## Flow Cytometric Immunophenotyping of nPOD Donors

Christopher Fuhrman, James Thompson, Howard Seay, and Todd Brusko
Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL

Purpose: Cells of both the innate and adaptive arms of the immune system influence the pathogenesis of type 1 diabetes. Certain cell subsets may be lost or phenotypically altered following density gradient centrifugation and the process of cryopreservation (e.g., dendritic cells, macrophages, etc). In an effort to gather and preserve this data for all nPOD users, we set out to develop SOPs to monitor major cellular subsets from freshly isolated peripheral blood and tissues derived from nPOD donors prior to cryopreservation and distribution.

Methods: We have developed polychromatic flow cytometric panels to gather information related to cell frequency, phenotype, and viability (initial and post-thaw) from peripheral whole blood, spleen, pancreatic draining lymph nodes, and non-pancreatic draining lymph nodes. While not comprehensive, these measurements include the analysis of monocytes, plasmacytoid and myeloid dendritic cells, B-lymphocytes, CD8+ T cells, CD4+ conventional and regulatory T cells, NK cells, and iNKT cells. In addition to defining these key populations, we have also added phenotypic markers commonly used to survey MHC class II expression, co-stimulatory ligands, and chemokine receptors.

Summary of Results: Analysis of whole blood cell populations has shown a considerable degree of heterogeneity between nPOD donor samples and normal healthy control peripheral blood samples. Notably, there is a paucity of CD11c+ or CD123+ dendritic cells present in the circulation of nPOD donor subjects. In addition, we have noted tissue-specific alterations in the cytokine profile of Tregs derived from peripheral blood, spleen, and lymph nodes in their capacity to produce IFN-γ and IL-10 upon activation with PMA and ionomycin.

Conclusions: Understanding the viability and functional state of immune cell subsets prior to cryopreservation may have important implications for interpreting nPOD investigator data. Information regarding cell viability and data related to basic immunophenotyping will be provided to nPOD program staff for distribution to program investigators. We expect this data to provide the community of nPOD investigators critical data related to the phenotype and viability of immune cell subsets, and will hopefully facilitate key insights into the etiology of type 1 diabetes.