Research Assessment Exercise 2020
Impact Case Study

University: *City University of Hong Kong*
Unit of Assessment (UoA): *02 - Pre-Clinical Studies*
Title of case study: *Novel antiviral strategies*

1. Summary of the impact

Researchers at City University of Hong Kong lead by Professor He Mingliang have developed antiviral agents for the detection and treatment of virus infections, including Hepatitis B (HBV), SARS-coronavirus and Enterovirus A 71 (EV-A71) [1]-[3]. Here we would like to present a special example. Based on his research, novel pharmaceutical agents were developed by several Chinese pharmaceutical companies during the assessment period. Today these agents are being used throughout China for fast and reliable detection of HBV replication intermediates, contributing significantly to the prevention of potentially deadly chronic liver disease.

2. Underpinning research

In 2002 Prof He Ming-Liang published a study [4], describing a novel way to detect HBV replication intermediates, so called cccDNA. The authors cloned Chinese HBV DNA of genotype C and isolated its cccDNA. The authors designed and employed several types of primers for those stretches of the cccDNA, which are highly conserved across different HBV genotypes. Quantification of cccDNA was achieved by real-time PCR. The results demonstrated that this novel method was highly sensitive to levels of HBV cccDNA in hepatic cells and could potentially be used as a diagnostic tool for determining the end point of antiviral chemotherapies.

In 2003, Prof He and his co-workers demonstrated that replication of HBV could also be suppressed by interfering with its cccDNA stage by RNA interference. Currently, three US-based companies (Roche, Arbutus, Johnson & Johnson) are conducting clinical trials by using RNAi agents. In 2009, Prof He and his co-workers demonstrated that stress protein GRp78 responds to HBV replication and stimulates the nature immunity of hepatocytes to limit HBV infections, breakthrough the theory that hepatocytes are incapable of innate immunity (Ma et al., *Mol Cell Proteomics.* 2009;8(11):2582-94). In 2016, Prof. He revealed that HBV oncoprotein HBx induces hepatocarcinogenesis through lncRNA UCA1/EZH2-p27Kip1 axis, and a potential target of liver cancer [5].

3. References to the research

[1] Zhou F, Wan Q, Lu J, Chen Y, Lu G, He ML Pim1 Impacts Enterovirus A71 Replication and Represents a Potential Target in Antiviral Therapy. iScience 2019 Sep 27; 19: 715-727.

[2] Dong Q, Men R, Dan X, Chen Y, Li H, Chen G, Zee B, Wang MHT, He ML*. Hsc70 regulates the IRES activity and serves as an antiviral target of enterovirus A71 infection. Antiviral Res. 2018 Feb;150:39-46.

[3] Li C, Huang L, Sun W, Chen Y, He ML, Yue J, Ballard H. Saikosaponin D suppresses enterovirus A71 infection by inhibiting autophagy. Signal Transduct Target Ther. 2019 Feb 22;4:4. doi: 10.1038/s41392-019-0037-x. eCollection 2019.

[4] He ML, Wu J, Chen Y, Lin MC, Lau GKK, Kung HF. A new and sensitive method for the quantification of HBV cccDNA by real-time PCR. Biochem Biophys Res Commun. 2002 Aug 2;295(5):1102-7.

[5] Hu JJ, Song W, Zhang SD, Shen XH, Qiu XM, Wu HZ, Gong PH, Lu S, Zhao ZJ, He ML, Fan H. HBx-upregulated lncRNA UCA1 promotes cell growth and tumorigenesis by recruiting EZH2 and repressing p27Kip1/CDK2 signaling. Sci Rep. 2016 Mar 24;6:23521.

4. Details of the impact

While vaccines against HBV have been available since 1982, HBV remains a global health issue. According to the World Health Organisation (WHO) an estimated 257 Million people worldwide live with a Hepatitis B (HBV) infection. However, only 9 percent of those infected were aware of their diagnosis — with deadly consequences: in 2015 alone, 887.000 people have died of complications of HBV, including liver cirrhosis and cancer. As these complications emerge mainly in chronic HBV patients, early detection and antiviral management of the disease is of paramount importance[A].

Researchers at City university of Hong Kong have developed a highly sensitive, fast and cheap method for the detection of one of the subcellular markers of an HBV infection. Socalled covalently closed circular DNA (cccDNA) is an intermediate of the viral replication in the nucleus of hepatocytes[B]. After infection the viral DNA first gets translated into cccDNA, which in turn serves as a template for viral RNA transcription. Once, the RNA is encapsulated by the virion core particle it gets reverse transcribed into viral DNA, completing the viral replication cycle. cccDNA is believed to be responsible for relapse after discontinuation of antiviral therapy as well as for antiviral drug resistance. Consequently, a fast and sensitive method of cccDNA detection is of paramount importance to prevent chronic liver disease from HBV and its life threatening complications. There was no sensitive method to quantify HBV replication intermediates, although it was urgently needed for years.

In 2002 Prof He Mingliang presented a novel method for the detection of HBV infections (He et al. 2002). The approach uses real-time PCR to specifically amplify the cccDNA.

Today several Chinese companies are selling HBV test kits based on the cccDNA method [E]. As no patent was filed, the method could not be licensed or otherwise monetised by Prof He's group. Nevertheless, the history of the literature demonstrates that Prof He's method of cccDNA amplification for HBV was the first to be described.

Prof He also demonstrated that cccDNA can be used as a target for HBV treatment. For the acute phase of HBV there are currently no available treatments. Though oral antiviral drugs can slow the progression of liver cirrhosis and reduce incidences of liver cancer, there is currently no cure

for HBV. Several lines of evidence suggest that successful treatment of HBV likely depends on complete eradication of cccDNA from liver cells. Research from Prof He's group (Chen et al., 2003) demonstrated that the replication HBV can be inhibited with short hairpin RNA (shRNA). Two US patents for this approach were granted in 2006 and 2007, respectively. Today, based on the research on cccDNA interference, several potential RNA interference agents are being tested in human clinical trials [C][D]. High efficacy and good tolerability are observed without any side-effects or toxicity observed. For example, Johnson & Johnson has licensed Arrowhead Pharmaceutics's ARO-HBV for market development.

Professor He and his team at City University of Hong Kong continue to develop methods for detection and treatment of different viral infections [H][I]. Apart from HBV the pathogens studied by his group are SARS, Coronavirus and Enterovirus A71. Several patents have been granted for testing methods for these pathogens [F][G].

5. Sources to corroborate the impact

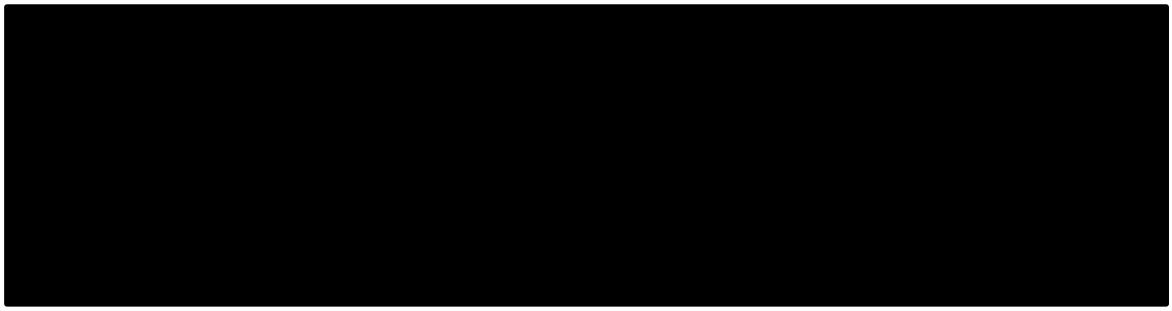
[A] WHO Hepatis B Key Facts:
https://www.who.int/news-room/fact-sheets/detail/hepatitis-b

[B] Allweiss & Dandri, The Role of cccDNA in HBV Maintenance, Viruses. 2017 Jun; 9(6): 156.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5490831/

[C] Flisiak et al., siRNA drug development against hepatitis B virus infection, Expert Opin Biol Ther. 2018 Jun;18(6):609-617
https://www.ncbi.nlm.nih.gov/pubmed/29718723

[D] Clinical Data on JNJ-3989 (ARO-HBV) presented by Arrowhead
http://ir.arrowheadpharma.com/news-releases/news-release-details/arrowhead-presents-clinical-data-jnj-3989-aro-hbv-international

[E] Companies selling the HBV cccDNA test：



[F] US Patent: He ML, Kung HF Inhibition of Hepatitis B virus (HBV) replication by RNA interference (US patent No. US 7,067,249 B2, issued on June 27, 2006)

[G] US Patent: Lin MC, He ML, Kung HF Gene Therapy of HBV Infection Via Adeno-Associated Viral Vector Mediated Long Term Expression of Small Hairpin RNA (shRNA). (U.S. patent No. US 2007/0027099 A1)

[H] US Patent: He ML Methods of treatment of viral infection and uses of anti-Hsc70 inhibitors (US Patent Application No. 15/966,121)

[I] US Patent: He ML, Chen Y, Zhou F, Wan Q Methods of Treatment of Viral Infection and Uses of Pim1 Inhibitors (US Provisional Patent Application No. 62/840,819)