**Treating Patient Xenographed Laryngeal Squamous Cell Carcinoma (LSCC) in Humanized Mice with an IL-10 Antagonist**

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**Summary:** This research proposal discusses Head & Neck Squamous Carcinoma with a tumor microenvironment including regulatory T cells of type: {CD4+,CD25+,Foxp3+, IL-10(high secretion), TGF-b+(low),CD127+(low)} and proposes using humanized mice with human laryngeal squamous carcinoma xerographs from biopsy or samples from surgical removal to test the viability of IL-10 antagonism in restoring effector T cell function to the cancer immune response.

**I) Head & Neck Squamous Cell Carcinoma(HNSCC)**

Head & Neck Squamous Cell Carcinoma(HNSCC) has a 5 year overall disease free survival of about %50().To put this into perspective, modern therapy for cancer in general results in 5 year survival rates ranging from almost %0 to almost %100(). According to the U.S. National Library of Medicine, "HNSCC is the seventh most common cancer worldwide. Approximately 600,000 new cases are diagnosed each year, including about 50,000 in the United States. HNSCC occurs most often in men in their 50s or 60s, although the incidence among younger individuals is increasing". (2) This roughly translates to 25,000 people dying each year in the United States. Since surgery and chemotherapy only provide a 50/50 chance of surviving from HNSCC, it is worthwhile seeking new methods of treating this cancer.

A major risk factor for HNSCCs are aromatic amines (AAs). The family of AAs from tobacco smoking and their metabolic derivatives fit this profile (reference &PHECM reference for AAs). A few different routes for oxidizing-detoxification of the AAs can determine what happens to the AAs as they come into contact with the epithelium of the main respiratory & sinus cavities. I) The N-Acetyl Transferase alleles' metabolic protein product can oxidize the Nitrogen of the AAs and induce them into phase II detoxification (). II) CYP450 is found on epithelial cells in smaller amounts, relative to the liver () III) Drug efflux pumps might have binding affinity and subsequent efflux of some AAs(). Regardless of these factors, AA driven mutations can eventually lead to cancer cells with immunosuppressive or evasive phenotype. Of these exploits of the immune system, IL-10 has shown up in clinical statistics as a poor prognostic marker in laryngeal squamous cell carcinoma (LSCC) () while also being a good prognostic marker in other cancers ().

IL-10 in general is an anti inflammatory signaler, therefore; infections and cancer cells that benefit from cytokine production suppression should show IL-10 as a poor prognostic marker while dysfunctional IL-10 should be a good prognostic marker for a patient. In LSCC IL-10 producing Tr1 cells and poor cytotoxic T cell tumor infiltration results in poor prognosis().

**II) IL-10's Bioactivity in HPV-- Laryngeal Squamous Cell Carcinoma(LSCC) Tumor Microenvironment by Immunosuppression via CD4+, CD25+(high), CD127+(low), Foxp3+, TGF-β+ , IL-10+ (high secretion) Regulatory T-cells of Type 1 (Tr1)**

IL-10 transcription promotion in T cells in general differ from the less potent promotion of it in macrophages and dendritic cells (DCs) in one way in that it is likely extracellular-signal-regulated kinase (ERK) dependent and p38 independent(). Additionally, the Tr1 promotion of IL-10 can be induced by cancer cells presenting antigens to antigen presenting dendritic cells(APDC) which can recruit Tr1 cells in an immune response to propagate tolerance to these tumor antigens(a). IL-10 production is also promoted through autocrine based STAT3 signaling through IL-10 receptors on IL-10+ Tr1 cells(IL-10 TRIAD).

Immune regulation by Tr cells include: secretion of soluble or membrane bound immunosuppressive molecules, direct cytolytic activity (lysis), metabolic disruption, and suppression of dendritic cells (DCs)(). IL-10/TGFB signaling or Tr1-DC interaction mediated by lymphocyte-activation gene 3 (LAG3) has been shown to reduce the capacity for APDCs to activate effector T cells(a).

IL-10 has at least two receptors, IL-10R1 and IL-10R2, which can act individually or complex and function in junction with each other. Binding of IL-10R2 to the 1:1 IL-10/IL-10R forms the 1:1IL-10/IL-10R/IL-10R2 complex. This complex uses the IL-10R2 to bind the extracellular section of the TYK2 signal transducer and uses IL-10R1 to bind the extracellular section of the JAK1 signal transducer. These transductions relay to activate Signal Transducer and Activator of Transcription (STAT) 1, 3, & 5().STATs can then bind to DNA through DNA binding sequence found in STAT proteins (DNA binding sequences vary) where it can then, by complex with other factors or directly, regulate transcription of genes where it has bound to its promoter sequence STAT's DNA binding sequence is complementary to.

Given IL-10 signaling's possible keystone role in LSCC development and maintenance through APDC suppression and STAT1,3,&5 signaling, IL-10 antagonists could help restore effector T cell function while stopping propagation of tolerance to tumor antigens.

**III) Xenotransplant of Surgically Removed or Biopsied LSCC Tissue & Control Laryngeal Epithelium Tissue to Humanized Mice**

The humanized mouse model known as Hu-PBL-SCID, is created by injection of human peripheral blood leukocytes, resulting in engraftment of human CD3+ T cells by the end of the first week. This naive population can then develop into a pseudo human immune system with limited amounts of limitations () A downside of this method is the development of lethal xenogeneic graft-versus-host disease (GVHD), usually within 4–8 weeks(). In this window of time, after CD3+ T cells have engrafted, xenotransplantation of the LSCC and control tissues from the patient to the humanized mouse must be engrafted; as to achieve a model of the patient's in vivo LSCC tissue. Once LSCC has developed in the non control humanized mice, a fraction of the LSCC+ mice will be treated with surgery and an IL-10 antagonist.

**IV) Antagonist for IL-10**

This antagonist used in this experiment would bind to the site on IL-10 which would normally bind the IL-R1 receptor, thus preventing it from carrying out its IL-10 dependent STAT 1,3,5 signaling. A risk factor in using an IL-10 antagonist includes uncontrolled inflammation. However, if the patient or mouse being treated is kept in a sterile environment and is at least HPV negative, this may be manageable.

**V) Experiment**

The experiment takes the following steps:

I) Biopsy or sampling from patient of surgically removed LSCC+ larynx or esophageal tissue and LSCC- tissue of those types in those same LSCC+ patients.

II) Humanizing mice by injecting them with human peripheral blood leukocytes from patient, resulting in CD3+ T cell engraftment and subsequent human immune development in the humanized mouse.

III) Once the mice have been humanized, run cytometry to get the initial LSCC count by fluorescent tagging of the antigen that was presented from the tumor to APDCs in the patient (methods for determining antigens 'held' by FDC extracellular ligaments). Once the 3 groups receive their treatment and the same amount of time has elapsed, run cytometry again to see if LSCC was reduced.

Control: CD3+T, LSCC-, IL-10 agonist not added

Group1: CD3+T, LSCC+, IL-10 agonist not added

Group2: CD3+T, LSCC+, IL-10 agonist added

**VI) Discussion**

Some respected results include a decrease in LSCC count or death of mice by xerograph rejection disease.

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