Metabolism as checkpoint: Induction of anti-tumor immune response with the novel glutamine antagonist JHU-083

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Introduction

- Hypoxia, acidosis, nutrient depletion, and elevated adenosine are metabolic characteristics of the tumor microenvironment (TME)[1-2]
- The metabolic characteristics of the TME present a significant hurdle for immunotherapy[2]
- Dysregulated glutamine metabolism plays a crucial role in supporting tumor metabolism[3]
- JHU-083, a prodrug of 6-diazo-5-oxo-L-norlecuine (DON), markedly disrupts the metabolism of tumors with minimal toxicity[4]

Hypothesis: Disruption of tumor glutamine metabolism with JHU-083 will condition the TME for enhanced immunotherapy

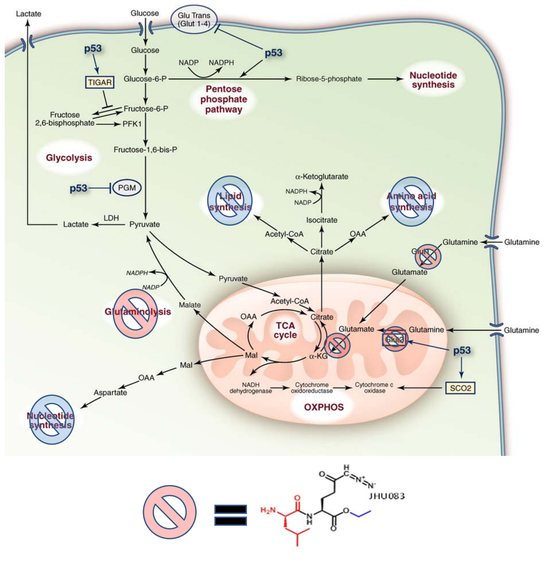

Figure 1: Cancer cells utilize glycolysis as a primary energy source even in the presence of oxygen. They also rely on glutamine and its conversion to alpha-ketoglutarate to support the TCA cycle and serve as an energy source, as well as a carbon source for nucleotides, lipids and amino acids. JHU-083 is a pro-drug of DON that, upon activation, serves as a non-selective, irreversible inhibitor of glutamine-requiring pathways (red symbols). This results in the reduced ability of cells to generate substrates for growth and proliferation (blue symbols).

Objectives

1. Characterize the metabolic changes in murine tumor models induced by JHU-083
2. Evaluate changes in the tumor immune infiltrate after treatment with JHU-083
3. Evaluate the ability of JHU-083 to enhance immunotherapy with anti-PD1 checkpoint blockade and adoptive cellular therapy (ACT)
4. Evaluate the effect of JHU-083 on effector T cells

Results

JHU-083 reprograms the metabolism of the tumor microenvironment

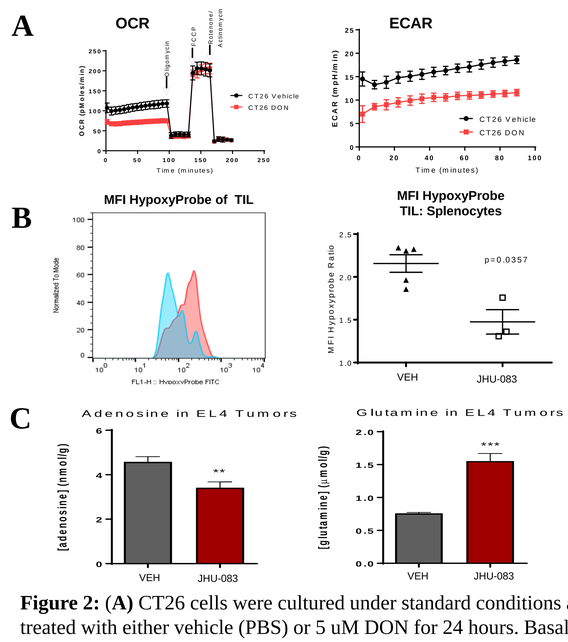

Figure 2: **(A)** CT26 cells were cultured under standard conditions and treated with either vehicle (PBS) or 5 uM DON for 24 hours. Basal O_2 consumption rates (OCR) and extracellular acidification rates (ECAR) were measured on a Seahorse XF96 extracellular flux analyzer. **(B)** BALB/C mice (n=3-5 per group) were injected subcutaneously with CT26 cells and treated on days 7-20 with either vehicle (PBS) or JHU-083 (0.3 mg/kg) qod. TIL and splenocytes stained using Hypoxia-Probe™. **(C)** C57BL/6 mice (n=5 per group) were injected with EL4 cells subcutaneously and treated on days 7-10 with either vehicle (PBS) or JHU-083 (1 mg/kg) daily. Tumors harvested on day 10 and flash frozen in liquid N_2 before metabolite quantification by MS.

Glutamine antagonism suppresses T_{reg} and MDSC response to tumors

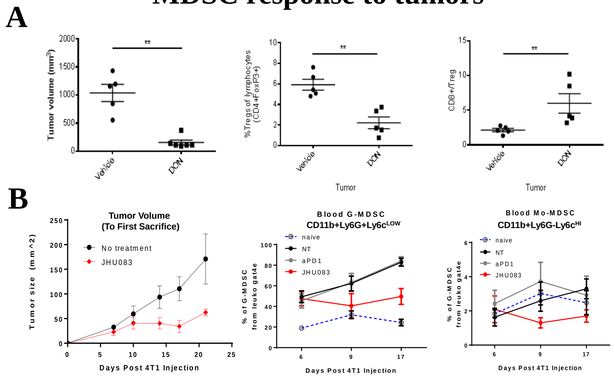

Figure 3. (A) Mice were injected with B16-F10 cells and treated with either DON or vehicle via i.p. injection for 3 days starting on day 7. DON treatment resulted in smaller tumors and significantly reduced the population of CD4+/FoxP3+ Tregs (day 10). **(B)** BALB/C mice (n=4-5 per group) were injected subcutaneously with 4T1 cells and treated on days 7-16 with either vehicle (PBS) or JHU-083 (1 mg/kg) daily. Treated mice showed reduced tumor growth and reduced MDSC in blood.

Results

JHU-083 overcomes resistance to anti-PD-1

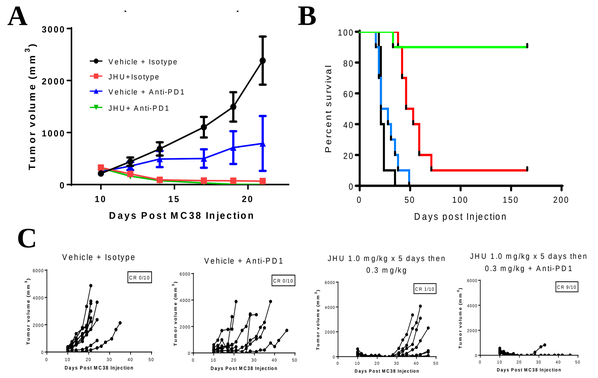

Figure 4. C57BL/6 mice (n=10 per group) were injected subcutaneously with MC38 cells. The mice started treatment on day 10 with either vehicle (PBS), αPD1 (RMP1-14, 5mg/kg on days 10, 12, 14, 16), JHU-083 at 1mg/kg daily for 5 days followed by 0.3 mg/kg daily for 9 additional days, or combination JHU-083 + αPD1. Tumor growth until first sacrifice (**A**), survival (**B**), and spider plots (**C**) are shown.

Anti-tumor response to JHU-083 monotherapy is immune mediated

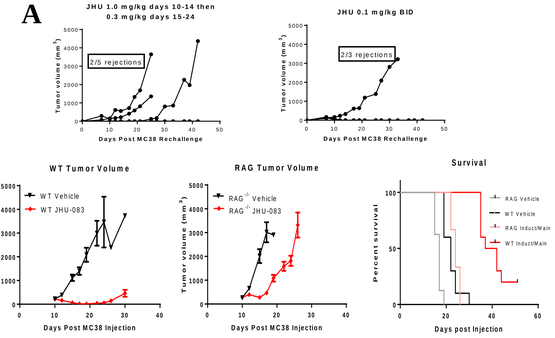

Figure 5. (**A**) Mice that had rejected MC38 tumor in the setting of JHU-083 monotherapy (as in Fig 4a) were re-challenged with an equal number of MC38 cells in opposite flank. Mice were not re-treated. (**B**) WT and RAG-/- mice challenged with MC38 (s.c.) and treated with vehicle or daily JHU-083 monotherapy (as described in Fig 4a)

JHU-083 enhances adoptive cellular therapy

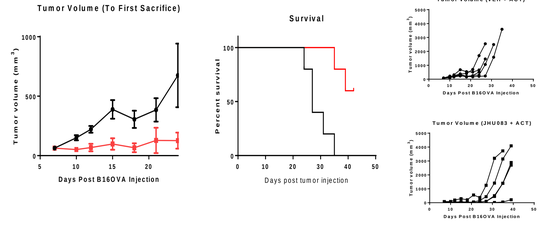

Figure 6. C57BL/6 mice (n=5 per group) were injected (s.c.) with $2x10^5$ B16-OVA tumor cells and received adoptive transfer of $1.5x10^6$ activated OT1 cells on day 10. Mice received vehicle (PBS) or JHU-083 (1 mg/kg) daily for 3 days prior to adoptive transfer.

Results

Glutamine antagonism enhances generation of CD8 memory phenotype

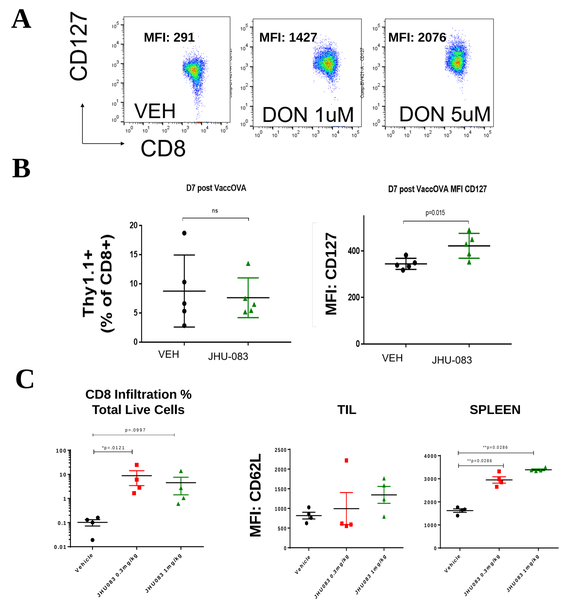

Figure 7. (A) Flow cytometric analysis of CD8+ splenocytes activated *in vitro* with αCD3 and αCD28 for 24 hrs, rested for 4 days in IL-2 with increasing concentrations of DON. **(B)** WT mice received $2x10^6$ OT1 cells with 10^6 PFU vaccinia OVA on day 0, then vehicle or JHU-083 (0.3 mg/kg) on days 1-7. PBMCs analyzed on day 7. **(C)** 10^6 EL4 cells (s.c.) were allowed to grow for 14 days in C57BL/6 mice +/- JHU083 on days 10-14 (n=4 mice per group).

Conclusions

1. JHU-083 disrupts the metabolism of the TME, limiting oxygen consumption, lactate production, adenosine generation, and glutamine depletion
2. Glutamine antagonism reduces MDSC and regulatory T cell response to tumor
3. JHU-083 markedly enhances tumor immunotherapy in combination with checkpoint blockade and ACT
4. JHU-083 monotherapy unleashes endogenous immune response to tumors, capable of establishing complete rejection and immunologic memory
5. JHU-083 enhances memory phenotype of CD8 T cells
6. JHU-083 should be pursued in early clinical trials as part of an immunotherapy regimen

References

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