

## **Combination Treatment Of PD-1 Inhibition And Metformin May Stimulate An Anti-Tumorigenic Immune Response In Head And Neck Squamous Cell Carcinoma.**

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**Introduction:** Head and Neck Squamous Cell Carcinoma (HNSCC) is the 6th most common cancer worldwide. Despite intensive therapy, up to 50% of patients will relapse, hence, improved treatments are needed. Metformin has been shown to have a direct anti-tumor effect via mTOR inhibition and lymphocyte stimulation. We have shown that metformin therapy in HNSCC patients may induce peripheral blood mononuclear cells (PBMCs) to secrete antitumorigenic cytokines. The programmed cell death ligand 1 (PD-L1) and programmed cell death 1 (PD-1) interaction is a key immunoinhibitory function. PD-L1 has been found to be up-regulated in HNSCC, and treatment with the anti-PD-1 inhibitors has been shown to decrease tumor burden in up to 46% of patients with recurrent and metastatic HNSCC. Targeting multiple aspects of the cancer immunity cycle could provide even more successful therapeutic outcomes.

**Hypothesis:** Combination treatment of anti-PD-1 antibody, Nivolumab, with mTOR inhibitor, Metformin, ex-vivo will result in an increased anti-cancer immune response.

**Methods:** Metformin IC50 dose for HNSCC cell lines was determined via a MTS cell proliferation and cytotoxicity assay. PBMC activation conditions were determined by flow cytometric analysis of activation marker CD69 and exhaustion markers PD-1, TIM-3, and LAG-3. Intracellular and secreted levels of cytokines were evaluated to confirm PBMC activation by flow cytometry and ELISAs. Patient PBMCs were co-cultured with HNSCC cell lines in the presence and absence of metformin and Nivolumab. Supernatant from co-culture was collected, and anti-tumorigenic cytokine levels were evaluated.

**Results:** Metformin IC50 dose was determined to be 50mM. CD69+ PBMCs had the lowest expression of exhaustion markers when activated with CD3/CD28 for 48 hours. Additionally, intracellular and secreted IL-2 levels were upregulated under those activation conditions.

**Conclusions:** Preliminary experiments have identified appropriate Metformin dosage and PBMC activation conditions for future experiments. There is evidence that combination therapy of metformin and a PD pathway blocker could be therapeutic in HNSCC.

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