

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 catabolizing 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 1mM 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 epacadostat 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 Tumor 1

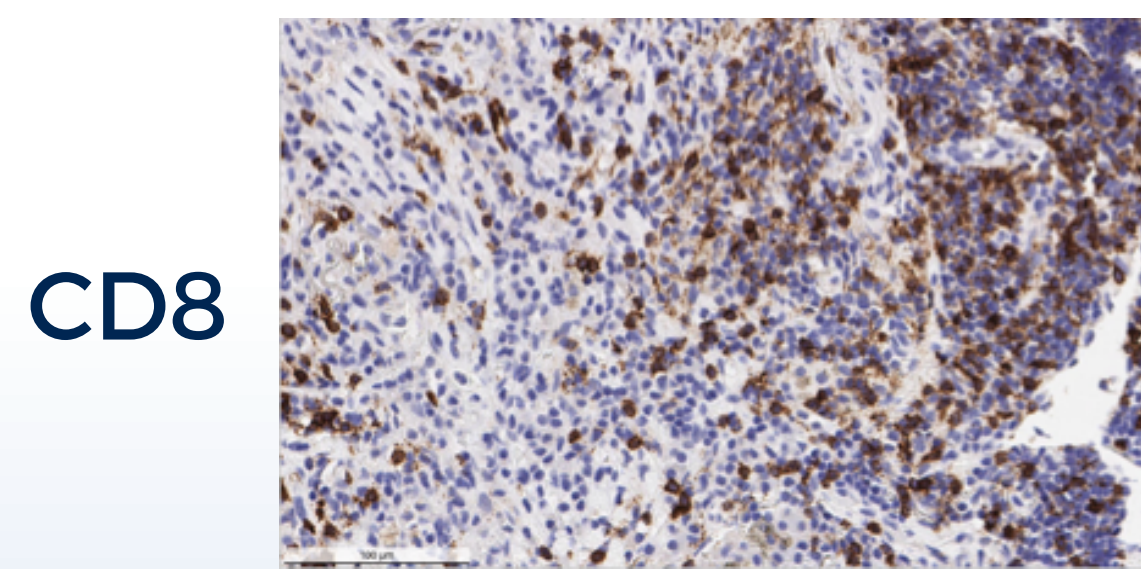
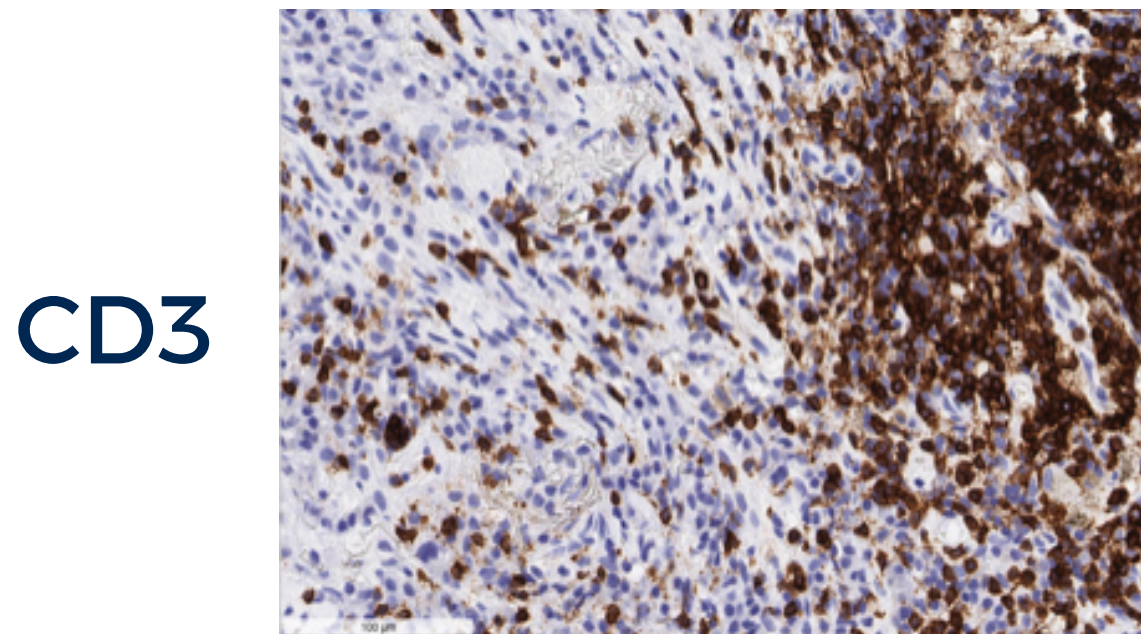
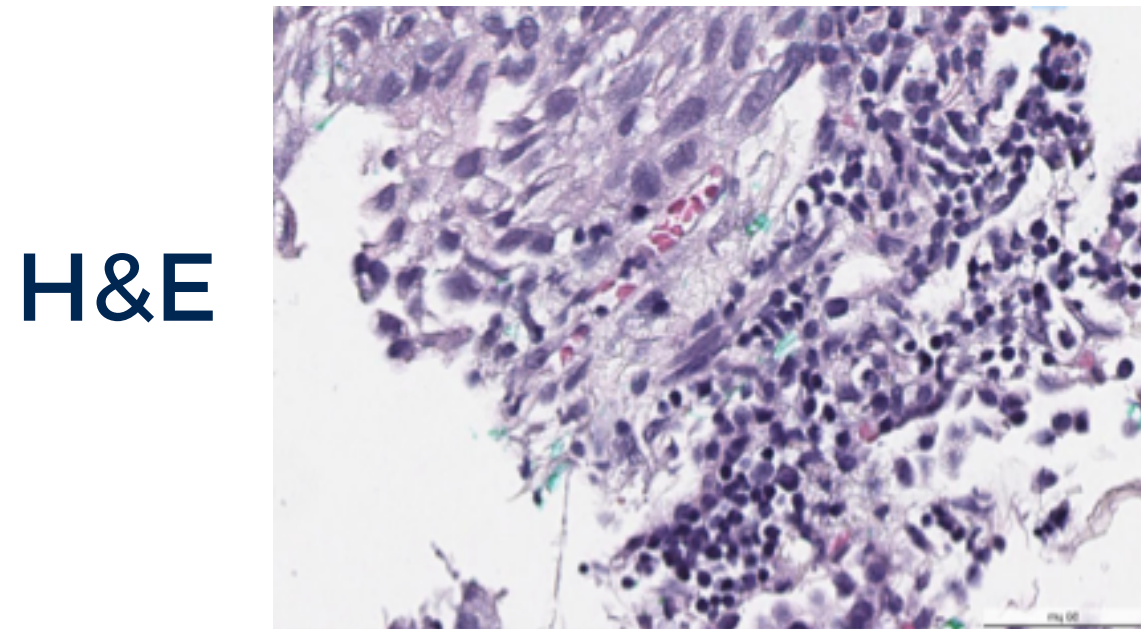


Figure 1. Characterization of immune checkpoint markers in lung cancer patient tumor samples. H&E, CD3 and CD8 analysis of fresh tissue from two patients with adenocarcinoma of the lung.

Tumor 2

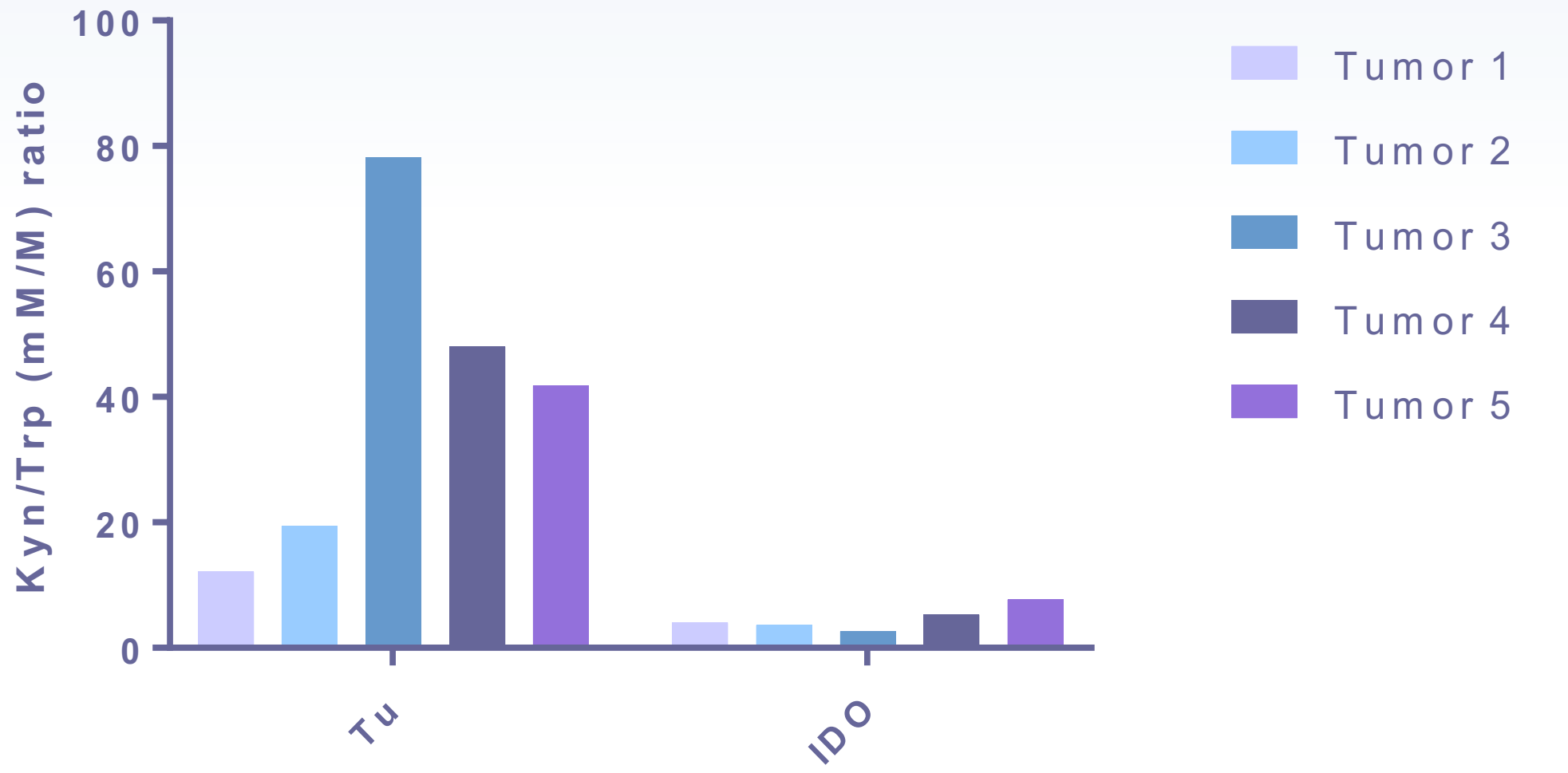
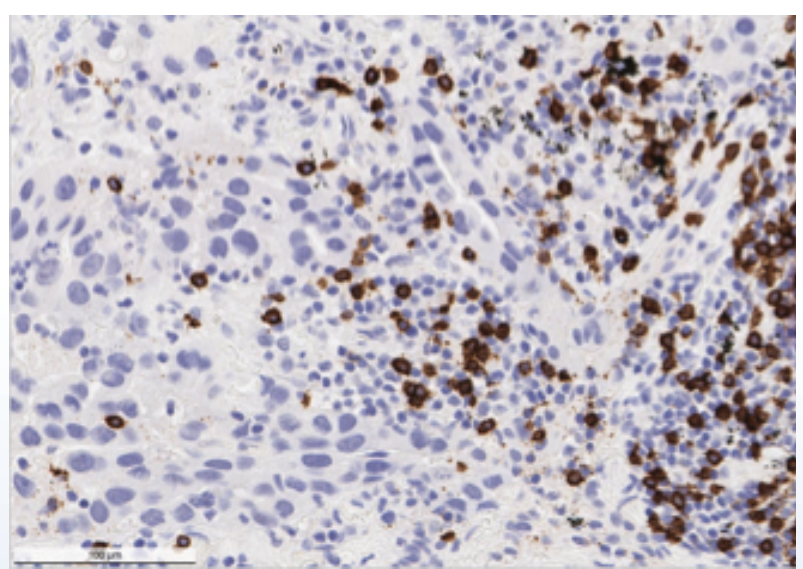
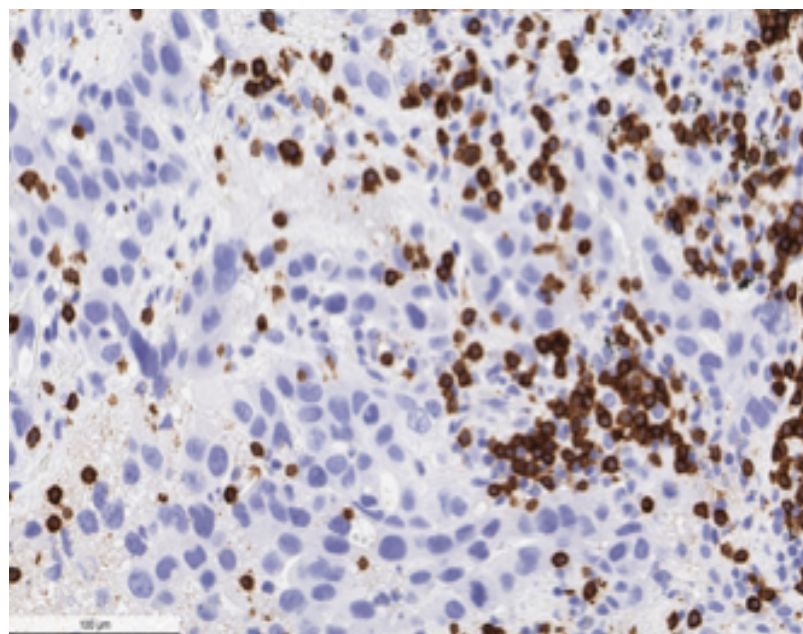
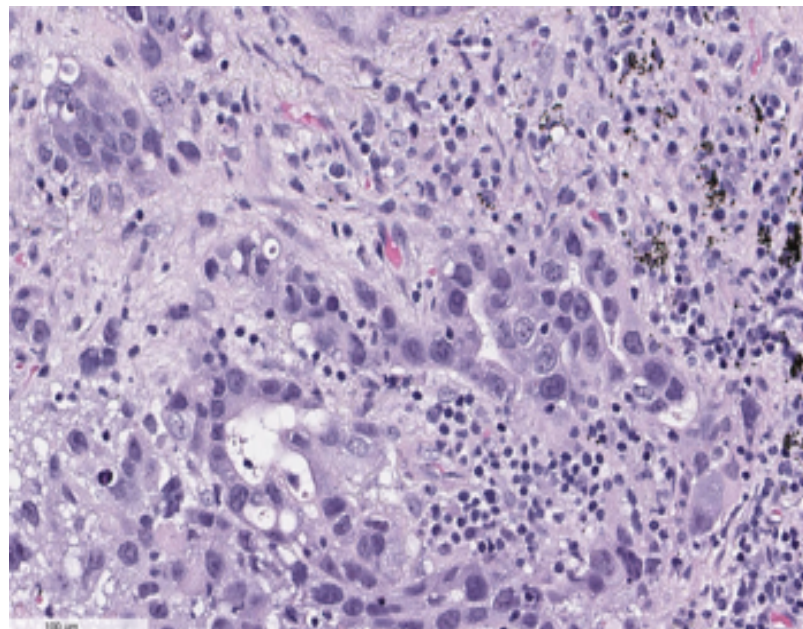
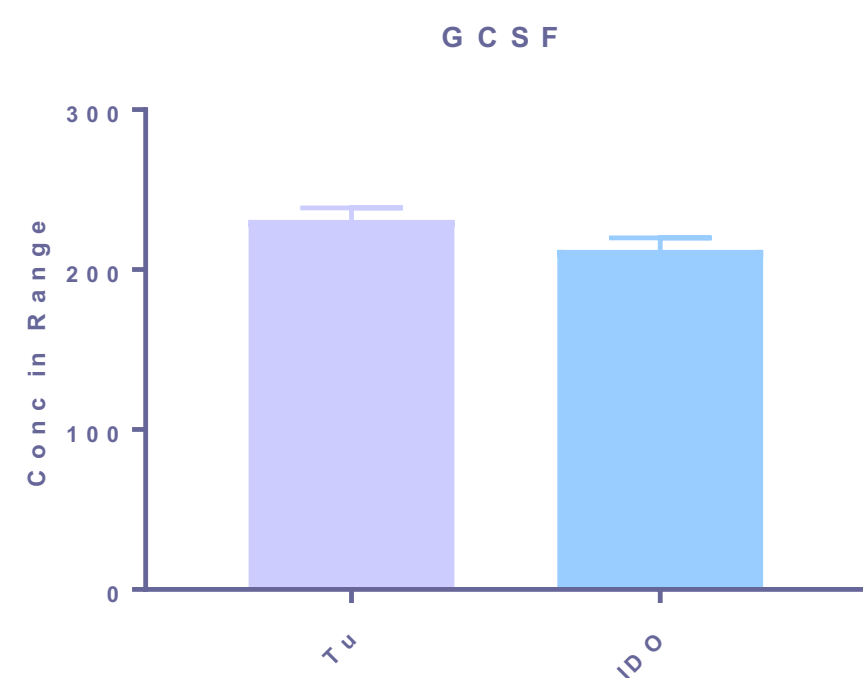
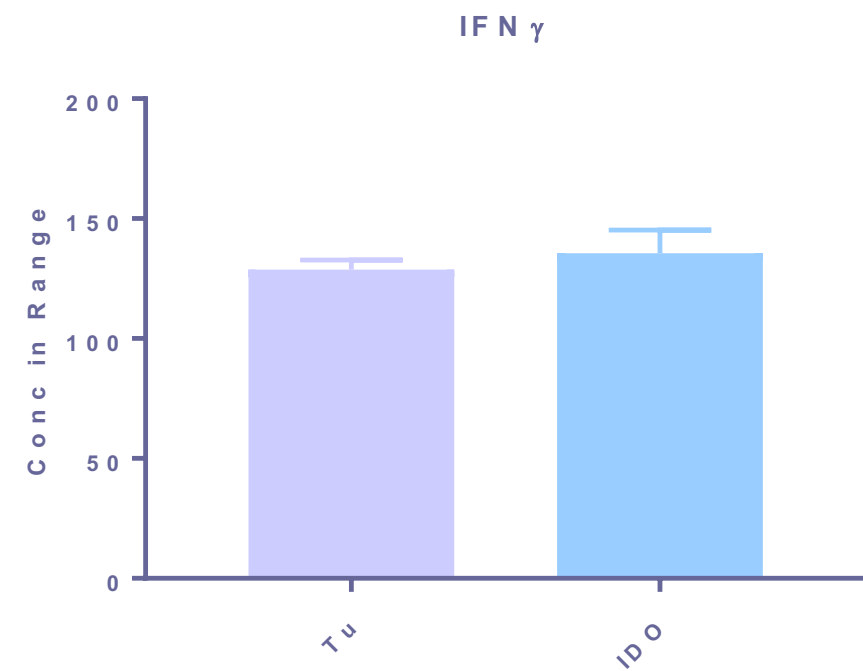


Figure 2. HPLC analysis of kynurenine and tryptophan. HPLC analysis was performed in supernatants after treatment with 1μM of epacadostat (IDO) for 48 hours in a 3D ex-vivo system. Decreased Kyn/Trp ratio demonstrates that epacadostat effectively inhibiting IDO activity in all tumors *ex vivo*.

Tumor 1



Tumor 2

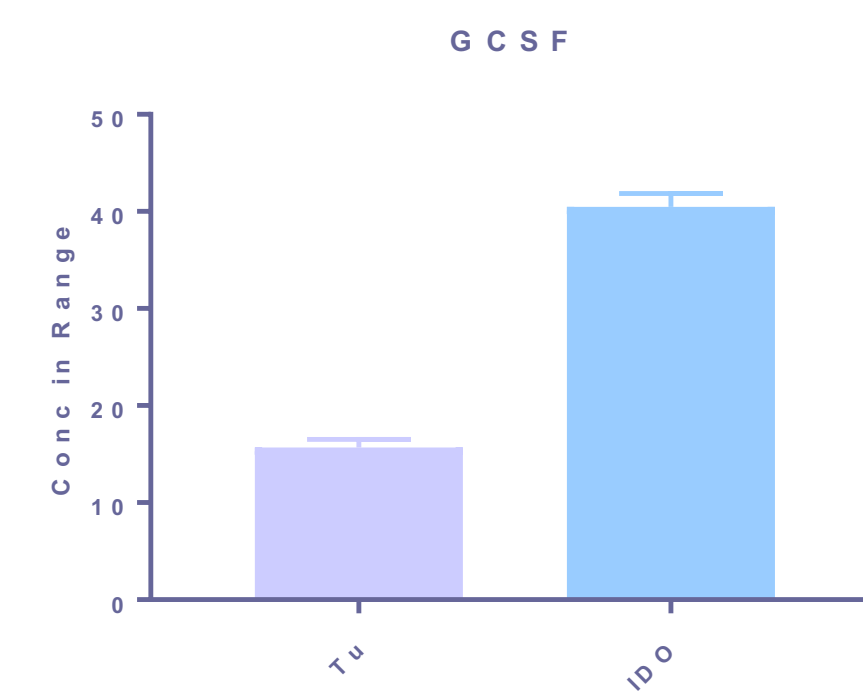
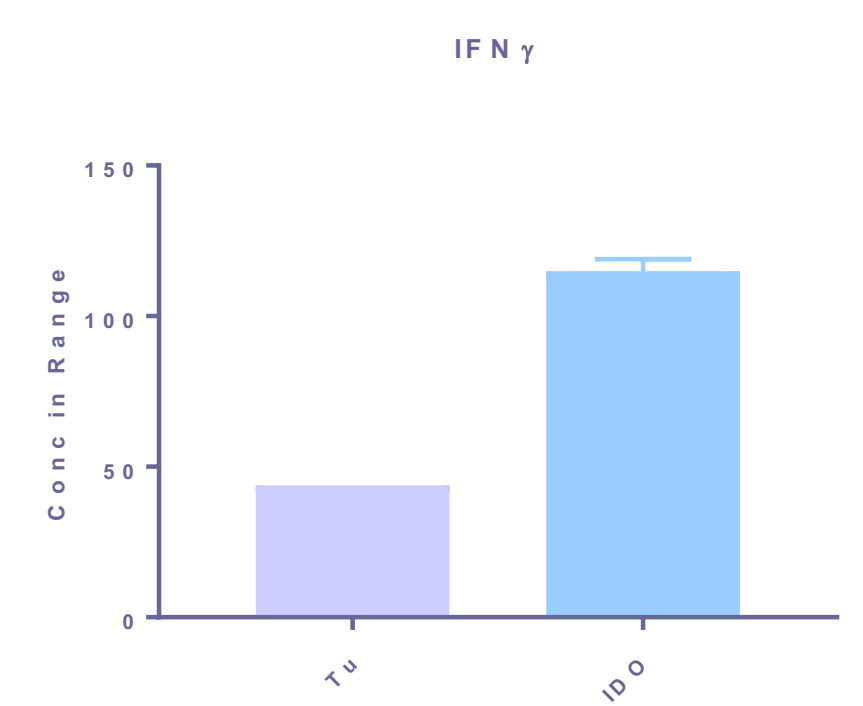


Figure 3. Multiplex analysis of cytokines in epacadostat (IDO) treated 3D microspheroids. 17-plex cytochemokine analysis was performed. Expression of IL1β, G-CSF, IFNγ and MIP1β in two patients is shown. Culture media obtained from *ex vivo* experiments were analyzed using the Bioplex Multiplex Assay 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).

Tumor 1



Tumor 2



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

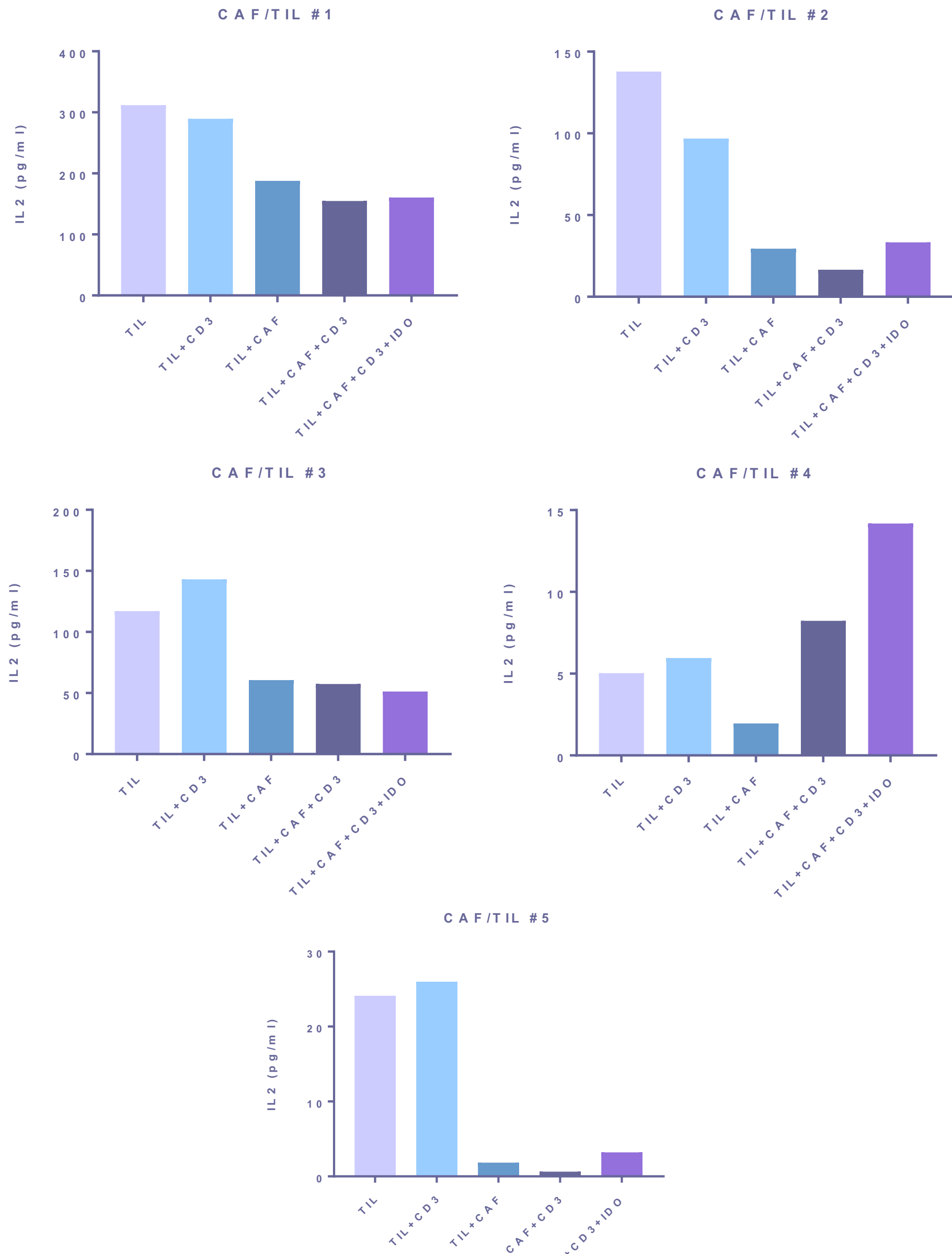


Figure 5. In vitro assessment of epacadostat's effect on CAF immunosuppression. Autologous CAF and TILs were co-cultured in the presence of CD3 and 1μM epacadostat (IDO) for 48 hours. IL-2 ELISA was performed to observe if epacadostat overcame the CAFs immunosuppressive mechanism.

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 IDOi are contextual and efforts to understand this are planned.