Targeting Glutamine Metabolism Enhances Immune Checkpoint Inhibitors Efficacy Captured by FDG PET

Hoon Choi[1], Hasan Babazada[1], Roderick O'Connor[2], Michael Farwell[1], David Mankoff[1,3] and Rong Zhou[1,3]

[1]Department of Radiology and [2]Department of Pathology and Laboratory Medicine, [3]Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA, USA

Introduction

OBJECTIVES: Glutaminolysis is a metabolic pathway deployed by aggressive cancers including triple-negative breast cancer (TNBC) to support their growth and progression. Immune checkpoint inhibitors (ICI) are FDA approved treatment modalities for TNBC; however, treatment responses are modest and variable [1-2]. To overcome the heterogeneity in response, there is a critical need to understand the unique characteristics underlying immune activity responding to ICI. Our prior study has shown that TNBC tumors are enriched with myeloid-derived suppressor cells (MDSCs), which were significantly reduced by a pan-glutamine metabolic inhibitor JHU083, a pool compound of DRP-104/Sirpiglenastat being evaluated in clinical trials. JHU083/DRP-104 is the prodrug form of DON (6-Diazo-5-oxo-L-norleucine) [3]. Here we examined the impact of glutamine antagonism in cancer versus immune cells traversing the tumor microenvironment. PET imaging is an important tool to identify patients most suited to the therapeutic promise of ICI. Leveraging the recent development of a radio-labeled antibody against CD69 [4], a well characterized early-activation cell surface marker, we tested whether this immune-PET marker can detect the enhanced activation of T cells in the TNBC tumor immune microenvironment.

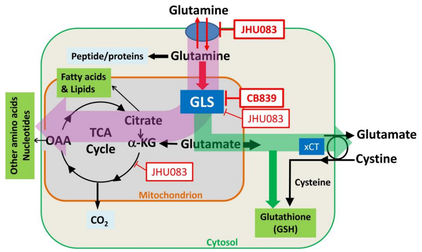


Figure 1 Cancer glutaminolysis pathway follows the purple arrow, starting with glutamine uptake via cellular glutamine transporters. Steps blocked by two drugs are marked. Glutamate produced by GLS is essential for GSH synthesis (green arrows).

Method

Murine (4T1, EO771, MMTV-PyMT) and human (HCC1806) TNBC cell lines, as well as an ER$^+$ cell line (MCF7), were treated DON (0.1 μM or 1 μM) to assess ROS and lipid ROS levels using Dihydroethidium and C11-BODIPY assays. T cells and MDSCs were isolated from the spleen. The effect of MDSCs on T-cell activation was evaluated by co-culturing T cells with MDSCs for one day in media containing 0.05 μg/ml PMA and 0.1 μg/ml ionomycin, with or without 0.1 μM DON. For in vivo studies, 4T1 cells were inoculated into the mammary fat pad of BALB/c mice. When tumors reached approximately 100 mm^3, mice were randomized into three groups: control (no treatment), ICI (PD-1 at 250 μg/mouse and CTLA-4 at 100 μg/mouse, administered i.p. twice weekly), or ICI plus JHU083 (1.82 mg/kg i.p. every other day starting on day 0). After one week of treatment, each mouse received 100–300 μCi of [^{89}Zr]-DFO-H1.2F3 (anti-CD69 mAb) via tail-vein injection, followed by PET imaging 48 hours later. FACS analysis was performed to characterize tumor-infiltrating immune cells. G-CSF and GM-CSF levels in the tumor interstitial fluid of 4T1 tumors were also measured.

Results

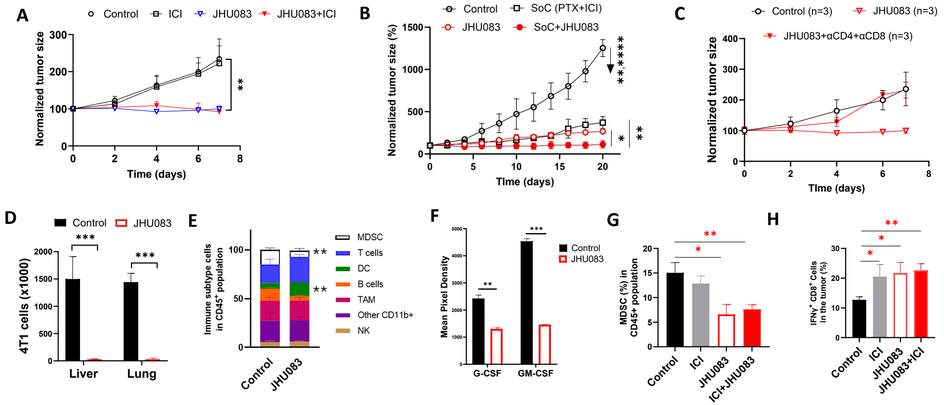


Figure 2 Glutamine Blockade–Driven Tumor Growth Delay and T-Cell Efficacy in 4T1 TNBC Tumor growth by ICI, JHU083 and ICI plus JHU083 (A) and standard of care treatment (SoC) for TNBC (B), Tumor growth by T cell depletion with αCD4 and αCD8 in 4T1 tumor (C); metastasis in liver and lung (D), immune cell population (E) and G-CSF and GM-CSF levels in the tumor interstitial fluid (F) in 4T1 tumors by JHU083; MDSC levels (G) and IFNγ-secreting CD8+ T cells (H) in 4T1 tumor by ICI, JHU083 or ICI plus JHU083.

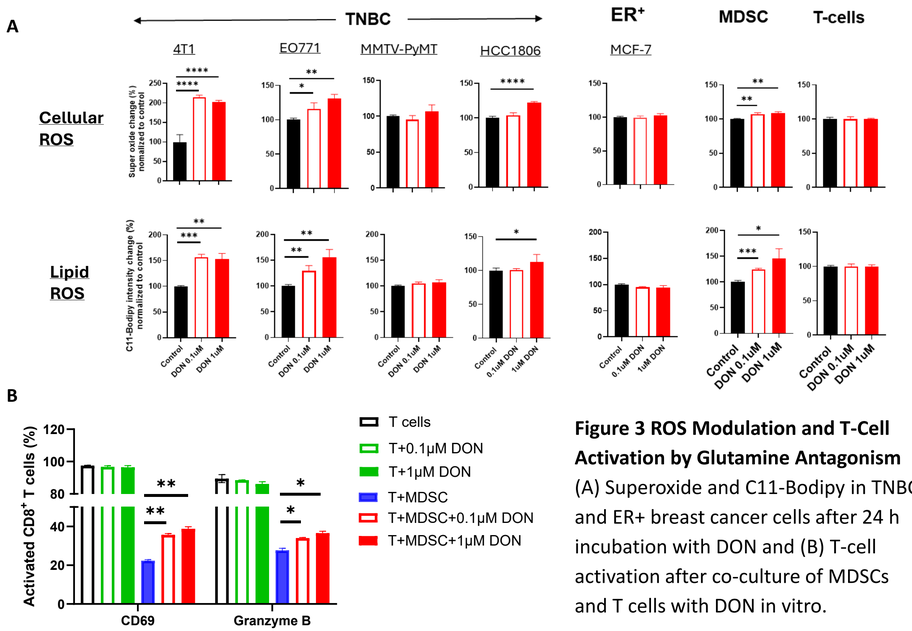


Figure 3 ROS Modulation and T-Cell Activation by Glutamine Antagonism (A) Superoxide and C11-Bodipy in TNBC and ER+ breast cancer cells after 24 h incubation with DON and (B) T-cell activation after co-culture of MDSCs and T cells with DON in vitro.

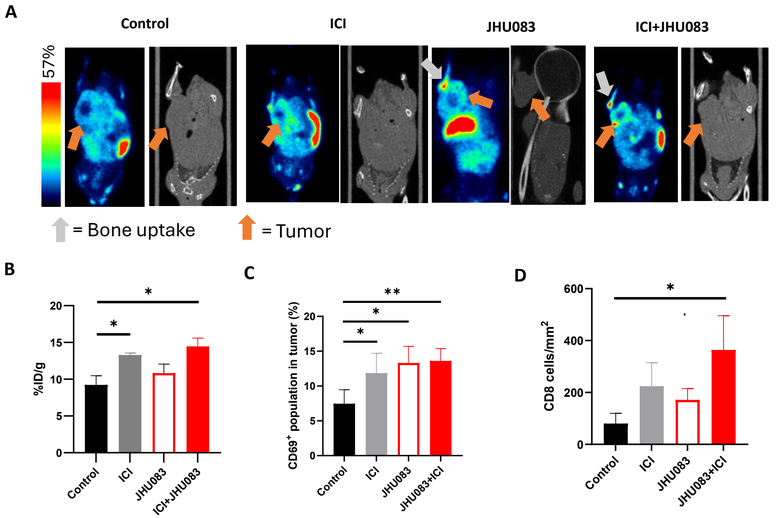


Figure 4 [89Zr]CD69 PET imaging for Tumor Immune Activation (A) [89Zr]CD69 PET image at baseline and 48h with control, ICI, JHU093 and ICI plus JHU083,(B) PET ROI quantification of [89Zr]CD69 in mean % ID/g, (C) CD69 (early immune activation marker) detection on immune cells in 4T1 tumors and (D) the quantification of CD8 cells from IF staining in 4T1 tumor by ICI, JHU083 or ICI plus JHU083.

Discussion

Selectively disrupting metabolism in cancer cells and sparing T cells that provide anti-tumor control has immediate translational relevance for adoptive immunotherapies. Targeting cancer glutamine metabolism by JHU083 /DON exerts distinct effects in cancer cells and MDSCs versus T cells in TNBC tumors. [89Zr]CD69 PET is able to detect enhanced activation of tumor immune microenvironment after ICI or ICI plus JHU083 treatment. ICI therapy fails to inhibit tumor growth likely due to predominant presence of MDSCs in the tumor and their suppressive effects on CD8+ T cells as demonstrated by co-incubation data. Combining JHU083 with ICI reduces infiltrated MDSCs in the tumor, leading to enhanced anti-tumor immunity of ICI.

Reference

1. Nederlof I, Horlings HM, et al. *Cancers.* 2021;13(2) 316.
2. Voorwerk L, Slagter M, et al. Nat Med. 2019; 25(6) 920
3. Rais R, Lemberg KM, Tenora L, et al. *Sci Adv.* 2022;8(46):eabq5925.
4. Edwards KJ, Chang B, et al. Cancer Immunol Res. 2022; 10(9): 1084.

Acknowledgement

We thank Dr. Barbara Slusher for insightful discussion and Johns Hopkins Drug Discovery Program for providing JHU083. This study was supported by NIH/NCI R01CA233771, R01CA211337 and R01CA266285, and a grant from Ludwig Institute for Cancer Research.