Northwestern Medicine | Robert H. Lurie Comprehensive Cancer Center of Northwestern University | Lou and Jean Malnati Brain Tumor Institute

NON-ENZYME IDO1 ACTIVITY AND ITS IMMUNOSUPPRESSIVE EFFECTS IN GLIOBLASTOMA

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INTRODUCTION

- Glioblastoma (GBM) is the most common primary malignant brain tumor in adults with a median survival of 15-20 months.
- GBM-induced immunosuppression is a significant obstacle that prevents patients from immunotherapy-related survival benefits[1].
- Indoleamine 2,3 dioxygenase 1 (IDO1) is highly expressed in GBM[2] and historically characterized as an immunosuppressive enzyme that converts the essential amino acid, tryptophan (Trp), into downstream metabolites referred to as kynurenines (Kyn)[3].
- Our previous work demonstrates that syngeneic, immunocompetent mice genetically knocked down for IDO1 in mouse GBM cells, ablates tumor-infiltrating Treg accumulation and increases overall survival[4].
- Mouse GBM cells do not engage in Trp metabolism, even through they possess endogenous IDO1 expression[6,7].
- Independent work has shown that mouse plasmacytoid dendritic cells increase Treg levels and enhance immunosuppression independent of IDO1 metabolism[7].
- These data collectively question the role of IDO1 enzyme activity in contributing toward GBM-induced immunosuppression.

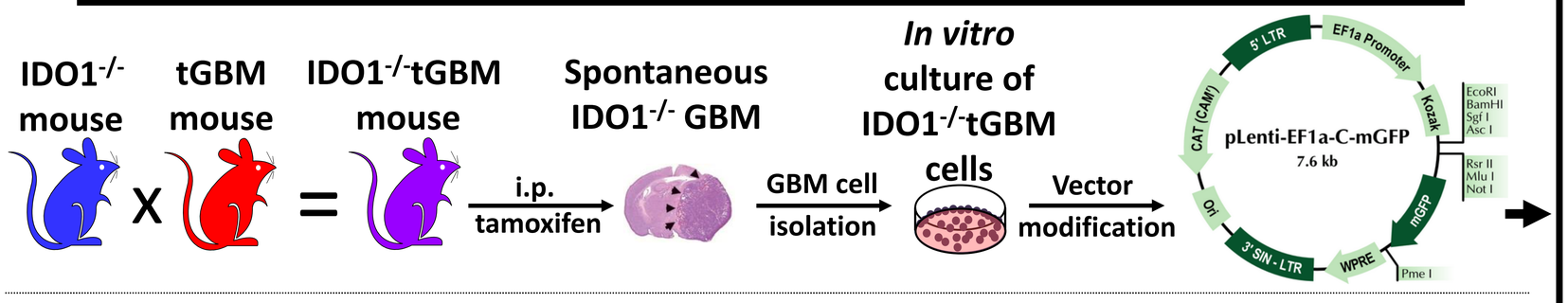
AIMS & SIGNIFICANCE

Create wild-type and enzyme null IDO1-expressing glioma cell lines to re-evaluate the REQUIREMENT FOR IDO1 ENZYME ACTIVITY in GBM-induced immunosuppression.

METHODS

- Transgenic mice that spontaneously develop glioma [tGBM; *GFAP*(*ERT2*)→*Cre*$^{+/-}$; *Pten*$^{fl/fl}$; *Rb*$^{fl/fl}$; *p53*$^{fl/fl}$] were provided by Dr. Suzanne Baker, PhD (St. Jude Children's Research Hospital, Memphis, TN). *Ido1*$^{-/-}$ mice were initially mated with the tGBM founder line to obtain *Ido1*$^{-/-}$ tGBM mice.
- The spontaneous mouse model of GBM was induced by 2 intraperitoneal injections of tamoxifen (TMX, 135mg/kg body weight) at +21, +22 days post-natal into [GFAP(ERT2)→Cre$^{+/-}$; pTEN$^{fl/fl}$; Rb$^{fl/fl}$; p53$^{fl/fl}$; ± IDO1$^{-/-}$)] mice.
- The orthotopic mouse model of GBM was performed by intracranial (ic.) injection of tGBM cells (2x10^5 cells) into syngeneic mice.
- Wild-type mIDO1-GFP encoding lentiviral plasmid was purchased from GeneCopoeia. Amino acid mutagenesis was performed using QuickChage II XL site-mutagenesis kit (Agilent).
- Cell proliferation was measured by MTT Cell Proliferation Assay Kit (Trevigen)
- Plasma and tissue Trp and Kyn. were analyzed by high-performance liquid chromatography (HPLC) using an ESA Coulochem II detector with a 5041 Enhanced Analytical cell containing a glassy carbon electrode (+600 mV).

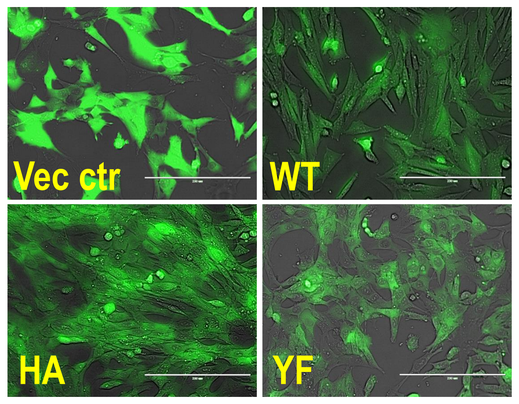
Schema for creating and testing enzyme null IDO1-expressing mouse GBM cells



Vector descriptions

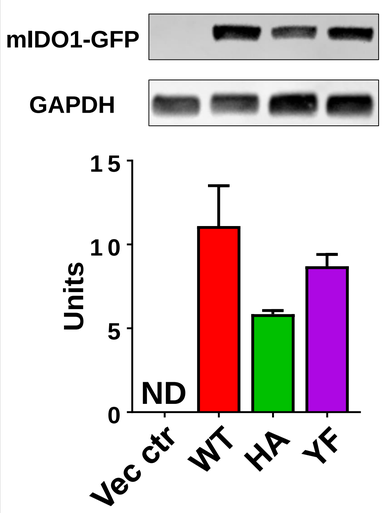
- Vector control (Vec ctr)
- Wild-type mIDO1 (WT)
- H350A mIDO1 (HA)
- Y115/253F mIDO1 (YF)

Vector transduction → *In vitro* culture of IDO1$^{-/-}$tGBM cells

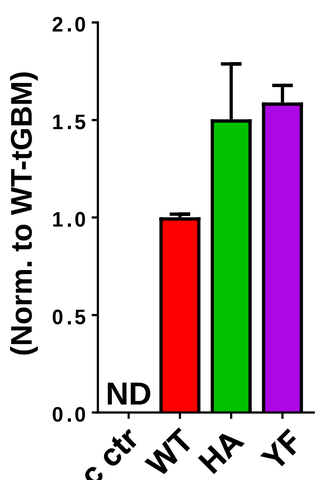


Intracranial injection of genetically modified IDO1$^{-/-}$tGBM cells → IDO1$^{-/-}$tGBM mouse

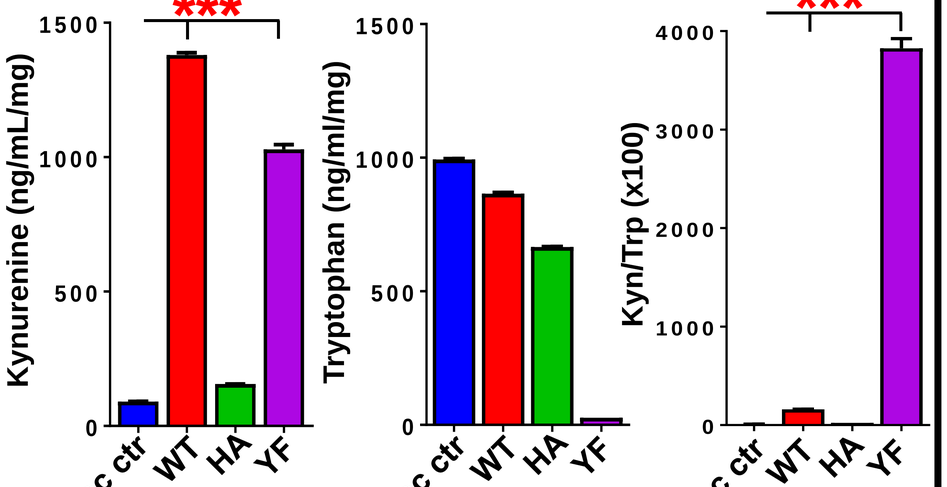
IDO1 Protein (*In vitro*)



IDO1 mRNA (*In vitro*)

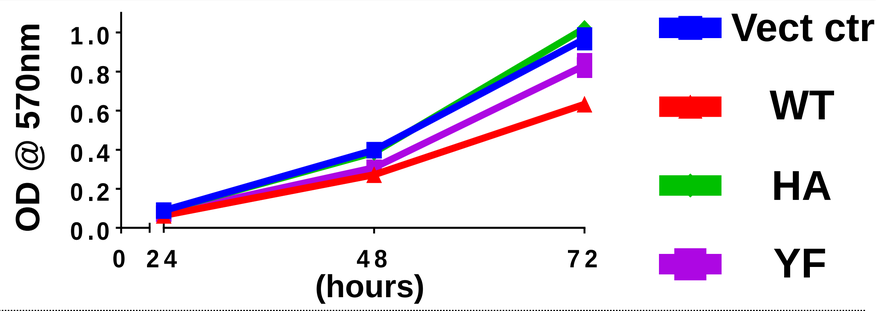


IDO1 Metabolism (*In Vitro*)



IDO1 increases GBM-infiltrating Tregs independent of enzyme activity

***In Vitro* cell growth of tGBM cells genetically modified for IDO1 expression**



GBM-infiltrating lymphocyte (GIL) levels

Intracranial injection of genetically modified IDO1$^{-/-}$tGBM cells → IDO1$^{-/-}$tGBM mouse → Orthotopic GBM → 4-wk post-ic. → GILs

CONCLUSIONS

- **Novel mouse GBM cell lines expressing GFP-tagged mIDO1 WT, enzyme-null (HA), and ITIM-null (YF) were created, for syngeneic, intracranial xenografting into IDO1$^{-/-}$ immunocompetent mice.**
- **The GFP-tag fused to mIDO1 does not interfere with Trp metabolism in IDO1$^{-/-}$tGBM cells.**
- **Substitution for mIDO1 histidine 350 to alanine disabled enzyme conversion of tryptophan into kynurenine.**
- **mIDO1 has no effect on tGBM cell growth.**
- **Mouse GBM cell IDO1 increased Treg accumulation in brain tumors independent of Trp metabolism, *in vivo*.**

FUTURE PLAN

- **Evaluate the *in vivo* metabolism of mIDO1 WT, enzyme-null (HA), and ITIM-null (YF) tumors in IDO1$^{-/-}$, syngeneic immunocompetent mice.**
- **Evaluate overall survival in syngeneic immunocompetent mice with intracranial mIDO1 WT, enzyme-null (HA), and ITIM-null (YF) tumors.**
- **Interrogate the effect(s) of WT, enzyme-null (HA), and ITIM-null (YF) tGBM cells on Treg differentiation in an *in vitro* co-culture system.**

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