Doi: 10.1038/aps.2017.67

S4.1

Naturally existing oncolytic virus M1 is nonpathogenic for the nonhuman primates after multiple rounds of repeated intravenous injections

Hai-peng ZHANG¹, Yuan LIN¹, Kai LI¹, Jian-kai LIANG¹, Xiao XIAO¹, Guang-mei YAN^{1,*}, Yuan LIN^{1,*}

SUN Yat-sen University, Guangzhou 510000, China

*To whom correspondence should be addressed.

E-mail: liangjk5@mail.sysu.edu.cn

Cancers figure among the leading causes of morbidity and mortality worldwide. The number of new cases is expected to rise by about 70% over the next 2 decades. Development of novel therapeutic agents is urgently needed for clinical cancer therapy. Alphavirus M1 is a Getah-like virus isolated from China with a genome of positive single-strand RNA. We have previously identified that alphavirus M1 is a naturally existing oncolytic virus with significant anticancer activity against different kinds of cancer (eg, liver cancer, bladder cancer, and colon cancer). To support the incoming clinical trial of intravenous administration of alphavirus M1 to cancer patients, we assessed the safety of M1 in adult nonhuman primates. We previously presented the genome sequencing data of the cynomolgus macaques (*Macaca fascicularis*), which was demonstrated as an ideal animal species for virus infection study. Therefore, we chose cynomolgus macaques of either sex for the present safety study of oncolytic virus M1. In the first round of administration, five experimental macaques were intravenously injected with six times of oncolytic virus M1 (1×10^9 PFU/dose) in 1 week, compared with five vehicle-injected control animals. The last two rounds of injections were further completed in the following months in the same way as the first round. Body weight, temperature, complete blood count, clinical biochemistries, cytokine profiles, lymphocytes subsets, neutralizing antibody, and clinical symptoms were closely monitored at different time points. Magnetic resonance imaging was also performed to assess the possibility of encephalitis or arthritis. As a result, no clinical, biochemical, immunological, or medical imaging or other pathological evidence of toxicity was found during the whole process of the study. Our results in cynomolgus macaques suggested the safety of intravenous administration of oncolytic virus M1 in cancer patients in the future.

Keywords: oncolytic virus; cynomolgus macaques; safety; M1 virus

Acknowledgements: This work was supported by National Natural Science Foundation of China (No 81273531 and 81573447), the Guangdong Provincial Science and Technology project (No 2015B020211003), the Guangdong Provincial Natural Science Foundation Grant (No 2015A030313081), and the Fundamental Research Funds for the Central Universities (No 13ykpy07).

S4.2

Therapeutic role of GMSC in autoimmune diseases

Ju-lie WANG, Nancy OLSEN, Song-guo ZHENG*

Milton S. Hershey Medical Center at Penn State University, USA

*To whom correspondence should be addressed.

E-mail: szheng1@hmc.psu.edu

Rheumatoid arthritis (RA) is a chronic symmetrical autoimmune disease characterized by synovial inflammation that affects primarily the small diarthrodial joints. None of the current treatments can cure the disease. Mesenchymal stem cells have been shown in maintaining immune homeostasis and preventing autoimmunity, and may be a potential therapeutic approach for RA. Recently, we observed that gingiva-derived mesenchymal stem cells (GMSCs) also have the capacity to inhibit immune responses and control the development and severity of collagen-induced arthritis (CIA) in mice that is dependent on CD39/CD73 signaling pathway and partially on the induction of CD4⁺CD39⁺FoxP3⁺ Treg cells. Moreover, GMSCs dramatically and directly inhibited NF- κ B and RANKL-mediated osteoclast formation, as well as bone erosion in CIA. To evaluate their clinical translational value, we have developed a humanized animal model, xeno-GVHD, to demonstrate that the infusion of GMSC can markedly inhibit human PBMCs-initiated xenogenic graft-versus-host-disease (GVHD) and this effect requests the CD39/CD73 and IDO signals. More importantly, the effect of GMSCs is significantly better than bone marrow-derived mesenchymal stem cells (BMSCs). Taken together, the manipulation of GMSCs could provide a promising approach for curing autoimmune diseases, such as rheumatoid arthritis and xeno-Graft-versus-host-disease.

Keywords: GMSC; rheumatoid arthritis; Foxp3; GVHD; CIA

Acknowledgements: This work was in part supported by grants from the NIH R01 (No AR059103) and NIH STAR project.

S4.3

Potent and selective inhibition of pathogenic viruses by engineered ubiquitin variants

Wei ZHANG¹, Ben A BAILEY-ELKIN², Robert CM KNAAP³, Baldeep KHARE², Tim J DALE-BOU³, Garrett G JOHNSON², Puck B van KASTEREN³, Nigel J McLEISH², Jun GU¹, Wenguang HE², Marjolein KIKKERT³, Brian L MARK², Sachdev S SIDHU^{1,*}

¹Donnelly Centre for Cellular and Biomolecular Research, Banting and Best Department of Medical Research, and Department of Molecular Genetics, University of Toronto, 160 College Street, Toronto, Ontario M5S3E1, Canada; ²Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, R3T2N2, Canada; ³Department of Medical Microbiology, Leiden University Medical Center, 2333 ZA Leiden, the Netherlands

*To whom correspondence should be addressed.

E-mail: sachdev.sidhu@utoronto.ca

The recent Middle East respiratory syndrome coronavirus (MERS-CoV), Ebola and Zika virus outbreaks exemplify the continued threat of (re-)emerging viruses to human health, and our inability to rapidly develop effective therapeutic countermeasures. Many viruses, including MERS-CoV and the Crimean-Congo hemorrhagic fever virus (CCHFV) encode deubiquitinating (DUB) enzymes that are critical for viral replication and pathogenicity. They bind and remove ubiquitin (Ub) and interferon stimulated gene 15 (ISG15) from cellular proteins to suppress host antiviral innate immune responses. A variety of viral DUBs (vDUBs), including the MERS-CoV papain-like protease, are responsible for cleaving the viral replicase polyproteins during replication, and are thereby critical components of the viral replication cycle. Together, this makes vDUBs highly attractive antiviral drug targets. However, structural similarity between the catalytic cores of vDUBs and human DUBs complicates the development of selective small molecule vDUB inhibitors. We have thus developed an alternative strategy to target the vDUB activity through a rational protein design approach. Here, we report the use of phage-displayed ubiquitin variant (UbV) libraries to rapidly identify potent and highly selective protein-based inhibitors targeting the DUB domains of MERS-CoV and CCHFV. UbVs bound the vDUBs with high affinity and specificity to inhibit deubiquitination, deISGylation and in the case of MERS-CoV also viral replicative polyprotein processing. Co-crystallization studies further revealed critical molecular interactions between UbVs and MERS-CoV or CCHFV vDUBs, accounting for the observed binding specificity and high affinity. Finally, expression of UbVs during MERS-CoV infection reduced infectious progeny titers by more than four orders of magnitude, demonstrating the remarkable potency of UbVs as antiviral agents. Our results thereby establish a strategy to produce protein-based inhibitors that could protect against a diverse range of viruses by providing UbVs via mRNA or protein delivery technologies or through transgenic techniques.

Keywords: MERS; CCHFV; deubiquitinases; phage display; ubiquitin variants; protein engineering; inhibitor design; antiviral drugs

Acknowledgements: MK is supported by the Division of Chemical Sciences of the Netherlands Organization for Scientific Research (NWO-CW) through Excellent Chemical Research (ECHO) grant (No 700.59.008). BLM is a Manitoba Research Chair and is supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) grant (RGPIN-2015-05310). BBE holds an NSERC Postgraduate Scholarship (PGS-Doctoral). WZ is supported by Mitacs Elevate program with funds from Mitacs Inc. and Centre for the Commercialization of Antibodies and Biologics (CCAB). SSS is supported by Canadian Institutes of Health Research (CIHR) grants (MOP-136956 and PJT-148510). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

S4.4

The relationship between biofilm formation ability and antibiotics resistance of *Acinetobacter baumannii*

Fei LIN¹, Bing-jie DU¹, Gao CAN¹, Xu JIA², Bao-dong LING^{1,*}

¹Small Molecule Drugs Sichuan Key Laboratory, Chengdu Medical College, Chengdu 610500, China; ²Department of Biomedical Sciences, Chengdu Medical College, Chengdu 610500, China

*To whom correspondence should be addressed.

E-mail: lingbaodong@cmc.edu.cn

Biofilm formation ability of *A. baumannii* is associated with long-term survival in hospital environments or equipment and provides resistance to antibiotics. The present study aimed to observe the biofilm formation ability of *A. baumannii* clinical isolates as well as the infection disease therapy, and the influence of biofilm formation for the antimicrobial therapy. A total of 47 *A. baumannii* clinical isolates were collected. The biofilm ability of *A. baumannii* was tested by the crystal

violet staining assay. In the same time, the biofilm ability on the surface of glasses and polypropylene which in the condition of shaking and stable. The minimal inhibitory concentrations of 12 antibacterial to the planktonic and biofilm for the biofilm formation isolates were tested by using the microdilution method. Thirteen isolates (27.66%) had biofilm formation ability which contains 3 multi-drug resistance isolates. Compared with the MIC of planktonic and biofilm, the tigecycline and colistin resistance ratio was increased from 0 to 15.38% and 100%, respectively. Meanwhile, the other 10 antibiotics resistance ratio increased to 100%. The fold change of MIC values for 12 antibacterial ranged from 1 to 1024. The clinical isolates have strong biofilm ability on the surface of polypropylene than glasses, showing the strong biofilm ability on the shaking condition than stable. The biofilm formation could lead to the bacterial resistance to antibiotics and clinical therapy failure.

Keywords: *Acinetobacter baumannii*; biofilm

Acknowledgements: This work was supported by the National Natural Science of Foundation of China (NSFC) (No 81373454).

S4.5

Molecular characterization of reduced susceptibility to biocides in *Acinetobacter baumannii*

Bao-dong LING^{1*}, Fei LIN^{1,2}, Ying XU³, Yao-wen CHANG^{1,2}, Chao LIU^{1,2}, Xu JIA²

¹Small Molecule Drugs Key Laboratory of Sichuan Province, Institute of Materia Medica, Chengdu Medical College, Chengdu 610500, China; ²Non-coding RNA and Drug Discovery Key Laboratory of Sichuan Province, Chengdu Medical College, Chengdu 610500, China; ³Clinical Laboratory, the First Affiliated Hospital, Chengdu Medical College, Chengdu 610500, China

*To whom correspondence should be addressed.

E-mail: lingbaodong@cmc.edu.cn

The present study aimed to investigate the molecular resistant characteristics of *A. baumannii* to biocides. Forty-seven clinical isolates were collected and their susceptibility to commonly used biocides was tested. The incidence of biocide resistance genes was analyzed through polymerase chain reaction and sequencing. In addition, the isolates that exhibited higher minimal inhibitory concentrations (MICs) of biocides were further determined by multilocus sequence typing (MLST), pulsed field gel electrophoresis (PFGE), reverse transcriptase quantitative PCR. The results revealed that *A. baumannii* showed different levels of reduced susceptibility to chlorhexidine, benzalkonium bromide, ethanol, hydrogen peroxide, especially extensive resistance to triclosan. The ratios of 11 reported biocide resistant genes in *A. baumannii* were above 68.09%, and 100% for *adeI*, *abeD*, *fabI* and *acel*. Sequence type (ST) 92, ST195, ST184, ST618 and 4 novel STs were identified in 14 clinical isolates which exhibited higher MICs to triclosan or ethanol. ST92 and ST195 belonged to clone complex (CC) 92 which was the major clone spread in hospitals. Two distinct PFGE patterns were identified, of which 2 isolates belonged to pulsotype A, 3 isolates belonged to pulsotype B, and the other 9 clinical isolates belonged to other pulsotypes. Simultaneously, CC92 was corresponded to the pulsotype B. The gene expressions showed that the active efflux did not appear to be a major reason for acquired triclosan and chlorhexidine resistance. Meanwhile, *fabI* and *acel* displayed the different expressions between higher and lower isolates. In conclusion, the reduced susceptibility to biocides of *A. baumannii* was extremely severe in this hospital. The findings suggested that it is urgent to monitor the biocide susceptibility to *A. baumannii* and to rationalize biocide usage in clinic.

Keywords: *Acinetobacter baumannii*; biocides; molecular characterization; MLST; PFGE

Acknowledgements: This work was supported by the National Natural Science of Foundation of China (NSFC) (No 81373454).

S4.6

$\alpha 7$ nAChR mediate Fas demethylation and upregulated Fas apoptotic pathway contribute to prenatal nicotine exposure-induced programmed thymocyte apoptosis

Han-xiao LIU, Sha LIU, Hui-yi YAN, Wen QU, Xiao WEN, Ting CHEN, Li-fang HOU, Jie PING*

Wuhan University, Wuhan 430071, China

*To whom correspondence should be addressed.

E-mail: pingjie@whu.edu.cn

Prenatal cigarette smoke exposure has been reported to increase thymocyte apoptosis and result in postnatal immune impairments. Nicotine is one of the leading candidates in cigarette smoke for causing immune cell apoptosis. However, whether prenatal nicotine exposure (PNE) induce thymocyte apoptosis and the mechanisms remain unclear. This study was designed to investigate the effects of

PNE on immune impairments in offspring and observe the programming changes of thymocyte apoptosis *in vivo*, and further explore the epigenetic mechanisms of the pro-apoptotic effect of nicotine *in vitro*. The results showed that PNE caused immune impairments in female offspring on postnatal day (PND) 49, manifested as increased IL-4 production and IgG₁/IgG_{2a} ratio in serum. Enhanced apoptosis of total thymocyte and CD4 single positive thymocyte were observed both in fetus and in female offspring on PND 49. Further, by exposing thymocyte to 0–100 μ mol/L of nicotine *in vitro* for 48 h, we found that nicotine increased $\alpha 7$ nicotinic acetylcholine receptor (nAChR) expression, activated Fas apoptotic pathway, and promoted thymocyte apoptosis in a concentration-dependent manner. In addition, nicotine could induce Tet methylcytosine dioxygenase (TET) 2 expression and Fas promoter demethylation, which can be abolished by TET2 siRNA transfection. Moreover, the $\alpha 7$ nAChR specific antagonist α -bungarotoxin treatment can abrogate nicotine-induced TET2 increase, and the following Fas demethylation and Fas-mediated apoptosis. In conclusion, our findings firstly reported that $\alpha 7$ nAChR activation could induce TET2-mediated demethylation of Fas in thymocyte and result in up-regulation of Fas apoptotic pathways, which provide evidence for elucidating the PNE-induced programmed thymocyte apoptosis.

Keywords: prenatal nicotine exposure; $\alpha 7$ nAChR; thymocyte apoptosis; Fas; TET2; immune impairments

Acknowledgements: This work was supported by Grants from the National Natural Science Foundation of China (No 81673215, 81273107), the Outstanding Youth Science Fund of Hubei Province (No 2012FFA017).

S4.7

The role of MANF in inflammation

Yu-xian SHEN*

School of Medical Sciences, Anhui Medical University, Hefei 230032, China

*To whom correspondence should be addressed.

MANF is an ER stress inducible secretory protein and has protective effects against various neural injuries. However, the involvement of MANF in inflammation has not been illustrated yet. In this study, we found that MANF mRNAs were up-regulated in the peripheral blood leukocytes (PBWC) of the patients with rheumatoid arthritis (63 cases), systemic lupus erythematosus (65 cases), and ankylosing spondylitis (26 cases) by using of qPCR. Meanwhile, MANF transcription levels were elevated in PBWC and synovial tissues in rabbit osteoarthritis induced by methylated bovine serum albumin and rat rheumatoid arthritis induced by Freund's complete adjuvant. BIP and CHOP were also up-regulated in the inflammatory synovium. These results suggested that inflammation induces ER stress and MANF expression. We cloned 5'-UTR of MANF and constructed a series of its mutations within the transcription factors binding sites. We found that XBP1, AP-1, or SP-1 bound to the ER stress response element (ERSE) that sited in the upstream of MANF promoter and activated MANF transcription by using of luciferase reporter gene technology and CHIP. We investigated the interaction between MANF and p65 by using Co-IP, pull-down, and double immunofluorescent labeling. We found that tunicamycin, LPS, or TNF α caused MANF to re-localize to the nuclei, where MANF interacted with the DNA binding domain of p65 through its C-terminal SAP-like domain. MANF consequently inhibited p65-mediated transcriptional activation by interfering with the binding of p65 to its target genes promoters. However, MANF did not affect the total levels of cellular p65 and I κ B. Consistently, MANF suppressed the expressions of NF- κ B-dependent target genes and the proliferation of inflammatory synoviocytes. These findings suggest that MANF may be a negative regulator of inflammation and mediate the crosstalk between the NF- κ B pathway and ER stress. Therefore, up-regulation of MANF is an adaptive response under inflammatory condition.

S4.8

The investigation of the turning point of over-inflammation to immunosuppression in CLP mice model and its application for drug study

Sheng-lan SHANG, Xiao-li LI, Dong-mei DENG, Hong ZHOU*

Department of Pharmacology, College of Pharmacy, the Third Military Medical University, Chongqing 400038, China

*To whom correspondence should be addressed.

E-mail: zhouh64@163.com

Immunosuppression is the predominant cause of sepsis mortality. Due to the lack of effective treatments, investigations of new drugs based on an animal sepsis model that closely resembles the clinical situation are important. Here, CLP immunosuppression mice model was carried out, and then the influence of artesunate (AS) and its underlying mechanisms were investigated using this model.

The colon tissue 1.0 cm distal to the cecum was ligated and punctured once with a No. 16 steel needle to establish CLP mice model. The results showed Day 1 after the CLP surgery, mice presented low levels of both pro-inflammatory and anti-inflammatory cytokines, as well as high bacterial loads. CLP group challenged with PA on Day 1 had higher sensitivity than mice in Sham group. The result showed that Day 1 after the CLP surgery was the turning point of over-inflammation to immunosuppression. AS obviously increased pro-inflammatory cytokine levels and decreased bacterial loads in the lung, spleen and blood as well as the

mortality of CLP immunosuppression mice challenged with PA. AS protects CLP immunosuppression mice against sepsis by increasing pro-inflammatory cytokine release and bacterial clearance. In addition, AS increase the expression of TLR4 and TRAF6 protein and the activation of NF- κ B; autophagy is also implicated in this effect. In conclusion, Day 1 after the CLP surgery is the turning point of over-inflammation to immunosuppression. AS represents a putative candidate for sepsis treatment.

Keywords: sepsis; immunosuppression; cecal ligation and puncture; artesunate