PI: Elizabeth Krieger, MD
Academic Rank: Assistant Professor
Title: Understanding Donor Lymphocyte Infusion Response: A Longitudinal Molecular Examination of T cell Receptor Diversity and Activation

Cancer is a chronic disease often termed as "wounds that do not heal." Developmental pathways such as the Wnt pathway acts co-ordinately to establish the body plan, and disruptions of the Wnt pathway result in cancer. Our previous findings identified that the Wnt antagonist, Dickkopf1 (DKK1), promotes pathological inflammation. Previous reports showed the systemic or local elevation of DKK1 protein expression in various human cancer patient samples. However, how DKK1 modulates the tumor microenvironment (TME) is unknown. Natural Killer (NK) cell is a subset of innate lymphoid cells (ILCs) with tumoricidal activity. Tumor cells evade NK cell-mediated immune surveillance by multiple mechanisms. The high expression of DKK1 is correlated to higher morbidity of Head and neck cancer (HNSCC) in human patients. Recently, the importance of NK cells in the TME has been investigated. The current proposal addresses how DKK1 dysregulates tumoricidal NK cells in head and neck cancer. We propose two aims as below.

In Aim 1, we will first investigate the importance of NK cells in head and neck cancer (HNSCC) using an orthotopic 602 oral squamous carcinoma cell line 602 and intrabuccal injection model. We will use NK cell-deficient mice that have NK cell-specific deletion of DKK1 receptor LRP5 and LRP6. Tumor growth will be monitored by bioluminescence imaging and conventional tumor volume measurement using a caliper. The excised tumors will be analyzed by flow cytometry to measure the infiltration of immune cells and their functions. Second, we will test whether the abrogation of DKK1-mediated signaling in NK cells restores the tumoricidal activity of NK cells in the orthotopic model. To this end, splenic NK cells from Nkp46iCre-Lrp6fl/fl or their littermate control mice will be adoptively transferred to NK cell-deficient mice. Tumor growth will be determined after multiple times of adoptive transfers during the two weeks. Next, we will address the source of DKK1 in the orthotopic HNSCC model. We will perform gain-offunction and loss-of-function studies using DKK1-overexpressing transfectants and DKK1 shRNAexpressing transfectants with the 602 cell orthotopic model.We will use Pf4Cre-Dkk1fl/fl mice to test whether platelet-derived DKK1 is a primary source of DKK1 in the orthotopic model.

In Aim 2, we will first test whether DKK1 abrogates NK cell-mediated cytotoxicity, promoting lung metastasis. To this end, we will first use DKK1-expressing 602 cells. 602 cells will be injected intravenously per NK cell-deficient mice. Tumor size will be monitored. Next, we will test whether DKK1-mediated signaling in NK cells abrogates tumoricidal functions of NK cells in HNSCC metastasis. To this end, splenic NK cells from Lrp6NKO mice or their littermate controls will be injected intravenously per NK cell-deficient mice on the same day. NK cells will be injected. As stated above, we will harvest lungs and analyze them by qPCR, DKK1 IHC, and flow cytometry. Lastly, we will use Pf4Cre-Dkk1fl/fl (platlet-specific deletion of DKK1 gene) mice to test whether plateletderived DKK1 is a primary source of DKK1 in the host for HNSCC metastasis. By addressing these two aims, we investigate the importance of NK cells in HNSCC tumor growth and metastasis. We address the dysregulation of NK cells by DKK1 in these two processes, providing comprehensive insight into how tumor cells utilize DKK1 to evade NK cell-mediated immune surveillance. Thee proposal will allow us to compete in the national peer-reviewed grant applications with a solid understanding of a new therapeutic rationale in HNSCC cancer immunotherapy.