RGC Ref.: N_HKU 747/11 NSFC Ref. : 81161160572
(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

Induction of tolerance by alloantigen-specific regulatory T cells in humanized mice and non-human primates

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Wenwei Tu	Gang Chen
Post	Professor	Professor
Unit / Department / Institution	Dept of Paediatrics & Adolescent Medicine/University of Hong Kong	Institute of Organ Transplantation/ Huazhong University of Science and Technology
Contact Information	wwtu@hku.hk	gchentj@163.com
Co-investigator(s) *(with title and institution)*	Prof. Yu-Lung Lau Dr. Yinping Liu	Prof. Feili Gong

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	01/01/2012		01/01/2012
Project Completion date	31/12/2014		31/12/2014
Duration *(in month)*	36		36
Deadline for Submission of Completion Report	30/09/2015	31/12/2015	31/12/2015

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To determine the survival, migration and homing programs of alloantigen-specific Treg induced by CD40-activated B cells in humanized mice during GVHD. (performed by HK team)

2. To determine whether alloantigen-specific Treg induced by CD40-activated B cells can prevent GVHD and preserve their general immunity in humanized mouse model.(performed by HK team)

3. To determine the mechanisms underlying the induction of allograft antigen-specific tolerance in humanized mouse model. (performed by HK team)

4. To determine whether alloantigen-specific Treg induced by CD40-activated B cells can prevent allograft rejection in non-human primate kidney transplantation. (performed by Mainland team)

5. To determine the mechanisms underlying the induction of allograft antigen-specific tolerance in non-human primate. (performed by Mainland team).

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

1.
2.
3. ....

6. Research Outcome

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

1). We established a human allogeneic GVHD model in humanized mice to mimic GVHD after BMT in humans. We demonstrate that *ex vivo*-induced $CD8^{hi}$ Treg can control GVHD in an allo-specific manner by reduction of alloreactive T-cell proliferation, and inflammatory cytokine and chemokine secretion within target organs through a CTLA-4-dependent mechanism in humanized mice. Importantly, these $CD8^{hi}$ Treg can induce long-term tolerance effectively without compromising general immunity and graft-versus-tumor (GVT) activity. [One paper published in *Science Translational Medicine* (**IF: 15.843**), see detail in attachment 2].

2). We discovered a novel function of TLR5-related signaling in enhancing the proliferation of $CD4^{hi}CD25^{+}$ regulatory T cells by promoting S phase progress but not involved in the suppressive function of human CD40-activated B cell-induced $CD4^{hi}CD25^{+}$ regulatory T cells, suggesting a novel role of TLR5-related signals in the generation of induced regulatory T cells. [One paper published in *PLoS ONE* (**IF:4.092**), see detail in attachment 3].

3). We demonstrated that ICOS regulates the generation and function of human $CD4^{+}$ Treg in a CTLA-4 dependent manner. Our results indicated the beneficial role of ICOS-ICOSL signal pathway in the generation and function of $CD4^{hi}$ Treg and uncovered a novel relationship between ICOS and CTLA-4. [One paper published in *PLoS ONE* (**IF:4.092**), see detail in attachment 4].

4). One US patent has been filed: Human CD8 regulatory T cells inhibit graft-versus-host disease and preserve general immunity (US 14/596.368, attachment 5).

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

Our study provided proof-of-concept of using *ex vivo*-induced human Treg to control GVHD after BMT. This novel strategy could readily be extended to human clinical trials using human Treg alone or in combination with minimal conventional immunosuppression to control GVHD. The GVHD model established here may also provide a more relevant platform for further studies of human immunopathogenesis and therapeutics for GVHD after BMT. The next step should be focus on translating this novel treatment strategy to the clinic by clinical trial.

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)

Graft-versus-host disease (GVHD) is a lethal complication of allogeneic bone marrow transplantation (BMT). The current strategy of using immunosuppressive agents to control GVHD may cause general immune suppression and limit the effectiveness of BMT. Adoptive transfer of regulatory T cells (Treg) can prevent GVHD in rodents, indicating the therapeutic potential of Treg for GVHD in humans. However, the clinical application of Treg-based therapy is hampered by the low frequency of human Treg and the lack of a

reliable model to test their therapeutic effects *in vivo*. Recently we successfully generated human alloantigen-specific Treg in a large scale from antigenically-naïve precursors *ex vivo* using allogeneic CD40-activated B cells as stimulators. Here, we report a human allogeneic GVHD model established in humanized mice to mimic GVHD after BMT in humans. We demonstrate that *ex vivo*-induced Treg can control GVHD in an allo-specific manner by reduction of alloreactive T-cell proliferation, and inflammatory cytokine and chemokine secretion within target organs through a CTLA-4-dependent mechanism in humanized mice. Importantly, these Treg can induce long-term tolerance effectively without compromising general immunity and graft-versus-tumor (GVT) activity. Our results support testing of human Treg in GVHD in clinical trials.

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)*						
2013				Zheng Jian, **Liu Yinping**, Liu Ming, Xiang Zeng, Lam Kowk-Tai, Lewis B David, **Lau Yu-Lung***, **Tu Wenwei***	Human CD8 regulatory T cells inhibit graft-versus-host disease and preserve general immunity in humanized mice. ***Science Translational Medicine*** 2013 Jan 16;5(168): 168ra9.	No	Yes	Yes	Yes

2013				Chan Ping-Lung, Zheng Jina, **Liu Yinping**, Lam Kowk-Tai, Xiang Zheng, Mao Huawei, Liu Yuan, Qin Gang, **Lau Yu-Lung***, **Tu Wenwei***.	TLR5 Signaling Enhances the Proliferation of Human Allogeneic CD40-Activated B Cell Induced CD4(hi)CD25(+) Regulatory T Cells. ***PLoS One*** 8:e67969.	Yes	Yes	Yes	Yes
2013				Zheng Jian, **Liu** Chan Ping-Lung, **Yinping**, Gang Qin, Xiang Zheng, Lam Kwok-Tai, Lewis David B, **Lau Yu-Lung***, **Tu Wenwei***	ICOS regulates the generation and function of human $CD4^+$ Treg in a CTLA-4 dependent manner. *PLoS ONE* 2013 Dec 2; 8(12): e82203. doi:10.1371/journal.pone.0082203.		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)*

08/2013/Roma, Italy	Human $CD8^+$ regulatory T cells inhibit GVHD and preserve general immunity in humanized mice	15^{th} international Congress of Immunology		Yes	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

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

1). One US patent has been filed: Human CD8 regulatory T cells inhibit graft-versus-host disease and preserve general immunity (US 14/596,368,).
2). Established collaboration with Stanford University Medical School, USA and Huazhong University of Science and Technology, China.