Targeting glutamine metabolism disables Warburg physiology by inhibiting proximal glycolysis and Krebs cycle rewiring

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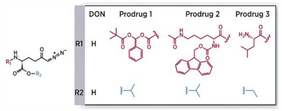
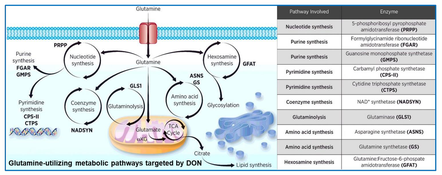
INTRODUCTION

Glutamine, the most abundant amino acid in plasma, plays a critical role in cancer metabolism. However, recent studies have challenged the utility of singularly targeting specific enzymes (e.g. glutaminase) for therapeutic gain. Instead, we hypothesized that broadly inhibiting multiple glutamine related pathways would more effectively shut down tumor growth. To test this hypothesis, we employed a novel prodrug of the glutamine antagonist 6-diazo-5-oxo-L-norleucine (DON) in a number of syngeneic mouse models of cancer including MC38 (derived from colon cancer) and B16 (derived from melanoma). In an attempt to explore the anti-tumor mechanisms in each of these models, we applied a MS-based stable isotope tracing strategy to profile [U-^{13}C]-glucose/glutamine derived metabolites. Our studies revealed that MC38 was highly sensitive to anti-glutamine treatment when compared to 3LL and B16. Interestingly, [U-13C]-glucose tracing study showed that treatment with the glutamine antagonist markedly decreased the flux into “proximal” glycolytic reactions, PPP pathway and serine synthesis pathway.



Prodrug of DON (JHU-083)

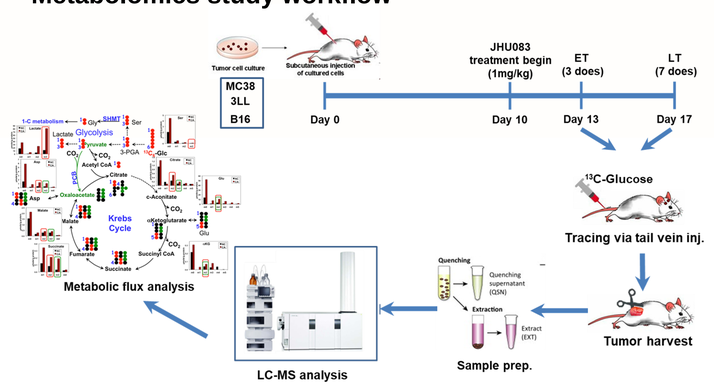


Pathway Involved	Enzyme
Nucleotide synthesis	5-phosphoribosyl pyrophosphate amidotransferase **(PPAT)**
Purine synthesis	Formylglycinamide ribonucleotide amidotransferase **(FGAR)**
Purine synthesis	Guanosine monophosphate synthetase **(GMPS)**
Pyrimidine synthesis	Carbamyl phosphate synthetase **(CPS-II)**
Pyrimidine synthesis	Cytidine triphosphate synthetase **(CTPS)**
Coenzyme synthesis	NAD^+ synthetase **(NADSYN)**
Glutaminolysis	Glutaminase **(GLS1)**
Amino acid synthesis	Asparagine synthetase **(ASNS)**
Amino acid synthesis	Glutamine synthetase **(GS)**
Hexosamine synthesis	Glutamine:Fructose-6-phosphate amidotransferase **(GFAT)**

Strikingly, our glucose tracing study revealed that the flux from succinate to fumarate was shut down following glutamine antagonist treatment. This finding implies that the glutamine antagonist used in our study may have direct or indirect inhibition of succinate dehydrogenase. Furthermore, by employing [U-^{13}C]-glutamine tracing we discovered the presence of a rewired Krebs cycle via the aspartate arginosuccinate shunt and GABA shunt in the MC38 model. Overall, our results highlight that broadly targeting tumor glutamine metabolism can markedly affect non-glutamine requiring metabolism essential for tumor growth.

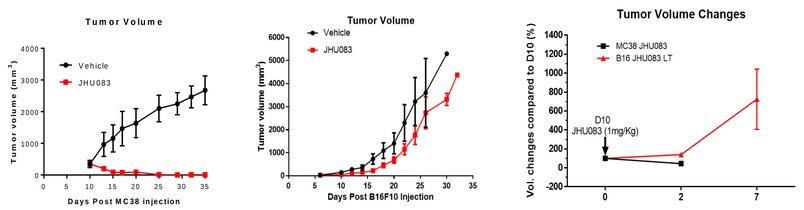
METHODS

- **Metabolomics study workflow**



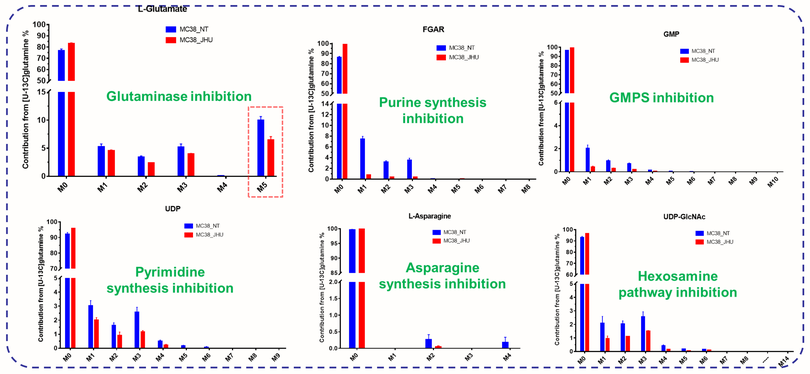
RESULTS

- **MC38 is sensitive to JHU-083, but B16 Melanoma is relatively resistant to JHU-083**

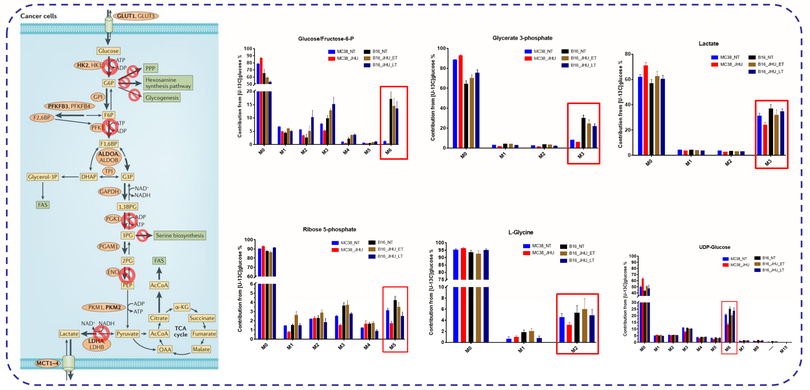


RESULTS (cont’d)

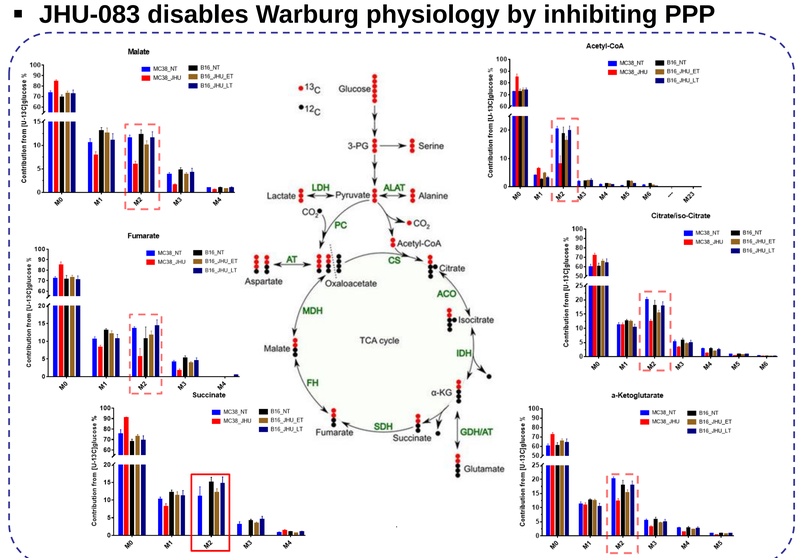
- **Prodrug of DON (JHU-083) targets glutamine metabolism**



- **JHU-083 disables Warburg physiology by inhibiting proximal glycolysis pathways**

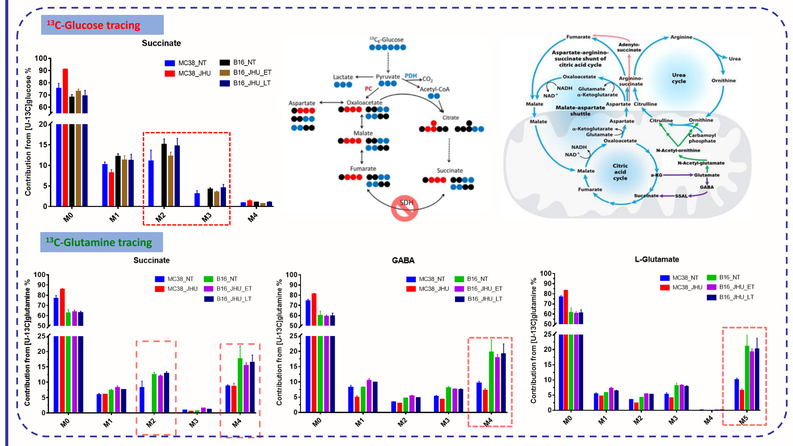


- **JHU-083 disables Warburg physiology by inhibiting PPP**

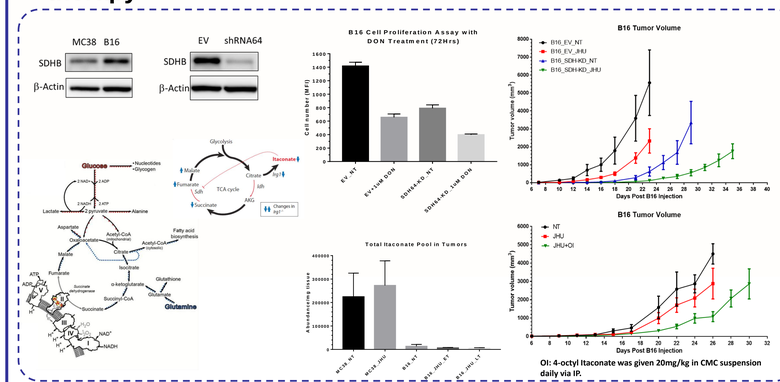


RESULTS (cont’d)

- **Disruption of flux to succinate and rewired TCA cycle via GABA shunt**

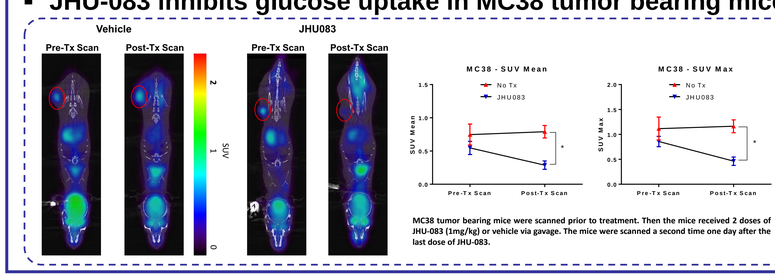


- **Combination of JHU-083 with SDHB inhibition improves B16 therapy**



OI: 4-octyl itaconate was given 20mg/kg in CMC suspension daily via IP.

- **JHU-083 inhibits glucose uptake in MC38 tumor bearing mice**



MC38 tumor bearing mice were scanned prior to treatment. Then the mice received 2 doses of JHU-083 (1mg/kg) or vehicle via gavage. The mice were scanned a second time one day after the last dose of JHU-083.

Summary & Future Experiments

- Targeting glutamine metabolism disabled Warburg physiology leads to inhibition of non-glutamine requiring proximal glycolysis pathways;
- Targeting glutamine metabolism disrupted the glucose derived flux into succinate. A rewiring of TCA cycle was identified with ^{13}C-glutamine tracing which showed the presence of an GABA shunt as an alternative source of succinate;
- Our results also revealed that SDH may play important role in tumor resistance to JHU-083 therapy. Combination therapy of JHU-083 with SDHB inhibition improved the B16 tumor therapy;
- Our study outlined potential future combination therapy to shut down tumor metabolism.