Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814-935-1081

5. **Grant SAP Number:** # 4100047645

6. **Project Number and Title of Research Project:** 35- Impact of iPS Cell-derived Highly Reactive Immune Cells on Cancer

7. **Start and End Date of Research Project:** 7/20/2010-6/30/2011

8. **Name of Principal Investigator for the Research Project:** Jianxun Song, PhD

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $ 46,350.00

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Haque	Post-Doctoral Fellow	25%	$11,340

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Lei	Research Assistant	10%
Song	PI	1%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____*x*_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No_____x_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert “not funded” in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____x_____ No_____

If yes, please describe your plans:

An NIH R01 application based on this research project was submitted in October 2012.

12. Future of Research Project. What are the future plans for this research project?

We submitted an R01 application to NIH proposing follow-on studies.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____x_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	1
Female			1	
Unknown				
Total			**2**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			2	1
Unknown				
Total			**2**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	
Black				
Asian			1	1
Other				
Unknown				
Total			**2**	**1**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____x_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes___X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This project expanded the scientific base for cancer research at PSCOM which has strengthened the research capacity of the Penn State Hershey Cancer Institute.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes___X_____ No_____

If yes, please describe the collaborations: This project enabled us to collaborate with the following investigator:

Richard A. Morgan, Ph.D., National Cancer Institute, Bethesda, MD

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____x_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____x_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Overview and Specific Aims from our Strategic Plan are as follows:

The *objective* of this project is to generate and characterize highly avid melanoma-specific cytotoxic T lymphocytes (CTLs) from human iPS cells. To address this objective, we will test the *working hypothesis* that lentivirally transduced human iPS cells with genes of melanoma-specific T cell receptor (TCR), and cultured via *in vitro* Notch signaling, will differentiate into highly avid melanoma-specific T lymphocytes. We will test our working hypothesis by using the *experimental approach* of lentivirus-mediated TCR transduction and stem cell culture with *in vitro* Notch signaling. Human iPS cells will be genetically modified with MART-1 (**M**elanoma **A**ntigen **R**ecognized by **T**-cells) /Melan-A (**mel**anocyte **an**tigen-A)-specific TCR and stimulation with *in vitro* Notch signaling.

1. Generation of highly avid MART-1/Melan-A-specific CTLs derived from human iPS cells. We will transduce human iPS cells with MART-1/Melan-A-specific-TCR by lentivirus-mediated transduction, and culture the cells with *in vitro* Notch signaling.

2 . Characterization of highly avid MART-1/Melan-A-specific CTLs derived from human iPS cells. We will characterize the generated MART-1/Melan-A-specific CTLs derived from human iPS cells:

(A) *In vitro analysis of the MART-1/Melan-A-specific CTLs*. We will stimulate the human iPS cell-derived CTLs *in vitro* with T2 cells pulsed with the cognate peptide MART-1/Melan-A $_{25\text{-}37}$ or the control peptide MAGE-3 $_{271–279}$ to determine the level of cytokine secretion, proliferation, expansion, and survival. After five days, melanoma-reactive CTLs will be isolated and restimulated to determine recall/memory responses. Cytotoxic potential of CTLs will be measured by *in vitro* chromium release assay.

(B) *In vivo testing of the MART-1/Melan-A-specific CTLs and ACT therapy in a murine melanoma model*. We will use NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ mice (NOD/SCID IL2Rγ^{null}, the Jackson Laboratory), a murine model to evaluate human iPS cell-derived MART-1/Melan-A-specific CTLs.

In this study, we have generated and characterized MART-1/Melan-A-specific CTLs from iPS cells by TCR gene transduction and stimulation with *in vitro* Notch ligand.

1. Generation of highly reactive MART-1/Melan-A-specific CTLs derived from human iPS cells. First, we have generated the construct that contains MART-1/Melan-A-specific TCR genes. We subcloned MART-1/Melan-A-specific TCR α and β chain genes (specific for the HLA-A2-restricted MART-1/Melan-A epitope $_{27\text{-}35}$: AAGIGILTV) into the lentiviral vector pLVX-IRES-mCherry (Clonetech). To ensure that TCR α and β chain genes were expressed equally in iPS cells, we used a 2A sequence to link the two genes. The created lentiviral construct (i.e., pLVX-TCRα-2A-TCRβ) was used to generate lentiviruses for TCR transduction that induced the development of MART-1/Melan-A-specific CTLs from human iPS cells.

Second, we have transduced human iPS cells with MART-1/Melan-A-specific-TCR by lentivirus-mediated transduction, and cultured the cells on OP9-DL1/DL4 cells, which express Notch ligands DL1 and DL4. After 50 days, the iPS cell-derived $CD8^+$ T lymphocytes were characterized by surface marker expression of TCRβ, CD3, CD8, CD1a, CD25, CD44 and CD69 (Figure 1). In addition, we observed to highly express MART-1/Melan-A-specific-TCR on iPS cell-derived $CD8^+$ T lymphocytes as determined by flow cytometry (Figure 2).

2. Characterization of highly avid MART-1/Melan-A-specific CTLs derived from human iPS cells. We have characterized MART-1/Melan-A-specific CTLs derived from human iPS cells:
(1) *In vitro analysis of the MART-1/Melan-A-specific CTLs*.
We found that $CD8^+$ T lymphocytes derived from human iPS cells were able to respond to antigen stimulation, proliferated, expanded, and secreted cytokines. In addition, these T cells could generate recall/memory responses when stimulated with antigen again. In addition, we have performed functional characterization of the MART-1/Melan-A-specific CTLs derived from human iPS cells. We found iPS cell-derived mature CTLs were functional in their ability to secrete cytokines and kill target-expressing target cells (Figure 3). Collectively, these data indicated that iPS cell-derived $CD8^+$ T lymphocytes were functionally mature.

(2) *In vivo testing of the MART-1/Melan-A-specific CTLs and ACT therapy in a murine melanoma model.*
First, we adoptively transferred the iPS cell-derived CTLs into NOD/SCID mice by an *i.v* injection through the tail vein. On the following day, we challenged mice by an *i.p.* injection of anti-CD3 plus CD28 Abs. At different time points (i.e., days 3, 7, 14, 35), we analyzed the population of $mCherry^+$ cells obtained from the spleens and lymph nodes by flow cytometry. To determine the extent of long-term T-cell persistence, we rechallenged a subset of mice with anti-CD3 plus Abs by *i.p.* injection 5 weeks after the first challenge, and determined the MART-1/Melan-A-specific CTLs still existed as described above.

Second, we used *s.c* injection in the flank to challenge NOD/SCID mice with the human melanoma cell line 526mel, which highly expresses MART-1/Melan-A protein. We analyzed the population of $mCherry^+$ cells obtained from the spleens, lymph nodes, and tumor sites by flow cytometry. We observed that adoptive transfer of iPS cell-derived CTLs significantly suppress melanoma growth (Figure 4).

Figures 1-4 contain the main results that we had expected. Figures 5-9 contain some additional results that we obtained.

Aim 1. Generation of Ag-specific TCR gene-transduced iPS cells
To generate Ag-specific TCR-transduced iPS cells for adoptive transfer, we used the mouse stem cell virus (MSCV)-based retroviral vector pMig in which MHC-I restricted OVA-specific TCR α and β chain genes were linked with a 2A peptide to deliver TCR genes into iPS cells. Since iPS cells express GFP as an endogenous stemness marker, we replaced GFP with DsRed as a new marker for monitoring gene integration, and named the new vector as MiDR (Figure5a). After transduction, DsRed expression was visualized by fluorescent microscopy on the TCR-transduced iPS cells (Figure5b). Although the transduction efficiency was low (< 3.5%), we were

able to sort for DsRed and GFP double positive cells (Figure 5c). Moreover, we confirmed expression of TCR Vβ5 mRNA and DNA integration in the sorted cells by RT-PCR and PCR (Figure 5d). We co-cultured the TCR gene-transduced iPS cells on feeder layers of irradiated SNL76/7 cells in culture plates as previously described, large numbers of TCR gene-harboring iPS cells were generated by using this culture system.

Aim 2. TCR gene-transduced iPS cells differentiated into $CD8^+$ T lymphocytes *in vivo*

To determine the outcome of MHC-I restricted OVA-specific TCR gene transduction in CTL differentiation from iPS cells *in vivo*, we adoptively transferred the OVA TCR gene-transduced iPS cells or bone marrow-derived $CD117^+$ Lin^- HSCs from OT-I TCR transgenic mice into C57BL/6 mice. After six to ten weeks, we analyzed OVA-specific $CD8^+$ T cells from the pooled lymph nodes and spleen by flow cytometry. We observed approximately 49% of $V\beta5^+$ cells in the $CD8^+$ population of mice receiving TCR gene-transduced iPS cells or bone marrow-derived $CD117^+$ Lin^- HSCs from OT-I TCR transgenic mice. In contrast, the $CD8^+$ $V\beta5^+$ cell population was less than 2% in mice receiving vector control gene-transduced iPS cells (Figure 6a). To assess the functionality of $CD8^+$ $V\beta5^+$ cells, we isolated $CD8^+$ T cells for phenotype analysis (Figure 6b), and stimulated *in vitro* with irradiated splenocytes from C57BL/6 mice pulsed with the $OVA_{257-264}$ peptide. We were able to observe IL-2 and IFN-γ production from $V\beta5^+$ cells gated on the $CD8^+$ population as detected by intracellular cytokine staining (Figure 6c). Furthermore, we performed an *in vivo* cytotoxicity assay to determine the proliferation/cytolytic effector potential of $CD8^+$ $V\beta5^+$ cells, which involves injection of CFSE-labeled, peptide-pulsed syngeneic splenocytes. Interestingly, target cell lysis was approximately 90 times greater in mice receiving TCR gene-transduced iPS cells than in control receiving vector control gene-transduced iPS cells (94% versus 1%) (Figure 6d). Collectively, these data indicate that MHC-I restricted OVA TCR gene-transduced iPS cells have the ability to develop into functional OVA-specific $CD8^+$ T lymphocytes *in vivo*.

A3. *In vivo* persistence of Ag-specific T cells derived from TCR gene-transduced iPS cells

To investigate whether *in vivo* programmed $CD8^+$ T cells from iPS cells are able to persist, we challenged two groups of mice with E.G7 lymphoma cells that express OVA protein: (i) six weeks after receiving TCR gene-transduced iPS cells; or (ii) one day before with $CD8^+$ T cells isolated from OT-I TCR transgenic mice. After fifty days, we found an increased number of OVA-specific $CD8^+$ T cells in the pooled lymph node and spleen cells in mice receiving TCR gene-transduced iPS cells than in mice receiving $CD8^+$ T cells from OT-I TCR transgenic mice (52.7% versus 12.8%) (Figure7). This result indicated that *in vivo* programmed $CD8^+$ T cells from iPS cells might generate from a time period (e.g., six weeks to twelve weeks after adoptive transfer), which is consistent with previous observations.

A4. Adoptive transfer of TCR gene-transduced iPS cells prevent tumor growth

Because TCR gene-transduced iPS cells are able to differentiate into fully functional Ag-specific $CD8^+$ T cells *in vivo*, we hypothesize that adoptive transfer of these iPS cells would generate tumor protection. To test this hypothesis, we adoptively transferred OVA TCR gene-transduced iPS cells into C57BL/6 mice, and challenged mice with E.G 7 tumor cells after six weeks of cell transfer to allow generation of OVA-specific $CD8^+$ T cells *in vivo*. In addition, we adoptively transferred $CD8^+$ T cells isolated from OT-I TCR transgenic mice into C57BL/6 mice, and challenged mice with the tumor cells on the following day as described in Figure 7. On day 30

after tumor challenge, we found fewer tumor cells in the peritoneal cavity of mice receiving TCR gene-transduced iPS cells than in mice receiving either $CD8^+$ T cells from OT-I TCR transgenic mice or control vector-transduced iPS cells (Figure 8b). On day 50, we observed 100% survival of mice receiving TCR gene-transduced iPS cells, compared to 55% survival of mice receiving $CD8^+$ T cells from OT-I TCR transgenic mice (Figure 8c). Moreover, we observed a large number of tumor-infiltrating OVA-specific $CD8^+$ T cells in mice receiving TCR gene-transduced iPS cells (Figs. 8a - 8c). Collectively, these data strongly support our hypothesis that adoptive transfer of tumor Ag-specific TCR gene-transduced iPS cells are able to induce tumor Ag-specific T-cell persistence, and result in tumor protection.

In conclusion, we developed a novel approach to generate Ag-specific T lymphocytes by programming iPS cells, and the iPS cell-derived CTLs have a great potential in the treatment of disease. One paper based on this study has been published in *Cancer Research* in the issue of July 15, 2011, with a specific introduction by a cover picture.

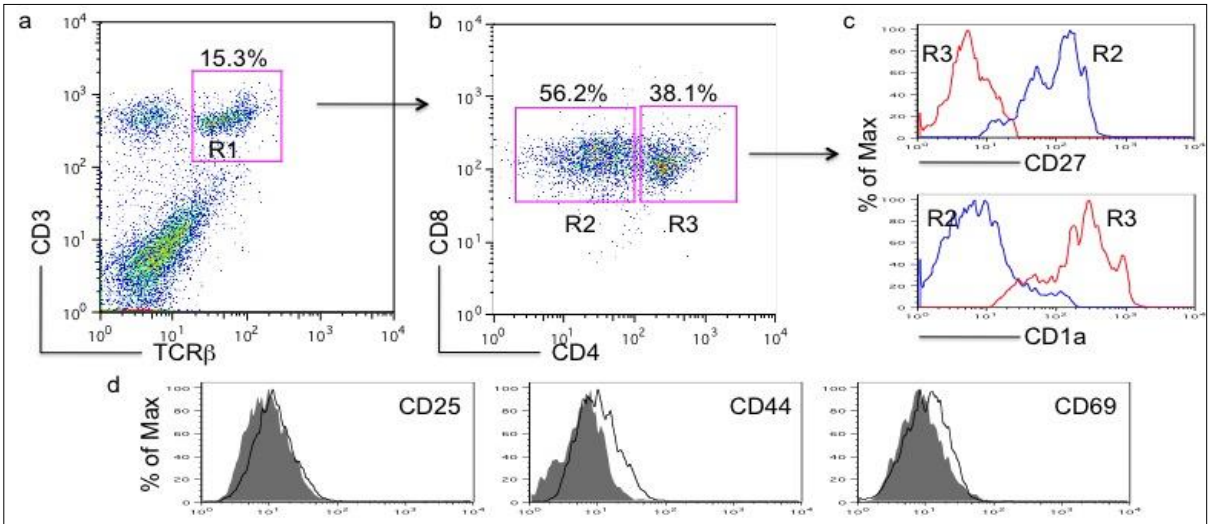

Fig. 1 Characterization of $CD8^+$ T cells generated *in vitro*. The data show combined TCR gene transduction and *in vitro* Notch signaling can give rise to mature $CD8^+$ T cells from human iPS cells. Method: Human iPS cells were transduced with a construct: pLVX-TCRα-2A-TCRβ containing MART-1/Melan-A-specific TCR genes. The gene-transduced cells ($mCherry^+$) were sorted and co-cultured on OP9-DL1/DL4 cells in the presence of rIL-7, Flt3L and SCF. (a) On day 50 the CD3 and TCRβ expression was analyzed by flow cytometric analysis. (b and c) $CD3^+$ $TCR\beta^+$ cells were gated (R1), and analyzed for the expression of CD4 and CD8, with CD27 and CD1a expression shown for cells gated as $CD4^-$ $CD8^+$ (R2) or $CD4^+$ $CD8^+$ (R3). (d) The expression of CD25, CD44 and CD69 for cells gated as R2 was shown. Shadowed histograms indicate isotype control staining. Data are representative of three experiments.

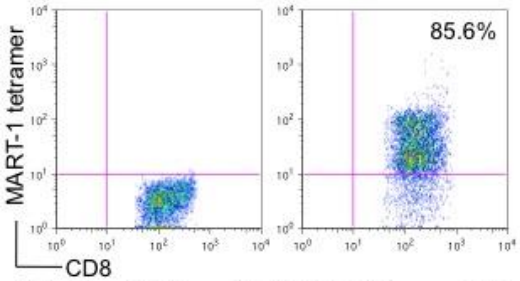

Fig. 2 TCR transduction and stimulation with *in vitro* Notch signaling promote MART-1/Melan-A-specific CTL differentiation from human iPS cells. The data show MART-1/Melan-A⁺ cells after gated on CD8⁺ cells by flow cytometry. Method: Human iPSB12-2 cells were transduced with the following construct: pLVX vector control (left) or pLVX--TCRα-2A-TCRβ construct that contains MART-1/Melan-A-specific TCR genes (right). mCherry⁺ cells were sorted and co-cultured on OP9-DL1/DL4 cells for seven weeks. Differentiated cells were analyzed for expression of MART-1/Melan-A⁺ by flow cytometry, after gating on the CD8⁺ population.

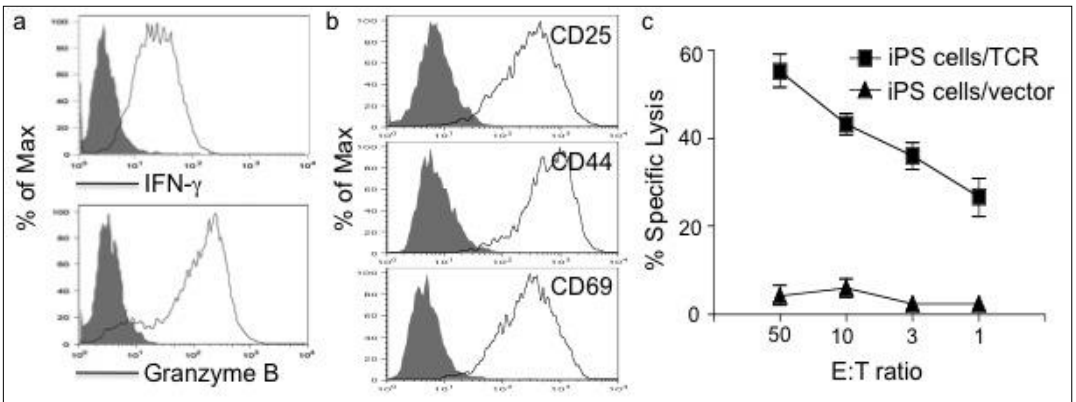

Fig. 3 Functional characterization on MART-1/Melan-A-specific CTLs derived from human iPS cells generated *in vitro*. The data show MART-1/Melan-A-specific CTLs derived from human iPS cells are functional. Method: Human IPS cell-derived mature CTLs (CD3⁺ CD8⁺ CD27⁺) as described in Fig. 1 were sorted and stimulated with plate-coated anti-CD3 antibody in the presence of rIL-2 and rIL-7 for three days. (a) CD8⁺ T cells were evaluated for IFN-γ and granzyme B expression by intracellular staining and flow cytometry. (b) CD8⁺ T cells were evaluated for expression of CD25, CD44, and CD69 by surface staining and flow cytometry. Shadowed histograms in a and b indicate isotype control staining. The activated CTLs were placed in a standard ^{51}chromium release assay utilizing MART-1/Melan-A $_{25-37}$ peptide coated T2 cells. Graph shows the specific lytic activity of cells at different effector to target cell ratio. Data are representative of three independent experiments.

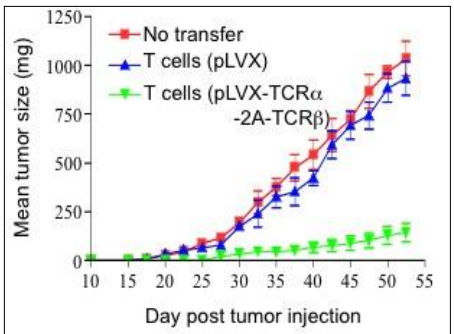

Fig. 4 Effects on human melanoma growth in a murine model. The data show adoptive transfer of iPS cell-derived MART-1/Melan A-specific CTLs derived from iPS cells inhibited human melanoma growth in NOD/SCID mice. Method: MART-1/Melan-A-specific CTLs derived from human IPS cells as described in Fig. 1 were adoptively transferred into NOD/SCID mice by *i.v.* injection (2×10^6 cells/per mouse). On the following day, mice were subjected to challenge with 5×10^5 526mel cells by *s.c.* injection. Tumor growth was measured three times a week and tumor weight was calculated. The histograms represent mean ± SD of tumor weight in 10 animals/point in three different experiments (■ : iPS cell-derived MART-1/Melan-A-specific T cells; ▲ : iPS cell-derived non-specific T cells; ▼: no T-cell transfer).

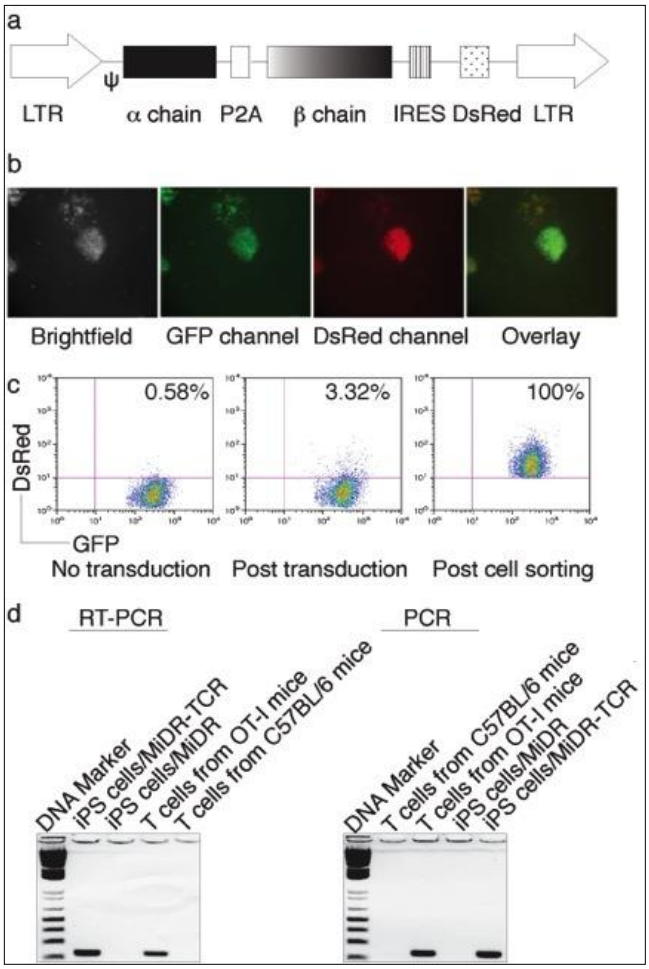

Figure 5. Lentivirus-mediated TCR transduction in iPS cells. iPS cells were transduced with the following constructs: vector control (MiDR), or Ag-specific TCR (MiDR-TCR). (a) Schematic representation of the construct. (b) TCR-transduced iPS cells were visualized by fluorescence microscopy. (c) GFP^+ iPS cells (left) were transduced with the retroviral construct MiDR-TCR, and GFP^+ $DsRed^+$ iPS cells (middle) were analyzed by flow cytometry and sorted by a high-speed cell sorter (right). (d) GFP^+ $DsRed^+$ iPS cells were sorted and total mRNA and DNA were analyzed for Vb5 gene expression by RT-PCR (left) and for the Vβ5 gene by PCR (right). The forward primer is ACGTGTATTCCCATCTCTGGACAT and the reverse primer is TGTTCATAATTGGCCCGAGAGCTG.

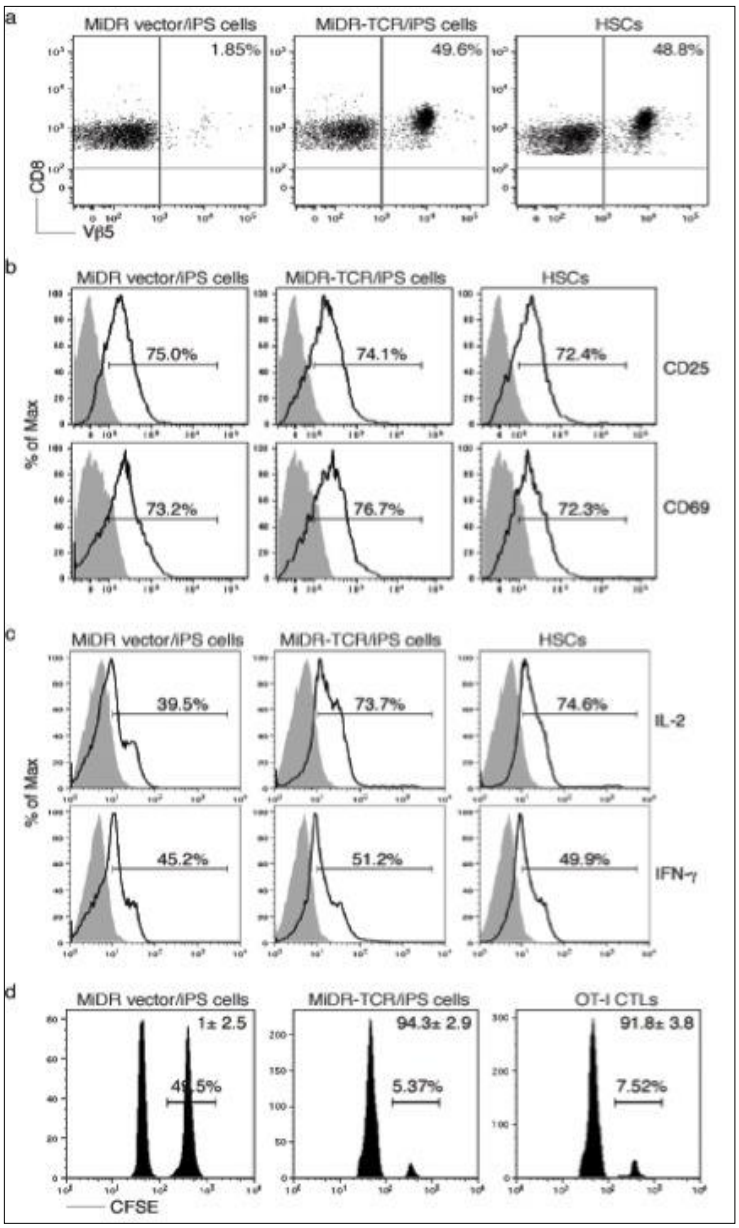

Figure 6. Ag-specific $CD8^+$ T-cell development from iPS cells *in vivo*. GFP^+ $DsRed^+$ iPS cells were injected *i.v* into C57BL/6 mice. After six to ten weeks, Ag-specific $V\beta5^+$ $CD8^+$ T cell development was determined. (a) $CD8^+$ $V\beta5^+$ T cells from pooled lymph nodes and spleen were analyzed by flow cytometry. (b) CD25 and CD69 expression was analyzed by flow cytometry, after gating on $CD8^+$ $V\beta5^+$ T cells (dark lines; shaded areas indicate isotype controls). (c) IL-2 and IFN-γ production from the $CD8^+V\beta5^+$ population (dark lines; shaded areas indicate isotype controls) were determined by intracellular cytokine staining. (d) *In vivo* proliferation/cytotoxicity assay. $CFSE^{hi}$ (right peaks) and $CFSE^{lo}$ (left peaks) target cells were pulsed with the peptide and the control, respectively, and were injected into mice ten weeks after iPS cell transfer or one day after OT-I CTL transfer. Data are representative of two or three independent experiments.

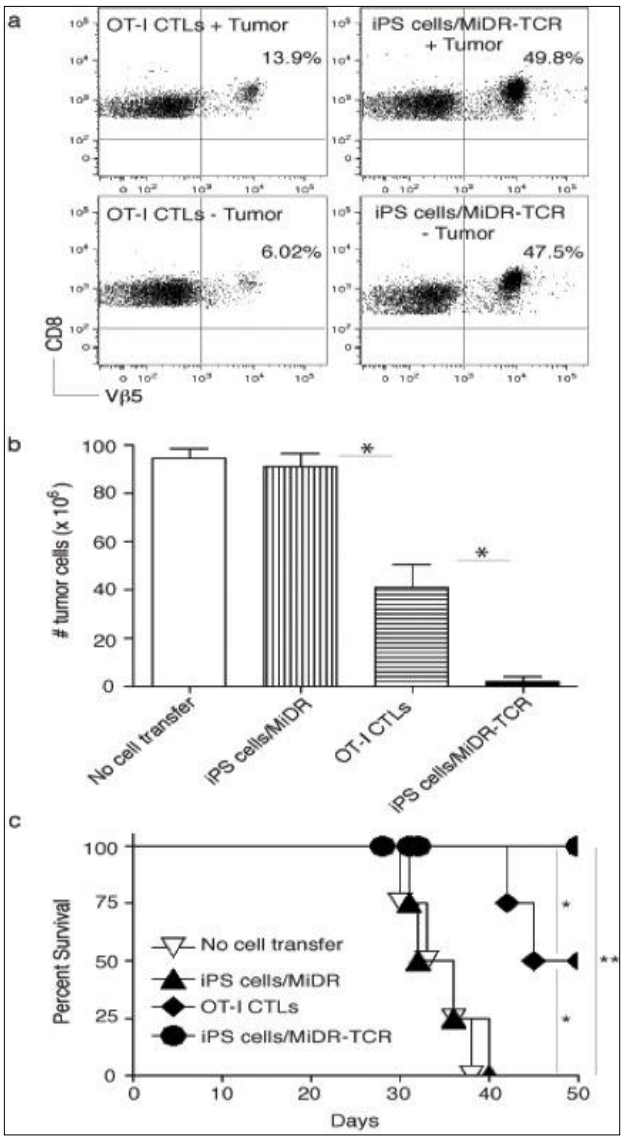

Figure 7. Adoptive transfer of TCR-transduced iPS cells suppresses tumor growth and sustains mouse survival. GFP^+ $DsRed^+$ cells were adoptively transferred into C57BL/6 mice. One group of mice were injected with Ag-reactive $CD8^+$ T cells from OT-I TCR transgenic mice, and one group of mice had no cell transfer. After either six weeks or on the following day after the cell transfer, mice were subjected to challenge with tumor cells. (a) Ag-specific T-cell persistence. Seven weeks post tumor challenge or thirteen weeks without tumor challenge, $CD8^+$ $V\beta5^+$ T cells from the pooled LNs and spleen were analyzed by flow cytometry. (b) On day 20, tumor cells in the peritoneal cavity were enumerated. Data represent mean (±SEM) tumor cell counts from six individual mice. One-way ANOVA test was used for statistical analyses between two groups (*: $p<0.05$). (c) Mouse survival on day 50. Kaplan-Meier survival curves are shown (n=6). *: $p<0.05$; **: $p<0.001$, One-way ANOVA with Newman-Keuls Multiple Comparison Test. Data are representative of three independent experiments.

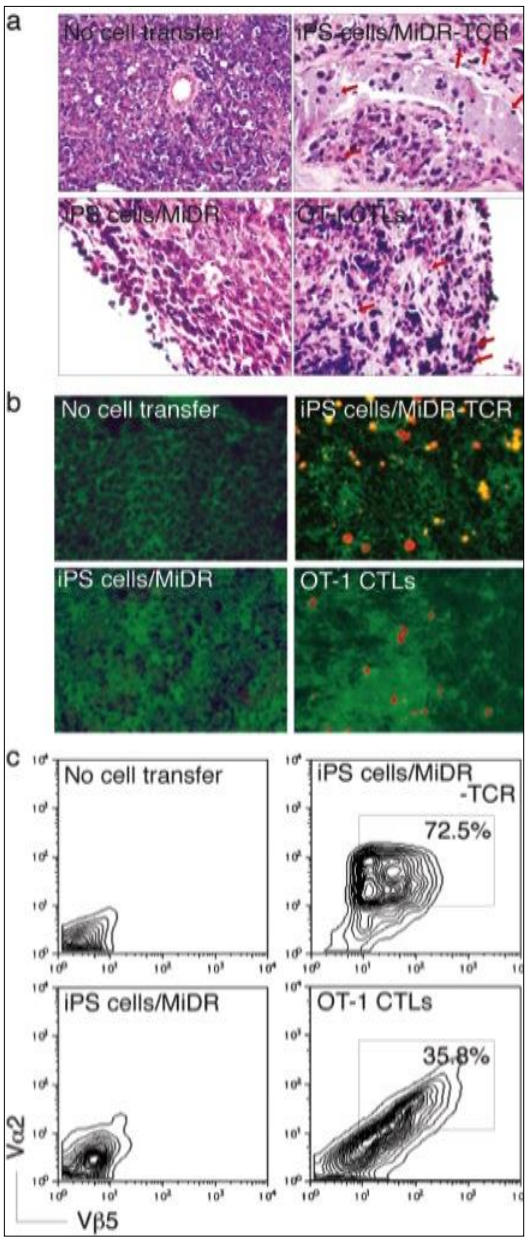

Figure 8. iPS cell-derived Ag-specific CTLs infiltrate into tumor tissues. On day 30 to 35 after tumor challenge, tumor tissues were examined for tumor-reactive T cell infiltration. (a) H &E staining. Inflammatory cells infiltrated in tumor tissues (↓). (b) Immunohistological staining. Ag-specific $V\alpha2^+$ CTLs (red) infiltrated in Ag-expressing tumor tissues (green). (c) Single-cell suspensions from tumor tissues were analyzed for expression of $V\alpha2^+$ and $V\beta5^+$ by flow cytometry, after gating on the $CD8^+$ population. Data are representative of three independent experiments.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes

__x___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__x___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__x__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed

acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. In vivo programming of tumor antigen-specific T lymphocytes from pluripotent stem cells to promote cancer immunosurveillance	Fengyang Lei, Baohua Zhao, Rizwanuel Haque, Xiaofang Xiong, Lynn Budgeon, Neil Christiansen, Yuzhang Wu, Jianxun Song	*Cancer Research*	05/15/2011	☐Submitted ☐Accepted ☒Published
2. Transgenic expression of survivin compensates the OX40-deficiency in driving Th2 development and allergic inflammation	Fengyang Lei, Jianyong Song, Rizwanuel Haque, Xiaofang Xiong, Deyu Fang, Yuzhang Wu, Susanne Lens, Michael Croft, Jianxun Song	*European Journal of Immunology*	12/22/2011	☒Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____x_____ No_____

If yes, please describe your plans:

Will submit a paper entitled "Programming of tyrosinase-specific CTLs from iPS cells for melanoma immunotherapy" to *Journal of Immunology* in May 2013.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

We developed a novel approach for generating therapeutic Ag-specific T lymphocytes from iPS cells.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___x_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?

f. Yes_____ No__
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

g. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No__

If yes, how many licenses were granted?_____

h. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes__ No__

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes___x_____ No_____

If yes, please describe your plans:

US patent - Generation of Ag-specific T lymphocytes by programming iPS cells (A novel approach for generating therapeutic Ag-specific T lymphocytes from iPS cells)

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Song, Jianxun	**Assistant Professor of Microbiology and Immunology**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Chongqing Normal University, Chongqing, China	B.Sc.	1989	Biology
The Third Military Med. Univ., Chongqing, China	M.Sc.	1993	Pathogenic Biology
The Third Military Med. Univ., Chongqing, China	Ph.D.	2001	Immunology
La Jolla Inst. for Allergy & Immunology, San Diego, CA	Post-doc	2004	Immunology

A. Positions and Honors.

Positions and Employment

2001-2007 Postdoctoral Fellow, Division of Molecular Immunology, La Jolla Institute for Allergy & Immunology, La Jolla, CA

2007-Present Tenure-track Assistant Professor, Department of Microbiology & Immunology and Penn State Cancer Institute, Penn State University College of Medicine, Hershey, PA

Other Experience and Professional Memberships

2003-present American Association of Immunologists (AAI)

1997-present Chinese Society of Immunology

Honors

2008 AAI Junior Faculty Travel Awards (San Diego)

2009 AAI Junior Faculty Travel Awards (Seattle)

2010 AAI Junior Faculty Travel Awards (Baltimore)

2011 Dean's Award for Excellence in Teaching (Penn State University College of Medicine)

2012 Dean's Award for Excellence in Teaching (Penn State University College of Medicine)

B. Selected peer-reviewed publications (in chronological order).

1. Haque R, Lei F, Xiong X, Bian Y, Zhao B, Wu Y and **Song J** (2012). Programming of regulatory T cells from pluripotent stem cells and prevention of autoimmunity. *J Immunol* 189 (3): 1228-1236.
2. Haque R, Lei F, Xiong X, and **Song J** (2012). Stem cell-based cellular therapy in rheumatoid arthritis. Autoimmune Diseases. InTech Europe.
3. Lei F, Haque R, Xiong X, and **Song J** (2012). Directed differentiation of induced pluripotent stem cells towards T lymphocytes. *J Vis Exp.* (63), e3986.
4. Lin Z, Yang H, Kong Q, Li J, Lee SM, Gao B, Dong H, Wei J, **Song J**, Zhang DD, and Fang D (2012). USP22 Antagonizes p53 Transcriptional Activation by Deubiquitinating Sirt1 to Suppress Cell Apoptosis and Is Required for Mouse Embryonic Development. *Mol Cell*, 46(4): 484-494.
5. Lei F, Haque R, Xiong X, Budgeon L, Christensen ND, Wu Y and **Song J** (2011). *In vivo* programming of tumor antigen-specific T lymphocytes from pluripotent stem cells to promote cancer immunosurveillance. *Cancer Res*, 71(14): 4742-4747.

6. Tian Y, Jia Z, Wang J, Huang Z, Tang J, Zheng Y, Tang Y, Wang Q, Tian Z, Yang D, Zhang Y, Fu X, **Song J**, Liu S, van Velkinburgh JC, Wu Y, Ni B (2011). Global mapping of H3K4me1 and H3K4me3 reveals the chromatin state-based cell type-specific gene regulation in human Treg cells. PLoS One. 2011; 6(11):e27770.
7. Haque R, Lei F, Xiong X, and **Song J** (2011). The regulation of FoxP3-expressing regulatory T cells. *Endocr Metab Immune Disord Drug Targets*, 11: 334-336.
8. Haque R, Lei F, Xiong X, Wu Y, and **Song J** (2010). FoxP3 and Bcl-xL cooperatively promote regulatory T cell persistence and prevention of arthritis development. *Arthritis Res Ther* 12(2): R66.
9. Lei F, Haque R, Weiler L, Vrana KE, and **Song J** (2009). T lineage differentiation from induced pluripotent stem cells. *Cell Immunol* 260 (1): 1-5.
10. Zhao B, Song A, Haque R, Lei F, Weiler L, Xiong X, Wu Y, Croft M, and **Song J** (2009). Cooperation between molecular targets of costimulation in promoting T cell persistence and tumor regression. *J Immunol* 182 (11): 6744-6752.
11. **Song J,** So T and Croft M (2008). Activation of NF-kappaB1 by OX40 contributes to antigen-driven T cell expansion and survival. *J Immunol* 180(11): 7240-7248.
12. **Song J,** Lei F, Xiong X and Haque R (2008). Intracellular signals of T cell costimulation. *Cell Mol Immunol* 5 (4): 239-247.
13. **Song J**, Salek-Ardakani S, So T, and Croft M (2007). The kinases aurora B and mTOR regulate the G1-S cell cycle progression of T lymphocytes. *Nat Immunol 8 (1)*: 64-73.
14. Song A, **Song J**, Tang X, Croft M (2007). Cooperation between CD4 and CD8 T cells for anti-tumor activity is enhanced by OX40 signals. *Eur J Immunol* 37(5): 1224-1232.
15. So T, **Song J**, Sugie K, Altman A, and Croft M (2006). Signals from OX40 regulate NFATc1 and Th2 lineage commitment. *Proc Natl Acad Sci U S A* 103(10): 3740-3745.
16. **Song J**, So T, Cheng M, Tang X and Croft M (2005). Sustained *Survivin* expression from OX40 costimilatory signals drives T cell clonal expansion. *Immunity* 22(5): 621-631.
17. Barouch-Bentov R, Lemmens EE, Hu J, Janssen EM, Droin NM, **Song J**, Schoenberger SP, Altman A (2005). Protein kinase C-theta is an early survival factor required for differentiation of effector CD8+ T cells. *J Immunol* 175(8): 5126-5134.
18. **Song J**, Salek-Ardakani S, Rogers PR, Cheng M, Parijs LV, and Croft M (2004). The costimulation-regulated duration of PKB activation controls T cell longevity. *Nat Immunol* 4(2): 150-158.
19. Lawson BR, Baccala R, **Song J**, Croft M, Kono DH, Theofilopoulos AN (2004). Deficiency of the Cyclin Kinase Inhibitor p21 (WAF-1/CIP-1) Promotes Apoptosis of Activated/Memory T Cells and Inhibits Spontaneous Systemic Autoimmunity. *J Exp Med* 199(4): 547-557.
20. Salek-Ardakani S, **Song J**, Halteman BS, Jember AG, Akiba H, Yagita H, Croft M (2003). OX40 (CD134) controls memory T helper 2 cells that drive lung inflammation. *J Exp Med* 198(8): 315-324.
21. Croft M, Salek-Ardakani S, **Song J**, So T, and Bansal-Pakala P (2003). Regulation of T cell immunity by OX40 and OX40L. The TNF Superfamily. Landes Bioscience, Texas.
22. **Song J**[1], Rogers PR[1], Gramaglia I, Killeen N and Croft M (2001). OX40 promotes *Bcl-xL* and *Bcl-2* expression and is essential for long-term survival of CD4 T cells. *Immunity* 14(9): 445-455 (1: joint first authorship).
23. **Song J**, Wang X, Huang F, Feng C, Zhang Z, Huo Y, and Pang Q. (1995). A preliminary study on the inhibitory action of target-primaqine on the exoerythrocytic forms of Plasmodium yoelii yoelii]. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* 13(2): 117-119.

BIOGRAPHICAL SKETCH

NAME	**POSITION TITLE**
Rizwanul Haque	Postdoctoral Scholar

EDUCATION/TRAINING

INSTITUTION AND LOCATION	**DEGREE** *(if applicable)*	**YEAR(s)**	**FIELD OF STUDY**
Aligarh Muslim University, Aligarh, India	B. Sc.	1992-1996	Biochemistry
Hamdard University, New Delhi, India	M.Sc.	1997-1998	Biochemistry
Hamdard University, New Delhi, India	Ph.D.	1998-2003	Toxicology
Penn State University College of Medicine, PA	Post-doc	2003-present	Immunology

A. Positions and Honors.

Positions and Employment

2003-present: Postdoctoral Scholar in the Department of Pediatrics and Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, PA.

2001-2003: Senior Research Fellow in the Department of Toxicology, Hamdard University, New Delhi, India.

1999-2001: Senior Research Fellow in the Department of Toxicology, Hamdard University, New Delhi, India.

1998-1999: Research Fellow in the Department of Biochemistry, VPCI, University of Delhi, Delhi, India.

Honors

American Society of Immunologists (AAI) Travel Award 2010 (14th International Congress of Immunology, August 22 -27, Kobe, Japan)

AAI Travel Award 2011 (IMMUNOLOGY 2011™, May 13-17, 2011, San Francisco)

B. Selected peer-reviewed publications (in chronological order).

1. **Haque R**, Bin-Hafeez B, Ahmad I, Parvez, Pandey S, and Raisuddin S (2001). Protective effects of *Emblica officinalis* Gaertn in cyclophosphamide-treated mice. *Human and Experimental Toxicology* 20: 643-50.
2. Bin-Hafeez B, Ahmad I, **Haque R,** and Raisuddin S (2001). Protective effect of *Cassia occidentalis* L. on cyclophosphamide-induced suppression of humoral immunity in mice. *Journal of Ethnopharmacology* 75: 13-18.
3. Pandey S, Ahmad I, Parvez S, Bin-Hafeez B, **Haque R,** and Raisuddin S (2001). Effect of endosulfan on anti oxidants of freshwater fish *Channa punctatus Bloch. 1.* Protection against lipid peroxidation in liver by copper pre-exposure. *Archives of Environmental contamination and Toxicology* 41: 345-352.
4. **Haque R**, Bin-Hafeez B, Parvez. S**,** Pandey S, Saeed I, Ali. M, and Raisuddin S. (2003). Aqueous extract of walnut (*Juglans regia* L.) protects mice against cyclophosphamide

induced biochemical toxicity. *Human and Experimental Toxicology* 22 (9): 473-480.

5. Bin-Hafeez B, **Haque R,** Parvez. S, Pandey. S, Saeed I, and Raisuddin S (2003). Immunomodulatory effect of *Trigonella foenum graecum* in mice. *Int Immunopharmacol* 3 (2): 257-265.
6. Parvez S, Sayeed I, Pandey S, Ahmad A, Ahmad I, Bin-Hafeez B, **Haque R,** and Raisuddin S (2003). Modulatory effect of copper on non-enzymatic antioxidants in freshwater fish *Channa punctatus* (Bloch.). *Biol Trace Elem Res* 93 (1-3): 237-48.
7. Pandey S, Parvez S, Sayeed I, **Haque R,** Bin-Hafeez B, and Raisuddin S (2003). Biomarkers of Oxidative Stress: Biomarkers of Oxidative Stress: A Comparative Study of River Yamuna Fish *Wallago attu* (Bl. & Schn.) *Sci Total Environ* 309 (1-3): 105-115.
8. Sayeed I, Parvez S, Pandey S, Bin-Hafeez B, **Haque R,** and Raisuddin S (2003). Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. *Ecotoxicol Environ Saf* 56 (2): 295-301.
9. **Haque R**, Umstead TM, Ponnuru P, Guo X, Hawgood S, Phelps DS, and Floros J (2007). The Effect of Acute Ozone Exposure on Lung Inflammation in Surfactant Protein-A-Deficient Mice. *Toxicol Appl Pharmacol* 220 (1): 72-82.
10. **Haque R**, Umstead TM, Freeman WM, Phelps DS and Floros J (2007). Quantitative Proteomic Profiles of BALF in Wild Type and SP-A KO Mice after Exposure to Ozone. *The FASEB Journal*, part 1, A9, Abst. 34.8.
11. Mikerov AN, **Haque R**, Gan X, Guo X, Phelps DS, and Floros J (2008). Ablation of SP-A has a negative impact on the susceptibility of mice to Klebsiella pneumoniae infection after ozone exposure: sex differences. *Respir Res* 9 (1): 77-86
12. Song J, Lei F, Xiong X, and **Haque R** (2008). Intracellular signals of T cell costimulation. *Cell Mol Immunol* 5 (4): 239-247
13. **Haque R**, Umstead TM, Freeman WM, Floros J, Phelps DS (2009). The impact of surfactant protein-A on ozone-induced changes in the mouse bronchoalveolar lavage proteome. *Proteome Sci* 7(1):12-22
14. Zhao B, Song A, **Haque R**, Lei F, Weiler L, Xiong X, Wu Y, Croft M, and **Song J** (2009). Cooperation between molecular targets of costimulation in promoting T cell persistence and tumor regression. *J Immunol* 182 (11): 6744-6752
15. Lei F, **Haque R**, Weiler L, Vrana KE, and Song J (2009). T lineage differentiation from induced pluripotent stem cells. *Cell Immunol* 260 (1): 1-5
16. **Haque R**, Lei F, Xiong X, Wu Y, and Song J (2010). FoxP3 and Bcl-xL cooperatively promote regulatory T cell persistence and prevention of arthritis development. *Arthritis Res Ther* 12 (2): R66
17. **Haque R**, Lei F, Xiong X, and Song J (2011). The regulation of FoxP3-expressing regulatory T cells. *Endocr Metab Immune Disord Drug Targets* 6 (3): *in press*

24. Lei F, Haque R, Xiong X, Budgeon L, Christensen ND, Wu Y and **Song J** (2011). *In vivo* programming of tumor antigen-specific T lymphocytes from pluripotent stem cells to promote cancer immunosurveillance. *Cancer Res*, 71(14): 4742-4747.