Novel prodrugs of the glutamine antagonist 6-diazo-5-oxo-norleucine (DON) as treatment for malignant peripheral nerve sheath tumor

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Highlights

- 6-diazo-5-oxo-norleucine (DON) is a broadly active glutamine antagonist that has been in >10 clinical trials in humans, but had dose limiting GI toxicity.
- Malignant peripheral nerve sheath tumor (MPNST) cell growth is inhibited by glutamine deprivation and by treatment with DON.
- DON affects glutamine-dependent metabolites in MPNST cells, including nucleotide and amino acid synthesis intermediates.
- JHU395 is a novel lipophilic prodrug of DON that improves delivery of DON to nervous system-associated tissues including MPNST.
- Oral administration of JHU395 as monotherapy to mice bearing flank tumors derived from *NF1*$^{+/-}$;*p53*$^{+/-}$ murine MPNST results in 40% smaller tumors compared to vehicle controls with no significant toxicity.
- Future studies will evaluate JHU395 in combination with clinically used sarcoma therapies and investigate MPNST glutamine dependence in animal models by stable isotope resolved metabolomics flux analysis using $^{13}C_5$- or $^{15}N_2$-glutamine.

Abstract

Neurofibromatosis Type I (NF1) is a heritable tumor predisposition syndrome in which up to 10% of patients develop malignant peripheral nerve sheath tumor (MPNST), an aggressive sarcoma. For MPNST that is incompletely resected at diagnosis, traditional cytotoxic chemotherapeutic strategies offer a 5 year event-free survival of less than 40% [1]; thus new therapeutic strategies are desperately needed. Reprogramming of energy metabolism, whereby tumor cells take up more glutamine than healthy cells and direct this substrate to replenish metabolites for proliferation, is a hallmark of several cancers that has not been effectively leveraged for treatment of MPNST [2]. Our group has recently described JHU395, a nervous system penetrant prodrug of the glutamine antagonist 6-diazo-5-oxo-norleucine (DON), which delivers DON preferentially to the brain [3] resulting in less gastrointestinal toxicity, which was the main toxicity of DON in past clinical trials [4]. The primary goals of this study were to evaluate glutamine antagonism and JHU395 activity in MPNST. Using immortalized healthy Schwann [5] and MPNST cell lines we investigated cell proliferation in culture under glutamine deprivation and antagonism. Mass spectrometry (MS)-based metabolomic profiling was used to characterize differences between MPNST cells treated with vehicle versus DON. MS-based bioanalytical methods were also used to investigate DON delivery to tumor cells by JHU395. We found that growth of MPNST cells in culture is preferentially inhibited by glutamine deprivation and DON treatment when compared to immortalized Schwann cells derived from non-tumored nerve (IC_{50} of 8-9 micromolar versus >30 micromolar). Targeted metabolomics analyses of DON treated human MPNST cells demonstrated multiple differences in downstream glutamine-dependent metabolites including intermediates in purine synthesis and amino acid synthesis, suggesting that DON acts broadly within the tumor cell to inhibit growth. While DON showed limited partitioning into MPNST cells versus plasma, JHU395 preferentially delivered DON into MPNST with over 5-fold higher cell-to-plasma ratio. In an MPNST murine flank tumor model [6], mice treated orally with JHU395 had a mean tumor volume >40% smaller than mice treated with vehicle. In conclusion, compared to healthy Schwann cells, MPNST cells have unique vulnerability to antagonizing glutamine utilization. The nervous system directed prodrug JHU395 enhances DON delivery to MPNST and represents a novel potential therapeutic approach for these aggressive tumors. Based on these results we have initiated additional preclinical therapeutic studies using JHU395 in combination with agents with known activity in MPNST.

Acknowledgements

We thank Verena Staedtke for sharing tumor cells extracted from *NPcis* (*NF1*$^{+/-}$;*p53*$^{+/-}$) murine MPNST. We thank Marc Ferrar of NCATS for supplying the immortalized peripheral nerve cell lines and Marigo Stathis (NTAP) for assistance with cell authentication. This work was supported by NIH T32CA060441 (KML) and a TEDCO Maryland Innovation Initiate Award (to BSS).

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2. Sheikh, T.N., et al. Oncotarget, 2017. **8**(55): p. 94054-94068.
3. Nedelcovych, M.T., et al. J Med Chem, 2017. **60**(16): p. 7186-7198.
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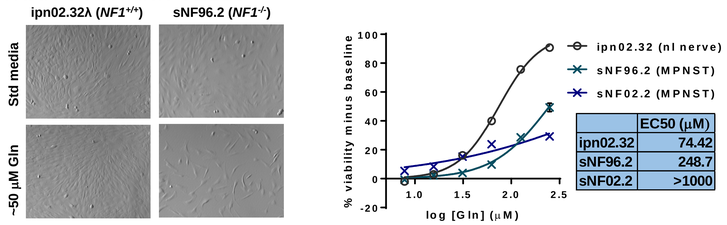
Results

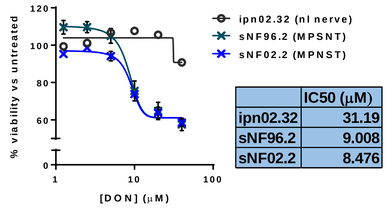
Glutamine Deprivation and 6-Diazo-5-Oxo-Norleucine (DON) Preferentially Inhibit MPNST Cells

A. MPNST CELLS ARE MORE SENSITIVE TO GLUTAMINE DEPRIVATION AND REQUIRE HIGHER GLUTAMINE CONCENTRATION TO RESCUE GROWTH THAN SCHWANN CELLS



	EC50 (µM)
ipn02.32	74.42
sNF96.2	248.7
sNF02.2	>1000

B. GLUTAMINE ANTAGONIST DON PREFERENTIALLY INHIBITS GROWTH OF MPNST CELLS

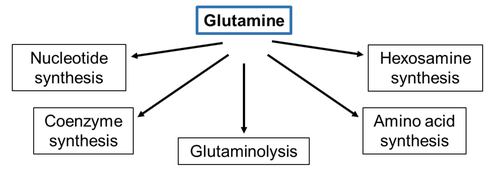


	IC50 (µM)
ipn02.32	31.19
sNF96.2	9.008
sNF02.2	8.476

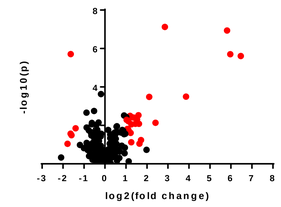
A) *Left:* sNF96.2 MPNST cells grow more sparsely at 72 hours in low glutamine compared to control media than immortalized Schwann cells (ipn02.32). *Right:* MPNST cells (sNF96.2, sNF02.2) require higher concentrations of glutamine added back to glutamine-free media to rescue growth compared to immortalized Schwann cells. **B)** The glutamine antagonist 6-diazo-5-oxo-norleucine (DON) inhibits growth of MPNST cells with IC50 over 3-fold less than for control cells. Corresponding IC50 values listed in table.

DON Treatment Alters the Metabolome of MPNST Cells

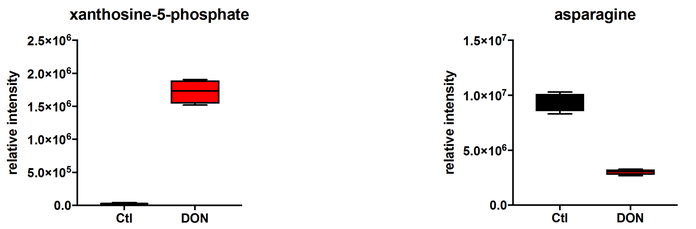
A. DON BROADLY ANTAGONIZES GLUTAMINE METABOLISM



B. TARGETED METABOLOMICS REVEALS DON SIGNIFICANTLY AFFECTS 28 METABOLITES



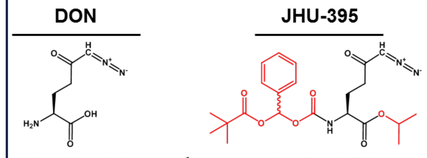
C. DON SIGNIFICANTLY AFFECTS NUCLEOTIDE AND AMINO ACID SYNTHESIS IN MPSNT CELLS



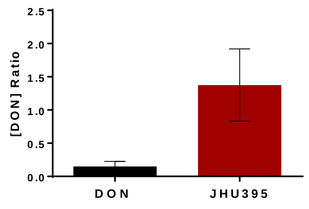
A) Schematic of glutamine utilizing synthetic processes affected by DON in mammalian cells. **B)** LC-MS targeted metabolomics identifies twenty-eight metabolites (red dots) from MPNST cells showing over two-fold change and p-value < 0.1 between DON- and vehicle-treated samples. **C)** Representative box plots comparing levels of XMP and asparagine from control (vehicle) and DON treated MPNST samples.

JHU395: A DON Prodrug that Improves Delivery of DON to Nervous Tissue

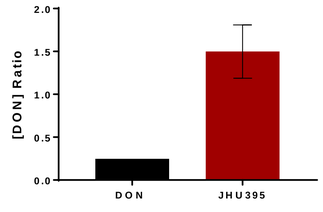
A. STRUCTURES



B. JHU395 ENHANCES DON DELIVERY TO BRAIN TISSUE 9-FOLD



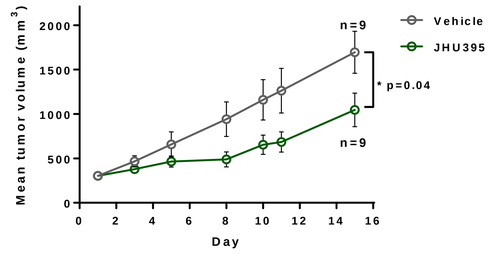
C. JHU395 IMPROVES DON DELIVERY TO MPSNT CELLS 6-FOLD



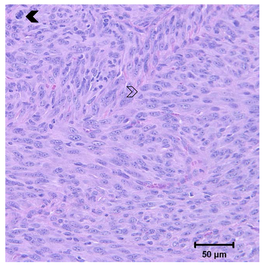
A) JHU395 is a DON prodrug that has increased lipophilicity (cLogP) versus DON. **B)** JHU395 delivers DON preferentially to nervous (brain) tissue compared to DON. DON and JHU395 were dosed by IV infusion (1.6 mg/kg equivalent doses for 1h) in swine and DON was quantified in plasma and brain samples. Adapted with permission from reference (3). Copyright 2017 American Chemical Society. **C)** MPNST cells (sNF96.2) in human plasma were incubated with DON or JHU395 (20 µM) for 60 minutes. DON was detected in separated cells and plasma by LC-MS.

JHU395 Is Active in a Mouse Model of MPNST-Derived Flank Tumors

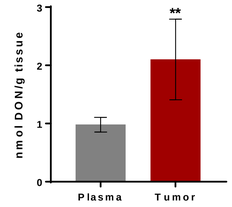
A. ORAL JHU395 DECREASES MPNST FLANK TUMOR GROWTH BY 40%



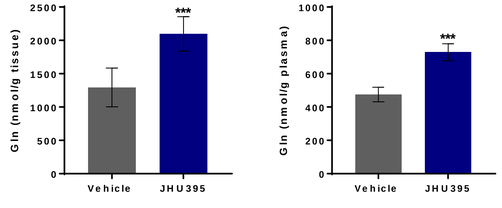
B. MPNST FLANK TUMOR HISTOLOGY



C. ORALLY ADMINISTERED JHU395 DELIVERS DON TO MPNST FLANK TUMOR



D. TUMOR AND PLASMA GLUTAMINE LEVELS INCREASE WITH JHU395 TREATMENT



A) Mice bearing flank tumors (initially n=10/arm) were treated daily for 14 days with oral vehicle or JHU395 (1.2 mg/kg x 5d; 0.5 mg/kg x 9d). Tumor volumes were calculated based on V=(L*(W^2))/2. **B)** Representative H&E stained image of untreated inoculated flank tumor generated from cells taken from an NPcis mouse MPNST. Spindle morphology, atypical nuclei (open arrow) and mitotic figures (closed arrow) are observed. **C)** JHU395 dosed orally (1.2 mg/kg) 30 minutes prior to tissue harvest delivers DON to tumor at twice the plasma level and **D)** increases both tumor and plasma glutamine levels measured quantitatively by LC-MS.