Scientific Progress Report Migrated - Scientific Progress Report Migrated

Award ID	**17POST33660597**	Project Title	**Functional consequences of antigen specificity in CMV-responsive T cells**
Grantee	**Higdon, Lauren**	Institution	**Stanford University**
Award Start	**07/01/2017**	Award End	**12/31/2019**

***1. Please provide a concise summary of progress for each specific aim of your project during this reporting period. If this is your final report, include an overall summary of the project achievements. Note: The suggested length for the Progress Report is approximately 2 pages. You can copy and paste from another document or type responses in the boxes below.**

1. For each aim, describe or summarize the following:
a) Major completed or ongoing activities;
b) Significant results, including major findings, developments, or conclusions (both positive and negative);
c) Discussion of stated goals not met or problems you have encountered and how they were resolved.

Specific Aim 1: Determine how specificity for CMV or alloantigen affects T cell function. The proposed experiments in this aim were to analyze specificity and function of CD8 T cells responsive to CMV or alloantigen from healthy volunteers. Proposed assays included single cell sequencing and mass cytometry.

I have completed characterization of gene expression and repertoire of CMV responsive T cells from 7 individuals through single cell TCR sequencing per the protocol developed in Han et al (DOI:10.1038/nbt.2938). Upon completion of sequencing of all samples, it became apparent that there were differences in sequencing between different batches of samples that significantly complicated interpretation of the data. To resolve this, I repeated some PCRs and the majority of sequencing in order to distribute samples across batches and include some samples in multiple batches. These changes were both chosen to improve batch correction efficacy. It took about 5 months to both determine the most appropriate approach to resolve the batch issue and complete the re-sequencing. It has taken about 1 month since that time to work with our bioinformatician collaborator, Dr. Steven Schaffert, to use those changes to update the batch correction. This process is now complete and we are proceeding with analysis of the data for publication.

In these data, CMV-responsive T cells tend to have an oligoclonal repertoire. Clonally expanded cells tend to be the most polyfunctional. In addition, clonally expanded cells tend to express the transcription factor BCL6, which has previously been associated with high levels of differentiation potential. This was unexpected, and may provide further avenues of study.

Specific Aim 2: Determine how transplantation and immunosuppression affect CMV-responsive T cells. The intent of this aim was to utilize the methodology described in Aim 1 to measure changes in CMV- and allo-responsive T cells after transplant. Proposed time points were during antiviral prophylaxis and 6 months after completion of prophylaxis.

In 5 of 6 transplant recipients, the TCR repertoire is dominated by one clone one year post-transplant. In 4 of the 5, that clone was already expanded prior to transplant. TCR repertoire diversity, calculated using the Gini coefficient, decreases in all patients. The degree of polyfunctionality of clonally expanded cells, defined as number of functions expressed, increases after transplant. Further analyses of these data are

ongoing in collaboration with Steven Schaffert and the Khatri lab. This collaboration allows us to apply unbiased high dimensional computational analyses to these data. We are using these analyses to define how gene expression of clonally expanded cells changes. This unbiased analysis has already identified that CMV-responsive T cells include a TGFbeta+ population, which may be deserving of future study given the connection between TGFbeta and fibrosis.

I have also started analyzing T cell function through a new approach not originally proposed. Specifically, CMV is known to age the immune system, and CMV-associated aging may be linked to heart disease. Our preliminary data suggest that immune aging may be accelerated after transplant. Immune aging leads to alterations of many cellular processes. This can result in altered T cell function. Thus, immune aging is most likely key to the affect of CMV-responsive T cells on transplanted hearts. To address this, I have optimized a flow cytometry protocol to concurrently stain cells with antibodies and a fluorescent telomere probe. With this assay I have measured telomere length of CMV-responsive T cells, and demonstrated shorter telomere length in this T cell population from the same individual two years later. I will soon extend this analysis to patient samples in order to understand whether premature aging is a factor in the post-transplant changes observed.

A second new approach is the use of peptide-MHC tetramer staining to analyze CMV-specific T cells. My approach thus far has relied upon identifying cells that produce cytokine in response to CMV. However, this introduces a bias towards cells that are sufficiently functional to produce cytokine. Comparing CMV-responsive cells to cells that specifically bind CMV antigen using a CMV-MHC tetramer reagent will identify cells that do not produce the cytokine. I have optimized a tetramer staining protocol and identified tetramers that should represent ~50% of the patient population, and will now analyze tetramer-binding cells in patient samples.

Given the time devoted to troubleshooting batch effects on the single cell sequencing data, my progress with alloantigen analysis has been limited. Because the analysis of CMV-responsive cells is near completion, I will focus in the near term on putting together that publication, and then on analysis of alloresponses.

I am currently in the process of writing a manuscript on the sequencing findings outlined above, which will most likely be submitted to the American Journal of Transplantation. The telomere experiments should be completed within the next 6-9 months, and we should be able to publish shortly after that.

Since my previous progress report, two AHA-funded papers have been published: a methods paper in Journal of Immunological Methods (previously accepted pending minor revisions), and a mini-review in American Journal of Transplantation (previously under preparation). I have also published an invited commentary in Science Immunology entitled ¿To debug or not to debug, a question worth asking.¿

In working on this project, I have consulted regularly with my sponsor, Dr Jonathan Maltzman, co-sponsor, Dr. Mark Davis, and collaborators Drs Purvesh Khatri and Holden Maecker. I have met regularly with these individuals and also presented my research to them periodically to obtain feedback. This includes a mentoring committee meeting in April that also included Drs Jane Tan and Sheri Krams, and a meeting with Drs Khatri and Maecker to resolve the batch correction issue described above.

Other progress:

Dr. Maltzman and I have worked closely on this project, meeting weekly to discuss experiments and analysis, and also to discuss long term plans. We had our most recent individual development plan

meeting to discuss longer-term plans on 11/25.

In the past year, I have presented my research as a poster at the Stanford conference Human Immune Monitoring Technology & Bioinformatics Conference and the AHA meeting Basic Cardiovascular Sciences, and as a poster and mini-talk at International Transplantation Science. My mini-talk received compliments from many people, both to myself and to Dr. Maltzman. I have submitted two career development award applications, and three internal grant applications within Stanford.

***3. Indicate what percent of the project you estimate has been completed (e.g. 30%).**

70

***4. Are there any significant changes or delay in your training program and/or research plan?**

Yes

***5. Are there any changes in Sponsor (on fellowship or mentored awards)?**

No

***6. Are there any changes in Principal Investigator or collaborators?**

No

***7. Are there any changes in location or facilities?**

No

***13. No Changes.**

No

***14. Please describe any significant changes that have occurred, as noted above, or any anticipated changes that represent a significant deviation from the original plan. Briefly discuss the reason(s) for the changes and the implications.**

There were two significant changes. First, the batch effects described in the progress report significantly delayed progress this year. While ultimately this had a minimal impact on the publication timeline for the analysis of CMV-responsive T cells, it did delay analysis of alloreactive T cells.

Second, additional experiments have been added to more directly address T cell function through measurement of aging (telomere assays) and CMV-specific T cells (MHC-peptide tetramer staining). The optimization of these assays has caused further delay, but ultimately this analysis will be crucial to understanding the impact of CMV-responses post-transplant.

***15. Expenditures: (not applicable to fellowship awardees.) If you have a low rate of expenditures this fiscal period, please provide a brief explanation.**