Research Assessment Exercise 2020
Impact Case Study

University: The University of Hong Kong (HKU)
Unit of Assessment (UoA): 02 - Pre-clinical Studies
Title of case study: Epidemiological investigation, antiviral and vaccine for influenza virus infection

(1) Summary of the impact

Influenza virus research from the University of Hong Kong has guided public health policy and generated novel antivirals and vaccines. We were the first to confirm that live poultry market (LPM) is the source of influenza A(H7N9) human infection and our finding has led to the closure of LPM which was proven to be an effective measure. Our groundbreaking work on nucleozin as an antiviral, imiquimod as a vaccine adjuvant, and DelNS1 as an influenza vaccine, have led to clinical trials by National Institute of Health, and commercial investment by Aptorum Group and Avalon Biomedical Group exceeding HKD$20,000,000.

(2) Underpinning research

Key HKU researchers at Department of Microbiology:
Professor H Chen: (Assistant Professor, 2003-2007; Associate Professor2007-2016 (tenured in 2012); Professor 2016 - present)
Dr RYT Kao: Associate Professor (Assistant Professor, 2007-2012; Associate Professor, 2012 - present)
Dr AJX Zhang, Research Assistant Professor (2011 - present)

Confirming the poultry-to-human transmission of avian influenza A(H7N9) virus

In 2013, avian influenza A H7N9 virus emerged to cause human infections, and subsequently became the most common avian influenza virus affecting humans with a mortality rate exceeding 30%. We were the first group to confirm poultry-to-human transmission of H7N9 virus based on epidemiological findings, which were supported by phylogenetic evidence (3.1). We have demonstrated that an influenza A(H7N9) virus isolated from a patient was almost identical to a virus isolated from an epidemiologically-linked market chicken.

Novel antiviral against influenza virus infection

There are only few antiviral drugs available for the treatment of influenza. Influenza virus strains resistant to these antivirals have been found. In 2010, we have discovered that nucleozin can protect mice from H5N1 infection by interacting with the viral nucleoprotein (3.2). This is the first study to show that nucleoprotein can be a drug target for influenza virus. This finding has led to the development of other antivirals targeting the viral nucleoprotein.

Improving influenza vaccine

Inactivated influenza vaccine and live-attenuated influenza vaccine (LAIV) have been recommended to prevent influenza virus infection. However, there are several problems associated with current influenza vaccines. First, these vaccines have low effectiveness, especially for antigenically-drifted viruses. Second, it takes about 2 weeks before sufficient antibody is elicited to confer protection. Third, currently approved LAIV are contraindicated in immunocompromised patients.

To overcome these problems associated with current influenza vaccines, we have designed two novel strategies. Our first strategy is to use imiquimod to improve currently available inactivated influenza vaccine. Imiquimod is an agonist of toll-like receptor 7, which is a key player in the innate immune response. Using a mouse model, we have demonstrated that imiquimod could augment and expedite the antibody production elicited by inactivated influenza vaccine. Using a lethal influenza virus challenge model with pandemic influenza A(H1N1) and avian influenza virus A(H7N9), we have demonstrated that mice given inactivated vaccine and imiquimod had a much

better survival rate than those given inactivated vaccine only (3.3, 3.4).

Our second strategy involves the use of influenza viruses with NS1 deletion (DelNS1 influenza viruses) as a LAIV. NS1 gene is a potent antagonist of host immune response. We have generated DelNS1 influenza viruses which can induce potent immune response but do not cause disease in mice (3.5, 3.6). Unlike other influenza virus with NS1 deletion, our DelNS1 influenza virus can grow to high titers in cell culture, facilitating vaccine production. We have shown that immunization using DelNS1 virus showed good protection against pandemic, seasonal and avian influenza viruses. Unlike currently available LAIV, our DelNS1 can offer broad protection against antigenically-drifted influenza viruses.

(3) References to the research

3.1 Chen Y, Liang W, Yang S, Wu N, Gao H, Sheng J, Yao H, Wo J, Fang Q, Cui D, Li Y, Yao X, Zhang Y, Wu H, Zheng S, Diao H, Xia S, Zhang Y, Chan KH, Tsoi HW, Teng JL, Song W, Wang P, Lau SY, Zheng M, Chan JF, To KK, Chen H, Li L, Yuen KY. Human infections with the emerging avian influenza A H7N9 virus from wet market poultry: clinical analysis and characterisation of viral genome. *Lancet* 2013;381(9881):1916-25. DOI: 10.1016/S0140-6736(13)60903-4

3.2 Kao RY, Yang D, Lau LS, Tsui WHW, Hu L, Dai J, Chan MP, Chan CM, Wang P, Zheng BJ, Sun J, Huang JD, Madar J, Chen G, Chen H, Guan Y, Yuen KY. Identification of influenza A nucleoprotein as an antiviral target. *Nat Biotechnol* 2010;28(6):600-5. DOI: 10.1038/nbt.1638

3.3 Zhang AJ, Li C, To KK, Zhu HS, Lee AC, Li CG, Chan JF, Hung IF, Yuen KY. Toll-like receptor 7 agonist imiquimod in combination with influenza vaccine expedites and augments humoral immune responses against influenza A(H1N1)pdm09 virus infection in BALB/c mice. *Clin Vaccine Immunol*. 2014 Apr;21(4):570-9. DOI: 10.1128/CVI.00816-13

3.4 To KK, Zhang AJ, Chan AS, Li C, Cai JP, Lau CC, Li CG, Jahan AS, Wu WL, Li L, Tsang AK, Chan KH, Chen H, Yuen KY. Recombinant influenza A virus hemagglutinin HA2 subunit protects mice against influenza A(H7N9) virus infection. *Arch Virol*. 2015 Mar;160(3):777-86. DOI: 10.1007/s00705-014-2314-x

3.5 Zheng M, Wang P, Song W, Lau SY, Liu S, Huang X, Mok BW, Liu YC, Chen Y, Yuen KY, Chen H. An A14U Substitution in the 3' Noncoding Region of the M Segment of Viral RNA Supports Replication of Influenza Virus with an NS1 Deletion by Modulating Alternative Splicing of M Segment mRNAs. J Virol. 2015 Oct;89(20):10273-85. DOI: 10.1128/JVI.00919-15

3.6 Wang P, Zheng M, Lau SY, Chen P, Mok BW, Liu S, Liu H, Huang X, Cremin CJ, Song W, Chen Y, Wong YC, Huang H, To KK, Chen Z, Xia N, Yuen KY, Chen H. Generation of DelNS1 Influenza Viruses: a Strategy for Optimizing Live Attenuated Influenza Vaccines. *MBio*. 2019 Sep 17;10(5). pii: e02180-19. DOI: 10.1128/mBio.02180-19

(4) Details of the impact

Impacts include: health and welfare, commercial and policy

Main beneficiaries include: patients and the public, industry, government, NGOs

Impact on the control of avian influenza virus A(H7N9) infection in humans

We have established the link between live poultry market (LPM) and human infection. This finding provided the important scientific basis for subsequent control measures, including closure of LPM. An editorial published in Lancet stated that our study provided the scientific basis for the closure of LPM as a measure to control the avian influenza virus outbreak [A]. The closure of LPM has reduced the number of infections by 97-99% in different cities in China [B]. Our findings also has significant economic impact, as influenza A(H7N9) is associated with a significant economic burden (mean direct cost of hospitalization estimated to be up to US $33728 per patient) [C]. Because of the impact of our findings, our team was rewarded the State Science and Technology

Award (outstanding award), State Council of the People's Republic of China [D]. The award announcement stated that our findings have provided the scientific basis for closure of poultry market, prevented the spread of disease, and reduce the economic impact [E].

Treatment of influenza virus infection

Our underpinning research on the discovery of nucleoprotein as an antiviral target has translated into a randomized controlled trial. We conducted a randomized controlled trial which included the use of naprosyn, a FDA-approved drug which targets nucleoprotein. The naprosyn-containing regimen was shown to be superior to oseltamivir alone in the treatment of influenza A H3N2 infection [F]. Our finding has led to the development of novel drugs targeting the influenza nucleoprotein [G].

We have obtained the USA non-provisional patent and worldwide patents for nucleozin [USA: US9212177B2; European: EP2462138China: ZL201080044361.6]. Nucleozin has been licensed to Acticule Life Sciences Limited, a university spin-off company financially supported by the Aptorum Group (http://acticule.com/), a NASDAQ list company. A full-time chemist at PhD level has been recruited to be the Senior Technical Manager and an administrative Officer. In addition, three sponsored research contracts worth a total of over HKD$11M sponsored by Acticule/Aptorum to HKU have been commenced as part of the endeavors for the commercialization of this first-in-the class influenza virus nucleoprotein inhibitor. Acticule Life Sciences Limited is in the process of optimizing nucleozin structure for further development of the compound into a drug candidate for toxicology studies and clinical trials. The improved drug candidate has been named ALS-1 in Aptorum's drug development pipeline [H].

Novel strategies on influenza vaccines

Our underpinning research has led to a double-blind, randomized controlled trial, which showed that the use of topical imiquimod prior to intradermal influenza vaccine could augment the immune response from influenza vaccine [I]. Furthermore, topical imiquimod also broadened the antibody response against antigenically-drifted virus. In the editorial of *Lancet Infectious Diseases*, the editor commented that "Imiquimod represents a promising candidate for topical vaccination treatments that use the skin-associated immune system to induce systemic immunity" [J]. The National Institute of Health of USA has started a clinical trial of topical imiquimod an influenza vaccine [K, L]. A patent has been obtained for the use of imiquimod intradermal vaccine (PCT/US2016042561) and has been licensed to Avalon Biomedical Group [M]. Two products (MedSpread™ and VaccineGrid™) have been developed by one of the subsidiaries of Avalon Biomedical Group, EMV Enhance (HK) Ltd (http://www.emvenhance.com/) for commercial production.

Our DelNS1-LAIV platform has been licensed to Emerging Viral Diagnostics (HK) Ltd for further development. Patents have been either issued or filed [US 9,827,304 B2]. Emerging Viral Diagnostics i) has successfully completed a Series A fundraising campaign of US $14 million; ii) has engaged several overseas CRO (Contract Research Organizations) to perform preclinical animal studies under GLP specifications in order to prepare for an IND application to the US FDA and iii) has started recruitment of staff for this project to build a team of up to 35 persons [N]. Furthermore, our findings have led us to the successful application of the Health@InnoHK grant for setting up the Centre for Virology, Vaccinology and Therapeutics.

(5) Sources to corroborate the impact

[A] Fournie G, Pfeiffer DU. Can closure of live poultry markets halt the spread of H7N9? Lancet. 2014;383:496-7. DOI: 10.1016/S0140-6736(13)62109-1

[B] Yu H, Wu JT, Cowling BJ, Liao Q, Fang VJ, Zhou S, Wu P, Zhou H, Lau EH, Guo D, Ni MY, Peng Z, Feng L, Jiang H, Luo H, Li Q, Feng Z, Wang Y, Yang W, Leung GM. Effect of closure of live poultry markets on poultry-to-person transmission of avian influenza A H7N9

virus: an ecological study. Lancet. 2014 Feb 8;383(9916):541-8. DOI: 10.1016/S0140-6736(13)61904-2

[C] Huo X, Chen LL, Hong L, Xiang LH, Tang FY, Chen SH, Gao Q, Chen C, Dai QG, Sun CW, Xu K, Dai WJ, Qi X, Li CC, Yu HY, Zhou Y, Huang HD, Pan XY, Xu CS, Zhou MH, Bao CJ. Economic burden and its associated factors of hospitalized patients infected with A (H7N9) virus: a retrospective study in Eastern China, 2013-2014. Infect Dis Poverty. 2016 Sep 1;5(1):79. DOI: 10.1186/s40249-016-0170-5

[D] State Science and Technology Award (outstanding award), State Council of the People's Republic of China

[E] Official announcement of the State Science and Technology Award (outstanding award), State Council of the People's Republic of China

[F] Hung IFN, To KKW, Chan JFW, Cheng VCC, Liu KSH, Tam A, Chan TC, Zhang AJ, Li P, Wong TL, Zhang R, Cheung MKS, Leung W, Lau JYN, Fok M, Chen H, Chan KH, Yuen KY. Efficacy of Clarithromycin-Naproxen-Oseltamivir Combination in the Treatment of Patients Hospitalized for Influenza A(H3N2) Infection: An Open-label Randomized, Controlled, Phase IIb/III Trial. Chest. 2017 May;151(5):1069-1080. DOI: 10.1016/j.chest.2016.11.012

[G] Hu Y, Sneyd H, Dekant R, Wang J. Influenza A Virus Nucleoprotein: A Highly Conserved Multi-Functional Viral Protein As A Hot Antiviral Drug Target. Curr Top Med Chem. 2017;17(20):2271-2285. DOI: 10.2174/1568026617666170224122508

[H] APTORUM corporate presentation

[I] Hung IF, Zhang AJ, To KK, Chan JF, Li P, Wong TL, Zhang R, Chan TC, Chan BC, Wai HH, Chan LW, Fong HP, Hui RK, Kong KL, Leung AC, Ngan AH, Tsang LW, Yeung AP, Yiu GC, Yung W, Lau JY, Chen H, Chan KH, Yuen KY. Topical imiquimod before intradermal trivalent influenza vaccine for protection against heterologous non-vaccine and antigenically drifted viruses: a single-centre, double-blind, randomised, controlled phase 2b/3 trial. Lancet Infect Dis. 2016;16(2):209-18. DOI: 10.1016/S1473-3099(15)00354-0

[J] Stein P, Radsak MP. The skin as an orchestrator of influenza immunity. Lancet Infect Dis 2016;16(2):139-40. DOI: 10.1016/S1473-3099(15)00413-2

[K] News release. Clinical trial testing topical cream plus influenza vaccine in progress. National Institutes of Health, USA

[L] Email from Dr. Chris Roberts (Acting Chief, Respiratory Diseases Branch, Division of Microbiology and Infectious Diseases, NIAID/NIH/DHHS) on the clinical trials on imiquimod.

[M] Letter from Avalon Biomedical Group regarding imiquimod as a vaccine adjuvant (dated 2nd July, 2019)

[N] Email from Avalon Biomedical Group regarding DelNS1-LAIV (Dated 24th September, 2019)