Targeting glutamine metabolism as a mean of treating a murine model of ovarian cancer and ascites development

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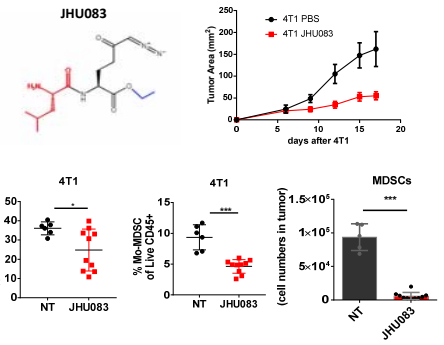
Abstract

Malignant ascites in ovarian cancer develops in the setting of recurrent and advanced metastatic disease and is associated with poor prognosis. Emerging evidence has demonstrated that ovarian cancer patients have altered metabolism and an immunosuppressive environment in the ascites fluid. Inasmuch as the rapid proliferation of ovarian cancer cells and development of ascites is dependent on glutamine metabolism, we hypothesized that targeting the glutamine metabolism can inhibit the development of ascites and enhance anti-tumor immunity in ovarian cancer. To test this hypothesis, we developed a novel small molecule glutamine antagonist (prodrug of 6-Diazo-5-oxo-l-norleucine, JHU-083). By employing JHU-083, we evaluated this hypothesis in mice bearing an aggressive and metastatic ovarian cancer (ID8 cell line with an overexpression of Defb29 and Vegfa). Targeting glutamine metabolism led to markedly reduced progression of ascites along with reduced tumor cell numbers. Furthermore, JHU-083 treatment also led to a marked diminution of the volume of fluid in mice with already established ascites. Inhibition of glutamine metabolism also led to a less suppressive tumor microenvironment by blocking the recruitment of myeloid derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs) to the ascites fluid. Functionally, both the TAMs and tumor cells from the JHU-083 treated group showed lower expression of PDL1 compared to the vehicle treated group. Additionally, we also observed reduced regulatory T cell (T regs) percentages and numbers with JHU-083 treatment. These findings are consistent with our previous observations that targeting glutamine metabolism can inhibit the generation and recruitment of MDSC and T regs by targeting both tumor cell and immune cell metabolism. Further, LC-MS based metabolite analysis of the ovarian cancer ascites fluid from JHU-083 treated mice revealed distinct differences in urea-ornithine pathway, nucleotide synthesis, and redox balance related metabolites. In light of these findings, ongoing experiments are examining the efficacy of JHU-083 in combination with epigenetic drugs, VEGF inhibitors, PARP inhibitors, and checkpoint blockade. These studies suggest that targeting glutamine metabolism may represent a novel approach to treating metastatic ovarian cancer and ascites.

Introduction

- **Together with the Johns Hopkins Drug Discovery Program, we developed a novel prodrug of 6-Diazo-5-oxo-l-norleucine (DON), to inhibit glutamine metabolism (JHU-083).**
- **Inhibition of glutamine metabolism blocks the generation and recruitment of MDSC within the primary breast tumor microenvironment as well as at sites of metastasis thus markedly inhibiting the development of metastatic foci.**



Objectives

We hypothesized that glutamine antagonism might inhibit the development of ascites and enhance anti-tumor immunity in ovarian cancer.

Results

Targeting glutamine using JHU-083 reduces tumor growth and progression of ascites

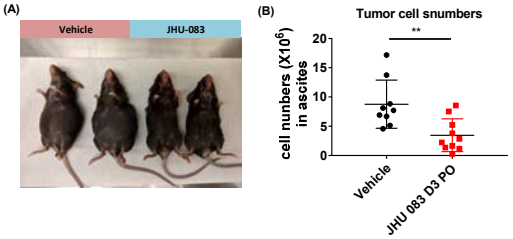


Fig 1. Targeting glutamine using daily JHU-083 PO (from day 3 post tumor inoculation) in the ID8-VD tumor (D8 cell line with an overexpression of *Defb29 and Vegfa*) bearing mice (A) delayed progression of ascites (B) reduced tumor growth

JHU-083 treatment leads to a marked diminution of the volume of fluid in mice with already established ascites

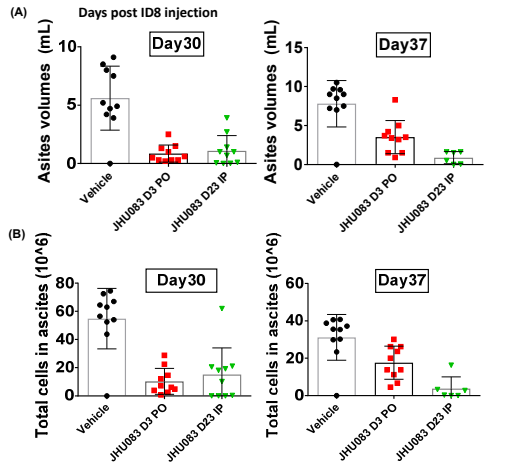


Fig 2. (A) Targeting glutamine using JHU-083 reduced tumor ascites volumes and (B) decreased total cells in ascites fluid from ID8 VD tumor bearing mice

Targeting glutamine reduces regulatory T cell infiltration in ascites fluids

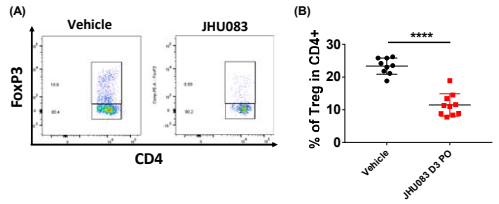


Fig 3. Reduced regulatory T cells (FoxP3+CD4+) in ascites fluids from the glutamine antagonist JHU-083 treated group. (A) representative plot (B) summary graph

The glutamine antagonist JHU-083 inhibits MDSCs and TAM infiltration in ascites fluids

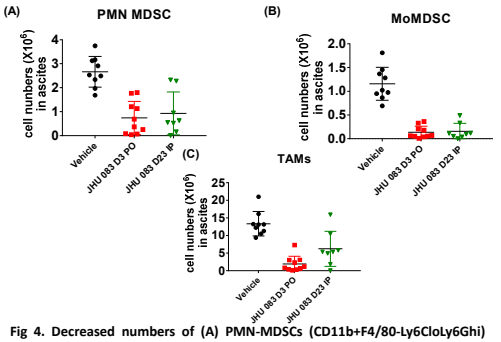


Fig 4. Decreased numbers of (A) PMN-MDSCs (CD11b+F4/80-Ly6CloLy6Ghi) and (B) Mo-MDSCs (CD11b+F4/80-Ly6ChiLy6Gneg) (C) TAMs (CD45+CD11b+ F4/80+Ly6C-Ly6G-) from ascites in JHU083 treated ID8 VD tumor bearing mice

Targeting glutamine using JHU-083 markedly reduces PDL1 expression on ovarian cancer cells and TAMs

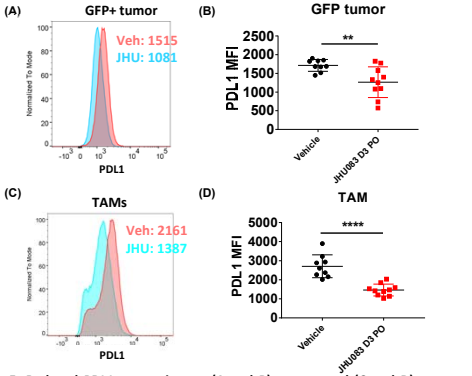


Fig 5. Reduced PDL1 expression on (A and B) tumors and (C and D) tumor-associated macrophages in ascites fluids from glutamine antagonist JHU-083 treated groups compared to the vehicle group

Glutamine antagonism reduces suppressive microenvironments for T cell proliferation

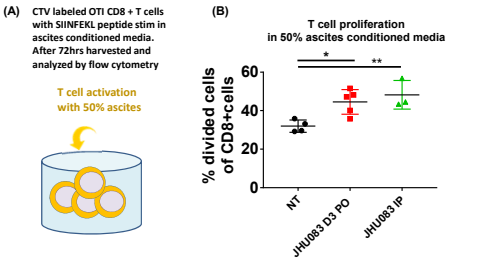


Fig 6. (A) Scheme of experiment for T cell proliferation in ascites conditioned media (B) increased dividing CD8+ cells in conditioned media with ascites from JHU083 treated mice

Glutamine antagonism changes metabolic TMEs in ascites fluids

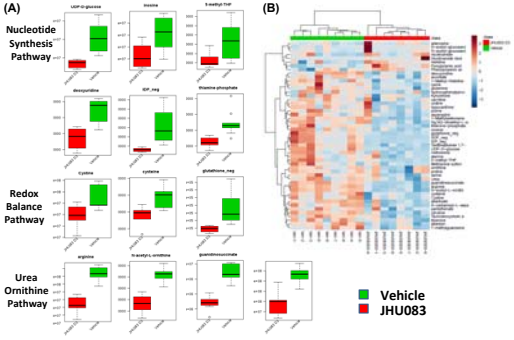


Fig 7. LC-Mass spec of metabolites of ascites fluids from the NT and glutamine antagonist JHU-083 groups revealed statistical differences in (A) nucleotide synthesis, redox balance pathway, and urea-ornithine pathways (B) two distinct metabolic clusters correlated between experimental groups. Presented as a heat map

Glutamine inhibition induces activation of STING pathway in P53/Brca mutant ovarian cancer cells.

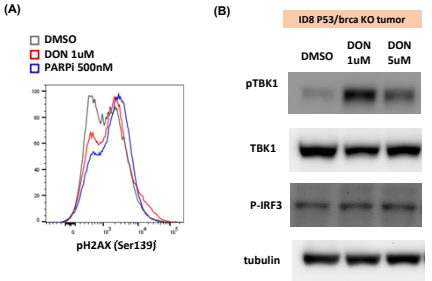


Fig 8. (A) Flow analysis using double strand break marker (pH2AX ser139) in ID8 P53/BRCA mutant cells in the presence or absence of DON1uM or PARP inhibitor (500nM) (B) STING related protein expression in the presence or absence of DON

Summary & Conclusion

- **Targeting glutamine metabolism is a novel means of treating ovarian cancer and inhibiting cancer induced ascites**
- **Both PO and IP drug delivery were effective**
- **Targeting glutamine metabolism leads to less immuno-suppressive tumor micro-environment by**
  - *** <u>decreasing</u> MDSCs, Tregs and TAMs**
  - *** <u>decreasing</u> PDL1 expression in both tumor and TAMs**
  - *** <u>increasing</u> T cell proliferation by changing the suppressive metabolic environment in ascites**
- **In the Brca mutated tumor, similar to PARP inhibition, glutamine antagonist enhances activation of STING pathway**
- **In future studies, we will combine glutamine antagonists with PARP inhibitors & immune-checkpoint blockade**