Targeting tryptophan catabolism in ovarian cancer

Lyndsey S. Crump[1], Jessica L. Floyd[2], Miriam D. Post[1], Li-Wei Kuo[1], Alan M. Elder[3], Bradley Corr[2], Elizabeth Woodruff[4], Jennifer K. Richer[1], Benjamin G. Bitler[4]

[1]Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, CO; [2] Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Colorado Anschutz Medical Campus; [3]Division of Medical Oncology, Department of Medicine; [4]Division of Reproductive Sciences, Department of Obstetrics and Gynecology, University of Colorado Anschutz Medical Campus, Aurora, CO

Abstract

Epithelial ovarian cancer (EOC) is the deadliest gynecologic malignancy, with few effective therapeutic options for chemo-resistant disease. Additionally, immunotherapy has had limited efficacy in ovarian cancer[1], and the 5-year survival rate is only ~30%[2]. Tumor metabolism and immune-modulatory metabolites 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[3]. 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 catabolize 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[4], 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.

Hypothesis

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

Methods

Cytokine profiling was performed using primary human ovarian cancer specimens and ascites from the CU Cancer Center Gynecologic Tumor and Fluid Bank. To generate knockdown (KD), OVCAR3s 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.

KYN is Detectable in Inflammatory EOC

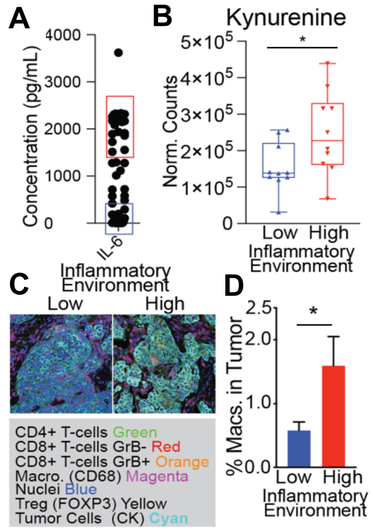

Figure 1. Inflammatory EOC tumor microenvironment is associated with altered metabolism. A) IL6 cytokine ELISA of ascites from 52 EOC patients. Red box = IL6 "high" and blue box = IL6 "low." **B)** Kynurenine metabolomics are shown from 10 IL6 high and 10 IL6 low ascites samples. **C)** Primary tumors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

TDO2 Dependency in Ovarian Cancer Cells

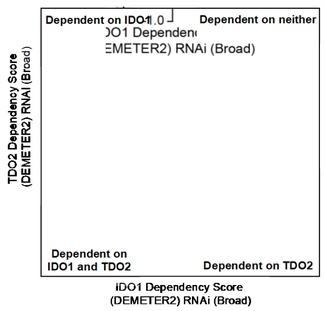

Figure 2. EOC cells are dependent on *TDO2*. DepMap data (The Broad Institute) from 551 cancer cell lines. TDO2 (y-axis) and IDO1 (x-axis) dependency scores. Red dots represent EOC cell lines. Black dots represent all other cancer cell lines.

TDO2 Promotes Cancer Hallmarks *In Vitro*

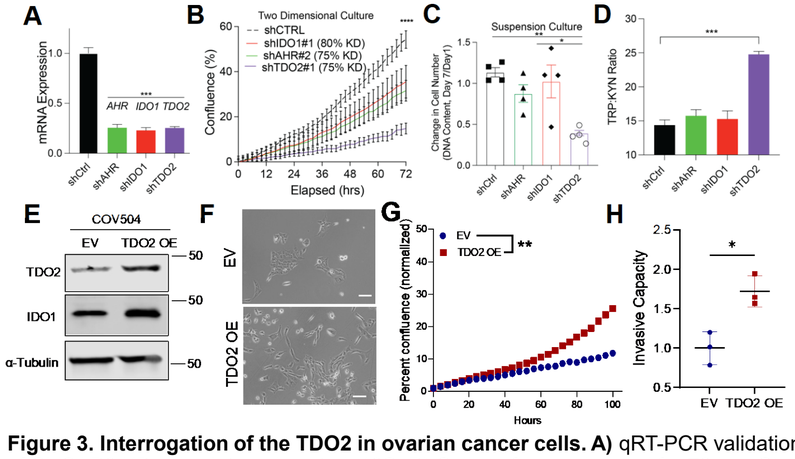

Figure 3. Interrogation of the TDO2 in ovarian cancer cells. A) qRT-PCR validation of OVCAR3 KD cells. Internal control, 18S. **B)** Cell confluence imaged for 72 hrs. **C)** Cells cultured in suspension for 7 days. DNA content measured at day 1 and 7. **D)** TRP and KYN levels in conditioned media (CM) from shCtrl and shKD cells over 24 hrs via mass spec. **E)** Immunoblot, **F)** live images, **G)** growth assay, and **H)** invasion assay of COV504 EV and TDO2 OE cells. *p<0.05, **p<0.01, ***p<0.001

Efficacy of TDO2/IDO Inhibition *In Vivo*

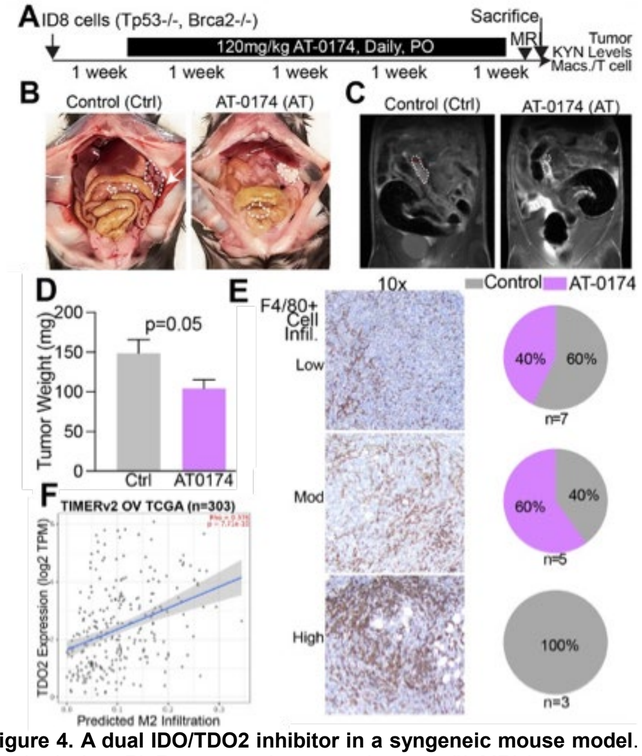

Figure 4. A dual IDO/TDO2 inhibitor in a syngeneic mouse model. A) Study design. **B)** Representative tumor images, **C)** MRI images (dotted lines), and **D)** tumor weight (n=7-9/group). **E)** Blinded binning of macrophage (F4/80+) infiltration. **F)** TIMERv2 analysis of TDO2 and "M2" pro-tumor macrophage infiltration.

TDO2/IDO Inhibition Alters Immune Cells

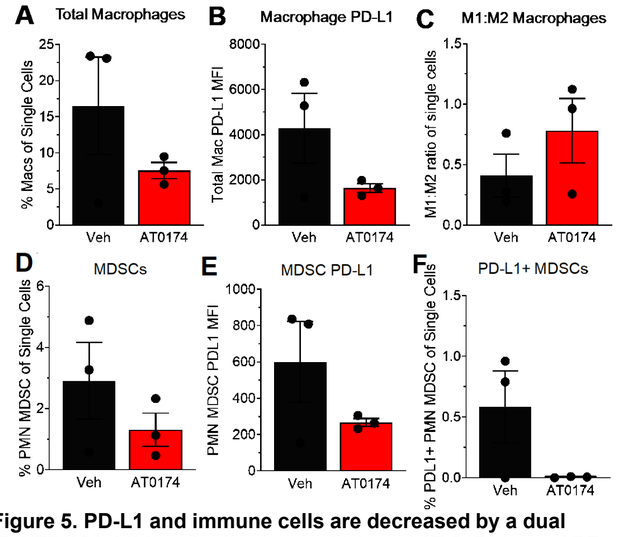

Figure 5. PD-L1 and immune cells are decreased by a dual IDO/TDO2 inhibitor. Ascites from vehicle and AT-0174 treated BR-Luc tumors was assessed via flow cytometry. Percentage of **A)** macrophages, **B)** PD-L1 macrophage MFI, **C)** ratio of MHCII+ "M1" to PD-L1+MerTK+ "M2" macrophages, **D)** % of 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

1. Zhu J, Yan L, Wang Q. Efficacy of PD-1/PD-L1 inhibitors in ovarian cancer: a meta-analysis. *J Ovarian Res*. 2021;14(1):112.
2. Zheng P, Zheng P, Chen G. Conditional Survival of Advanced Epithelial Ovarian Cancer: A Real-World Data Retrospective Cohort Study From the SEER Database. *Front Med*. 2021 Nov 23;8:756401.
3. Smith, L.P., et al., Tryptophan catabolism in epithelial ovarian carcinoma. *Trends Cancer Res*, 2019. 14: p.1-9.
4. Kristeleit, R., et al., A randomised, open-label, phase 2 study of the IDO1 inhibitor epacadostat versus tamoxifen as therapy for biochemically recurrent epithelial ovarian cancer, primary peritoneal carcinoma, or fallopian tube cancer. *Gynecol Oncol*, 2017. 146(3): p.484-490.

Acknowledgements

AT-0174 provided by Antido Therapeutics. This work was supported by the HERA Ovarian Cancer Foundation, the Cancer League of Colorado, The Department of Defense, The American Cancer Society, the NIH/NCI, and the Ovarian Cancer Research Alliance. We acknowledge contributions from the Kay L. Dunton Endowed Memorial Professorship, the McClintock-Addlesperger Family, Karen M. Jennison, the Mohler Johnson Family, Michael Intagliata, Duane and Denise Suess, Mary Normandin, and Donald Engelstad. We also acknowledge the CU Cancer Center, the Cell Technologies Shared Resource, the Flow Cytometry Shared Resource, and the Metabolomics Shared Resource.