Dr. Ran Reshef

Title: Identification of Effector and Suppressive T-Cell Clones in Graft-versus-Host Disease

FY17 PRCRP Topic Area: Immunotherapy

FY17 PRCRP Military Relevance Focus Area: Gaps in treatment of hematologic malignancies that have a profound effect on the health and well-being of active duty Service members, Veterans and their beneficiaries.

Background: Allogeneic hematopoietic stem-cell transplantation (SCT) is the oldest form of cancer immunotherapy and is effective in lymphoma, leukemia and other blood cancers due to the graft-versus-tumor (GvT) potential of the donor graft. However, successful allogeneic SCT is limited by graft-versus-host disease (GvHD), induced by alloreactive T-cells that encounter recipient antigens and migrate into tissues to cause damage and leading to significant morbidity and mortality. Existing modalities for treatment of GvHD have limited efficacy and may impair immunity against pathogens and tumor cells. Tools that allow early diagnosis or prognostication are also limited in GvHD. Identification of specific clones of tissue-infiltrating regulatory T-cells (Tregs) in tissues affected by GvHD is critical for the development of adoptive transfer strategies, where infusion of expanded tissue-infiltrating Tregs clones will effectively suppress the GvH response and lead to therapeutic benefit. However, existing approaches for identification of tissue-infiltrating Tregs are limited. Overcoming this barrier is necessary for the development of a strategy that induces tolerance in GvHD tissues while minimizing a detrimental effect on the GvT response and on immunity against pathogens.

Objective/Hypothesis: We hypothesize that specific clones of alloreactive Tregs can be identified in GvHD tissues and distinguished from effector T-cells that cause GvHD or tissue-resident T-cells that have a role in maintaining homeostasis and immune memory against pathogens. Our preliminary work demonstrated that donor alloreactive clones could be identified through mixed lymphocyte reaction (MLR) between donor and recipient cells. Using *TCRB* sequencing, we were able to identify and quantify circulating alloreactive T-cells and measure their presence in tissue biopsies from GvHD organs. Moreover, we developed a technique that characterizes the functional phenotype of *in situ* T-cell clones and links it to their clonal identity using single-cell RNA sequencing. This method allows the functional characterization of individual T-cell clones that infiltrate GvHD tissues and differentiation between effector T-cells that express high levels of effector molecules such as IFN-gamma, regulatory T-cells that express FOXP3, IL-10 and TGF-beta and tissue resident T-cells that express memory markers but are functionally quiescent. The precise identification of effector and regulatory tissue-infiltrating T-cell clones sets the stage for the development of a personalized adoptive cell therapy approach for induction of tissue-specific and antigen-specific tolerance and treatment of GvHD.

The primary objective of this proposal is to develop a platform to identify alloreactive tissue-infiltrating T-cell clones and characterize their effector function using single-cell techniques. Our secondary objective is to identify the diagnostic and prognostic potential of measuring the relative abundance and clonality of effector and regulatory T-cells in tissues.

Specific Aims and Study Design

Aim 1. Identify alloreactive T-cell clones in GvHD affected tissues and determine their individual function. I will use high-throughput single cell RNA sequencing of the T-cell receptor genes coupled with measurement of 36 immune genes by a combined platform of RNA sequencing and flow cytometry to determine the function of CD4+ and CD8+ T-cell clones that are present in gut, liver and skin biopsies from acute and chronic GvHD patients. I will confirm the alloreactivity of these clones using pretransplant MLR between donor and recipient cells. This will allow functional mapping of individual T-cell clones in GvHD.

Aim 2. Determine the diagnostic and prognostic properties of alloreactive T-cell clones in GvHD affected organs. I will quantify the abundance and ratio between effector and regulatory T-cells in GvHD tissue biopsies and correlate these variables with histologic and clinical features of GvHD. I will also examine whether high

abundance of alloreactive effector T-cells or high ratio between effector and suppressive cells have prognostic impact. This will allow the development of more accurate diagnostic and prognostic methods for GvHD.

Military Relevance: Blood cancers such as lymphoma and leukemia represent some of the more common cancer types and their high prevalence in young adults and in children makes it highly relevant for Service men and women and their family members. Allogeneic SCT is standard therapy in blood cancers and is performed in approximately 30,000 people worldwide each year, including many Military personnel.