RGC Ref.: A-HKU709/14
(please insert ref. above)

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

(Please attach a copy of the completion report submitted to the ANR by the French researcher)

Part A: The Project and Investigator(s)

1. Project Title (ANR Acronym)

A novel PDI DNA-based vaccination strategy to mimic Gag-specific responses found in HIV Elite controllers

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

	Hong Kong Team	French Team
Name of Principal Investigator *(with title)*	Prof. Zhiwei Chen	Dr. Lisa Chakrabarti
Post	Director / Professor	Associate Professor
Unit / Department / Institution	AIDS Institute / Microbiology / HKU	Viral Pathogenesis Unit / Pasteur Institute
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Co-investigator(s) *(with title and institution)*	Prof. Qiang Wei Institute of Laboratory animal sciences, Chinese Academy of Medical Sciences, Beijing	Dr. Victor Appay INSERM Unit, Pitié-Salpétrière Hospital, Paris, France
	Ms. Liye Chen Teresa Bio-Tech Co., Ltd, Shanghai	Prof. Olivier Lambotte INSERM UIOI2

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	1 April 2015		
Project Completion date	31 Mar 2019		
Duration *(in month)*	48 months		
Deadline for Submission of Completion Report	31 Mar 2020		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. Establish a benchmark for optimal Gag responses by studying HIV Elite Controllers;
2. Induce high-avidity Gag T cell responses by in vitro vaccination with sPD1-Gag vectors;
3. Determine the role of high avidity Gag-specific T-cells induced by sPD1-Gag vaccination in a mouse challenge model;
4. Evaluate the chosen sPD1-Gag vaccination strategy in a non-human primate model.

Revised Objectives

Date of approval from the RGC: N/A

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

The French team has characterized the CD4 T cell TCR repertoire specific for the most immunodominant epitope in HIV capsid, Gag293 in HIV elite controllers (ECs). A set of high-affinity TCR clonotypes preferentially shared by ECs was identified (Benati et al 2016). These high-affinity TCRs were essential for the cytotoxic capacity of EC CD4+ T cells, indicating that Gag-specific CD4+ T cells could directly kill HIV-infected cells for viral control. The French team has also determined crystal structures of three controller TCRs revealed a peptide-centric recognition of the Gag293 epitope, explaining how these TCRs could focus their binding

on the peptide and tolerate amino acid variations in HLA-DR helixes, and thus function in the context multiple HLA-DR genotypes (Galperin et al 2018). This broad HLA-DR cross-restriction will be an asset in vaccination strategies, as shared TCR clonotypes may be induced in a genetically diverse human population. The French team wrote a review on the key role of TCR clonotypes in determining the T cell efficacy in anti-HIV responses (Lissina et al 2016).

In addition, the French team has optimized an *in vitro* vaccination system to evaluate the capacity of CD8+ T cell priming using human cells and showed that ligands for pattern recognition receptors (PRR), such as TLR8L or cGAMP, were particularly efficient at priming CD8+ T cell responses, including enhanced cytokine secretion and cytotoxicity (Lissina et al 2016, Gutjahr et al 2019, Kuse et al 2019).Using this system, the team also found that HIV-infected patients consistently elicited lower de novo CD8+ T cell responses. The team is currently testing whether the use of PRR ligands and sPD1-tagged antigens could improve the quality and quantity of HIV patients' CD8+ T cells. At present, the results showed that type I IFN signaling stimulation can mount potent de novo CD8+ T cell responses using HIV-infected patient samples. These findings have direct relevant for the development of effective formulation of vaccinations in HIV infected patients.

For the sPD1-based vaccine evaluation, the HK team first demonstrated the efficacy of the vaccine design in mice. BALB/c mice vaccinated with sPD1-p24 vaccine via electroporation (device provided by our industrial partner Teresa Bio-Tech) were protected from an otherwise lethal challenge of mesothelioma AB1 cells expressing the p24 antigen (Tan et al 2014). Protection was associated with the induction of CD8+ T cells with polyfunctional cytokine secretion ability, overcome of the tumor immunosuppressive environment, and epitope spreading (Tan et al 2014, Yu et al 2015). These studies highlighted the potential of the sPD1 vaccine as an immunotherapeutic approach in cancer. The HK team also developed a modified DNA vaccine comprising two mosaic p41 Gag genes made of Gag p17 plus p24 sequences. These mosaic Gag genes were designed to cover the majority (70-96%) of HIV-1 strains circulating in China, based on the analysis of over 500 strains of HIV-1 subtypes B, C/CB', and CRF01_AE. A majority of HIV infected Chinese patients had T cell reactivity against the mosaic-p41 Gag peptides, validating the choice of this antigen for future human immunization studies. In addition, this mosaic-p41 construct induced broad and long-lasting T cell responses in BALB/c mice in a prime/boost regimen, using DNA vaccine as prime, and a Modified Vaccinia Tian Tan (MVTT) vector as boost (Liu et al 2018). We also generated a sPD1-fused mosaic-p41 DNA vaccine and demonstrated that this vaccine elicited broader T cells responses in BALB/c mice, compared to the non-targeting mosaic-p41 or the original sPD1-p24 constructs (Chen et al, in preparation). Importantly, the sPD1-mosaic-p41 vaccinated mice showed a greater decrease in viral load than non-targeting mosaic-p41 vaccinated mice after challenge with EcoHIV virus. The sPD1-mosaic-p41 design has been patented.

The immunogenicity of sPD1-based vaccine was then investigated in the non-human primate rhesus macaque model. Four rhesus macaques were immunised with the sPD1-mosaic-p41 vaccine via electroporation using the Teresa Bio-Tech device. The macaques received the vaccine at 0, 6, 12 and 25 weeks. The vaccination resulted in strong ELISPOT T cell responses (>1,000 IFN-γ+ cells/10^6 PBMCs) in the macaques at 2 weeks after second immunisation. More importantly, T cell responses were efficiently boosted in greater than 10-folds after the 4th vaccination, demonstrating the advantage of the DNA vaccination platform in allowing multiple boosts, due to the absence of anti-vector immunity. Importantly, the mosaic Gag sequences allowed the induction of a broadly directed response that cross-reacted against HIV-1 subtypes AE, B and C (Chen et al, in preparation). To test for the PD1-based vaccine efficacy in the SHIV macaque challenge model, we have constructed a rhesus sPD1-Gag p27 DNA vaccine, rhPD1-p27. Seven macaques were immunised with rhPD1-p27 via electroporation four times in 6-to 12-week intervals. All the vaccinated macaques showed suppressed setpoint viremia to undetectable levels following high-dose intravenous challenge with the highly pathogenic $SHIV_{SF162P3}$. Poly-functional effector-memory CD8+ T cells were induced by the sPD1-based

vaccination, and they were potently recalled upon the viral challenge. CD8+ T cell immunity is essential for suppressing viral replication as *in vivo* CD8+ T cell depletion resulted in transient viremia. Importantly, vaccinated macaques with sustained viremia suppression responded to boost PD1-based vaccination without viral escape. Manuscripts for the macaque studies are being prepared for submission. These very encouraging results demonstrate that sPD1-based vaccination induces virus-specific effector-memory CD8+ T cells that are effectively recalled during AIDS virus infection and provides sustained functional cure.

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

The TCR clonotyping analysis demonstrates the unusual properties of Gag-specific TCRs on T cells from HIV controllers. These works reinforced the rationale and importance for inducing similar T cell responses through vaccination. Using the in vitro vaccination system, we are testing the ability of sPD1-based vaccines to prime such immune responses. In addition, functionality and cytotoxicity of T cells also play a critical role for controlling viral infection. As such, we are testing the use of various stimuli, including TLR ligands and cGAMPs, in priming highly functional T cells. This will improve the formulations used for future vaccine candidates. Over this project, our industrial partner, TERESA Bio-tech, has been actively involved in study design and experimental procedure validation. The company has optimized its electroporation device TERESA-EPT-I for use in Rhesus macaques and it is pursuing the development of its next-generation electroporation device. During this project, we have also designed, constructed, and evaluated the efficacy of a human sPD1-tagged mosaic-p41 DNA vaccine. We have patented this novel vaccine design (Z Chen as an inventor). Based on its encouraging immunogenicity, we have established a new collaboration with a new industrial partner, namely ImmunoCure HK Ltd, in order to prepare for clinical trials. The vaccine, now called ICVAX, has been produced in a GMP facility in three lots of 3000 doses. We have recently started the safety, toxicity, and immunogenicity evaluations in the rhesus macaque models by a China FDA-certified third-party contract research organization. Results generated will be used for filing an Investigational New Drug (IND) approval from China FDA for clinical phase I clinical trials in Hong Kong and in China. We have already established collaborations with The University of Hong Kong Clinical Trial Center and the Shenzhen Third People's Hospital as potential clinical trial sites. We plan to pursue the France-HK collaboration to investigate the nature of the TCR clonotypes induced by vaccination in human subjects and explore the possible correlates of protection of ICVAX in future studies.

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)

This project aims to develop an innovative sPD1-based DNA vaccination strategy against HIV. Developing a vaccine that induces HIV Gag responses similar to those seen in HIV elite controllers (ECs) represents a key factor. Here, we first made an in-depth characterization of the Gag-specific T cell receptors (TCRs) induced in ECs and found that these very sensitive TCRs conferred the capacity to more efficiently kill HIV-infected cells. We then evaluated our sPD1-based vaccine platform in several mouse models and demonstrated the superiority of the sPD1-targeting Gag DNA vaccine against viral infections and tumorigenesis over non-targeted vaccines. We also assessed the immunogenicity and the protective efficacy of the sPD1-Gag DNA vaccine against a highly pathogenic chimeric SIV/HIV virus (SHIV) in rhesus macaques. All the vaccinated macaques rapidly controlled SHIV replication to undetectable levels. This viral control was sustained over 2 years and was, mechanistically, mediated by the vaccine-induced CD8+ T cell responses. Over this project, we have also designed and generated a highly immunogenic sPD1-based mosaic Gag DNA vaccine, aiming for human clinical trials in the near future. These encouraging results suggest that the sPD1-Gag DNA vaccination represents a straightforward approach to induce AIDS virus control.

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)*						
2018				Liu W, Wong YC, Chen SMY, Tang J, Wang H, Cheung AKL, **Chen Z***	DNA prime/MVTT boost regimen with HIV-1 mosaic Gag enhances the potency of antigen-specific immune responses. **Vaccine.** 2018 Jul	2020	Yes	Yes	Yes

					25;36(31):4621-4632.				
2017				Cheung AK, Kwok HY, Huang Y, Chen M, Mo Y, Wu X, Lam KS, Kong HK, Lau TCK, Zhou J, Li J, Cheng L, Lee BK, Peng Q, Lu X, An M, Wang H, Shang H, Zhou B, Wu H, Xu A, Yuen KY, **Chen Z***	Gut-homing Δ42PD1+Vδ2 T cells promote innate mucosal damage via TLR4 during acute HIV type 1 infection. Nature Microbiology. 2017 Aug.	2020	Yes	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)*
Sep/2015 Vienna, Austria	Enhanced protection of human PD1-based HIV-1 mosaic DNA vaccine	4th European Congress of Immunology	Mar/2017	No	yes	yes
Jul/2016 Durban, South Africa	Human sPD1-based HIV-1 Gag-fusion DNA vaccine induces high frequency of broadly reactive T cell responses in mice and rhesus macaques	International AIDS Conference 2016	Mar/2017	No	yes	yes

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
Chen Man Ying	3-year PhD	1 September 2013	31 August 2016
Liu Wan	4-year PhD	1 October 2013	30 September 2017

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

Nil