and release of lentiviral particles pseudotyped with hemagglutinin of avian influenza H5N1 viruses: implication for strain-specific H5 pseudotype development (Submitted to the **Research Fund for the Control of Infectious Diseases**).
4. Identification of cellular enhancing and restricting factors of dengue virus egress (Submitted to the **Research Fund for the Control of Infectious Diseases**).
5. The cellular interactome of the H5N1 influenza virus (submitted to the **Area of Excellence**).

### 2.1.5 Personnel

| Name | Position | End of contract (renewable) |
|---|---|---|
| Beatrice Nal-Rogier | Research Assistant Professor | 31 July 2008 |
| Francois Kien | PDF | 13 June 2008 |
| Dong Jiang Tang | PDF | 02 April 2008 |
| Pei Gang Wang | PDF | 28 March 2008 |
| Mateusz Kudelko | PhD student | 30 June 2010 |
| Jean Millet | PhD student | 31 August 2009 |
| Kim Tat Teoh | PhD student | 31 August 2009 |
| Lewis Siu | Research Technician | 31 December 2011 |
| Kid Chu | Laboratory Technician | 27 January 2008 |
| Yuet Wu | BSc student | |
| Sin Hang Siu | BSc student | |

## 2.2 PROGRAMME 2: VIRAL INFECTION & IMMUNITY

### 2.2.1 Objectives and Strategies

The host responds to infecting viruses by activating its innate immune system and mounting virus-specific humoral and cellular immune responses to control viral replication and eliminate the virus from the host. **The objective of the Viral Infection & Immunity program is to investigate specific mechanisms underlying the interplay between viral infection and the immune response,** innate and acquired. Our projects are at the interface of fundamental and applied research with the aim of contributing to the identification of novel therapeutic applications.

1. **Understanding innate immunity during viral infection**. The first project focuses on cells of the innate immunity system such as Natural Killer, macrophages, and dendritic cells, which are the frontline players in the event of a viral attack. **We are investigating the activation mechanism of Natural Killer cells by viral envelope proteins of Influenza, SARS-CoV, and dengue virus.**
2. **Stimulating adaptive immunity: The role of antibody dependent enhancement (ADE) in SARS vaccine development and safety**. While pursuing the project that led to develop a candidate vaccine against SARS-CoV (Kam et al., 2007) we have uncovered the role of ADE as a potential adverse side effect of such vaccines. These studies may also help **elucidate aspects of pathogenesis of SARS and gain insight into the pathways involved in viral entry into target cells**.

### 2.2.2 Achievements

**Understanding innate immunity during viral infection. We have discovered that envelope proteins of highly pathogenic avian influenza, SARS-CoV and dengue** virus but not hepatitis C virus and HIV **bind specific receptors on immune Natural Killer** (NK) cells, one of the first barrier of cellular defense against pathogens. It is known that the triggering of NK cells is delicately regulated by the balance between opposing signals delivered by inhibitory and activating receptors expressed on the NK cell membrane. Natural cytotoxicity receptors (NCRs), expressed by NK cells, trigger the lysis of tumor and virus-

infected cells upon interaction with cell-surface ligands of these target cells. Previously, it had been demonstrated that the activating NK cell receptors NKp44 and NKp46 are involved in the recognition of the hemagglutinins (HAs) expressed on the surface of infected cells by human influenza with H1- or H3-subtypes. It was relevant to define which (if any) of these receptors would recognize the avian influenza A H5 HA. **We have established** that (i) **recombinant NKp44 recognize H5-expressing cells**; (ii) **the interaction** between recombinant NKp44 and soluble H5 **is direct**; (iii) enhanced binding of H5pp is observed only with NKp44 expressing NK cells; and (iv) the **activation of NK cells** interacting with H5 pseudotyped particles or H5-expressing target cells **is mediated by NKp44**. Therefore, NKp44-H5 interactions and induced NK activation followed the same pattern observed for NKp44-H1 interaction. Similarly, NKp46 interact functionally with H5 (O. Mandelboim, unpublished results). To summarize, **the enhanced virulence of the influenza A H5N1 strain could not be attributed to the avian virus exploiting a "blind-spot" in the innate NK-cell defenses** of humans. These **results have recently been published** (Ho et al., 2008) in the **Journal of Virology**, the top specialty journal.

**Stimulating adaptive immunity: The role of antibody dependent enhancement (ADE) in SARS vaccine development and safety**. We already reported that specific antibodies induced by SARS-CoV infection (present in SARS convalescent patients) or induced by SARS vaccines can promote entry of SARS-CoV in human B cell lines that lack ACE-2 (but express FcRγII receptors for the Fc portion of immunoglobulins) via the so-called antibody-dependant enhancement (ADE). This may be also relevant in explaining the severe lymphopenia observed in patients with SARS. **To further address safety issues of SARS-CoV vaccines** we have compared humoral immune response elicited by different vaccine formulations and have observed a distinct pattern of antibody-mediated entry of SARS-CoV into hematopoietic cells despite comparable abilities of the sera to neutralize infection of cells that express ACE-2 (angiotensin-converting enzyme-2), the natural receptors for the virus. Interestingly, **immunization of mice with whole inactivated SARS-CoV virion elicited neutralizing antibody response lacking ADE potency**. A better **understanding of this phenomenon will be relevant to vaccine safety as well as to the pathogenesis of SARS**.

**Local and international contacts** have been actively pursued in 2007, with particular attention to the following collaborations:

- Professor Angel Porgador, Ben-Gurion University of the Negev, to further develop the ongoing projects on NK cells and molecular immunology;
- Dr Nicolas Escriou, Institut Pasteur, to map epitopes of Spike protein and Ig G isotypes inducing ADE of SARS-CoV;
- Dr Chung Cheung; Department of Microbiology at HKU, to investigate the occurrence of ADE with the live SARS-CoV;
- Professor Marc Daëron Institut Pasteur, with the goal to investigate the signaling cascade triggered through FcγR during ADE.

### 2.2.3 Current projects

The team is vigorously pursuing these two complementary projects that have already received extramural support.

Our first goal is to better define the **molecular basis of the interaction between viral envelope proteins and NK cell receptors** and to assess its role in the anti-viral immune response. Following our demonstration of a functional interaction between NKp44 and H5 **we are currently testing whether this binding could prevent both virus attachment and entry into human host cell**, or could act as a neutralization agent by binding to H5N1 infected target cells. These experiments will be carried out with the wild-type virus in BSL-3 containment (State Key Laboratory of Emerging Infectious Diseases of HKU). Since glycosylation of envelope proteins has been demonstrated to play an important role in virulence and immune interactions, we are investigating the relevance of the five unique glycosylation sites of the H5 hemagglutinin for its specific interaction with NKp44 and NK cells. **These experiments will form the basis to undertake a comprehensive study addressing the signaling pathways that are triggered in NK cells upon specific binding of H5 to NKp44**, which may further clarify the “helper” and “killer” role of NK cells in the event of viral clearance.

Since the exact mechanism by which the human immune system may react to a wide range of viral infections is still not well understood, **we are extending our observations to other viral envelope proteins**. In particular, many unknown questions still remains regarding the exact functions and cross-talks of players in the innate immunity, such as natural killer cells, dendritic cells, and macrophages upon viral infections. We have shown that **the binding of the NCRs NKp44 and NKp46 to different viral hemagglutinins requires the sialylation of NKp44** and NKp46 oligosaccharides. Given that members of **at least seven virus families utilize sialic acid as a receptor for virus entry** into host cells and that viral hemagglutinins are expressed on the surface of infected cells, it is likely that there is **a general strategy for NK cell recognition of a virus-infected cell** that triggered by a substantial subset of viruses. Thus **it is conceivable that NKp44 and related receptors may serve as molecules that neutralize viruses in a broadly reactive way** and this may have therapeutic potential. We are concentrating on the NK cell receptor interaction with viral envelope proteins, in particular, with E protein of dengue viruses, S, M and E of SARS-CoV.

**We are pursuing our efforts to define the biochemical pathways involved in ADE**, and to investigate their potential role in the pathogenesis of SARS-CoV and Avian Influenza infections. **Our initial data indicates differences in requirements in protease activity and endosome/lysosome acidity** during SARSpp infection mediated via the **natural receptor** angiotensin-converting enzyme-2 (ACE-2) or via **Fc receptors** for immunoglobulins (FcR). In addition, we are investigating the **mechanisms and effects of ADE of SARS-CoV infection in human B cells** in the presence of heat-inactivated serum from triSpike immunized mice or sera from convalescent SARS patients. This project will be extended to study the interaction between SARS-CoV and other haematopoietic cell types.

**Adaptation of an avian virus to transmission in humans is likely to involve multiple factors**. Because humans infected with the currently endemic, seasonal human influenza viruses H3N2 and H1N1 have heterosubtypic anti-influenza antibodies that can bind to the H5 hemagglutinin, although not necessarily neutralize it**, it is conceivable that such antibodies may in fact perversely**

**facilitate antibody-mediated infection of avian influenza viruses** into FcR bearing cells, even if the latter lack the natural receptor for binding such avian viruses. Studies done at HKU by our collaborators have shown that the FcR bearing macrophage is one target of H5N1 infection in the lung. **Such a phenomenon may provide an avian virus with a foothold** to establish itself in the human host, thus allowing the virus an opportunity to adapt to humans. Indeed, this may explain why only a minority of humans who are exposed to H5N1 virus get infected. **We have begun to study the potency of human sera collected from individuals infected by human seasonal flu** (e.g. H1N1, H3N2) or vaccinated with trivalent seasonal flu vaccine to cross-neutralize and cross-enhance entry of avian influenza pseudoparticles (e.g. H5pp). Preliminary data show that **cross neutralization of H5pp was observed with some human sera** presumably only exposed to H1N1 or H3N2 viruses implying the presence of hetero-subtypic antibody. In addition, cytokine induction from macrophages is hypothesized to be one mechanism explaining the pathogenesis of the severity of human H5N1 disease. It is known from other systems (e.g. Ross River virus) that entry via the FcR pathway can modulate host cell cytokine responses. **Therefore, in the event of an ADE of the avian H5N1 influenza virus, we will assess whether the pattern of cytokine production is perturbed;** this may be relevant to the pathogenesis of the disease.

### 2.2.4 Funding

These projects are financed by the following grants:

1. SARS CoV subunit vaccine: Mechanisms of antibody-mediated neutralisation and investigation of potential antibody-mediated enhancement (ADE) (**Research Fund for the Control of Infectious Diseases**, until June 16, 2008).
2. Interaction of NK activating receptors and hemagglutinin from avian flu – Potential implication of NKp44 in anti-flu therapy (**Research Fund for the Control of Infectious Diseases**, until September 30, 2009).
3. Analysis of interactions of NK inhibitory and activatory receptors and viral envelope proteins (Small Project Funding from the **Research Grants Council,** until September 30, 2009).

One grant is currently under review.

1. Investigation of Antibody-Dependent Enhancement (ADE) of influenza infection and its role in pathogenesis of avian flu (Submitted to the **Research Fund for the Control of Infectious Diseases**)

### 2.2.5 Personnel

| **Name** | **Position** | **End of contract (renewable)** |
|---|---|---|
| Joanna Ho | PDF | 30 June 2008 |
| Martial Jaume | PDF | 02 January 2009 |
| Cherry Xia | MPhil student | 31 December 2009 |
| Isabelle Dutry | Research technician (PhD candidate) | 31 May 2008 |
| Ping Hung Li | Research technician | 31 May 2008 |
| Simon Yip | Research Assistant (PhD candidate) | 31 October 2008 |
| Horace Lee | Research Assistant | 30 September 2008 |

## 2.3 PROGRAMME 3: SCREENING PLATFORM

### 2.3.1 Objectives

HKU-PRC has established the screening platform (SP) under the supervision of Dr Jean-Michel Garcia with three related programs:

1. **Development of a High throughput / high content (HT/HC) technology platform** based on state-of-the-art flow cytometry and open to HKU and Pasteur collaborators;
2. **Drug discovery against viral diseases**: identification of inhibitors of HIV, Influenza virus and dengue virus by screening an original compound library assembled by the Shanghai Institute of Materia Medica (SIMM, Chinese Academy of Sciences);
3. **Sero-epidemiology studies** on the highly pathogenic avian influenza (HPAI) H5N1. This project is part of the RESPARI program and conducted in close collaboration with Institutes of the Pasteur-Asia Pacific Network (mainly IP-Cambodia) and the Department of Microbiology of HKU.

### 2.3.2 Achievements

**Development of a high throughput/high content (HT/HC) technology platform.** Our team continues to test and evaluate different fluorescent proteins as well as toxicity markers for the flow cytometry. Nevertheless, **data management is now the main limiting factor for the development of HT/HC applications**. To overcome this bottleneck, **we have initiated collaboration with the Institut Pasteur of Montevideo** (Uruguay). The objective is to develop software to import/store/manage/treat the flow cytometry data while at the same time ensuring that the program design provides maximum flexibility and permit its applicability to other HT/HC approaches based on different technologies but facing the same problems. Our screenings will not only provide data for the test and validation of the software but also will generate new drug candidate or seroepidemiological data. **This project** has grown to include 2 other teams in IP-Paris and IP-Dakar through a grant proposal that **has been approved by the International Network of Pasteur Institutes** (**ACIP**, concerted action between Pasteur Institutes of the international network). The center has started to **open**

**this platform to HKU labs with the Department of Physiology** being the first beneficiary of this core service.

**Drug discovery against viral diseases**

*HIV.* **We have tested six of the most promising hits** from HIV-replication screening **against live, drug resistant viruses** and confirmed potent antiviral activity. The structure of **these compounds** and the results of in-silico docking studies suggested that they **belong to therapeutic group of nonnucleoside reverse transcriptase inhibitors** (NNRTi). Because this particular drug target is already well-studied and our molecules didn't show any obvious advantage compared to existing inhibitors of revere transcriptase, it was decided that pursuing these as commercial drug targets is unlikely to be productive. We have opted, therefore, to publish these results so that they are in the public domain and also to **prove the proof-of-principle of such a screening strategy** using pseudoparticles. **A manuscript is in preparation and will be submitted shortly for publication**.

*Highly Pathogenic Avian Influenza.* **The most potent inhibitors (n=6) of viral entry** that we had identified in our 2006 screening using lentiviral particles pseudotyped with the hemagglutinin **have now been tested against the live H5N1 virus** of a recently isolated highly pathogenic H5N1 isolate (H5pp). **Antiviral activity was confirmed for three** of these compounds. The data obtained to date suggest that **the mechanism of activity of these molecules is likely to be different from the currently exploited targets**, viz. viral uncoating and the viral neuraminidase activity. Thus, these compounds open up two new avenues of research that will address the mechanism of action of these drugs and, in parallel, aim at their pharmacological development.

In collaboration with the group of Mark von Itzstein (Griffith University, Australia), we have started to study the interaction of a wide range of glycans with H5. **These studies have implications for development of antiviral targets but have even more profound implications for understanding viral–host interactions and interspecies transmission of avian influenza viruses**. The receptor specificity for the influenza virus is controlled by the glycoprotein hemagglutinin (HA) on the virus surface. These viral HAs bind to host cell receptors containing complex glycans-carbohydrates that in turn contain terminal

sialic acids. For this project, H5-bearing virus-like particles (VLPs) were produced to investigate their binding in complex with α(2,3)- and α(2,6) sialyllactose using state-of-the-art NMR technology. The proof-of-concept of this approach, which will be amenable to large scale screening, has been demonstrated in the first round of experiences and **the results have recently been published in Angewandte Chemie, one of the highest impact journals in the field of chemistry** (Haselhorst et al., 2008). This major achievement has been the object of a press release from HKU and Griffith University and has been reported in the local media. Apart from a better understanding of influenza virus pathogenesis and inter-species transmission, **this research will help in the rational design of a much needed new class of drugs** directed against the virus HA. The need for novel drug targets for influenza is highlighted by reports of dramatically increased resistance in human seasonal influenza H1N1 to the current mainstay antiviral drug, oseltamivir.

*Dengue.* We **have completed the first phase of a project** developed in the context of the DENFRAME program **to identify new inhibitors of dengue replication**. The screening of 48,000 compounds from the SIMM library used in the other high-throughput screens (see above) led to identification of 20 hits that will be evaluated against the live virus. A Declaration of Invention is in preparation for the intellectual property protection of these molecules.

**Sero-epidemiology studies. We have published a manuscript** with a detailed characterization of viral particles pseudotypes with H5 hemagglutinin of avian influenza (Nefkens et al., 2007). We have provided evidence that H5pp replicate the entry mechanism of H5N1. Moreover, **we have demonstrated the feasibility of the use of the H5pp system in antibody-mediated neutralization studies** under BSL-2 conditions, suggesting that H5pp have great potential to be used as a safe, large-scale method for sero-diagnosis and sero-epidemiology studies. This approach has the additional advantage that functional (neutralization) serology assays can be developed without access to the original virus, or even viral RNA, but can purely be based on virus HA sequence. The description of these H5pp has already been protected by a declaration of invention. We have now **extended our studies and validated this approach both for sero-diagnosis and for sero-epipdemiology**. This assay has been used for the testing of thousands of

sera from several species and provides an excellent correlation with the "gold-standard" method of virus neutralization. The pseudotype strategy was recently adapted not only to the other subclades of H5 but also to seasonal influenza subtypes H1 and H3. During 2007 **we have successfully transferred our technology to the Hospital for Tropical Diseases** (Ho Chi Minh City, Vietnam) **and to the Institut Pasteur-Cambodia as part of the RESPARI project**.

**Industrial Partnerships. The potential of our pseudoparticle test** for serology testing **has been also recognized by pharmaceutical companies such as Sanofi-Pasteur, Baxter and GSK**, which have entrusted us for testing sera collected during clinical trial of candidate anti-influenza vaccines using the H5pp assay. These **industrial collaborations will not only generate income** (on a pay-for-service basis) **but also research output** in the form of publications. **Sanofi-Pasteur has also taken out an option for a non exclusive license** to have the right to use this technology for their vaccine development and evaluation studies. Furthermore, a Research Agreement was signed with CombinatoRx, (Singapore) to evaluate the efficacy of combination of drugs owned by the company with assays developed at HKU-PRC. **This collaboration** will not generate intellectual property but **will contribute to the funding of the platform** activities on a pay-for-service basis.

**Local and international contacts** have been actively pursued in 2007, with particular attention to the following collaborations:

- Dr Hugo Naya (IP Montevideo), Dr Amadou Sall (IP Dakar) and Dr Pierre-Olivier Vidalain (Institut Pasteur), to develop a network program with the goal establish a multi-application technical platform based on High-Content High-Throughput Flow cytometry;
- Dr Philippe Buchy (IP Cambodia) and Dr Menno de Jong (Wellcome Trust/ Oxford University Clinical Research Unit), to study the sero-epidemiology of avian influenza;
- Mark von Itzstein (Griffith University) and John Nicholls (Department of Pathology at HKU), with the goal of dissecting the molecular requirements of receptor preference of avian and human influenza virus hemagglutinins;

- Robert Webster (St Jude Children's Research Hospital) and Linda Lambert (DHHS) to evaluate the efficacy of the an H5N1 candidate vaccine in ferrets and humans;
- Sanofi-Pasteur, Baxter and GSK as collaborations and fee-for service programs using the pseudoparticle assay as a serological test;
- Paul Vanhoutte (Biopharmaceutical Development Centre at HKU) to monitor drug development of the hits identified from our screening campaigns (lead selection, preliminary toxicity);
- Jiang Ping Zuo (SIMM) and Michael Jacobs (University College London) to complete the drug discovery projects on avian influenza and dengue.

### 2.3.3 Current projects

Through the **ACIP project**, we want to **establish the nucleus of a multi-center technical platform within the Pasteur Institute International Network**. The first goal will be to explore the potential of flow cytometry as HT/HC technology for drug discovery, protein-protein interactions, diagnostic and sero-prevalence of infectious diseases. The **expected output is the generation of modular software for data management of HT/HC screenings** based on the use of flow cytometry.

In order **to enhance the high content screening (HCS) platform to the next level**, we propose to explore the possibility of **multiplexed testing using three different pseudoparticles bearing different fluorescent proteins as reporter genes** (e.g. CFP, YFP, HcRed), which would allow monitoring the effect on a number of viruses in one assay. This multiplex **approach** can be used for both **drug screening** and also for **serology testing**. In the first instance, the feasibility will be investigated for serology tests for the different subtypes of influenza ha-emagglutinin (H1, H3 or H5). This will not only measure the level of neutralizing antibodies for each sub-type but also evaluate the relative cross-neutralization in one experiment, saving time, reagents, and most importantly, the precious clinical specimen or drug being investigated. Two technical aspects are of particular importance: optical compatibility of reporter genes and management of multi-dimensional data sets. **If this approach is proved to be feasible**, it can be extended as a multiplex serology format to **serve other combinations of**

**pathogens** that need to be tested for syndromic or epidemiologically relevant reasons.

**We are continuing our efforts to search for new antiviral drugs**. We are **concentrating on the pharmaceutical development of H5N1 entry inhibitors** identified with the pseudoparticles based assay and will investigate their mechanism of action. **The quality of the hits** discovered in our last screening using the dengue replicon system and **confirmation of their action on the live virus** will determine the outcome of the project.

We are also **pursuing the development of the use of H5pp as a serological method** by producing H5pp from the different virus clades and in studies evaluating the H5 cross-clade reactivity of the neutralizing response of the pseudotyped virus in comparison with conventional neutralization tests. These studies will use human sera from patients with clade 1 H5N1 disease from Vietnam and from vaccinated individuals immunized with H5N1 vaccines with different clades (in collaboration with industrial partners Sanofi and Baxter). We also aim to extend these methods to evaluate the serology for other influenza subtypes, H9 and H6, which remain pandemic candidates. We will use these tests to investigate the sero-prevalence in humans and animals of avian H9 and H6 subtype viruses by comparing pseudotyped viruses, conventional neutralization tests and (for avian sera) hemagglutination inhibition tests.

We have initiated **a new project to explore the whether the viral neuraminidase can be detected in such pseudotype assays** and if so, its **potential use as a high-throughput serological method**. A recent study has demonstrated that antibodies against neuraminidase can also be neutralizing. We aim to exploit this to develop new assays for a DIVA (**D**ifferentiate **I**nfected from **V**accinated **A**nimals) strategy with potential application in veterinary diagnostics.

We are **collaborating** with **Griffith University** to **investigate the interaction of hemagglutinins with glycans** on the host cell surface by combining Saturation Transfer Difference-Nuclear Magnetic Resonance (STD-NMR) and virus-like-particles (VLPs) bearing hemagglutinins of different influenza strains. We will use this approach to re-e**valuate the spectrum of glycans which have the the ability to bind the avian H5-virus**. These will be correlated with parallel studies

ongoing at HKU (Dr JM Nicholls, Department of Pathology) to fully characterize the glycans on human cell surfaces in different tissues within the context of the Area of Excellence program. To this end, two sets of hemagglutinins have been synthesized: one derived from H5 and the other from H3. In each set, we have engineered naturally occurring point mutations that can potentially trigger a shift in receptor binding preferences, from avian to human [α(2,3) to α(2,6)-linked N-acetylneuraminic acid-containing glycans] in H5 and, conversely, from human to avian (α2,6 to α2,3) in H3. Interactions of different glycans with the HA constructs incorporated on the surface of the VLPs will be monitored by NMR at atomic level. **These systematic studies will lead not only to a better understanding of structural changes that may facilitate avian viruses to better interact with human cells and lead to human-to-human transmission and H5N1 pandemic emergence**. They will also **help** in the rational **design of a new therapeutic class of anti-influenza** drugs (hemagglutinin inhibitors).

### 2.3.4 Funding

These research programs are funded by the French Ministry of Health, the Li Ka Shing Foundation and the Institut Pasteur (through the ACIP). Three grants are currently under review.

1. Pseudotyped influenza viruses for serodiagnosis and sero-epidemiology (submitted to the **Area of Excellence**)
2. Investigation of the interactions of glycans and H5pp and H3pp VLPs to define virus host restriction and inter-species transmission (in collaboration with Dr JM Nicholls, submitted to the **Area of Excellence**)
3. Development of multiplex neutralization tests for avian and seasonal influenza using pseudotyped lentiviruses (Submitted to the **Research Fund for the Control of Infectious Diseases**)

## 2.3.5 Personnel

| Name | Position | End of contract (renewable) |
|---|---|---|
| Jean-Michel Garcia | PDF | 3 November 2008 |
| Joyce Choi | Junior Technician | 31 May 2007 |
| Nadege Lagarde | Research technician | 13 June 2007 |
| Jimmy Lai | Junior Technician | 2 July 2007 |
| Agnes Dumont | Research Technician (Fellow of the French Ministry of Foreign Affairs) | 30 September 2008 |
| Audrey Derveloy | Research Technician (Fellow of the Fondation de France) | 31 January 2009 |
| Kei Shuen Tang | BSc student | |

## 2.4 PROGRAMME 4: TEACHING AND TRAINING

### 2.4.1 Objectives

The Hong Kong University-Pasteur Research Center (HKU-PRC) aims to develop, in close synergy with HKU, a Teaching and Training Centre of excellence for Biomedical Research open to Asian students and scientists, in particular from HKU and the Institut Pasteur network. The Teaching and Training Centre will facilitate the organization of hands-on ("wet lab") teaching and training courses and workshops that require cell culture facilities and / or BSL-2 containment on the campus. At present, there is no laboratory space available for the conduct of such courses. For example, in 2006 the Department of Microbiology encountered difficulties to find a suitable place to hold the WHO Laboratory training course on Influenza for the Asian region. HKU-PRC conducts annual courses on Virology for the Asian region but has been unable to provide meaningful laboratory training because of the lack of such a dedicated training laboratory space.

### 2.4.2 Achievements

#### 2.4.2.1 Students

**The first Ph.D. student of HKU-PRC has successfully defended his research project.** Jason Kam has been awarded the degree of Doctor of Philosophy by the University of Hong Kong for his work entitled "Biochemical, functional and immunogenic characterization of the SARS spike glycoprotein: Implications for the development of a subunit vaccine". Dr Jason Kam joined the Center in the Fall of 2003 and has already published two scientific articles (a third manuscript is in preparation). **He has joined the laboratory of Dr Lisa Ng at the Singapore Immunology network located in Biopolis (Singapore) for his postdoctoral training**.

**Three new PhD students have started working at the center**: 2 in September 2006 and one in July 2007. In addition, **2 MPhil students have been recruited** at the beginning of 2008 and t**wo PhD candidates have joined the center** with temporary appointments as technical staff, **pending the result of their application**, which should be communicated next month.

**Three students of the Department of Biochemistry**, Ms Tang Kei Shuen, Ms Wu Yuet and Mr Siu Sin Hang **joined HKU-PRC as part of the "BIOC3614 Biochemistry Project"** to work under the supervision of Drs Jean-Michel Garcia, Pei Gang Wang and Dong Jiang Tang, respectively. This course, which is one of the requirements in the Sciences majors and minors offered by the Department of Biochemistry at HKU, enables students to acquire the basic skills in scientific research by actively participating in a research project in molecular life sciences.

We have welcomed **two summer students**, Ms Josephine Ng and Mr Johann Lok who worked for 6-8 weeks under the supervision of Drs Joanna Ho and Martial Jaume, respectively. They presented the results of their projects at a lab meeting at the end of their lab rotation.

**The Centre is a regular contributor of educational programs in science sponsored by the French International School**. We have hosted students from the French International School for a one-week studentship for two years in a row. The students had the opportunity to interact closely with their tutors and HKU-PRC staff and to discover various aspects of current research in virology. A PhD student in the Centre, **Jean Millet, has participated in the Career Forum (2007**) to offer information and answer questions about careers in biology; **Nadège Lagarde**, a Research Technician, has presented to French students the molecular biology of the Human Immunodeficiency Virus **on the occasion of the AIDS World Day 2007**.

#### 2.4.2.2 Courses

The Faculty Higher Degrees Committee (FHDC) and the Board of Graduate Studies (BoGS) of HKU have formally approved that the **Pasteur-Asia Virology Course be included in the coursework curriculum for Research Postgraduate studies**. MPhil and 4-year PhD students who have successfully completed this course will be considered as having fulfilled all the required coursework of the Medical Faculty.

**The 4$^{th}$ Pasteur-Asia Virology Course**, which consisted of lectures, bibliographic seminars and experimental demonstrations, proved to be once again **very successful**. The major focus of the program was to provide a comprehensive overview of epidemiology, molecular and cellular biology and prevention strategies

against respiratory viruses. **We received 54 applications and selected 29 students coming from 9 different countries.** Ten of them originated from the Pasteur Institute Network and, for the first time two were from African institutes. **The number of local applications was significantly increased**, due to the inclusion of the course in the Research Postgraduate curriculum of HKU.

#### 2.4.2.3 Croucher-Pasteur Exchange Program

The CROUCHER PASTEUR EXCHANGE PROGRAMME is jointly sponsored by the International Affairs Department of Institut Pasteur and the Croucher Foundation to encourage highly motivated students and post-doctoral fellows resident in Hong Kong to perform research work in laboratories of Institut Pasteur-Paris or one of the institutes of the International Network. There are currently two students selected through the Exchange Program working at IP-Paris.

The **Croucher-Pasteur Lecture Series has been organized for the third consecutive year**. For all four lectures organized we have attracted more than 70 students and scientists who have engaged in stimulating discussions with the invited speakers. Lecturers and potential candidates have now working closely with their project proposal (November 15 deadline) and two students have submitted an application.

### 2.4.3 Current projects

**The incorporation of the third floor of the HC Dexter Man Building into HKU-PRC allows us to propose to expand this training program** in the near future and offer more international courses mainly targeted at MPhil and PhD students **in Immunology, Cell Biology and related topics**. After carefully considering several options, we have decided to f**ocus on Immunology and Cell Biology**. **Immunology** appears as the logical partner to complement efforts in the field of infectious diseases and several groups at HKU are actively engaged in research programs in this area. However, **there are currently no specific courses devoted to this discipline** and, therefore, tour proposal is answering a recognized need. Similarly, **cell biology** is at the heart of all aspects of biological science from genomics to infectious disease and therefore **strengthening this area of research in Hong Kong will have broad impact**.

Proposals describing the goals and structure of these high-level courses, which will feature lectures from international experts and practical sessions for students and trainees across Asia, have be submitted to the FHDC and BoGS for inclusion into available **modules offered to students at HKU as coursework in the Research Postgraduate curriculum**. The first **Pasteur-Asia Immunology Course** has been tentatively **planned for October-November 2008**. It will be co-organized with **Dr Allan Lau** (Department of Pediatrics and Adolescent Medicine at HKU) and **Professor Jean-Marc Cavaillon** of Institut Pasteur. The first **Pasteur-Asia Cell Biology Course will be co-organized in 2009** with **Professor George Tsao** (Department of Anatomy at HKU), in collaboration with **Dr Chiara Zurzolo** (Institut Pasteur) and **Philippe Chavrier** (Institut Curie).

The development of the Teaching Center will further increase the quality of the Virology course and its impact in the region. The course should also become a cornerstone for HKU trainees in microbiology and we are considering alternative schemes that could be more attractive to the best local students. One possibility would be to build the program around week-long modules combined into a three-week course. This approach has been successfully tested in courses at Institut Pasteur-Paris (for example the Molecular Biology of the Cell course) and would give HKU students the possibility of selecting only the modules of their choice. Separate exam sessions will be included in the program.

We also envisage running **shorter theoretical and technical courses, which may include training sessions on high-throughput screening and cytometry** in a BSL-2 platform or the applications of microscopy to the study of virus-host cell interactions. We have submitted to the **Croucher Foundation** a first proposal to hold a **series of seminars on "Subcellular Structures and Cellular Dynamics"**, which will illustrate through several examples (cell polarization, differentiation, vesicular trafficking and gene regulation) how specialized compartments participate to the regulation of cellular functions in health and disease. The lectures will be opened to Scientists from all Academic Institutions of Hong Kong, including students and senior scientists and will help in promoting inter-institutional collaborations in the field.

Furthermore, **in close collaboration with Professor George Tsao** (Department of Anatomy), who is in charge of the Core Imaging Facility of the Faculty of

Medicine, we have planned to **host a workshop on "Frontiers in live cell and molecular imaging: application in biomedical research"**. **Live cell imaging is at the cutting edge of biomedical research**, thanks to important technical progress and many breakthroughs have been recently achieved with novel applications of live cell imaging in biomedical studies. The Faculty of Medicine at HKU and other local institutes have begun to install imaging facility in live cell and molecular imaging, **but the experience of local researchers is still limited. The proposed workshop will fill this gap by bringing world leaders in live cell imaging to Hong Kong to provide in-depth state-of-the-art training in live cell and molecular imaging technology**. In addition, we have secured the help of **key scientific staff from Institut Pasteur and Institut Pasteur-Korea** to provide their vast expertise in their established imaging centers to participate also in the practical sessions, which will take place in the laboratories of the Core Imaging Facility of the Faculty of Medicine and HKU-PRC. We have obtained the necessary support from the major confocal laser scanning microscope providers in Hong Kong (Carl Zeiss, Perkin-Elmer, Olympus, Nikon, Leica) to provide confocal laser scanning microscopes for the running of the practical and demonstration sessions. **An application to the Croucher Foundation to fund this activity as part of the Advanced Study Institute scheme has been submitted** (decision expected in April).

## 2.4.4 Funding

The 4rd Pasteur Asia Virology course has been funded by Institut Pasteur-Paris, the HKU-Faculty of Medicine, the Croucher Foundation, the French Chamber of Commerce, the RESPARI network (through the SISEA program), the French Consulate and Sanofi-Pasteur (see Annex 3).

With respect to the renovations planned to make room for a more spacious lecture room (1st floor), as well as for the teaching lab and its equipment (3rd floor), an initial budget of 780,000 HK$ was approved. Work on the first floor has been completed and the new lecture room has proved more than needed during the 4th Pasteur-Asia Virology course, with many lectures packed to the full capacity of seats (see Annex for budget). The renovations of the 3rd floor needed for the organization of hands-on ("wet lab") teaching and training courses and workshops

that require cell culture facilities and / or BSL-2 containment have been estimated at 0.6 million HK$ (see Annex 4). We are starting the tender process and expect that work will be completed by the end of May, thus enabling to include a practicum in the 5th Pasteur-Asia Virology course. To continue the development of this project in 2007 we have obtained an interest-free loan of 3.2 million HK$ from the Li Ka Shing Faculty of Medicine to equip the Teaching and Training Centre with state of the art material and reach the level of excellence targeted (see Annex 5). The loan will be repaid by 2011.

## 2.5 NETWORK PROJECTS

### 2.5.1 RESPARI

*2.5.1.1 Objectives of the program*

In December 2004, the Directors of the Pasteur Institutes from the Asia-Pacific region met in Hong Kong and decided to promote a large scale initiative dedicated to research on respiratory diseases. This network program has been called **RESPARI**, for **R**esearch-driven R**ESP**onse to **A**cute **R**espiratory **I**nfections (www.hku.hk/respari). The mission of RESPARI is t***o develop technologies and instruments that permit to discover viral threats in the region and develop a network with the capacity to respond in a timely manner***. This mission is implemented through the following strategies:

- Documenting the etiologies of pneumonia in children and adult, with special focus on preventable infections (through vaccine and antibiotics);
- Developing novel diagnostic tools that will strengthen both biological and epidemiological research methods for acute respiratory infections;
- Improving the understanding of the pathogenesis of ARI and develop novel therapeutic options;
- Teaching and training of young medical doctors and researchers with a focus on public health and biomedical science.

**The specific goals of RESPARI are to tackle several aspects of acute respiratory infections** and other emerging diseases through the implementation of four work packages (WP):

1. Development of molecular epidemiology and clinical microbiology (WP1);
2. Development of diagnostic tools and surveillance in adults and children (WP2);
3. Development of pathophysiology and therapy, small chemicals and vaccine (WP3);
4. Implementation of teaching and training (WP4).

Each work package is comprised of different activities that are being developed in coordination by the participating centers. This project will cover medical issues,

including surveillance and research in public health (WP1), development and standardization of new diagnostic tools (WP2), fundamental molecular research as well as innovative therapy development (WP3), and a continuing educational program (WP4).

**HKU-PRC is involved in** the **standardization of techniques** for the diagnostic and epidemiological studies based on our H5pp assay (**WP 2**), the understanding of **basic mechanisms of virus-host cell interactions** and the search for new molecules with anti-viral activity (**WP3**) and in the **implementation of training of researchers**, medical doctors and through top-tier courses that will become a reference for the region (**WP4**).

The RESPARI initiative should strengthen both research and intervention groups in the different institutes from the Asia-Pacific region, in particular clinical microbiologists, epidemiologists and molecular biologists working together on a common project. All theses interactions will allow a more efficient sharing of means and tools, and a more rapid valorization of the knowledge output. **RESPARI will promote an annual scientific meeting to discuss the progress of the project**.

*2.5.1.2 Achievements*

**We have taken up the Scientific Coordination of the program** and have put in place a Scientific Advisory Board (SAB). The composition of the SAB is the following whose members are:

Prof Yves CHAMPEY, Chair, Drugs for Neglected Diseases Initiative (DNDi), Geneva, Switzerland;

Prof. Jeremy FARRAR, Oxford University Clinical Research Unit, The Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam;

Prof. François FREYMUTH, Laboratoire de Virologie Humaine et Moléculaire, CHU, Caen, France;

Prof. Hans-Dieter KLENK, Institute of Virology, Philipps University, Marburg, Germany;

Prof. Charles MAYAUD, Service de Pneumologie et de Réanimation respiratoire, Hôpital Tenon, Paris, France.

**We have organized the first scientific meeting of RESPARI, which has taken place in Hong Kong, May 9-11, 2007**. The meeting was attended by approximately 40 scientists of the Pasteur network and was open to the public. Together, the presentations have provide an updated of ongoing activities and promoted this initiative among the scientific community. On the last day of the meeting the SAB discussed behind closed doors their report with at least one representative from each participating center. A second RESPARI workshop in the presence of all members of the SAB has been held in Shanghai on November 6-7, 2007. It was devoted to the evaluation of the activities included in the SISEA (**S**urveillance and **I**nvestigation of Epidemic **S**ituations in South **E**ast **A**sia).

**On both occasions the SAB was unanimous in its support for the activities of the RESPARI Network** and wished to extend its appreciation to everyone involved on the outstanding work that has already been achieved and the plans for the further development of the Network. The **SAB further noted that presentations were of the highest international standard and the external partners**, who have been very carefully selected, are clearly world class in their area of expertise and will undoubtedly make a major collaborative contribution. The SAB had some concerns about the planned Clinical Studies and suggested that the Network consider engaging with additional expertise in the planning of these studies. We are currently addressing those concerns with the preparation of a technical document that will form the basis for the clinical/epidemiological activities in the region. This should be produced through a collaborative process and in a timely manner to meet the recommendations of the SAB.

Besides achieving significant results in all activities in which we are involved, as reported above for each individual project, **we have successfully contributed to technology-transfer of the H5pp assay in the region**. Dr Jean-Michel Garcia went to the **Hospital for Tropical Diseases in Ho Chi Minh City**, Vietnam and to the **Institut Pasteur-Cambodia** to train local personnel (see above 2.3.2). These collaborations allow us to expand the sero-prevalence studies that are currently ongoing in the center.

*2.5.1.3 Funding*

Since the establishment of this program, RESPARI has been able to secure important funds from different sources, including the French Agency for

Development (SISEA Program), the Department of Human and Health Services of the United States, the Li Ka Shing Foundation and the French Ministry of Health. **HKU-PRC has received 3.9M HK$ from the French Ministry of Health and 1.7M HK$ from the Li Ka Shing Foundation**.

## 2.5.2 DENFRAME

*2.5.2.1 Objectives*

The main **aim of the DENFRAME project is to improve the management of dengue** disease in the human populations of Latin America and Asia. **The role of HKU-PRC in this program is to develop a cell-based assay** for screening of inhibitory compounds.

*2.5.2.2 Achievements*

We have unsuccessfully tried to develop a pseudotype assay for dengue virus (Dvpp). Although infectious Dvpp were produced from 293T human epithelial cells, the process was inefficient and titers were typically 2 log lower than type-I fusion proteins (Influenza HA, SARS Spike) and 1 log lower than type-II fusion proteins. We have, therefore changed our strategy and **developed an assay based on the production of viral-like particles (VLPs)**. We have established a dengue VLP producing stable cell line (HeLa-prME) by using a codon-optimized dengue 1 prME gene that highly increases expression level of prME. VLPs can readily form in cells expressing the glycoproteins of dengue virus without any other viral element. Our data shows that most E protein co-localizes with markers of the ER, which correlates with a previous report. Biochemical analysis of VLPs produced by HeLa-prME cells demonstrates that they contain dimerized E protein and M protein, the cleaved product or prM, indicating that VLPs have gone through the same secretion pathway and maturation process as wild type dengue viruses. Furthermore, we have set up a chemical luminescence dot-blot (CLDB) system to quantify VLP production by HeLa-prME cells. Thus, **the VLP producing HeLa-prME cell line constitutes a safe and convenient tool to study prM/E interaction and assembly of empty virus-like particles that will be amenable to screening for cellular factors interfering with viral egress**.

**Our second goal** in this program is to develop a drug screening strategy to **identify inhibitors of dengue virus infection**. We have completed **the first**

**phase of a high-throughput screening** of the 48,000 compounds of the SIMM library, used also for the HIW and avian influenza projects, to identify new inhibitors of dengue replication. The screening **led to the identification of** 35 confirmed active compounds, **20 of which were not toxic and are now ready to be tested against live type 2 dengue viruses**.

*2.5.2.3 Funding*

The DENFRAME consortium has been funded for three years (November 2005-October 2008) by the European Union within the 6th Framework Programme. HKU-PRC has received 960K HK$.

## 2.5.3 Area of Excellence

*2.5.3.1 Objectives*

**The University Grants Committee**, in the fourth round of its Areas of Excellence (AoE) scheme, **has awarded HK$76 million to fund a research project entitled "Control of Pandemic and Inter-pandemic Influenza",** to be led by a HKU research team comprising over 30 scientists. Capitalizing on the niche advantage and international excellence achieved in influenza research, this network **aims to develop a synergistic program with broad scope and global impact** which will address questions about the **emergence, transmission and pathogenesis of pandemic and seasonal influenza**. **The** mission of the **AoE**, which **is chaired by Professor Malik Peiris**, is:

- To enhance global influenza pandemic preparedness and control of inter-pandemic (seasonal) influenza;
- To consolidate Hong Kong's status as a centre of research excellence in influenza and infectious diseases, to promote industry-partnerships and enhance the development of a "knowledge-based-economy" in Hong Kong;
- To nurture the next generation of bio-medical scientists and promote Hong Kong as a regional and global hub for education and research in the bio-medical sciences.

This mission is **implemented through five major work-packages** (WP):

1. To define the ecology and molecular evolution of animal influenza viruses in southern China and other parts of Asia so as to identify viruses of potential risk to human health (WP1);
2. To identify viral and host determinants of the pathogenesis of human H5N1 disease (WP2);
3. To identify the inter-species barriers restricting the transmission of avian influenza viruses to humans and other mammals, and those restricting transmission between different avian species (WP3);
4. To identify evidence-based options for the control of influenza in animals and humans by defining the transmission dynamics and quantifying the impact of interventions (WP4);
5. To develop novel options for diagnosis, vaccines and therapy (WP5).

**HKU-Pasteur Research Centre's efforts are concentrated on pathogenesis**, **host response** and **innate immunity** (WP2) and **translational research** (WP5).

*2.5.3.2 Funding*

The program will distribute funds on a competitive basis. Three projects have been submitted in the first call for proposals:

**The cellular interactome of the H5N1 influenza virus**, with the objective to characterize enhancing and restricting cellular factors of the highly pathogenic H5N1 virus using large-scale genomic screens, with a particular emphasis on early and late stages of the replication cycle (PI: Beatrice Nal-Rogier).

**Pseudotyped influenza viruses for sero-diagnosis and sero-epidemiology**, with the aim to develop and validating an assay for sero-diagnosis and sero-epidemiology of avian and human influenza viruses by testing a comprehensive set of influenza pseudotypes for potentially pandemic subtypes such as H5-and H9 and for human seasonal influenza (PI: Jean-Michel Garcia).

**Investigation of the interactions of glycans and H5pp and H3pp VLPs**, with the aim to define virus host restriction and inter-species transmission (in collaboration with Dr JM Nicholls, Department of Pathology).

# 3 SCIENTIFIC OUTPUT

## 3.1 Publications (since September 2006)


1. Buchy P, Mardy S, Vong S, Toyoda T, Aubin JT, Miller M, Touch S, Sovann L, Dufourcq JB, Richner B, Tu PV, Tien NT, Lim W, Peiris JS, van der Werf S (2007) Influenza A/H5N1 virus infection in humans in Cambodia. *J Clin Virol* **39**:164-168.
2. Chui SS, Peiris JS, Chan KH, Wong WH, Lau YL (2007) Immunogenicity and safety of intradermal influenza immunization at a reduced dose in healthy children. *Paediatrics* **119**:1076-1082.
3. Peiris JS, de Jong MD, Guan Y (2007) Avian influenza virus (H5N1): a threat to human health. *Clin Microbiol Rev* **20**:243-267.
4. Nefkens I, Garcia JM, Chu SL, Lagarde N, Nicholls J ,Tang DJ, Peiris M, Buchy P, Altmeyer R. (2007) Hemagglutinin pseudotyped lentiviral particles: Characterization of a new method for avian H5N1 influenza sero-diagnosis. *J Clin Virol* **39**:27-33.
5. Kam YW, Kien F, Roberts A, Cheung YC, Lamirande EW, Vogel L, Chu SL, Tse J, Subbarao K, Peiris M, Nal B, Altmeyer R. (2007) Antibodies against trimeric S glycoprotein protect against SARS-CoV challenge despite their capacity to mediate FcγRII-dependent entry into B cells in vitro. *Vaccine* **25**:729-740.
6. Ho JW, Hershkovitz O, Peiris M, Zilka A, Bar-Ilan A, Nal B, Chu K, Kudelko M, Kam YW, Achdout H, Mandelboim M, Altmeyer R, Mandelboim O, Bruzzone R, Porgador A. (2008) H5-type influenza hemagglutinin is functionally recognized by the natural killer activating receptor, NKp44. *J Virol* **82**:2028-2032.
7. Haselhorst T, Garcia JM, Islam I, Lai JC, Rose FJ, Nicholls JM, Peiris M, von Itzstein M (2008) An investigation of the host-cell receptor specificity of avian H5N1 influenza virus-like particles (VLP's) by STD NMR spectroscopy. *Angew Chem Int Ed* **47**:1-4.


## 3.2 Seminars, Invited Lectures and Oral Presentations at Meetings (since September 2006)

### *2006*

1. Garcia JM. Fourth ICAV International and First Pacific Rim ICAV Meeting, God Coast, Australia (Oral Presentation).
2. Garcia JM. Journées Départementales de Virologie de l'Institut Pasteur, Paris, France (Oral Presentation).
3. Millet J. 11th Research Postgraduate Symposium of the Li Ka Shing Faculty of Medicine at HKU, Hong Kong SAR (Oral Presentation).
4. Nal-Rogier B. Journées Départementales de Virologie de l'Institut Pasteur, Paris, France. (Oral Presentation).
5. Tang DJ. Scientific conference of the 38th Board of Institutes Pasteur Directors, National Institute of Hygiene and Epidemiology. Hanoi, Vietnam. (Oral Presentation).

*2007*

1. Bruzzone R. Singapore Immunology Network and Genome Institute of Singapore, Singapore (Invited Lecture).
2. Bruzzone R. IBRO 2007, World Congress of Neuroscience, Melbourne, Australia (Invited Speaker).
3. Bruzzone R. VIII European Meeting on Glial Cell Function in Health and Disease, London, UK (Invited Speaker).
4. Garcia JM. IX International Symposium on Respiratory Viral Infections, Hong Kong SAR (Oral Presentation).
5. Garcia JM. 1st Meeting of the Pasteur Asia Network on Emerging Respiratory Diseases, Hong Kong SAR (Oral Presentation).
6. Ho JW. 1st Meeting of the Pasteur Asia Network on Emerging Respiratory Diseases, Hong Kong SAR (Oral Presentation).
7. Jaume M. Vaccines and enhancement of viral entry. 3rd Mediterranean Immunology Course, Marrakech, Morocco (Oral Presentation).
8. Nal-Rogier B. DENFRAME Workshop on work packages 5 and 6. Shanghai, China (Oral Presentation).
9. Nal-Rogier B. 1st Meeting of the Pasteur Asia Network on Emerging Respiratory Diseases, Hong Kong SAR (Oral Presentation).

*2008*

1. Nal-Rogier B. Imaging Workshop of HKU-Pasteur Research Centre and the University Core Imaging Facility, HKU, Hong Kong SAR (Oral Presentation).

## 3.3 List of Posters (since September 2006)

*2006*

1. GARCIA JM, I NEFKENS, J CHOI, N LAGARDE, DJ TANG, JP ZUO, F NAN, J LI, P VANHOUTTE, B NAL-ROGIER, R BRUZZONE, M PEIRIS, R ALTMEYER. High Throughput Screening technological platform: Application to drug discovery, sero-diagnosis and cellular biology research. Scientific conference of the 38th Board of Institutes Pasteur Directors, National Institute of Hygiene and Epidemiology, Hanoi, Vietnam.
2. HO JW, A PORGADOR, R ALTMEYER. Identification of novel interactions between viral envelop proteins and activating receptor family of natural killer cells. Scientific conference of the 38th Board of Institutes Pasteur Directors, National Institute of Hygiene and Epidemiology, Hanoi, Vietnam.
3. JAUME M, F KIEN, YW KAM, P CORBY, PH LI, K CHU, J TSE, YC CHEUNG, JSM PEIRIS, M DAERON, B NAL-ROGIER, R ALTMEYER. FcγRII-mediated SARS-CoV entry in human B cell lines. 16th European Congress of Immunology, Paris, France
4. JAUME M, F KIEN, YW KAM, PH LI, YC CHEUNG, JSM PEIRIS, M DAERON, B NAL-ROGIER, R ALTMEYER. FcγRII-mediated SARS-CoV entry in human B cell lines. Scientific conference of the 38th Board of Institutes Pasteur Directors, National Institute of Hygiene and Epidemiology, Hanoi, Vietnam.

5. KAM YW, F KIEN, YC CHEUNG, K CHU, J TSE, M PEIRIS, B NAL, R ALTMEYER. Development of subunit vaccine against SARS-CoV using truncated soluble S glycoprotein with balanced Th1/Th2 immune responses. Scientific conference of the 38th Board of Institutes Pasteur Directors, National Institute of Hygiene and Epidemiology. Hanoi, Vietnam.
6. WANG PG. Development of dengue pseudotyped viruses. Scientific conference of the 38th Board of Institutes Pasteur Directors, National Institute of Hygiene and Epidemiology. Hanoi, Vietnam.

## *2007*

1. GARCIA JM, I NEFKENS, N LAGARDE, K CHU, P BUCHY, R ALTMEYER, M PEIRIS. H5 pseudotyped lentiviral particles: A New tool for sero-diagnosis of influenza H5N1 infection. IX International Symposium on Respiratory Viral Infections, Hong Kong SAR.
2. JAUME M, YW KAM, F KIEN, PH LI, YC CHEUNG, R ALTMEYER, R BRUZZONE, JSM PEIRIS, B NAL-ROGIER. SARS-CoV subunit vaccine: Mechanisms of antibody-mediated neutralization and investigation of potential antibody-mediated enhancement. Health Research Symposium, Hong Kong SAR.
3. TEOH KT, YL LAU, M PEIRIS, R, B NAL. Identification of a cellular factor which interacts with SARS-CoV E protein: Insight for function during SARS-CoV infection. 12th Research Postgraduate Symposium of the Li Ka Shing Faculty of Medicine at HKU, Hong Kong SAR.

## *2008*

1. GARCIA JM, N LAGARDE, E MA, M DE JONG, VM HIEN, DQ HA, P BUCHY, E GOVORKOVA, M PEIRIS. Evaluation of H5 pseudoparticle-based assay for influenza serology testing in humans and animals. X International Symposium on Respiratory Viral Infections, Singapore.
2. HO JW, O HERSHKOVITZ, M PEIRIS, A ZILKA, A BAR-ILAN, B NAL, K CHU, M KUDELKO, YW KAM, H ACHDOUT, M MANDELBOIM, R ALTMERYER, O MANDELBOIM, R BRUZZONE, A PORGADOR. H5-type influenza hemagglutinin is functionally recognized by the natural killer activating receptor, NKp44. Keystone Meeting on NK and NKT Cell Biology, Keystone, USA.
3. KIEN F, L SIU, J MILLET, DJ TANG, J TSE, M CHAN, R CHAN, R BRUZZONE, M PEIRIS, B NAL. Development of molecular tools for SARS-CoV single-virus tracking. Keystone Meeting on Cell Biology of Virus Entry, Replication and Pathogenesis, Fairmont Empress Victoria, Canada.

## 3.4 Current grants

***RESPARI, Li Ka Shing Foundation***

| | |
|---|---|
| Principal Investigator: | Dr Roberto Bruzzone |
| Amount: | RMB 1,777,000.00 |
| Start Date: | 26/Apr/2006 |
| Completion Date: | 25/Apr/2009 |

***RESPARI, French Ministry of Health,***

| | |
|---|---|
| Principal Investigator: | Dr Roberto Bruzzone |
| Amount: | Euro 430,000.00 |
| Start Date: | 01/Mar/2006 |
| Completion Date: | 29/Feb/2009 |

***DENFRAME, Commission of the European Communities***

| | |
|---|---|
| Principal Investigator: | Dr Beatrice Nal-Rogier |
| Amount: | Euro 117,000.00 |
| Start Date: | 01/Nov/2005 |
| Completion Date: | 31/Oct/2008 |

***Research Fund for the Control of Infectious Diseases***

| | |
|---|---|
| Principal Investigator: | Dr Beatrice Nal-Rogier |
| Amount: | HK$750,720.00 |
| Start Date: | 17/Jul/2006 |
| Completion Date: | 16/Jun/2008 |

***Research Fund for the Control of Infectious Diseases***

| | |
|---|---|
| Principal Investigator: | Dr Joanna W Y Ho |
| Amount: | HK$800,000.00 |
| Start Date: | 01/Oct/2007 |
| Completion Date: | 30/Sep/2009 |

***Research Grants Council, Small Project Funding***

| | |
|---|---|
| Principal Investigator: | Dr Joanna W Y Ho |
| Amount: | HK$58,840.00 |
| Start Date: | 01/Oct/2007 |
| Completion Date: | 30/Sep/2009 |

***Actions Concertees InterPasteuriennes (ACIP)***

| | |
|---|---|
| Principal Investigator: | Dr Jean-Michel Garcia |
| Amount: | Euro 10,000.00 |
| Start Date: | 01/Apr/2008 |
| Completion Date: | 31/Mar/2010 |

## 3.5 Pending Grant Applications

***Research Grants Council***

Principal Investigator: Dr Roberto Bruzzone
Amount: HK$1,110,480.00
Date Submitted: October 2007
Project Duration: 24 months

***Research Fund for the Control of Infectious Diseases***

Principal Investigator: Dr Beatrice Nal-Rogier
Amount: HK$724,000.00
Date Submitted: November 2007
Project Duration: 24 months

***Research Fund for the Control of Infectious Diseases***

Principal Investigator: Dr Martial Jaume
Amount: HK$789,840.00
Date Submitted: November 2007
Project Duration: 24 months

***Research Fund for the Control of Infectious Diseases***

Principal Investigator: Dr Pei Gang Wang
Amount: HK$343,000.00
Date Submitted: November 2007
Project Duration: 24 months

***Research Fund for the Control of Infectious Diseases***

Principal Investigator: Dr Dong Jiang Tang
Amount: HK$774,101.00
Date Submitted: November 2007
Project Duration: 24 months

***Research Fund for the Control of Infectious Diseases***

Principal Investigator: Dr Jean-Michel Garcia
Amount: HK$800,000.00
Date Submitted: November 2007
Project Duration: 24 months

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

Principal Investigator: Dr Beatrice Nal-Rogier
Amount: HK$802,920.00
Date Submitted: January 2008
Project Duration: 24 months

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

Principal Investigator: Dr Jean-Michel Garcia
Amount: HK$780,000.00
Date Submitted: January 2008
Project Duration: 24 months