Efficacy of TDO2/IDO Inhibition

Dysregulated metabolism and immunomodulatory functions in the tumor microenvironment (TME) can directly affect immune cell function by depleting nutrients and activating immune suppressive programs. We identified tryptophan (TRP) catabolism as a potential contributor to EOC progression. TRP is catabolized by two structurally distinct rate-limiting enzymes: Indoleamine 2,3-Dioxygenase (IDO1/2) and Tryptophan 2,3-Dioxygenase (TDO2). IDO1 and TDO2 catalyze TRP into the immune-suppressive metabolite kynurenine (KYN), which then binds to AHR to mediate transcriptional programs. To date, IDO1 inhibitors in clinical trials have had limited efficacy for EOC, but those inhibitors did not target TDO2. We found that EOC cells are more dependent on TDO2 than IDO1. TDO2 knockdown significantly inhibited EOC growth and reduced TRP catabolism, and TDO2 overexpression was sufficient to promote cell growth and invasion. In a syngeneic EOC mouse model, a first-in-class orally available dual IDO/TDO2 inhibitor (AT-0174) inhibited tumor progression, reduced tumor-associated immune cells, and reduced expression of immune-suppressive proteins, including PD-L1. These studies demonstrate the importance of targeting TDO2 and the potential of using AT-0174 to overcome the immune-suppressed ovarian TME.

Efficacy of TDO2/IDO Inhibition In Vivo

• PD-L1 and immune cells are decreased by a dual IDO/TDO2 inhibitor. Ascents from vehicle and AT-0174 treated BR-Luc tumors was assessed via flow cytometry. Percentage of A) total macrophages, B) PD-L1 macroage MFI, C) ratio of MHCII+ M1 to PD-L1+MertK+ M2 macrophages, D) % of PMN-MDSCs, E) PD-L1 MFI on PMN-MDSCs, and F) % PD-L1+ PMN-MDSCs.

Conclusions

• Inflammatory tumors, which are associated with poor outcomes, have high levels of the tryptophan catabolite KYN.
• Ovarian tumors are highly dependent on the tryptophan-catabolizing enzyme TDO2.
• TDO2 drives pro-metastatic ovarian cancer cell survival, growth, and invasion in vitro.
• Dual targeting of TDO2 and IDO reduces ovarian tumor growth in vivo.
• Dual targeting of TDO2 and IDO reduces immune-suppressive cells and protein expression (PD-L1) in the TME.

Future Directions

• Verify efficacy of TDO2 inhibition and AT-0174 in additional ovarian cancer mouse models.
• Identify the mechanism of PD-L1 regulation that is driven by TDO2/KYN.
• Perform single cell sequencing of TDO2-inhibited tumor and immune cells to characterize global changes mediated by TDO2 in ovarian cancer.

References


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TDO2 Dependency in Ovarian Cancer Cells

TDO2 drives pro-metastatic ovarian cancer cell function and immune-suppressive alterations, which can be targeted with TDO2 inhibition.

Methods

Cytokine profiling was performed using primary human ovarian cancer specimens and ascites from the UC Cancer Center Gynecologic Tumor and Fluid Bank. To generate knockdown (KD) OVCA3s were transduced with shRNA against AHR, IDO1, and TDO2. To generate TDO2 overexpression (OE), COV504 cells were transduced with an empty vector (EV) or TDO2 OE lentivirus. Forced suspension cultures were grown on plates coated with 12μg/mL poly-HEMA. Invasion assays were performed with transwell filters coated with 200μg/mL Cultrex matrix. A student's t-test was used to compare 2 groups, and an ANOVA was used to compare >2 groups. Error bars show standard deviation for in vitro studies and SEM for in vivo studies.

Figure 3. Interrogation of the TDO2 in ovarian cancer cells. A) qRT-PCR validation of OVCA3 KD cells. Internal control, 18S. B) Cell confluence imaged for 72 hrs. C) Cells cultured in suspension for 7 days. DNA content measured at day 1 and 7. D) TRP and KYN levels in conditioned media (CM) from shCtrl and shKD cells over 24 hrs via mass spec. E) Immunofluorescence live images. F) Growth assay, and H) Invasion assay of COV504 EV and TDO2 OE cells. *p<0.05, **p<0.01, ***p<0.001.

Figure 4. A dual IDO/TDO2 inhibitor in a syngeneic mouse model. A) Study design. B) Representative tumor images, C) MRI images (dotted lines), and D) tumor weight (n=7/group). E) Blinded binning of macrophage (F4/80+) infiltration. F) TIMER2 analysis of TDO2 and M2 pro-tumor macrophage infiltration.

Figure 5. PD-L1 and immune cells are decreased by a dual IDO/TDO2 inhibitor. Ascents from vehicle and AT-0174 treated BR-Luc tumors was assessed via flow cytometry. Percentage of A) total macrophages, B) PD-L1 macroage MFI, C) ratio of MHCII+ M1 to PD-L1+MertK+ M2 macrophages, D) % of PMN-MDSCs, E) PD-L1 MFI on PMN-MDSCs, and F) % PD-L1+ PMN-MDSCs.

Figure 1. Inflammatory EOC tumor microenvironment is associated with altered metabolism. A) IL6 ELISA of ascites from 52 EOC patients. Red box = IL6 “high” and blue box = IL6 “low.” B) Kynurenine metabolomics are shown from 10 IL6 high and 10 IL6 low ascites samples. C) Primary tumors were analyzed via multispectral IHC. IL6 low and high tumors shown. D) IHC: analyses of tumor-associated macrophages (MacPs.) from IL6 low and high tumors. *p<0.05 unpaired t-test.