RGC Ref.: N_HKU 709/11 NSFC Ref.:
(please insert ref. above)

The Research Grants Council of Hong Kong
NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Blocking HIV Infection by Gene-encoding Neutralizing Antibodies
用基因编码的中和抗体阻断艾滋病毒的感染

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Professor Zhiwei Chen	Dr. Paul Zhou
Post	Professor	Professor and Unit Chief
Unit / Department / Institution	The University of Hong Kong / Microbiology / AIDS Institute	Institut Pasteur of Shanghai/ Chinese Academy of Sciences
Contact Information	zchenai@hku.hk 3917 9825	blzhou@sibs.ac.cn
Co-investigator(s) *(with title and institution)*	Dr. Liu Li, RAP AIDS Institute, Microbiology The University of Hong Kong	

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	01 Jan 2012		
Project Completion date	31 Dec 2014		
Duration *(in month)*	36		
Deadline for Submission of Completion Report	31 Dec 2015		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To test synergistic effect of GPI-anchored antibody derivatives and secretory immunoadhesins against large panels of multiclade HIV-1 pseudotypes and primary isolates in transduced TZM.bl cells.

2. To test synergistic effect of GPI-anchored antibody derivatives and secretory immunoadhesins against a large panel of multiclade HIV-1 primary isolates and SHIV in transduced human T cell line or primary CD4 T and B cells.

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

6. Research Outcome: Major findings and research outcome.
(maximum 1 page; please make reference to Part C where necessary)

1). Development of GPI-VHH JM4: JM2 and JM4 are two recently isolated variable regions (VHH) of heavy chain only antibodies from llamas that have been immunized with a trimeric gp140 bound to a CD4 mimic. JM2 binds the CD4-binding site of gp120 and neutralizes HIV-1 strains from subtypes B, C and G. JM4 binds gp120 and neutralizes HIV-1 strains from subtypes A, B, C, A/E and G in a CD4-dependent manner. Crystal structure of JM4 in the complex of Yu-2 gp120 core and a CD4-mimic shows that JM4 binds to an epitope spanning the gp120 bridge sheet, V3 loop, β19 strand, the CD4-binding loop and the glycan at Asn386 residue. The JM4 epitope overlaps with b12 epitope in the CD4BS and 17b, 48d, X5 and 412d epitopes in co-receptor binding site of gp120. Thus, JM4 targets a hybrid epitope on gp120 that combines elements from both the CD4-binding and the co-receptor binding sites. In the study, we constructed GPI-VHH JM2 and JM4 along with E4 control. We show that by genetically linking the VHHs with a GPI attachment signal, VHHs are targeted to the lipid rafts of the plasma membranes through a GPI anchor. GPI-VHH JM4, but not GPI-VHH E4 and JM2, on the surface of transduced TZM.bl cell lines neutralizes multiple subtypes of HIV-1 isolates with a high degree of potency including transmitted founder viruses, quasispecies and viruses that are resistant to soluble VHH JM4. Moreover, transducting human CD4 T cell line CEMss-CCR5 with GPI-VHH JM4, but not with GPI-VHH E4, confers resistance to HIV-1 infection via both cell-free and T cell-T cell transmissions as well as HIV-1 envelope-mediated cell-cell fusion. Finally, human primary CD4 T cells transduced with GPI-VHH JM4, but not with GPI-VHH E4, are resistant to both cell-free and T cell-T cell transmission of HIV-1.

2). Development of Bi-specific Immunoadhesins: Since no broadly neutralizing antibody responses can be elicited by active immunization so far, passive immunization with braodly neutralizing antibodies or immunoadhesins is a viable alternative. Development of bispecific antibodies and immunoadhesins has many practical advantages than monospecific antibodies and immunoadhesins. Towards this goal, we first compared neutralization synergy among antibodies PGT128, Hu5A8, PG16 and VRC01. Since PGT128 and Hu5A8 is the best combination, we made two different formats of immunoadhesins of antibodies PGT128 and Hu5A8, bi-IA-dual and bi-IA-mono. Bi-IA-dual was encoded by two individual gene constructs; while bi-IA-mono was encoded by a single gene construct. After transiently transfected into 293 T cells, the expression of bi-IA-dual and bi-IA-mono in the culture supernatants was detected by Western blot analysis. We then used a single cycle of infectivity assay to compare neutralization activity of bi-IA-mono, bi-IA-dual, PGT128, Hu5A8 and VRC01 against a panel of 40 HIV-1 pseudotypes including CRF01_AE, B, CRF07/08_BC, and C as well as VSV-G control. A manuscript based on these findings was submitted for publication.

3). Testing of GPI antibody derivatives and bi-specific immunoadhesins in human primary CD4 T cells *ex vivo* and in humanized mice *in vivo*: Having demonstrated that GPI anchored antibody derivatives and bispecific immunoadhesins block HIV-1 in transduced cell lines, we next tested their anti-HIV-1 activity in human primary CD4 T cells ex vivo and humanized mice in vivo. Towards this goal, we constructed lentiviral transfer vectors that express GPI-scFv X5 or AB65-2A-eGFP fusion genes. After optimizing recombinant lentivirus production and transduction protocols, we obtained 90% or higher transduction efficiency in human primary CD4 T cells. Then we challenged GPI-scFv X5 or AB65-transduced human primary CD4 T cells with dual tropic HIV-1 R3A (the HIV-1 strain will be used in our follow-up humanized mouse study). GPI-scFv X5-transduced human primary CD4 T cells, but not GPI-scFv AB65-transduced primary CD4 T cells, are completely resistant to HIV-1 infection. Besides ex vivo testing human primary CD4 T cells, we will use humanized mouse model to evaluate GPI-scFv X5-transduced human primary CD4 T cells in vivo. Our results show that GPI anchored antibody derivatives and bispecific immunoadhesins can block HIV-1 infection, which will be published in near future.

Potential for further development of the research and the proposed course of action (maximum half a page)

In humanized mouse models, we have obtained some promising results with GPI-scFv X5 and bi-specific immunoadhesins, which warrants future clinical trials of potential products. For example, passive immunization using bi-specific bnAbs/IAs becomes an attractive strategy for human immunodeficiency virus (HIV) prevention and immunotherapy. The conventional bi-specific bnAb (BiNab) consists of one single-chain variable fragment (scFv) from each of two parental bnAbs using "knobs-in-holes", which has limitations for bnAbs that require a pair of parental scFvs for complete function. In this study, we discover a bi-specific immunoadhesin encoded in a single gene that expresses both scFvs of two parental bnAbs. Compared with parental and knobs-in-holes IAs, BiIA-SG has functional properties of both parental bnAbs with enhanced breadth and potency. Importantly, it neutralizes 100% of viruses tested, including the transmitted/founder viruses of major subtypes and multiple viruses naturally resistant to parental IAs, and is capable of protecting humanized mice from live virus challenge. Therefore, Our BiIA-SG can be further developed for bnAb-gene-based immunoprophylaxis and treatment. Similar approaches can also be taken for testing GPI-VHH JM4 with or without combination with BiIA-SG.

7. The Layman's Summary

(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

During the past three years, we have jointly carried out three areas of research. First, we developed GPI-VHH antibody derivatives from llama single domain antibody JM4 and demonstrated that GPI-VHH JM4 neutralizes multiple subtypes of HIV-1 isolates with a high degree of potency including transmitted founder viruses, quasispecies and viruses that are resistant to soluble sdAb JM4. Moreover, we demonstrated that the transduction of human CD4 T cell lines with GPI-VHH JM4 confers resistance to both cell free and T cell-to-T cell transmission of HIV-1 infection and blocks HIV-1 envelope-mediated cell-to-cell fusion. Second, we developed bi-specific immunoadhesins and demonstrated that in most HIV-1 strains bi-specific immunoadhesins neutralize more potently than parental neutralizing monoclonal antibodies and in some cases they also neutralize strains that parental antibodies fail to neutralize. Third, we started testing these GPI-anchored antibody derivatives and bi-specific immunoadhesins in primary T cells *ex vivo* and in humanized mouse models. We have developed a protocol to efficiently deliver genes into human primary T cells *ex vivo* and transfuse transduced T cells and soluble bi-specific immunoadhesins into humanized mice. Although this area of study is still on-going, our preliminary results are very promising.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each 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.)

The Latest Status of Publications				Author(s) *(**bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	Title and Journal/ Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*						
			✓	L.H. Liu, W.M. Wang, J. Matz, S. Li, J.T. Kimata, **Z. Chen**, S. Benichou and **P. Zhou**	Glycosyl Phosphatidylinositol (GPI)-Anchored Variable Region of Heavy Chain Only Antibody JM4 Blocks Both Cell-Free and Cell-to-Cell Transmission of Human Immunodeficiency Virus Type 1		No	Yes	No
2015	2015			J. Guo, T. Zuo, L. Cheng, X. Wu, J. Tang, C. Sun, L. Feng, L. Chen, L. Zhang, and **Z. Chen**,	Simian Immunodeficiency Virus Infection Evades Vaccine-Elicited Antibody Responses to V2 Region		Yes	Yes	Yes http://www.ncbi.nlm.nih.gov/pubmed/25622057

		✓		J. Guo, M. An, X. Wu, H. Zhang, L. Cheng, H.Y. Tang, H. Wang, H. Shang, **P. Zhou** and **Z. Chen**	Single gene-encoded bispecific immunoadhesin enhances significantly the breadth and the potency of neutralization against diverse HIV-1 strains		Yes	Yes	

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)*

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
February, 2014	Human Monioclonal Antibodies	Keystone Symposium on "Human Monioclonal Antibodies"		No	Yes	Yes
July/2014/ Australia	A single-gene encoded bi-specific neutralizing antibody against diverse HIV-infection	20th International AIDS Conference		Yes	Yes	Yes http://pag.aids2014.org/session.aspx?s=1122#1

A manuscript based on these findings is almost completed and will be submitted soon. The findings were also presented in the Keystone Symposium on "Human Monioclonal Antibodies" Keystone, CO. February, 2014.

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Jia GUO	PhD	01-09-2009	31-08-2013

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

One patent application in Mainland China in 2014, entitled: "Single-gene encoded bivalent or multivalent specific anti-HIV (human immunodeficiency virus) immunoadhesin". Application number: 201410245945.X.