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JHU395, a nervous tissue penetrant glutamine antagonist, restricts growth of malignant peripheral nerve sheath tumor with inhibition of nucleotide synthesis

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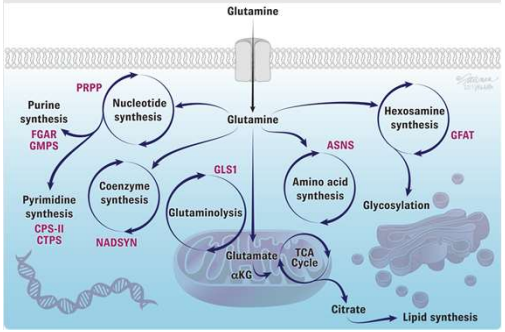
Background

- Malignant peripheral nerve sheath tumor (MPNST) is a deadly sarcoma that occurs in up to 15% of people with the cancer predisposition syndrome neurofibromatosis type I (NF1) and for which there are no effective medical treatments [1].
- Metabolic inhibitors have been underexplored in MPNST; we and others have found that glutamine deprivation inhibits growth of human MPNST cells in culture [2].
- 6-diazo-5-oxo-L-norleucine (DON) is a broadly acting irreversible glutamine antagonist (GA) that inhibits at least 9 glutamine utilizing enzymes in mammalian cells.
- Previous clinical oncology trials of DON in humans were hampered due to dose-limiting gastrointestinal (GI) toxicity [3,4].
- JHU395 is a novel lipophilic DON prodrug that is stable in plasma but delivers active GA to nervous tissue, increasing the brain-to-plasma ratio of DON nearly 10-fold [5].
- We hypothesized that JHU395 would provide a novel, robust, and well-tolerated means to investigate the effect of broad glutamine antagonism on MPNST.

GLUTAMINE: **DON:** clogP = -2.5 **JHU395:** clogP = 2.75



Future Directions

- Investigate JHU395 effect on glutamine-derived nitrogen incorporation to pyrimidines
- Investigate JHU395 combination strategies with nucleotide synthesis inhibitors
- Investigate glutamine utilization and JHU395 sensitivity in additional MPNST models including patient-derived samples

Acknowledgements & Disclosures

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JHU395 is licensed to Dracen Pharmaceuticals, a company pursuing small molecule glutamine antagonists for oncology and immunometabolic applications. Drs. Slusher, Rais, and Majer are co-founders of and hold equity in Dracen Pharmaceuticals, Inc. Under license agreements between Dracen Pharmaceuticals, Inc., the Johns Hopkins University, and the IOCB, Drs. Slusher, Rais, Majer, Tenora and Mr. Alt are entitled to royalty distributions related to technology used in the research described in this presentation. Dr. Nedelcovych served as a consultant to Dracen Pharmaceuticals. These arrangements have been reviewed and approved by the Johns Hopkins University and the IOCB in accordance with institutional conflict of interest policies. The remaining co-authors declare no conflicts of interest.

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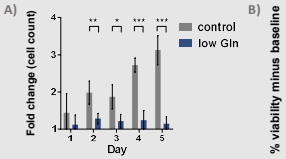
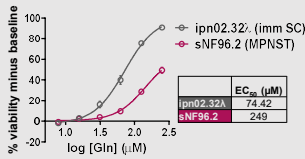
Results

MPNST cells were sensitive to glutamine deprivation and antagonism

MPNST growth was abrogated in low glutamine

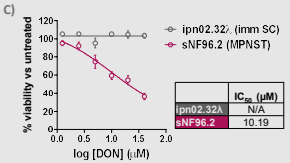


>3-fold higher glutamine required to rescue MPNST



	EC_{50} (μM)
ipn02.3λ	74.42
sNF96.2	249

DON selectively inhibited MPNST growth at μM levels

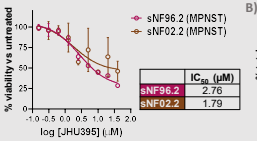


	IC_{50} (μM)
ipn02.3λ	N/A
sNF96.2	10.19

A) Human MPNST cells (sNF96.2) were grown in control (>2mM glutamine) or low (50μM) glutamine media. Live cells were counted daily by trypan blue exclusion assay and normalized to number of cells seeded. **B)** Immortalized Schwann cells from a non-NF1 individual (ipn02.3λ; [6]) and human MPNST cells (sNF96.2) were grown in glutamine-free media and glutamine was titrated in at increasing concentrations. Viable cells were measured at 72 hours by alamar blue fluorescence. **C)** Immortalized Schwann cells (ipn02.3λ) and human MPNST cells (sNF96.2) were treated with DON. Viability was measured at 72 hours by alamar blue fluorescence.

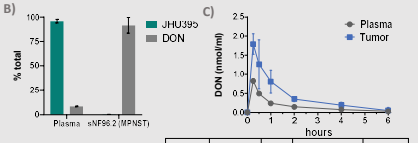
JHU395 delivered glutamine antagonist to MPNST

JHU395 inhibited MPNST growth at μM levels



	IC_{50} (μM)
sNF96.2	2.76
sNF02.2	1.79

JHU395 was stable in plasma and delivered GA to MPNST in vitro and in vivo

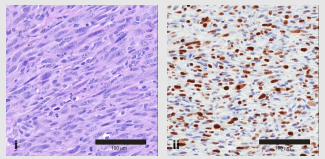


	C_{max} (nmol/ml or nmol/g)	T_{max} (hr)	AUC_{0-t} (hr*nmol/ml or hr*nmol/g)	Ratio (tumor: plasma)
Plasma	0.826	0.25	0.9767	2.57
Tumor	1.793	0.25	2.496	

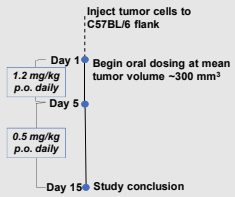
A) Viable MPNST cells (sNF96.2, sNF02.2) following 72h treatment with JHU395 based on alamar blue fluorescence normalized to untreated controls. **B)** JHU395 and DON levels quantified by LC-MS in human plasma and MPNST cells (sNF96.2) following one hour incubation of 20 μM JHU395. Values were normalized to total pool of compound. **C)** DON quantification by LC-MS following oral administration of 1.2 mg/kg JHU395 to flank MPNST mice. Tumor and plasma were harvested at indicated times.

Orally administered JHU395 slowed MPNST growth in a murine flank tumor model without overt GI, liver, or neurotoxicity

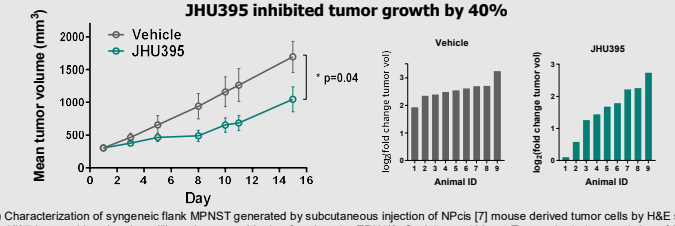
Murine flank tumors capture features of human MPNST



Experimental Design

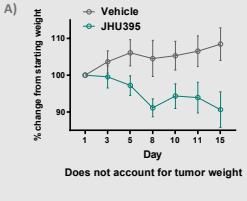


JHU395 inhibited tumor growth by 40%

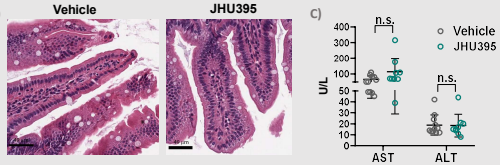


A) Characterization of syngeneic flank MPNST generated by subcutaneous injection of NPcis [7] mouse derived tumor cells by H&E stain (i), Ki67 immunohistochemistry (ii), and western blotting for phospho-ERK1/2. Scalebar = 100μm. Tumors had characteristics of high-grade peripheral nerve sheath tumors with high cellularity, marked nuclear pleomorphism, and brisk mitotic activity, and exhibited many Ki67 positive cells. Tumor cells were positive for phospho-ERK. **B)** Schematic of experimental design for efficacy studies. Tumor volumes were measured ~3x/week after initiation of dosing. **C)** Mean tumor volumes in mice treated daily with vehicle or JHU395 (1.2 mg/kg p.o. x 5 days, 0.5 mg/kg p.o. x 9 days). Right panels show log_2 (fold change) of individual animal tumor volumes for Day 15 vs. Day 1.

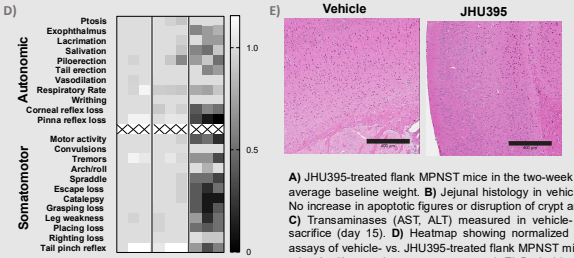
Does not account for tumor weight



No overt GI or liver toxicity observed



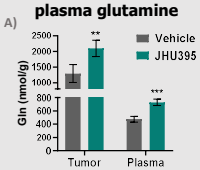
No neurotoxicity observed



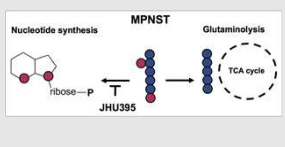
A) JHU395-treated flank MPNST mice in the two-week efficacy experiment remained within 10% of average baseline weight. **B)** Jejunal histology in vehicle- and JHU395-treated flank MPNST mice. No increase in apoptotic figures or disruption of crypt architecture was observed. Scalebar = 49μm. **C)** Transaminases (AST, ALT) measured in vehicle- or JHU395-treated flank MPNST mice at sacrifice (day 15). **D)** Heatmap showing normalized mean scores on modified Irwin behavioral assays of vehicle- vs. JHU395-treated flank MPNST mice. Muscarinic agonist oxotremorine-treated mice (n=5) served as an assay control. **E)** Brain histology in vehicle- (n=3) and JHU395-treated (n=3) flank MPNST mice. No gross changes and no cortical vacuolization were observed. Scalebar = 400 μm. **F)** Normalized forelimb grip strength in vehicle- and JHU395-treated flank MPNST mice.

In vivo JHU395 inhibited glutamine utilization for purine synthesis in MPNST

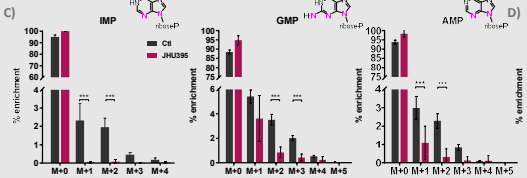
Oral JHU395 increased tumor and plasma glutamine



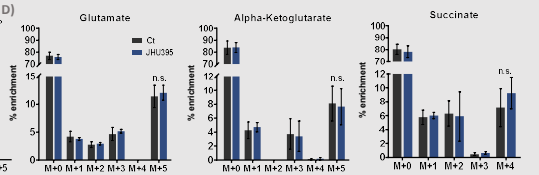
Schematic of stable isotope labeled glutamine flux analysis



$^{15}N_2$-glutamine labeling of purines in MPNST was inhibited by JHU395



$^{13}C_5$-glutamine labeling of TCA cycle intermediates in MPNST was not affected by JHU395



A)Tumor and plasma glutamine quantified in vehicle- and JHU395-treated tumors. **B)** Schematic of in vivo stable isotope labeling using $^{15}N_2$- or $^{13}C_5$-glutamine tracing downstream metabolites in purine synthesis ($^{15}N_2$-glutamine) or to the TCA cycle ($^{13}C_5$-glutamine). **C)** $^{15}N_2$-glutamine labeled JHU395-treated tumors showed significantly lower ^{15}N-enrichment of isotopologues from m+1, m+2 (IMP, AMP) and m+2, m+3 (GMP) pools compared to vehicle-treated tumors. **D)** $^{13}C_5$-glutamine labeled JHU395-treated tumors do not show a difference in m+5 isotopologue enrichment in glutamate or alpha-ketoglutarate or m+4 isotopologue enrichment in succinate compared to vehicle-treated tumors. Data shown is representative of 6-7 animals per treatment over two independent experiments.