Side-by-side comparison of small molecule IDO1 inhibitors in biochemical and cell-based assays and development of an *IDO1*-expressing mouse model to evaluate target modulation

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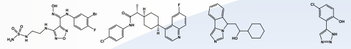
Introduction

- Indoleamine 2,3-dioxygenase (IDO1) is an important drug target for cancer immunotherapy and is associated with resistance to PD-L1-targeted therapies [1, 2].
- IDO1 converts L-tryptophan into N-formylkynurenine (NFK). Depletion of L-tryptophan levels induces immune tolerance by suppression of effector T-cells and natural killer cells, and activation of regulatory immune cells [1].
- Five small molecule inhibitors are currently investigated in clinical phase III (linrodostat/BMS-986205), phase II (epacadostat/INCB024360) or phase I (MK7162, KHK2455 and LY3381916).
- Here, we compare the *in vitro* potency and selectivity of important IDO1 inhibitor classes, and study two inhibitors in a mouse model to evaluate target modulation.

Methods

- Inhibitors were evaluated in biochemical assays using recombinantly expressed human and mouse IDO1 and TDO, and in cell-based assays with cancer cell lines and cell lines stably overexpressing either the human or mouse *IDO1* or *TDO2* gene.
- To evaluate the effect of IDO1 expression on tumor growth, a syngeneic mouse model using mouse *IDO1*-overexpressing B16F10 melanoma cells was developed in collaboration with Charles River. Modulation of L-tryptophan and L-kynurenine levels upon treatment with IDO1 inhibitor was determined with liquid chromatography - tandem mass spectrometry (LC-MS/MS).

Table 1: Overview of IDO1 inhibitor potencies of clinical and reference IDO1 inhibitors in a panel of biochemical and functional cell-based assays.



Inhibition of NFK formation (IC_{50} in nM)	epacadostat	linrodostat	navoximod analog	compound 6 [3]
IDO1				
Human IDO1 biochemical assay	20	> 31600	76	220
IFNγ-stimulated A375 melanoma cell line	19	2.1	160	12
IFNγ-stimulated human whole blood	79	67	2200	1700
Human IDO1-overexpressing HEK293 cell line	11	1.7	330	28
Patient-derived ovarian cancer cells (ASC 009)	5.4	0.69	360	100
Mouse IDO1 biochemical assay	53	> 31600	42	33
Mouse IDO1-overexpressing B16F10 cell line	230	24	55	4.7
TDO				
Human TDO biochemical assay	36	> 31600	17	9200
SW48 colon cancer cell line	4200		530	> 31600
Human TDO-overexpressing HEK293 cell line	21000	7900	2700	> 31600
Mouse TDO biochemical assay	200	> 31600	22	29000
Mouse TDO-overexpressing GL-261 cell line	16000	> 31600	710	> 31600

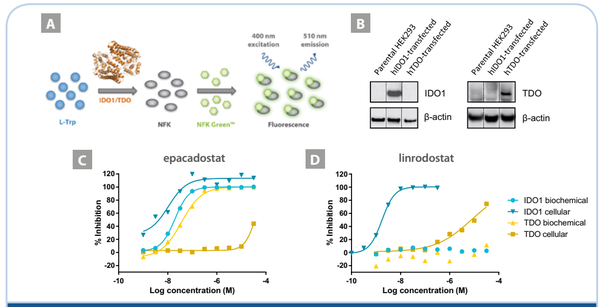


***Figure 1: A**: NFK Green™ assay technology for biochemical and cellular IDO1- and TDO-based assays [4]. **B**: Western blot analysis of hIDO1- and hTDO-transfected HEK293 cell lines used for cellular assays. **C-E**: Inhibition profiles of IDO1 inhibitors in hIDO1 and hTDO biochemical and HEK293 cell-based assays.*

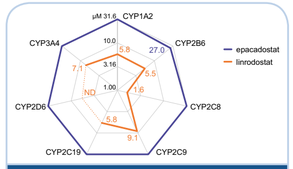


***Figure 2:** Relative inhibitory potencies in LC-MS/MS-based CYP inhibition assays. Data points at the graph border represent > 31.6 μM.*

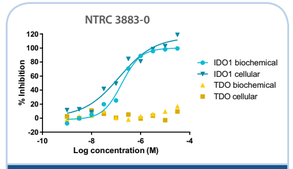


***Figure 3:** Dose-response curves of the IDO1 inhibitor NTRC 3883-0 [5] in hIDO1 and hTDO biochemical and HEK293 cell-based assays.*

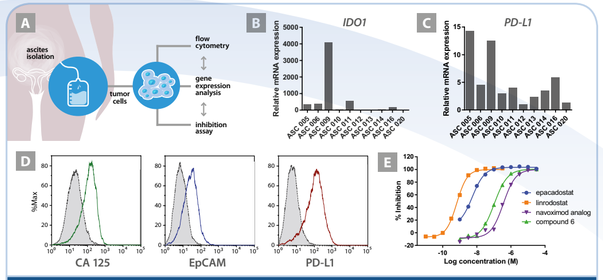


***Figure 4:** IDO1 expression and inhibition in tumor cells isolated from ascites of ovarian cancer patients. **A**: Schematic workflow of experiment. **B and C:** Analysis of IDO1 and PD-L1 mRNA expression in tumor cell samples by quantitative PCR (qPCR). **D**: Analysis of tumor cell markers and PD-L1 in a sample with high IDO1 and PD-L1 expression (ASC 009). **E**: Inhibition of IDO1 activity in sample ASC 009.*

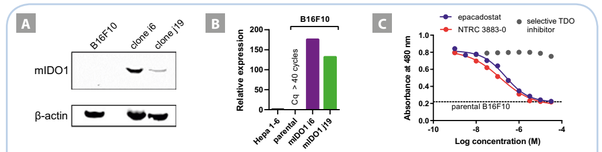


***Figure 5:** Characterization of a subline of the mouse melanoma cell line B16F10 stably expressing mouse IDO1 (mIDO1). **A**: Western blot of the parental B16F10 cell line and two mIDO1-transfected clones. **B**: Analysis of mIDO1 mRNA expression by qPCR. Hepa 1-6 is included as a positive control. **C**: Inhibition of the mIDO1-transfected clone i6 by IDO1 and TDO inhibitors.*

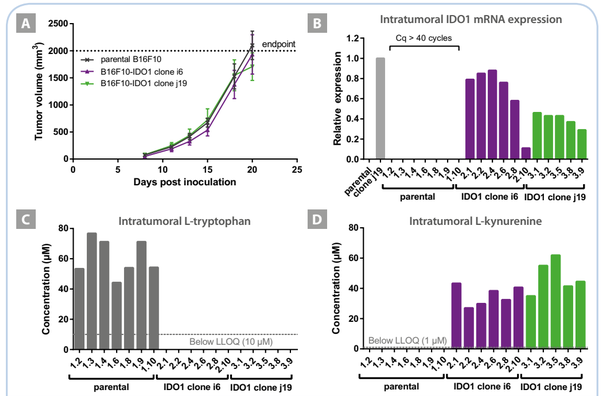


***Figure 6:** Syngeneic mouse model based on IDO1-overexpressing B16F10 cell line. **A**: Expression of IDO1 in B16F10 cells does not affect tumor growth. **B**: Analysis of mIDO1 mRNA expression in tumor tissues by qPCR. **C and D**: IDO1 expression leads to reduced intratumoral L-tryptophan levels and increased L-kynurenine levels in vivo as determined by LC-MS/MS. LLOQ = lower limit of quantification.*

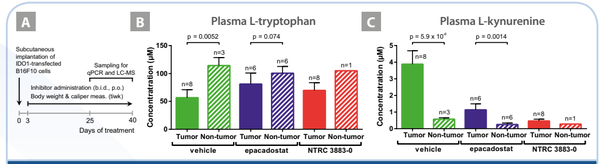


***Figure 7:** Prophylactic administration of IDO1 inhibitors in the IDO1-overexpressing syngeneic mouse model developed using the B16F10 clone j19. **A**: Experimental schedule. **B and C**: Plasma L-tryptophan and L-kynurenine levels are indicative of tumor presence as determined by LC-MS/MS. Significance was tested using a two-sided Student's t-test.*

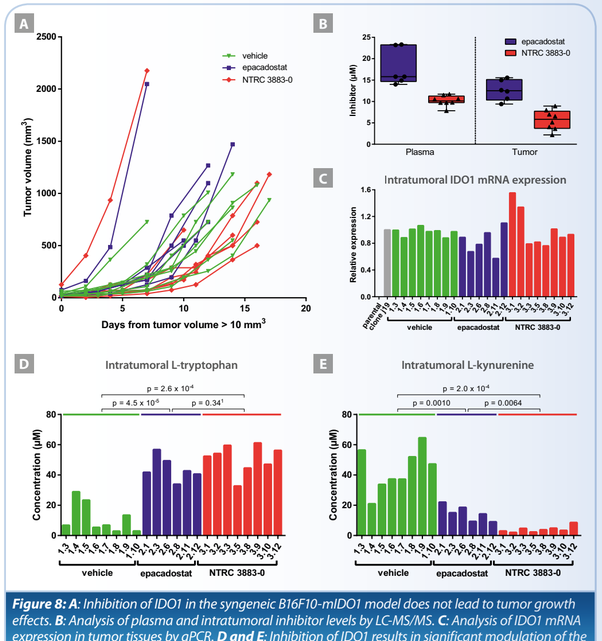


***Figure 8: A**: Inhibition of IDO1 in the syngeneic B16F10-mIDO1 model does not lead to tumor growth effects. **B**: Analysis of plasma and intratumoral inhibitor levels by LC-MS/MS. **C**: Analysis of IDO1 mRNA expression in tumor tissues by qPCR. **D and E**: Inhibition of IDO1 results in significant modulation of the intratumoral L-tryptophan and L-kynurenine levels as determined by LC-MS/MS. Significance was tested using Welch's ANOVA followed by Games-Howell post hoc analysis.*

Conclusion

- The most advanced IDO1 inhibitors - epacadostat and linrodostat - differ significantly in their potency and selectivity over TDO and CYP enzymes.
- IDO1 is expressed in primary cancer cells of a subset of ovarian patients and can be inhibited by IDO1 inhibitors.
- Engraftment of *IDO1*-overexpressing B16F10 melanoma cells in syngeneic mice resulted in reduced L-tryptophan and increased L-kynurenine levels in B16F10-derived tumors and in plasma.
- Treatment with the IDO1 inhibitors epacadostat or NTRC 3883-0 restored intratumoral L-tryptophan and decreased L-kynurenine levels.

References: [1] Holmgaard *et al.* (2015) *Cell Rep.* **13**: 412-424. [2] Gomes *et al.* (2018) *Mol. Cancer. Ther.* **17**:2530-2542. [3] Röhrig *et al.* (2016) *Bioorg. Med. Chem. Letters* **26**: 4330-4333. [4] Seegers *et al.* (2014) *J. Biomol. Screen.* **19**: 1266-1274. [5] de Man *et al.* (2017) WO2017/153459.