HIGHLY POTENT AND SELECTIVE INHIBITORS OF INDOLEAMINE 2,3-DIOXYGENASE (IDO)

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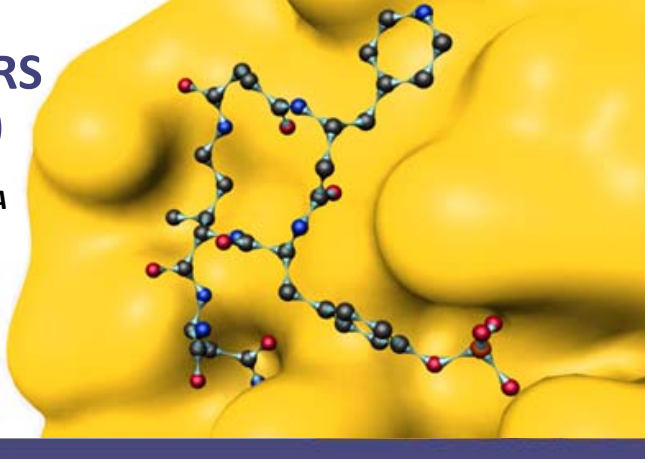
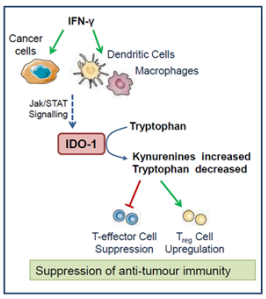
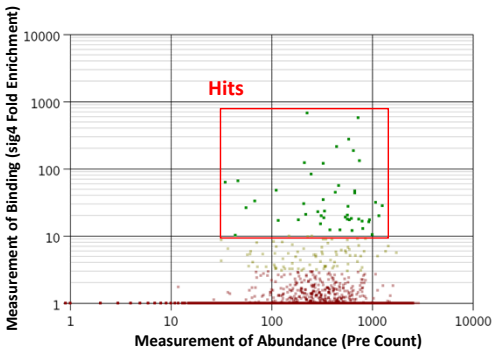


Introduction

Indoleamine 2,3-dioxygenase 1 (IDO-1) is an intracellular immune checkpoint protein that catalyzes the breakdown of tryptophan to *N*-formyl kynurenine (NFK). Dysregulation of this enzyme can result in the suppression of an appropriate immune response to cancer by macrophages and T-cells. Inhibition of IDO-1 has been shown to reinvigorate the natural immune response to cancer cells, and could have utility in diverse forms of cancer such as metastatic melanoma and ovarian cancer.

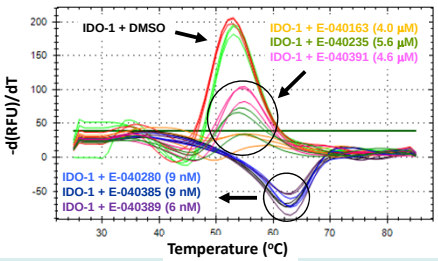


Affinity Based Selection



- Libraries of DNA-encoded molecules were screened against IDO-1 in highly sensitive affinity selection assays.
- PCR and next generation sequencing of results identified 'outliers' that are several fold more prevalent in the post selection sample than in the original library.
- A hit series was chosen based on its enrichment and ability to maintain enrichment in the presence of a competitor (tryptophan).
- The hit compounds were synthesized without DNA tether and tested in a number of *in-vitro* assays.

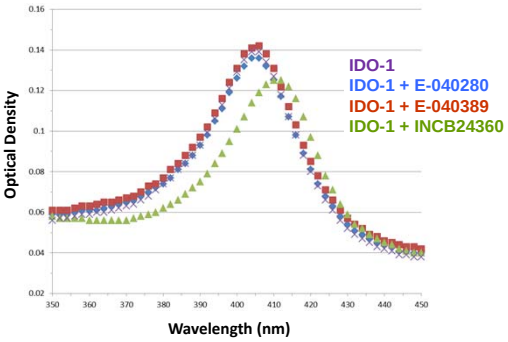
Thermal Stability Assay



A thermal stability assay was undertaken to confirm binding characteristics to IDO-1.

- Clear shift in the temperature of denaturation from 45 °C to 60 °C in the presence of very potent inhibitors E-040280, E-040385 and E-040389.
- No shift in the temperature of denaturation was observed for weakly active inhibitors E-040163, E-040235 and E-040391
- Results confirm that the compounds are binding to IDO-1.

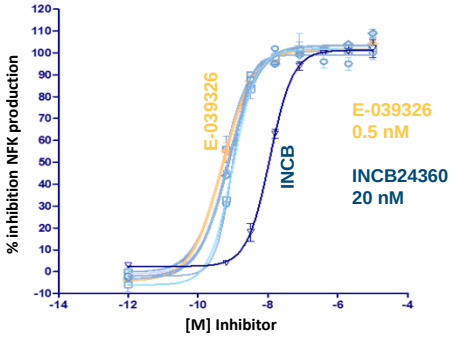
Detection of Heme Binding



Compounds were evaluated for heme binding. A redshift in A_{max} of IDO-1 + inhibitor relative to that of the enzyme itself indicates compound is binding at the heme.

- A clear redshift is observed with INCB24360, a known heme-binding inhibitor of IDO-1.
- No apparent shift in absorbance spectrum is observed for any Ensemble compounds. Results are consistent with our hypothesis of allosteric inhibition.

Hela Cell Assay



Compounds are evaluated for reduction of NFK production in IFN-γ stimulated Hela cells.

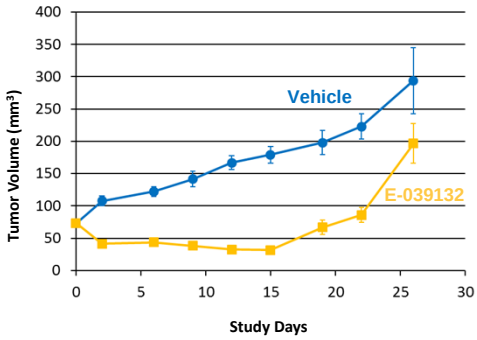
- 40 fold more potent than INCB24360 (Phase II)
- Compounds are the most potent IDO-1 inhibitors reported.
- Lead series demonstrates excellent and predictable structure activity relationships.

Selectivity Profile

Compound	IDO IC_{50} (µM)	TDO IC_{50} (µM)	Fold Selectivity	PanLabs Hit Profiler
E-039132	0.0009	120	133,000	Negative
INCB24360	0.015	5	333	

- IDO inhibitors in the clinic also demonstrate the ability to inhibit the complementary enzyme tryptophan 2,3-dioxygenase (TDO). While the therapeutic value of TDO inhibition is unclear, we believe a highly selective inhibitor of IDO-1 will reduce the toxicity profile. Additionally, an allosteric inhibitor will not be susceptible to non-specific heme interactions, further decreasing the likelihood of off target effects.
- Further investigation into the selectivity of E-039132 revealed no inhibition of 30 protein targets in a Cerep PanLabs Hit Profiling assay at 10 µM.

PD Study: Pan02 Syngeneic Mouse Model



Pharmacodynamic study of E-039132 was undertaken in mice inoculated with a Pan02 (pancreatic cancer) cell line.

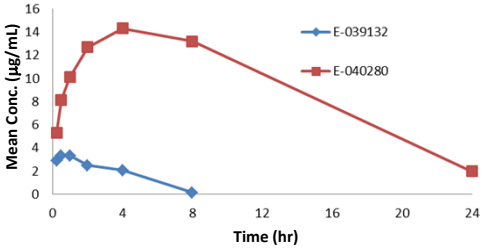
- Dosing: 100 mg/kg PO, BID
- Formulation: 10 mg/mL in 33% solutol in water
- Significant suppression of tumor growth maintained for >2 weeks
- No change in body weight relative to vehicle
- Histopathology revealed no toxic effects to all major organs

Identification of a Development Candidate

	Original Hit		Development Candidate
Cell Activity (nM)	708		9
Solubility (µM)	42		128
Caco-2 AB Papp (10^{-6} cm/sec)	<0.1	10 months	23
%F Rat PO, 10 mg/kg	--		50
Cl Rat IV, 1 mg/kg (mg/mL/kg)	--		9

The program has advanced rapidly to reduce the MW, improve permeability and efflux, while addressing metabolic liabilities.

Advanced candidate demonstrates improved PK



At 100 mg/kg PO in mice, E-040280 has an oral exposure in mouse >15 fold higher than E-039132.
A pharmacodynamic study of E-040280 in mice inoculated with a Pan02 (pancreatic cancer) cell line is currently underway.
The program is currently on track toward nominating a development candidate in Q3 2016.

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