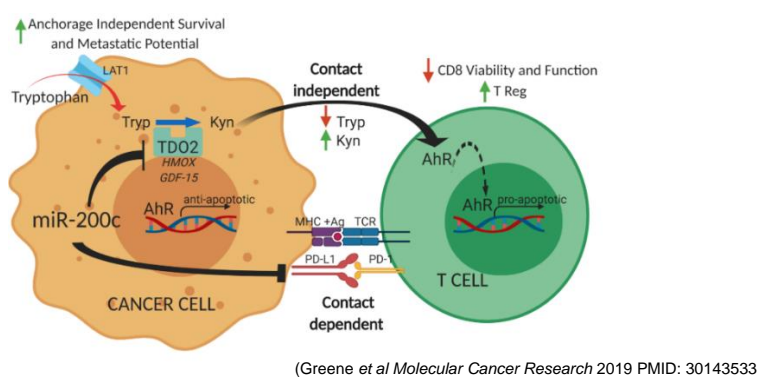


Abstract

Triple negative breast cancers (TNBCs) account for 10-15% of all BC cases and has a rapid rate of relapse as metastatic disease in the first five years after diagnosis. TNBC cell lines exhibit better anchorage independent survival than estrogen receptor alpha (ER) positive lines and are thus resistant to anoikis (or death induced by detachment) exhibited by normal epithelial cells. TNBCs exhibit higher infiltration of immune cells, but also higher expression of PD-L1 and other signs of immune-suppression. The Richer lab reported an increase in tryptophan catabolism in TNBC lines under anchorage independent conditions via the tryptophan metabolizing enzyme Tryptophan 2,3-Dioxygenase (TDO2), which increases in TNBC under anchorage independent conditions and with NFkB activation^{1,2,3}. In addition, tryptophan catabolism was associated with high androgen receptor (AR) expression in TNBC⁴, which correlates to a poor response to chemotherapy. The regulation of the tryptophan catabolism under anchorage independent conditions and how it promotes TNBC progression is poorly understood. Drugs targeting TDO2 are in early stages of development. Here, we conduct tryptophan tracing in a high AR TNBC cell line in attached or suspended conditions and following activation of NFkB via inflammatory cytokines. We find that a novel TDO2 inhibitor significantly reduced production of the tryptophan catabolites kynurenine and formyl-kynurenine that are known to decrease the viability of cytotoxic T cells. Single cell RNA sequencing identified surface markers CD9 and CD24 as positively associated with TDO2 expression. Our results reveal new ways to study and target tryptophan catabolism in TNBC.

Working Hypothesis

Tryptophan catabolism promotes anchorage independence and immune suppression in TNBC, and targeting this pathway may reduce metastasis and enhance immune function.



Background

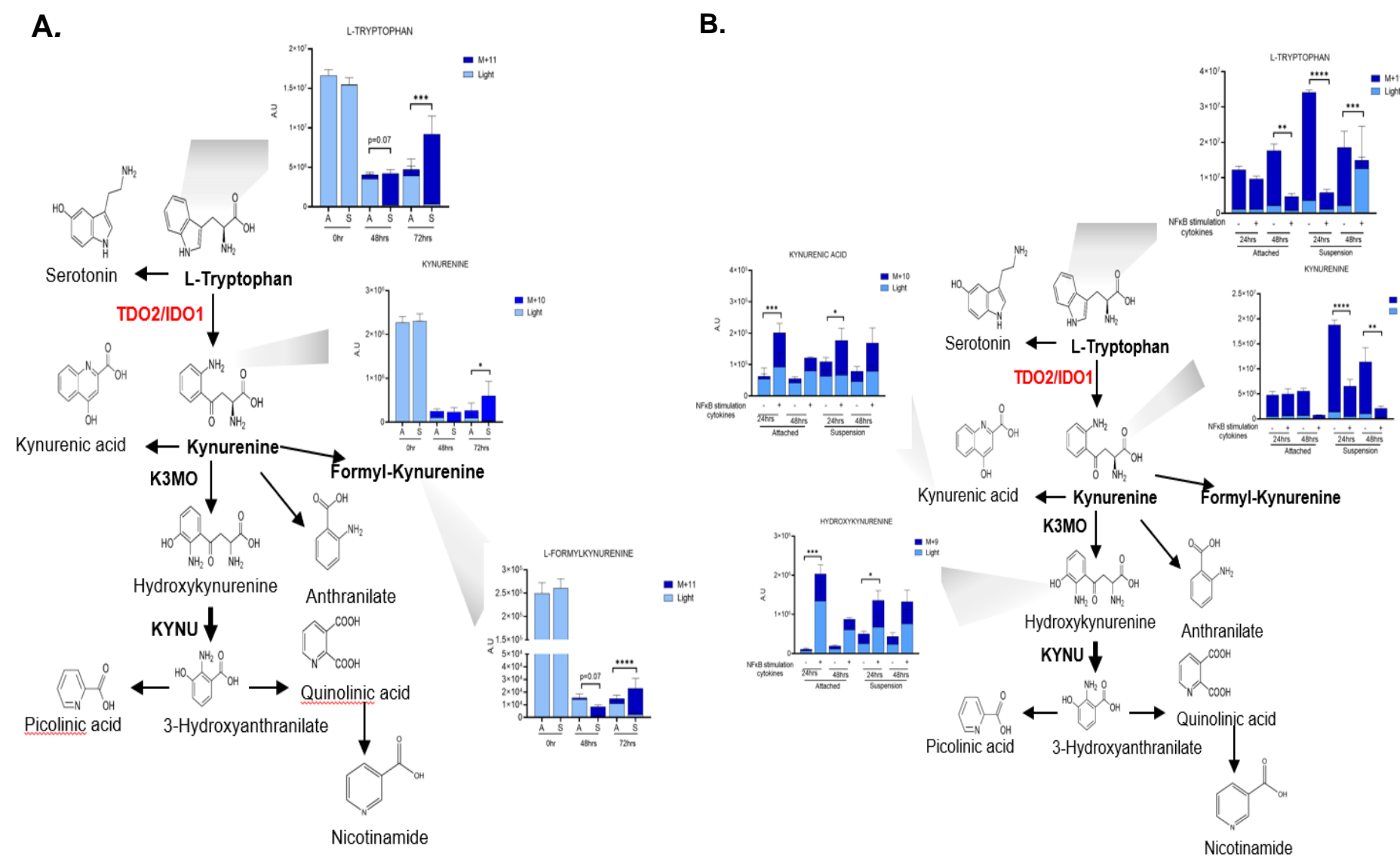
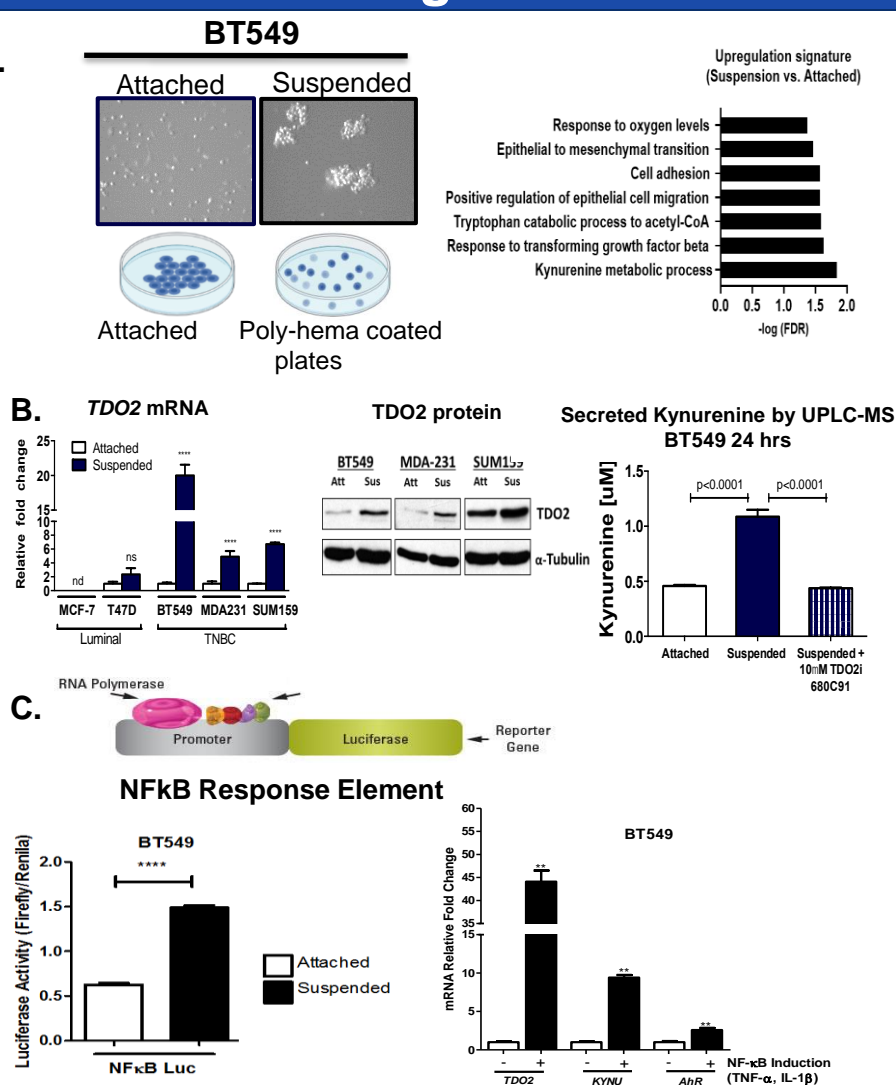


Figure 1. Tracing of ¹³C labeled tryptophan demonstrates that tryptophan catabolism increases when TNBC cells are cultured under anchorage independent conditions and increases with NFkB activating cytokines. A. MDA-MB-453 were cultured in tryptophan depleted media in attached versus suspended conditions and cells harvested at 0, 48 and 72 hours after addition of ¹³C labeled tryptophan. B. MDA-MB-453 were cultured in regular attached and suspension then treated with the NFkB activating cytokines TNFα and IL1β. Statistical analysis was conducted by Two-way ANOVA defined *: p<0.05, **:p<0.01, ***p<0.001, ****p<0.0001 per formed biological triplicates. The composited graph with dark blue: heavy/labeled isotope and light blue: light/unlabeled.

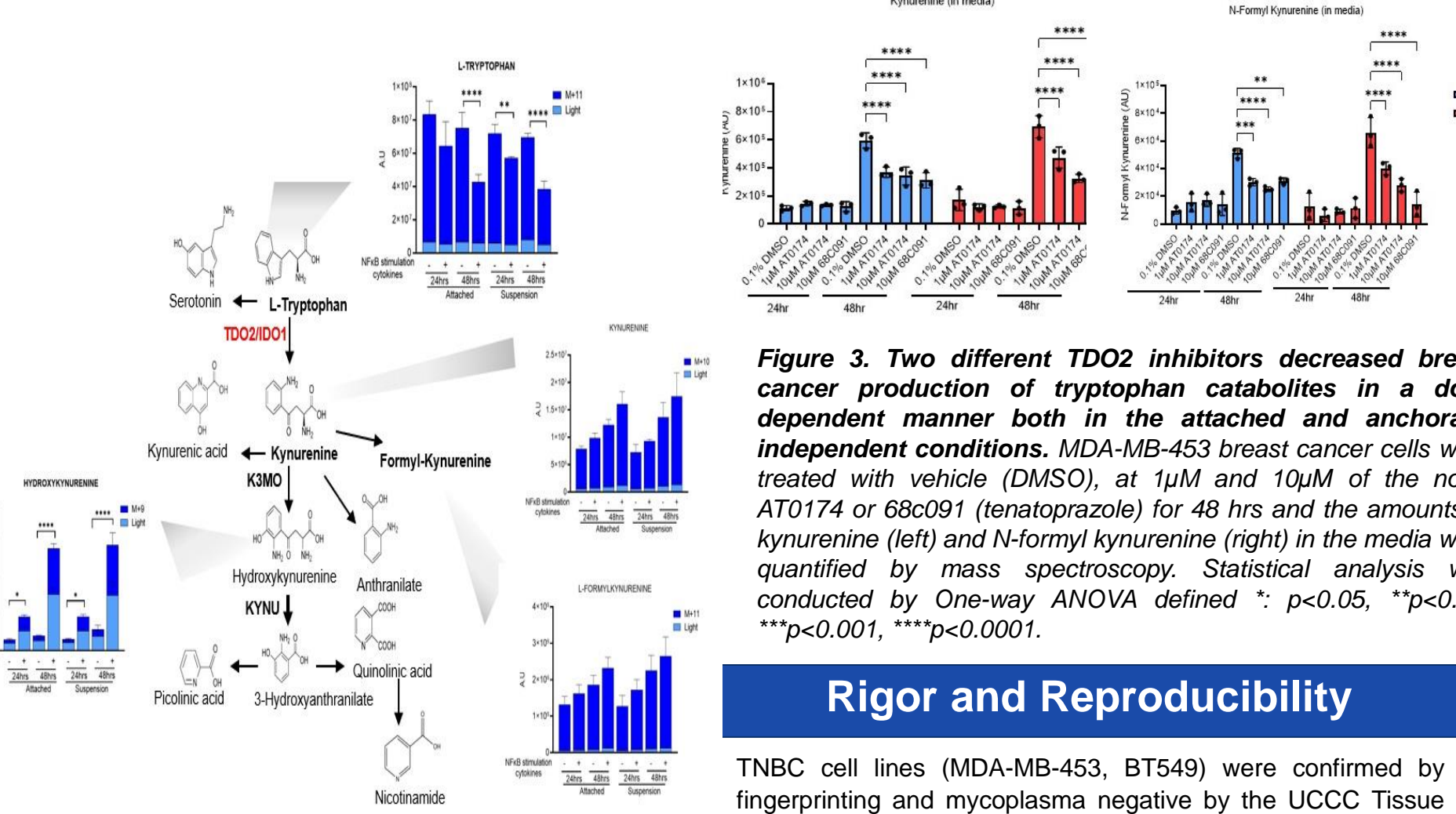


Figure 3. Two different TDO2 inhibitors decreased breast cancer production of tryptophan catabolites in a dose dependent manner both in the attached and anchorage independent conditions. MDA-MB-453 breast cancer cells were treated with vehicle (DMSO), at 1μM and 10μM of the novel AT0174 or 68c091 (tenatoprazole) for 48 hrs and the amounts of kynurenine (left) and N-formyl kynurenine (right) in the media were quantified by mass spectrometry. Statistical analysis was conducted by One-way ANOVA defined *: p<0.05, **:p<0.01, ***p<0.001, ****p<0.0001.

Rigor and Reproducibility

TNBC cell lines (MDA-MB-453, BT549) were confirmed by fingerprinting and mycoplasma negative by the UCCC Tissue Culture Shared Resource. Experiments were conducted in biological triplicate and analyze by One-way ANOVA or Two-way ANOVA if group >2.

Acknowledgements and Funding

We acknowledge the UCCC Mass Spectrometry Metabolomics Shared Resource Facility at CU Anschutz Medical Campus- Dr. Angelo D'Alessandro, Dr. Julie Haines and Micaela Roy for their contribution to analyze the samples. The novel TDO2/IDO dual inhibitor-AT0174 was provided by Antido Therapeutics. The single cell RNA sequencing was conducted in the UCCC Functional Genomic Core Facility and analyzed by Andrew Goodspeed. This project is supported by DOD BCRP- W81XWH-20-1-0617 (to JKR).

Results

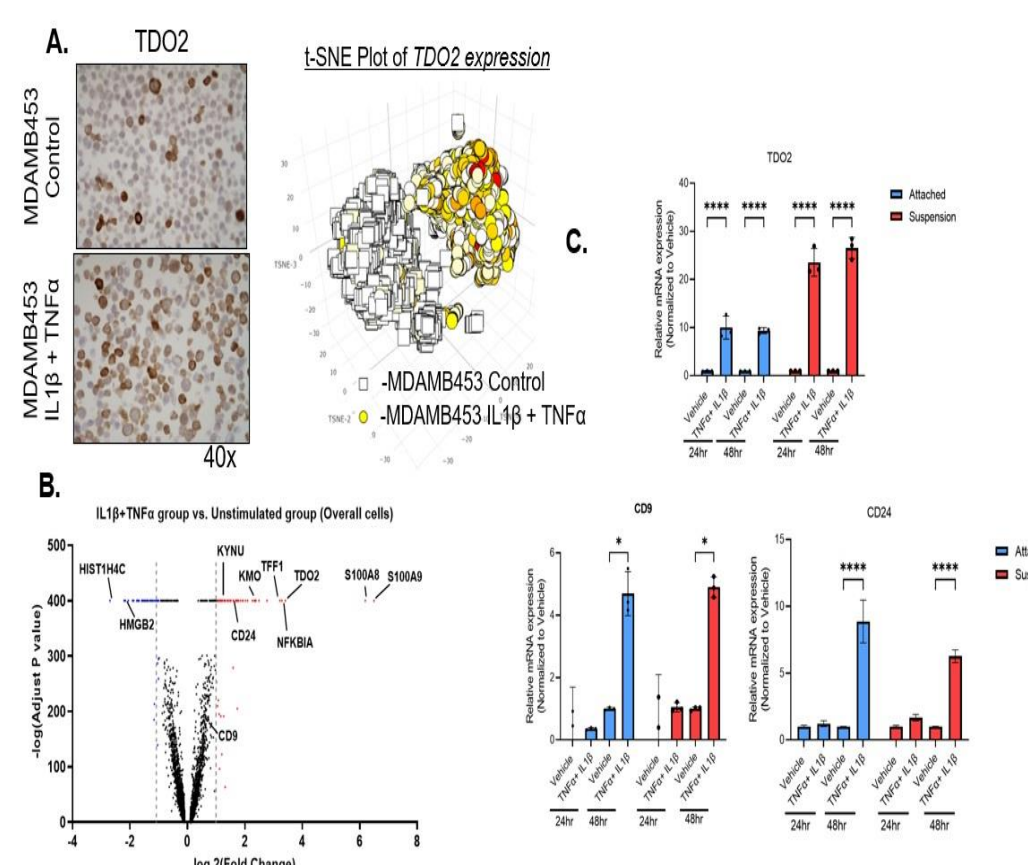


Figure 4 TDO2 increases in TNBC cell lines following NFkB stimulation, but the protein is only expressed in specific cells and CD9 and CD24 are elevated in cells with high TDO2. A. MDA-MB-453 cells treated with 10 ng/mL IL1β + 10 ng/mL TNFα or vehicle were harvested at 24 hours and IHC for TDO2 performed. Over 3,000 cells were captured/sample and sequenced at a depth of ~70,000 reads/cell and tSNE plot colored for TDO2 (square = control treated cells; circle = IL1β + TNFα treated cells). B. A volcano plot shows that CD9, CD24 and TFF1 increase significantly with TDO2 in the MDA-MB-453 cells. C. qRT-PCR validation for CD9 and CD24.

Conclusions

- MDA-MB 453 cells increased uptake of tryptophan and production of immune suppressive catabolites under anchorage independent conditions and NFkB activation.
- The TDO2/IDO inhibitor AT0174 reduced uptake of tryptophan and production of secreted downstream metabolites.
- CD24 or CD9 may serve as potential surface markers to isolate and study TDO2+ TNBC cells.

Future directions

- Explore the tryptophan transporter- LAT1 (SLC7A5) in TNBC in attached versus anchorage independent conditions and with and without NFkB activation.
- Determine the effects of tryptophan depletion and kynurenine production on macrophage levels, polarization and function.
- Test the novel TDO2 inhibitor (AT0174) in vivo in syngeneic mice and humanized mice, alone and with checkpoint inhibitor.

References

- Rogers, T.J. et al. Reversal of Triple-Negative Breast Cancer EMT by miR-200c Decreases Tryptophan Catabolism and a Program of Immunosuppression. Molecular Cancer Research 17, 30-41 (2019).
- Greene, L.I. et al. A Role for Tryptophan-2,3-dioxygenase in CD8 T-cell Suppression and Evidence of Tryptophan Catabolism in Breast Cancer Patient Plasma. Molecular Cancer Research 17, 131-139 (2019).
- D'Amato, N.C. et al. A TDO2-AhR Signaling Axis Facilitates Anoikis Resistance and Metastasis in Triple-Negative Breast Cancer. Cancer Research 75, 4651-4664 (2015).
- Lehmann, B.D. et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. Journal of Clinical Investigation 121, 2750-2767 (2011).