Project number: **T11-707/15-R**

RESEARCH GRANTS COUNCIL
THEME-BASED RESEARCH SCHEME (TRS)

Completion Report on Funded Project

Project start date: *Nov 2015*
Project completion date: *Oct 2020*

1. Project Title: Molecular basis for interspecies transmission and pathogenesis of Middle East Respiratory Syndrome Coronavirus

2. Names and Academic Affiliations of Project Team Members[#]

Project team member	Name / Post	Unit / Department / Institution	Average number of hours per week spent on this project in the current reporting period
Project Coordinator (PC)	WOO, Patrick CY	Microbiology, HKU	8
Co-Principal Investigator(s)	LAU, Susanna KP	Microbiology, HKU	8
	JIN, Dong-yan	Biomedical Sciences, HKU[1]	8
	YUEN, Kwok-yung	Microbiology, HKU	8
	TSUI, Kwok-wing	Biomedical Sciences, CUHK	4
	CHEN, Hong-lin	Microbiology, HKU	8
	KAO, Richard	Microbiology, HKU	6
	ZHENG, Bo-jian	Microbiology, HKU	6
	SHAM, Pak-Chung	Centre for Genomic Sciences, HKU	2
	CAI, Zong-wei	Chemistry, BU	6

	LAU, Terrence CK	Biomedical Sciences, CityU	6
Co-Investigator(s)	TENG, Jade LL	Microbiology, HKU	12
	CHAN, Jasper FW	Microbiology, HKU	8
	TSE, Herman	Microbiology, HKU	6
	ZHANG, Anna JX	Microbiology, HKU	8
	KOK, Kin-hang	Microbiology, HKU	8
	LAM, Yun-wah	Bio & Chem, CityU	5
	SZE, Kong-hung	Microbiology, HKU	8
	TANNER, Julian A.	Biomedical Sciences, HKU[1]	2
	YEUNG, Man-lung	Microbiology, HKU	8
	ZHOU, Jie	Microbiology, HKU	8
	CHU, Hin	Microbiology, HKU	8
	TO, Kelvin KW	Microbiology, HKU	6
Collaborators	WERNERY, Ulrich	Central Veterinary Research Lab, UAE	N.A.
	WANG, Ming Prof	Guangzhou CDC	N.A.
	ZHANG, Hailin	Yunnan Institute of Endemic Diseases Control & Prevention	N.A.
	LI, Wenhui	National Institute of Biological Sciences, Beijing	N.A.
	QIN, Chuan	Chinese Academy of Medical Sciences, Beijing	N.A.
	KHABAR, Khalid SA	King Faisal Specialist Hospital & Research Centre, Saudi Arabia	N.A.

 # Please highlight the approved changes in the project team composition and quote the date when the RGC granted approval of such changes. For changes in the project team composition, please submit a separate request, together with the justification and the curriculum vitae of the new member(s), to the RGC three months prior to the intended effective date of the change.

[1]Department of Anatomy, Biochemistry and Physiology were amalgamated into School of Biomedical Sciences

3. Project Objectives

Summary of objectives addressed/achieved:

Objectives*	Percentage achieved	Remarks**
1. To understand the origin, transmission pathways and evolution of MERS-CoV.	100%	
2. To enhance public health measures for MERS by identifying primary reservoir and intermediate hosts.	100%	
3. To improve prevention and management of CoV infections through identification of novel antiviral and vaccine targets.	100%	

Objectives*	Percentage achieved	Remarks**
4. To produce a consortium of world-leading researchers in CoVs, train PhDs, PDFs, clinician-scientists and coronavirologists to tackle future CoV outbreaks and large epidemics.	100%	
5. To hold workshops and symposia on CoV infections.	100%	

* *Please highlight the approved changes in objectives and quote the date when the RGC granted approval of such changes.*

****** *Please provide reasons for significantly slower rate of progress than originally planned.*

6. Research Highlights and Outputs

6.1 What are the most exciting research accomplishments of the project?

(Please list five or more of the team's best research accomplishments, such as journal and conference papers, software codes, research infrastructure, etc. For each item, please clearly justify how it has achieved international excellence (e.g. best paper award, invited presentation, citations, product licensed to industry, etc.))

- We have successfully isolated Ty-BatCoV HKU4 from lesser bamboo bats using CaCo-2 cells for the first time. While a number of human coronaviruses are believed to be originated from ancestral viruses in bats, it remains unclear if bat coronaviruses are ready to cause direct bat-to-human transmission. Ty-BatCoV HKU4 replicates efficiently in CaCo-2 and Huh7 cells with cytopathic effects, and the exogenous expression of human-dipeptidyl-peptidase-4 (hDPP4) and dromedary camel-DPP4 (dcDPP4) rendered non-permissive cell lines to become susceptible for Ty-BatCoV HKU4 infection, suggesting the use of hDPP4 and dcDPP4 as the receptors for cell entry. Flow cytometry, co-immunoprecipitation and surface plasmon resonance assays show that Ty-BatCoV HKU4-receptor-binding-domain (RBD) can bind hDPP4, dcDPP4, and Tylonycteris pachypus-DPP4. We have successfully developed a hDPP4-transgenic mouse model, which is useful in studying MERS-CoV pathogenesis as well as for evaluating MERS vaccines and drug candidates' efficacy. This transgenic mouse model can also be infected by Ty-BatCoV-HKU4 through intranasal inoculation, with virus and inflammatory changes detected in lungs and brains of infected mice, suggesting that MERS-related bat coronaviruses may overcome species barrier by utilizing DPP4 and potentially emerge in humans by direct bat-to-human transmission.
- Besides Ty-BatCoV HKU4, we have also identified a novel MERS-CoV-related betacoronavirus, Hp-BatCoV HKU25, from Chinese pipistrelle bats in southern China. Although it is closely related to MERS-CoV in most genome regions, its spike protein occupies a phylogenetic position between that of Ty-BatCoV HKU4 and Pi-BatCoV HKU5. The HKU25-RBD could bind to hDPP4 protein and hDPP4-expressing cells, though with a lower efficiency than MERS-RBD. Pseudovirus bearing HKU25-spike was shown to be capable of using hDPP4 for entry to hDPP4-expressing cells, but less efficiently than pseudovirus bearing MERS-spike and HKU4-spike. These suggest that bat CoV spike proteins may have evolved in a stepwise manner for binding to hDPP4. In addition to bats, we have also discovered a novel MERS-related CoV in hedghogs from China, with the spike gene most closely related to African bat MERS-related CoVs genetically. Furthermore, we have detected MERS-CoV antibodies in the two-humped Bactrian and hybrid camels in Dubai, the United Arab Emirates for the first time, suggesting that these camels can be another possible reservoir for MERS-CoV. All these findings give insights on the evolutionary origin and the interspecies transmission mechanisms of MERS-CoV. We have already established the reverse genetic system for MERS-CoV, which can be used to further study the virulence, host tropism and interspecies transmission mechanism of MERS-CoV.
- We found that MERS-CoV M protein uses its N-terminal transmembrane domains to interact with TRAF3 and disrupt TRAF3-TBK1 association leading to reduced IRF3 activation. Our findings suggest a common and conserved mechanism through which highly pathogenic MERS-CoV and SARS-CoV harness their M proteins to suppress type I IFN expression at the level of TBK1-dependent phosphorylation and activation of IRF3 resulting in evasion of the host innate antiviral response.
- We identified and characterized type I IFN antagonism of MERS-CoV ORF8b accessory protein. ORF8b was abundantly expressed in MERS-CoV-infected cells. When ectopically expressed, ORF8b inhibited IRF3-mediated IFNβ expression induced by Sendai virus and poly(I:C). ORF8b was found to act at a step upstream of IRF3 to impede the interaction between IRF3 kinase IKKε and chaperone protein HSP70, which is required for activation of IKKε and IRF3. Infection study using recombinant wild-type and ORF8b-deficient MERS-CoV further confirmed the suppressive

role of ORF8b in type I IFN induction and its disruption of the colocalization of HSP70 with IKKε. Ectopic expression of HSP70 relieved suppression of IFNβ expression by ORF8b in an IKKε-dependent manner. Enhancement of IFNβ induction in cells infected with ORF8b-deficient virus was erased when HSP70 was depleted. Taken together, HSP70 chaperon is important for IKKε activation and MERS-CoV ORF8b suppresses type I IFN expression by competing with IKKε for interaction with HSP70.

- We discovered the host trafficking protein AP2M1 which interacts with the virus protein YxxØ-motif as an universal virus dependency factor for the completion of the viral replication cycle. Furthermore, we have found the chemical ACA which has broad spectrum and potent antiviral activity against coronaviruses, influenza virus, Zika virus and HIV. This finding is now patented for further research and development.
- We also demonstrated that protein kinase R-like endoplasmic reticulum kinase (PERK) signaling mediated the proapoptotic signals in Middle East respiratory syndrome coronavirus (MERS-CoV) infection, which converged in the intrinsic apoptosis pathway. Chemical inhibitors of PERK signaling or intrinsic apoptosis both alleviated MERS pathogenesis in vitro and in vivo. Our study provides the first evidence that virus-induced apoptosis is an important disease determinant of highly pathogenic coronaviruses and demonstrates that this process can be targeted to attenuate disease severity. We have continued to work in this direction and are now submitting a paper to Nature.

6.2 What was the added value of the TRS funding, rather than standard project grant funding?
(For example, could this work have been achieved with other funding scheme, such as the General Research Fund or Collaborative Research Fund? If not, why?)

The TRS funding allowed a consortium of world-leading researchers in coronaviruses to merge their individual knowledge and skills and work together as a team to elucidate the molecular basis of interspecies transmission and pathogenesis of MERS-CoV as a whole programme. The TRS team was able to effectively use the funding provided by TRS to extensively answer three different aspects of MERS-CoV, including 1) how MERS-CoV emerged, i.e., animal origin and transmission pathways and viral determinants of interspecies transmissibility; 2) mechanisms by which the MERS-CoV modulate our innate immunity; and 3) mechanisms by which MERS-CoV induce severe pulmonary and extrapulmonary damages. This would not have been possible with other funding schemes which would not be able to provide sufficient resources and funding to carry out the vast amount of research completed in this project. Moreover, we succeeded in training a group of young researchers who have become budding scientists in the field of viruses. The team that we have built up using the TRS funding was also a timely team that was perfect for coping with the COVID-19 pandemic. Our rapid response to COVID-19 has led to numerous high impact publications as well as secured a new TRS funded project.

6.3 If the project has not met its original objectives, why?

All objectives of the project were met.

6.4 (a) Peer-reviewed journal publication(s) arising directly from this project:
(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference. Please mark the symbol "#" next to the publications involving inter-institutional collaborations)

Below is a list of publications which were published from June 2020 to September 2021 with results arising directly from this project. Publications arising from this project which were published before June 2020 are not included and have been listed in previous progress reports (last progress report submitted in June 2020).

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessi-ble from the institu-tional repository *(Yes or No)*
Year of publica-tion	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under prepara-tion *(optional)*						
2021				Chu H, Shuai H, Hou Y, Zhang X, Wen L, Huang X, Hu B, Yang D, Wang Y, Yoon C, Wong BH, Li C, Zhao X, Poon VK, Cai JP, Wong KK, Yeung ML, Zhou J, Au-Yeung RK, Yuan S, Jin DY, Kok KH, Perlman S, Chan JF*, Yuen KY*#	Targeting highly pathogenic coronavirus-induced apoptosis reduces viral pathogenesis and disease severity. Sci Adv. 2021 Jun 16;7(25):eabf8577.	No	Yes (Appendix A)	Yes	No
2021				Yeung ML*, Teng JLL, Jia L, Zhang C, Huang C, Cai JP, Zhou R, Chan KH, Zhao H, Zhu L, Siu KL, Fung SY, Yung S, Chan TM, To KK, Chan JF, Cai Z, Lau SKP, Chen Z, Jin DY, Woo PCY*, Yuen KY*#	Soluble ACE2-mediated cell entry of SARS-CoV-2 via interaction with proteins related to the renin-angiotensin system. Cell. 2021 Apr 15;184(8):2212-2228.e12.	No	Yes (Appendix B)	Yes	No
2021				Lau SKP*, Lung DC, Wong EYM, Aw-Yong KL, Wong ACP, Luk HKH, Li KSM, Fung J, Chan TTY, Tang JYM, Zhu L, Yip CCY, Wong SCY, Lee RA, Tsang OTY, Yuen KY, Woo PCY*#	Molecular Evolution of Human Coronavirus 229E in Hong Kong and a Fatal COVID-19 Case Involving Coinfection with a Novel Human Coronavirus 229E Genogroup. mSphere. 2021 Feb 10;6(1):e00819-20.	No	Yes (Appendix C)	Yes	No

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessi-ble from the institu-tional repository *(Yes or No)*
Year of publica-tion	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under prepara-tion *(optional)*						
2021				Lau SKP*, Fan RYY, Zhu L, Li KSM, Wong ACP, Luk HKH, Wong EYM, Lam CSF, Lo GCS, Fung J, He Z, Fok FCH, Au-Yeung RKH, Zhang L, Kok KH, Yuen KY, Woo PCY*#	Isolation of MERS-related coronavirus from lesser bamboo bats that uses DPP4 and infects human-DPP4-transgenic mice. Nat Commun. 2021 Jan 11;12(1):216.	No	Yes (Appendix D)	Yes	No
2020				Lau SKP*, Wong ACP, Luk HKH, Li KSM, Fung J, He Z, Cheng FKK, Chan TTY, Chu S, Aw-Yong KL, Lau TCK, Fung KSC, Woo PCY*#	Differential Tropism of SARS-CoV and SARS-CoV-2 in Bat Cells. Emerg Infect Dis. 2020 Dec;26(12):2961-2965.	No	Yes (Appendix E)	Yes	No
2020				Wong CK, Luk HK, Lai WH, Lau YM, Zhang RR, Wong AC, Lo GC, Chan KH, Hung IF, Tse HF, Woo PC, Lau SK*, Siu CW*#	Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes Platform to Study SARS-CoV-2 Related Myocardial Injury. Circ J. 2020 Oct 23;84(11):2027-2031.	No	Yes (Appendix F)	Yes	No
2020				Wong LR, Ye ZW, Lui PY, Zheng X, Yuan S, Zhu L, Fung SY, Yuen KS, Siu KL, Yeung ML, Cai Z, Woo PC, Yuen KY, Chan CP*, Jin DY*#	Middle East Respiratory Syndrome Coronavirus ORF8b Accessory Protein Suppresses Type I IFN Expression by Impeding HSP70-Dependent Activation of IRF3 Kinase IKKε. J Immunol. 2020 Sep 15;205(6):1564-1579.	No	Yes (Appendix G)	Yes	No
2020				Yuan S, Chu H, Huang J, Zhao X, Ye ZW, Lai PM, Wen L, Cai JP, Mo Y, Cao J, Liang R, Poon VK, Sze KH, Zhou J, To KK, Chen Z, Chen H, Jin DY, Chan JF*, Yuen KY*#	Viruses harness YxxØ motif to interact with host AP2M1 for replication: A vulnerable broad-spectrum antiviral target. Sci Adv. 2020 Aug 28;6(35):eaba7910.	No	Yes (Appendix H)	Yes	No
2020				Zhu L, Fung SY, Xie G, Wong LR, Jin DY, Cai Z*#	Identification of Lysine Acetylation Sites on MERS-CoV Replicase pp1ab. Mol Cell Proteomics. 2020 Aug;19(8):1303-1309.	No	Yes (Appendix I)	Yes	No
2020				Chow FW, Chan TT, Tam AR, Zhao S, Yao W, Fung J, Cheng FK, Lo GC, Chu S, Aw-Yong KL, Tang JY, Tsang CC, Luk HK, Wong AC, Li KS, Zhu L, He Z, Tam EWT, Chung TW, Wong SCY, Que TL, Fung KS, Lung DC, Wu AK, Hung IF, Woo PC*, Lau SK*#	A Rapid, Simple, Inexpensive, and Mobile Colorimetric Assay COVID-19-LAMP for Mass On-Site Screening of COVID-19. Int J Mol Sci. 2020 Jul 29;21(15):5380.	No	Yes (Appendix J)	Yes	No

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessi-ble from the institu-tional repository *(Yes or No)*
Year of publica-tion	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under prepara-tion *(optional)*						
2020				Lau SKP*, Luk HKH, Wong ACP, Li KSM, Zhu L, He Z, Fung J, Chan TTY, Fung KSC, Woo PCY*#	Possible Bat Origin of Severe Acute Respiratory Syndrome Coronavirus 2. Emerg Infect Dis. 2020 Jul;26(7):1542-1547.	No	Yes (Appendix K)	Yes	No

(b) Recognised international conference(s) in which paper(s) related to this project was/were delivered:

(Please attach a copy of each conference abstract)

Month/Year/ Place	Title	Conference name	Submitted to the RGC	Attached to this report	Acknowledged the support of the RGC	Accessible from the institutional repository
June/2020/ Virtual	Antiviral strategy for COVID-19 (KYYuen)	COVID-19 and Beyond – Solutions in Science	No	No (No abstract available)	Yes	No
June/2020/ Virtual	Overview of COVID-19 (KYYuen)	ISV Virtual Congress on COVID 19 Vaccines	No	No (No abstract available)	Yes	No
June/2020/ Virtual	Laboratory test and antiviral treatment for COVID-19 talk (KYYuen)	An online Forum on COVID-19 presented by Hong Kong Academy of Sciences	No	No (No abstract available)	Yes	No
July/2020/ Virtual	Options of antiviral therapy for COVID-19 (KYYuen)	The Cell Press Life Science Week on COVID-19	No	Yes (Appendix L)	Yes	No
August/2020/ Virtual	Controlling SARS CoV-2 – Lessons from Hong Kong (KYYuen)	Virtual COVID-19 Grand Rounds	No	No (No abstract available)	Yes	No
September/ 2020/Virtual	Updates on COVID-19: HK perspectives (KYYuen)	Asian Pacific Congress of Nephrology (APCN)	No	Yes (Appendix L)	Yes	No

Month/Year/ Place	Title	Conference name	Submitted to the RGC	Attached to this report	Acknowledged the support of the RGC	Accessible from the institutional repository
September/ 2020/	From SARS-03 to COVID-19 (KYYuen)	HKUSZH 2020 Neonatal Transition Summit: Virus & Infection Control	No	No (No abstract available)	Yes	No
September/ 2020/Virtual	Zoonotic origins of human coronaviruses (DYJin)	The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Conference on Coronavirus Disease (ECCVID)	No	No (No abstract available)	Yes	No
September/ 2020/Virtual	Combined steroid and antiviral therapy COVID-19 in the hamster model (DYJin)	International Online Conference of the World's Leading Virologists on COVID-19. Foundation for the Support of International Projects	No	No (No abstract available)	Yes	No
October/2020/ Virtual	From 2003 SARS to 2019 COVID: Hong Kong perspectives (KYYuen)	The Annual Meeting of the International Conference on Genomics	No	No (No abstract available)	Yes	No
October/2020/ Virtual	Beneficial effect of combinational methylprednisolone and remdesivir in the hamster model of SARS-CoV-2 infection (DYJin)	21st Virtual COVID-19 Symposium - Columbia University organized by Drs. Andrea Califano, Eric Greene, Stephen Goff and Andy Marks	No	No (No abstract available)	Yes	No
November/2020/Virtual	Antiviral options for SARS-CoV-2 (KYYuen)	Cold Spring Harbor Asia COVID19/SARS CoV2 Rapid Research Reports	No	No (No abstract available)	Yes	No
December/ 2020/Virtual	Activation of NLRP3 inflammasome by SARS-CoV and SARS-CoV-2 (DYJin)	The 3rd Symposium of the Association of Chinese Virologists in America (ACVA) and Virology Division of the Society of Chinese Bioscientists in America (SCBA)	No	Yes (Appendix M)	Yes	No
January/2021/ Virtual	Inflammasome activation by SARS-CoV and SARS-CoV-2	Emerging Microbes and Infections (EMI) Symposium 2021: COVID-19 – Retrospectives and Perspectives	No	No (No abstract available)	Yes	No
February/ 2021/Virtual	Rapid genomic sequencing for management of COVID-19 (KYYuen)	COVID-focused symposium of CROI 2021 - Conference on Retroviruses and Opportunistic Infections (CROI)	No	Yes (Appendix L)	Yes	No

Month/Year/ Place	Title	Conference name	Submitted to the RGC	Attached to this report	Acknowledged the support of the RGC	Accessible from the institutional repository
April/2021/ Virtual	Global trends in novel coronavirus variants (KYYuen)	The 2nd Infection Control Conference of Guangdong Medical Association 广东省医学会医院预防与控制学分会	No	No (No abstract available)	Yes	No
May/2021/ Virtual	From SARS-2003 to COVID-2019 (KYYuen)	Hospital Authority Convention 2021	No	Yes (Appendix L)	Yes	No
May/2021/ Virtual	Pathogenesis & Clinical presentation of COVID-19 (KYYuen)	17th Conference of the International Society of Travel Medicine	No	No (No abstract available)	Yes	No
May/2021/ Virtual	Virology and Diagnostics for Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2) (KYYuen)	25th Hong Kong Medical Forum	No	Yes (Appendix L)	Yes	No
June/2021/ Virtual	Mechanism of cell entry by SARS-CoV-2 (KYYuen)	XVth International Nidovirus Symposium (NIDO2021)	No	No (No abstract available)	Yes	No
June/2021/ Virtual	Poster presentation: 'Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes Platform to Study Pathogenic Human Coronaviruses Related Myocardial Injury'	XVth International Nidovirus Symposium (NIDO2021)	No	Yes (Appendix L)	Yes	No
June/2021/ Virtual	From SARS03 to COVID19 (KYYuen)	Infection and Immunity symposiums at the Amsterdam University Medical Centers (AUMC)	No	No (No abstract available)	Yes	No

(c) RGC funding should have been acknowledged in all publication(s)/conference papers listed in (a) and (b) above. If no acknowledgement has been made in any of the publications/ papers, please indicate and provide explanations.

N/A

6.5 To what extent this project has strengthened inter-institutional collaborations and other partnerships?

The TRS project has been an important driver in creating strong inter-institutional collaborations and collaborations with international researchers. This is highlighted by the fact that more than 90% of our publications were prepared with inter-institutional and/or international research collaborators.

6.6 Research students trained (registration/awards):

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Vidyanath Chaudhary	PhD	Sept 2013	Graduated 2018
Kitty Fung	PhD	Sept 2013	Graduated 2018
Chun-Kit Yuen	PhD	Sept 2013	Graduated 2018
Vivian Shuai	PhD	Sept 2013	Graduated 2018
Hinson Cheung	PhD	Sept 2014	Graduated 2019
Cun Li	PhD	Sept 2014	Graduated 2018
Hayes Luk	PhD	Sept 2014	Graduated 2019
Roy Wong	PhD	Sept 2014	Graduated 2018
Kah-Meng Tee	MPhil	Jan 2015	Graduated 2017
Long-Fung Mak	PhD	Sept 2015	Graduated 2017
Jessica Tsang	PhD	Sept 2016	Graduated 2020
Zhu Zheng	PhD	Sept 2016	Graduated 2020
Longchao Zhu	PhD	Sept 2016	Graduated 2020
Lilong Jia	PhD	Jan 2017	Graduated 2021
Antonio Wong	PhD	Jan 2017	Graduated 2021
Kenn Chik	PhD	Jan 2017	Graduated 2021
Dong Yang	PhD	Jul 2017	N/A
Xi Zhang	PhD	Sept 2017	N/A
Terance Tsang	MPhil	Sept 2017	N/A
Zirong He	PhD	Sept 2018	N/A
Lei Wen	PhD	Sept 2018	N/A
Raymond Chiu	PhD	Sept 2018	N/A
Joshua Fung	MPhil	Sept 2018	N/A
Tsz Tai Yuen	MPhil	Sept 2018	N/A
Yixin Wang	PhD	Aug 2019	N/A
Tony Chan	PhD	Sept 2019	N/A
Jianli Cao	PhD	Sept 2019	N/A
Chris Chan	MPhil	Sept 2019	N/A
Yuxin Hou	PhD	Dec 2019	N/A

6.7 Specific products (e.g. software or netware, instruments or equipment developed):

- A patent “Compositions And Methods For Broad-Spectrum Antiviral Therapy” was registered arising from results related to this project. This patent claims the usage of AM580, a retinoid derivative and RAR-α agonist, and its analogues (in particular Tamibarotene) for broad-spectrum antiviral therapy. These results were published in Nature Communications.
- Two monoclonal antibodies against the NP protein of MERS-CoV have been generated with high specificity for the detection of MERS-CoV.
- A capture ELISA has been developed using the two specific monoclonal antibodies against MERS-CoV NP with 100% specificity for MERS-CoV in human and dromedary samples and no reactivity towards other human CoVs such as HCoV-229E and HCoV-OC43.
- A rapid MERS-CoV NP test using the lateral flow immunoassay technique has been developed based on the capture ELISA and has been shown to be highly specific for MERS-CoV in human and dromedary samples with negative reactivity towards other human CoVs (HCoV-OC43, HCoV-229E, HCoV-NL63, and HCoV-HKU1) or dromedary CoV UAE-HKU23. The rapid

MERS-CoV NP test has been further verified to be able to detect lineage C betaCoVs. This test allows on-site detection of MERS-CoV infection and can be used to screen animal samples collected from across the world to track the transmission patterns and identify ancestors and potential animal sources of MERS-CoV.

6.8 Other education activities and/or training programmes developed:

A total of ten local knowledge exchange activities for secondary school students were organized during the project period. These included a seminar presented in June 2017 by Professor Patrick Woo (PC) on "Virus Discovery Journey in Dubai" and nine KE sessions held in 11th November 2017 (2 sessions), 27th January 2018, 19th May 2018, 27th April 2019 (2 sessions), 11th May 2019 (2 sessions) and 5th October 2020 by next-generation leaders Dr Jade Teng, Dr Philip Yeung, Dr Raven Kok, and Dr Jasper Chan. The topic of the 2017-2018 KE sessions was on the "Rapid method for the early diagnosis of MERS", the topic of the 2018-2019 KE sessions was on the "Development of Animal Models for the study of Middle East Respiratory Syndrome Coronavirus" and the topic of the 2020 KE session was on the "Coronavirus Journey at HKU: Rapid Diagnosis and Mass On-site Screening of COVID-19". The first two KE topics were held at the Department of Microbiology, HKU, while the last KE topic was held at the Tsing Yi campus of the Technological and Higher Education Institute of Hong Kong (THEi).

6.9 Please highlight any deliverables indicated in the project implementation timetable endorsed by the RGC which have not been covered or achieved as per sections 6.1 to 6.8 above, and explain/ elaborate.

N/A

Project Management

6.10 Please elaborate how the PC has played his/her role in coordinating and managing the project.

The main role of the PC is to coordinate between all the Co-PIs and Co-Is of the project, particularly team members who are not situated at HKU. The PC chair all committee meetings and is responsible for maintaining communication with the international advisory board as well as many of the collaborative partners of the project, such as contact with Dubai collaborator Ulrich Wernery to update on progress and discuss results, and securing a subaward grant of USD390,000 with Professor Paul Young for vaccine work on MERS infection. The PC is also responsible for the incubation and development of research ideas for the project and maintains close monitoring of the project progress as well as the performance of individuals, assessing work duties arising from each part and delegating tasks to maximize efficiency. In addition, the PC, with the help of next-generation leaders, personally supervises core projects that serve as the foundation of all three parts of this TRS, such as the generation of transgenic mice and infectious virus clones. The PC also designed and coordinated the knowledge exchange program for secondary school students and was the speaker of one of the seminars.

7. Awards and Recognition

7.1 Have any research grants been awarded that are directly attributable to the results obtained from this project?

Based on results obtained from this project, we have been successful in applying for research grants from Health and Medical Research Fund (HMRF), Collaborative Research Fund, an additional TRS fund and an Innovation and Technology Fund (ITF) (details in Section 9).

7.2 Have any project team members participated as invited speakers in or organisers of international conferences as a result of this project?

Section 6.4(b) lists the conferences in which Prof Kwok-yung Yuen (KYYuen) and Prof Dong-yan Jin (DYJin) were invited as presentation speakers.

7.3 Have any project team members taken leadership positions in editorial boards, scientific and professional organisations?
N/A

7.4 Any documentary proof of the application of technologies arising directly from this project?
N/A

7.5 Other awards and recognitions as a result of this project (please specify):
N/A

8. Impacts

8.1 What are the current and expected impacts of the project on the long-term development of Hong Kong (social or economic development, e.g. patent, technology transfer, collaboration with external organisations, etc.)?

- Since the start of this project, we have published a total of 75 publications that are supported by this TRS. These publications includes two publications in Lancet (impact factor 59.102), one publication in Nature Medicine (impact factor 30.641), one publication in Nature Microbiology (impact factor 14.174), one publication in Journal of Clinical Investigation (impact factor 13.251), two in Nature Communications (impact factor 12.353), three in Science Advances (impact factor 11.51), one in FASEB Journal (impact factor 5.595), four in Journal of Infectious Diseases (impact factor 5.186), one in Nature Reviews Drug Discovery (impact factor 57.000), one in Cell Research (impact factor 15.606), one in Trends in Microbiology (impact factor 11.776), two in Clinical Infectious Diseases (impact factor 9.055) and one in Journal of Virology (impact factor 4.663). In addition, we have one publication submitted to Nature Microbiology (impact factor 14.174), one publication submitted to Science Advances (impact factor 11.51) and one publication submitted to Clinical Infectious Diseases (impact factor 9.055) that are currently under review.
- We have proven well to have successfully trained a consortium of world-leading researchers in CoVs to tackle future CoV outbreaks and large epidemics (Objective 4). Our research group have been particularly valuable in the current situation of the COVID-19 outbreak and are recognised as one of the leading experts to face this global health emergency. With the knowledge which we have gained from our study of MERS-CoV, we have been able to apply the information to SARS-CoV-2 and are making accomplishments on the study of this novel CoV. Our team is now putting extra efforts in the development of rapid diagnostic reagents, treatment modalities and vaccines, as well as in understanding the origin, pathogenicity, and transmissibility of SARS-CoV-2. This work will be valuable to Hong Kong and globally to combat the ongoing outbreak of COVID-19.
- We have successfully filed a patent “Compositions And Methods For Broad-Spectrum Antiviral Therapy” arising from results related to this project. This patent claims the usage of AM580, a retinoid derivative and RAR-α agonist, and its analogues (in particular Tamibarotene) for broad-spectrum antiviral therapy. These results were published in Nature Communications.
- We held two very successful CoV Symposiums in September 2017 and September 2019. These meetings were a great opportunity for TRS team members to interact with experienced coronavirologists and carry out in depth discussion of progress and results. Solid relationships which were established in the previous meeting have stayed strong and have been particularly

beneficial for the establishment of our MERS infectious clone systems.

- We have established a collaboration with Prof Paul Young from the Australian Infectious Diseases Research Centre which apply leading technologies to identify, understand and prevent infectious diseases. Our collaboration involves a project titled "Rapid Response Pipeline for Stabilized Subunit Vaccines" with a subaward grant of USD390,000. The work will involve testing of a vaccine developed by their research group with mice vaccination, immune response assays, mice MERS challenge protection experiments and passive transfer of human sera for mice protection study. We have obtained the vaccine candidate from the collaborator and are currently testing it with our hDPP4 knock-in mice model.
- We have successfully developed a DPP4-transgenic mouse model which has been shown to be useful for susceptibility and pathogenesis studies for MERS-CoV.
- We have developed a novel viral vaccine system based on the influenza viral vector. This system has been extensively tested in various subtypes of flu virus and has been shown to be avirulent and effective. This vaccine can be further evaluated for its application in animals, such as camels, and in humans. In particular, we are in discussion with Dr Wernery in using their camel farm in Dubai as a field study for the testing of this vaccine.
- Prof Woo (PC) and Prof Lau (Part 1 leader) were invited by Prof Martin Schwemmle to write a chapter on Human Coronaviruses for the Elsevier Reference Module in Biomedical Sciences. This module provides biomedical and life science researchers access to trustworthy reference content updated as science progresses and gives recognition to our work as one of the authoritative, peer-reviewed reference of Elsevier.
- We have identified lopinavir-ritonavir and betaferon as potential drugs to protect against MERS-CoV infection. The combination of lopinavir-ritonavir and betaferon with or without ribavirin was said to be extensively used in the Korean outbreak and the Middle East and there is an ongoing Phase 2/Phase3 clinical trial on treating MERS patients with lopinavir at King Abdullah International Medical Research Center in Saudi Arabia (clinical trial no: NCT02845843). These drugs are a valuable resource and can be developed commercially into an antiviral option against MERS-CoV infection for local and international markets.

8.2 Others (please specify):
N/A

9. Sustainability of the Project

9.1 Whether there are new ideas evolved directly from this project?

The work supported by the TRS has formed a solid basis for research on coronaviruses. A large number of ideas have evolved from results from this project, including vaccine development, drug therapy and treatments, and in particular, the application of our gained knowledge in our response to the COVID-19 pandemic. Numerous high impact publications have since been published as a result of ideas branching from the TRS project.

9.2 Whether there are new projects evolved directly from this project?

- Using the results from the TRS, we have new funding from HMRF to develop a new potential vaccine candidate against MERS-CoV by utilizing the reverse genetic system platform.
- Our work on inflammasome activation by SARS-CoV and MERS-CoV has paved the avenue for our studies on inflammasome activation by SARS-CoV-2, which is now supported by funding from a One-off Collaborative Research Fund Coronavirus Disease and Novel Infectious Disease Exercise.
- Our work supported by the TRS has laid the foundation for our work on SARS-CoV-2 and a new

TRS project, titled "Ecology, Molecular Virology and Pathogenesis of SARS-CoV-2: From Bedside to Bench and Back" has been initiated.

- Based on our findings from the TRS project, we have successfully obtained an ITF for further work on emerging infectious disease agents including coronaviruses.

9.3 Whether there are new collaborations developed directly from this project?

In this project, we have established collaboration with the following parties:

- Ulrich Wernery, Central Veterinary Research Laboratory (CVRL), Dubai – for obtaining animal samples from Middle East, evaluation of the capture ELISA and rapid MERS-CoV test and potential evaluation of our vaccine candidate using dromedaries in their camel farm
- Hailin Zhang, Yunnan Institute of Endemic Diseases Control and Prevention, China – for obtaining animal samples from Yunnan, China
- Ming Wang, Guangzhou Centre for Disease Control and Prevention, China – for obtaining animal samples from Southern China
- Libiao Zhang, Guangzhou Entomological Institute, China – for obtaining bat samples from various provinces in China
- Yixin Chen, Xiamen University, China – for development of monoclonal antibodies for MERS diagnosis
- Wenhui Li, National Institute of Biological Sciences, Beijing, China – for defining binding affinity of bat virus RBDs to DPP4; and providing a three months training to our PDF for the development of protein-protein interaction experiments
- Chuan Qin, Chinese Academy of Sciences, Beijing, China – for providing Biosafety Level 3 facility for conducting experiments with Primate models
- Lim Lee Sim, Universiti Sains Malaysia, Malaysia – for obtaining bat samples from Malaysia
- Christian Drosten, University of Bonn, Germany – for his expertise on MERS-CoV replication in human intestines
- Hans Clevers, Hubrecht Institute, Holland – for providing a two weeks training to our RAP to learn about the generation of lung organoids
- David Markovitz, University of Michigan, USA – for his expertise on banana lectin (protein developed by his laboratory) as an antiviral option
- Jincun Zhao, Guangzhou Medical University, Guangzhou, China – for obtaining materials for established MERS infectious clone systems and state-of-the-art DPP4 knock-in mice via humanizing mouse locus with plasmid-mediated gene targeting strategy
- Alain Didier Missoup, University of Duoala, Africa – for extending our search for ancestors of MERS-CoV in African bats
- Theam Soon Lim, Universiti Sains Malaysia, Malaysia – for the purification of monoclonal antibodies to the MERS nucleoprotein
- Paul Young and Keith Chappell, University of Queensland, Australia – a newly established collaboration to test their developed vaccine technology using our transgenic animal model. This project is based on the "molecular clamp" vaccine platform which is funded by The Coalition for Epidemic Preparedness Innovations (CEPI) to synthesize the viral surface proteins of MERS-CoV while "clamping" them into shape, making it easier for the immune system to induce a response that recognizes them as on the virus surface. This synthetic antigen can then be purified and rapidly manufactured into a vaccine, within 16 weeks from pathogen identification. We have obtained the vaccine candidate from the collaborator and are currently testing it with our hDPP4 knock-in mice model.

9.4 Please give details on how much money and from which sources has been obtained/requested for the specific purpose of continuing the work started under this project.

We have been successful in applying for the following research grants based on results obtained from this project:

- HMRF Commissioned Research on Control of Infectious Diseases (Phase IV) (HKD1,800,000)
- One-off Collaborative Research Fund Coronavirus Disease and Novel Infectious Disease Exercise C7142-20GF (6/2021-5/2024) on Mechanism of Inflammasome Activation by SARS-CoV-2 (HKD8,431,247)
- RGC Theme-based Research Scheme 11th Round T11-709/21-N (1/2022-12/2026) on Ecology, Molecular Virology and Pathogenesis of SARS-CoV-2: From Bedside to Bench and Back (HKD42,278,000)
- Innovation and Technology Fund (ITF) (HKD405,000,000)

10. Statistics on Research Outputs

(Please ensure the statistics in this section are consistent with the information presented in other sections of this report.)

	Peer-reviewed journal publications	Conference papers	Scholarly, books, monographs and chapters	Patents awarded	Other research outputs (please specify)	
					Type	No.
No. of outputs arising directly from this research project	75	38	1	1		

11. Public Access of Completion Report

(PCs of projects funded in 2010/11 academic year or onwards are required to release the completion reports to the public through the RGC website. Completion reports containing information such as abstracts in non-technical terms, objectives, research output including the list of conference papers / publications / journals and research findings and contact information of PCs should be open to public access.)

Please specify the information, if any, that cannot be provided for public access and give the reasons.

Information that cannot be provided for public access	Reasons
Nil	

12. The Layman's Summary

(describe in layman's language the abstracts and research impact of the project.)

Middle East Respiratory Syndrome (MERS) is a severe and highly lethal respiratory disease caused by the novel human coronavirus (CoV) MERS-CoV. It has high case fatality of over 35% but therapeutic options are limited. As a potential pandemic agent, we sought to understand the origin, molecular pathogenesis and mechanisms of MERS-CoV. The TRS project asked three important questions: how did MERS-CoV emerge to infect camels and humans; what are the mechanisms by which MERS-CoV modulate our innate immunity; and what are the mechanisms by which MERS-CoV induce severe pulmonary and extrapulmonary damages.

As a result, we have elucidated the evolutionary origin and interspecies transmission mechanism of MERS-CoV. Novel coronaviruses related to MERS-CoV were discovered from different animals including bats and hedgehogs, providing insights on the receptor usage of spike protein during the evolution of MERS-CoV-related bat coronaviruses. We have successfully cultured a bat coronavirus,

Ty-BatCoV-HKU4, from lesser bamboo bats for the first time, which can utilize cell receptor human-dipeptidyl-peptidase-4 (hDPP4) for cell entry in the same way as MERS-CoV does. A hDPP4-transgenic mouse model was developed for studying MERS-CoV pathogenesis as well as vaccine and drug evaluation. This model can also be infected by Ty-BatCoV-HKU4, implying the potential of direct bat-to-human transmission of bat coronavirus. We have developed twelve in-house bat cell lines to determine the bat cell tropism of MERS-CoV, which was also later used to study the potential bat host tropism of SARS-CoV-2. These findings will provide us with a better understanding on the origin and transmission of coronaviruses in preparation for the next pandemic like COVID-19.

Moreover, how infection with MERS-CoV suppresses antiviral protein production remains incompletely understood. We speculate that potent suppression of host innate antiviral response could be a cause of severe disease. We showed that structural protein M and accessory protein ORF8b of MERS coronavirus are antagonists of host antiviral protein known as interferon. M protein interacts with a cellular protein named TRAF3 to prevent it from activating interferon production. ORF8b-deficient MERS coronavirus induces more interferon in infected cells. ORF8b was further found to be capable of binding and inhibiting a molecular chaperone, thereby crippling a cellular enzyme that requires the chaperone to turn on the molecular switch of interferon production. Our findings provide new mechanisms for suppression of interferon production by MERS-CoV and have implications in rational design of new antivirals and vaccines.

Finally, we have elucidated important pathways by which coronaviruses replicate, including their hijacking of the cargo protein AP2M1 for their intracellular transportation, and their targeting of the cell death pathway for their replication. These are previously undiscovered pathways which markedly increase our understanding of how coronavirus multiply and cause damage to our cells. They also represent important targets for the development of antiviral agents.

The work supported by TRS has resulted in a total of 75 publications in international peer-reviewed journals including high impact journals such as Lancet, Nature Medicine, Nature Microbiology, Nature Communications and Journal of Clinical Investigation, as well as a book chapter for the Elsevier Reference Module in Biomedical Sciences on Human Coronaviruses. We have successfully filed a patent “Compositions And Methods for Broad-Spectrum Antiviral Therapy” using results related to this project, and have generated two monoclonal antibodies against the NP protein of MERS-CoV which have been used to develop a rapid MERS-CoV NP test using the lateral flow immunoassay technique. This test allows on-site detection of MERS-CoV and can be used to screen animal samples collected from across the world to track the transmission patterns and potential animal sources of MERS-CoV. We have also developed a novel viral vaccine system based on the influenza viral vector which will be further evaluated for its application in animals and in humans.

In terms of the impact of the TRS project on teaching and knowledge transfer, we have successfully built a consortium of world-leading researchers in CoVs, including experienced clinician-scientists, coronavirologists and budding young researchers, which have been particularly valuable in coping with the COVID-19 pandemic. We are recognised as one of the leading experts in this global health emergency and our rapid response to COVID-19 has led to numerous high impact publications. During the project period, two CoV Symposiums and numerous TRS seminars were held with presentations by international speakers and provided an excellent opportunity for the TRS team to interact with experienced coronavirologists. Results from the project were also presented by TRS team members at more than 38 international and local conferences. Solid collaborations were established with researchers from Dubai, China, Australia, Malaysia, Germany, Holland, USA and Africa, and include a breakthrough collaboration to test a newly developed vaccine technology on our hDPP4 knock-in mice model. In addition, we have conducted knowledge exchange activities for secondary school students which have aroused their interest in scientific research and highlighted the importance of science for Hong Kong, China, and the world.

In summary, the objectives of the TRS project were successfully completed. We have successfully secured four research grants using results from the TRS project, totalling more than HKD450 million, which will allow us to continue the research on coronaviruses. This will benefit us tremendously to gain knowledge in preparation for any future coronavirus epidemics.