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 13C5- or 15N2-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 nucleotide and amino acid synthesis, is a hallmark of cancers that has not been effectively leveraged for treatment of MPNST (2). Our group has recently described JHU395, a novel nervous system-targeted 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 cells (sNF96.2) and MPNST cell lines we investigated cell proliferation in culture under glutamine deprivation and antagonism. Mass spectrometry (MS)-based metabolic profiling was used to characterize differences between MPNST cells treated with vehicle versus DON. MS-based biochemical 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 (NC3, 8-9 mmicrometer versus >30 micrometer). 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 immortalized Schwann cells versus plasma, JHU395 preferentially delivered DON into MPNST cells with over 5-fold higher cell-to-plasma ratio. In an MPNST murine flank tumor model (5), 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 pharmacological studies using JHU395 in combination with agents with known activity in MPNST.

Results

DON Treatment Alters the Metabolome of MPNST Cells

A. DON BROADLY ANTAGONIZES GLUTAMINE METABOLISM

B. TARGETED METABOLICOS REVEALS DON SIGNIFICANTLY AFFECTS 28 METABOLITES

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

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

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

B. MPNST FLANK TUMOR HISTOLOGY TUMOR GROWTH BY 40%

C. ORALLY ADMINISTERED JHU395 DELIVERS DON TO MPNST FLANK TUMOR

D. TUMOR AND PLASMA GLUTAMINE LEVELS INCREASE WITH JHU395 TREATMENT

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