Results

ECAR

C R 9/10

C R 0/10

3. Evaluate the ability of JHU-083 to enhance generation substrates for growth and proliferation (blue symbols).

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 prodrug 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).

Figure 2: (A) CT26 cells were cultured under standard conditions and treated with either vehicle (PBS) or 5 mM DON for 24 hours. Basal O2 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 HypoxyProbeTM. (C) CT26L6 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 N2 before metabolite quantification by MS.

Glutamine antagonism suppresses Treg 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 immunotherapy with anti-PD1 checkpoint blockade and adoptive cellular therapy (ACT).

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

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) or anti-PD1 (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 + anti-PD1. 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 WT Veh + Anti-PD1 or JHU+Isotype, died from tumor rejection on day 60. (B) JHU083 0.3mg/kg alone resulted in 2/3 rejections.

Figure 6. C57BL/6 mice (n=5 per group) were injected (s.c.) with 2x10^6 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.

Figure 7. (A) Flow cytometric analysis of CD8+ splenocytes activated in vitro with eCD3 and eCD28 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 +/- JHU-083 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