Providence Portland Medical Center
Role of Epigenetic Modulation in Anti-OX40 Immunotherapy Resistance
PI: Chi Viet, DDS, MD, PhD
Co-PI: R. Bryan Bell, MD, DDS

As surgeons we struggle with poor outcomes in head and neck squamous cell carcinoma (HNSCC) patients, with no significant improvement in survival in the past 40 years. We have previously shown that epigenetic dysregulation (i.e.,methylation) is central to HNSCC development and chemotherapy resistance [2,3]. Similarly, a dysregulated immune system contributes to HNSCC progression and poor treatment outcome [4]. Recently immunotherapies that enhance T-cell function have been shown to improve survival. Our group has developed anti-OX40, an agonist to OX40, a receptor in the tumor necrosis factor (TNF) family. We have shown that OX40 is overexpressed on T cells in HNSCC tumors as well as metastatic cervical nodes [4]. Anti-OX40, currently in phase Ib/II trials at our institute for solid tumors, acts by promoting positive T cell signaling, cytotoxic function and cytokine production, and shows a promising anti-tumor effect [5]. However some HNSCC patients die from cancer progression despite anti-OX40 treatment. We hypothesize that anti-OX40 resistance in these patients is epigenetically modulated, whereby HNSCC cells hypermethylate and silence genes that produce the antigen presenting machinery (APM), thereby escaping recognition by T cells. We propose that combining the demethylating agent decitabine with anti-OX40 treatment will enhance anti-OX40 efficacy by forced re-expression of APM genes. Our proposal will proceed based on the following Aims:

Aim 1: Determine the effect of combining decitabine with anti-OX40 on cancer growth in a HNSCC mouse model.

We hypothesize that the combination of decitabine and anti-OX40 will produce a greater anti-tumor effect in HNSCC than anti-OX40 alone. We have shown in a HNSCC mouse model that decitabine alone inhibits cancer growth by re-expressing silenced genes [3]. Anti-OX40 treatment can enhance CD8 T cell function in the cancer resulting in prolonged survival of solid cancer models [5]. We will use an established syngeneic HNSCC mouse model to determine whether this combination treatment enhances anti-tumor immunity leading to increased tumor cell destruction.

Aim 2: Determine the effect of decitabine on antigen presenting machinery (APM) molecules in HNSCC.

We hypothesize that HNSCC cells silence APM genes through methylation, leading to anti-OX40 resistance. We will perform expression studies of tumors from Aim 1 that receive decitabine and anti-OX40 to compare them to tumors that receive anti-OX40 alone. Specifically, we will analyze calnexin, calreticulin, β2-microgloblin, latent membrane protein 2 (LMP2), LMP7, transporter associated with antigen processing (TAP) 1, and TAP2 by Methylight, RT-PCR and immunohistochemistry.

Aim 3: Establish a gene signature for HNSCC patients who will benefit from the addition of decitabine to their anti-OX40 regimen.

Based on the APM expression data in Aim 2 we will choose the 3 genes with the most disparate expression between the anti-OX40 responsive and resistant mouse groups. We will then quantify expression and methylation of these 3 genes in cancer tissues of our cohort of HNSCC patients who have been enrolled into the phase Ib/II anti-OX40 trial. We will correlate the change in methylation and mRNA/protein expression after anti-OX40 treatment and compare this with clinical outcome in these

patients. Our goal is to establish a gene signature to determine which patients are resistant to anti-OX40 treatment and could benefit from combination treatment of anti-OX40 with decitabine.