Inhibition of IDO activity by epacadostat (INCB024360) activates tumor infiltrating lymphocytes in a patient-derived 3D ex vivo system of lung cancer and alleviates stromal immunosuppression

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Abstract

Introduction: Immune evasion is one of the major hallmarks of cancer and identifying mechanisms by which cancer cells evade the immune system have become a major strategy against cancer. IDO (indoleamine 2,3-dioxygenase) is a tryptophan catalyzing enzyme expressed constitutively by tumor cells and different components of immune cells present within the tumor microenvironment. It has been shown that high expression of IDO increases the number of Tregs and blocks the proliferation of effector T cells. Thus, inhibiting the IDO pathway is a promising strategy to restore immune system responses to more easily identify and destroy cancer cells. This study evaluates the immunomodulatory effect of an IDO inhibitor epacadostat (INCB024360) on the immunosuppressive effect of cancer-associated fibroblasts and activation of tumor infiltrating lymphocytes in a 3D ex vivo assay utilizing fresh patient tumor samples.

Materials and Methods: 3D ex vivo studies were performed with fresh tumor tissue obtained from consented NSCLC patients. Tumor samples were treated with epacadostat at 1µM for 48 hours. HPLC analysis on kynurenine and tryptophan was performed to verify target inhibition in the ex vivo model. A multiplex human cytokine assay was used to simultaneously analyze the differential release of cytokines in culture media. Additionally, NanoString PanCancer Immune Profiling platform containing probes to quantitate 770 immune function genes was used to determine positive and negative associations between expression of immune function genes and TIL activation by ex vivo treatment. Furthermore, autologous patient-derived cell lines (CAF and TILs) were utilized in an in vitro assay to determine the role of IDO inhibition on CAF-mediated immunosuppression.

Results and Conclusions: 3D ex vivo studies showed a significant decrease in kynurenine demonstrating that epacadostat effectively inhibited the enzymatic activity of IDO in the tumor microenvironment accompanied by increased release of pro-inflammatory cytokines such as IFNγ. Treatment with epacadostat demonstrated decreased expression of genes involved in tumor growth (CCL25) and increased expression of antitumor immune response genes (CXCL14, CCL19 and CCL21). These studies showed epacadostat at an effective concentration of 1nM induced specific changes in the microenvironment and increased immune response. Furthermore, the autologous patient derived cell line in vitro assay determined that epacadostat overcame CAF induced inhibition of TIL activity. This patient-derived 3D ex vivo approach demonstrated the immunomodulatory activity of epacadostast in NSCLC and indicates that inhibition of IDO activity may overcome stroma-induced immunosuppression in lung cancer. Studies on the effects of epacadostat in combination with anti-PD1 in the same culture systems are currently ongoing.

Results Table

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Figure 1. Characterization of immune checkpoint markers in lung cancer patient tumor samples. H&E, CD3 and CD8 analysis of fresh tissue from two patients with adenocarcinoma of the lung.

Figure 2. HPLC analysis of kynurenine and tryptophan. HPLC analysis was performed in supernatants after treatment with 1µM of epacadostat (IDO) for 48 hours in an 3D ex vivo system. Decreased Kyn/Trp ratio demonstrates that epacadostat effectively inhibiting IDO activity in all tumors ex vivo.

Figure 3. Multiplex analysis of cytokines in epacadostat (IDO) treated 3D microspheroids. 17-plex cytokine/chemokine analysis was performed. Expression of IL1β, C-CSF, IFNγ, and IP10 in two patients is shown. Culture media obtained from ex vivo experiments were analyzed using the Bioplex Multiplex Assay for cytokine secretion. All experiments were performed in duplicate, and the means and standard deviations were plotted. Combination of Phorbol myristate acetate (PMA) and Ca2+ ionophore (I) was used as positive control to activate TILs (data not shown).

Figure 4. Immune gene expression analysis in epacadostat (IDO) treated 3D microspheroids. Increased expression of genes involved in antitumor immune response genes were observed.

Figure 5. In vitro assessment of epacadostat's effect on CAF immunosuppression. Autologous CAF and TILs were co-cultured in the presence of CD3 and 1µM epacadostat (IDO) for 48 hours. IL2 ELISA was performed to observe if epacadostat overcame the CAFs immunosuppressive mechanism.

Summary

- Epacadostat treatment led to an increased proinflammatory tumor microenvironment including T-cell activation in two of five fresh NSCLC patient tumors in the 3D ex vivo assay.
- In all tumors tested ex vivo epacadostat treatment significantly decreased Kyn/Trp ratio, thus proving the drug inhibits IDO activity at 1µM.
- Inhibition of IDO activity overcame immunosuppressive effect of cancer-associated fibroblasts indicating epacadostat acts on tumor stroma.
- Gene expression changes following IDO inhibition are contextual and efforts to understand this are planned.