Targeting glutamine metabolism enhances tumor specific immunity by inhibiting the generation and function of suppressive myeloid cells

Min-Hee Oh1, 2, Im-Hong Sun1, Liang Zhao1, Im-Meng Sun3, Wei Xu2, Ching Pate1, Robert Leone1, Ada Tam1, Judd Engler1, Pavel Majer1, Rana Rais1, Barbara Slusher1, Maureen Horton2, Jonathan D. Powell1,3

1 Bloomberg Kimmel Institute for Cancer Immunotherapy, Johns Hopkins Drug Discovery, Department of Medicine, Johns Hopkins School of Medicine, 2 Medimmune, LLC, Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic

Abstract

In order to sustain their invasive growth, tumors have specialized reprogrammed metabolism. This metabolism creates an acidic, hypoxic and nutrient deprived tumor microenvironment (TME). Such an environment inhibits anti-tumor effector cells while recruiting the differentiation and function of immune cells such as T regulatory cells, Myeloid Derived Suppressor Cells (MDSC) and Tumor Associated Macrophages (TAM). We hypothesized that by targeting tumor metabolism we could alter the TAM and “condition” tumors to be more susceptible to immunotherapy. To this end, along with the Johns Hopkins Drug Discovery Program, we developed a novel prodrug of 6-Diazo-5-oxo-L-norleucine, inhibitor of glutamine metabolism (JHU-083). Recently, it has been shown that MD2 macrophages require glutamine metabolism for differentiation and function. In light of the similarities between MD2 macrophages and suppressive myeloid cells, we hypothesized that JHU-083 might inhibit the generation and function of MDSC and TAMs. We tested this hypothesis in the 4T1 breast cancer model and 3LL lung carcinoma model. These tumors are relatively resistant to immunotherapy and are characterized by increased generation of MDSC and distant spontaneous metastasis. JHU-083 treated mice suppressed tumor growth compared to the vehicle treated group. Immunologically, we observed markedly reduced numbers of MDSCs in circulating blood within 3 days of drug treatment compared to vehicle group, leading to favorable CD8+ T cell responses. Consistently, JHU-083 treated group displayed significantly decreased percentages and numbers of MDSCs in the tumor, and increased tumor infiltrating CD8+ T cell numbers. Interestingly, JHU-083 treatment reduced TAM macrophagization. While the TAM from the vehicle treated group displayed increased M2 markers and arginase, the JHU-083 treated tumor infiltrating cells showed increased TAMs and reduced M2-like macrophage phenotypes compared to vehicle group. These Th1-eliciting cells were negatively correlated with tumor size. Notably, JHU-083 treatment not only controlled primary tumor growth but also drastically reduced spontaneous lung metastases. The decrease of MDSCs infiltration in the lung was also observed in JHU-083 treated group. Importantly, our data suggests that JHU-083 inhibits CSF2/CSF production and survival of the tumor itself as well as directly affects macrophage metabolism and signaling. Also, LC-AH based metabolomics analysis from JHU-083 treated mice revealed reduced kynurenine:tryptophan ratios compared to the control group, indicating the metabolic modulation of the tumor microenvironment. Overall, our data suggest a novel role for glutamine inhibitor, JHU-083 in enhancing tumor specific immunity by targeting suppressive myeloid cells.

Introduction

Increased monocyte derived suppressive cells are responsible for poor responses to immunotherapy and spontaneous metastasis in 4T1 (mammary) tumors

M2 macrophages rely on glutamine metabolism for differentiation and its function

Monocyte derived suppressive cells (MDSCs) share metabolic characteristics and markers with M2 macrophages

Together with the Johns Hopkins Drug Discovery Program, we developed a novel prodrug of 6-Diazo-5-oxo-L-norleucine, to inhibit glutamine metabolism (JHU-083).

Objectives

We hypothesized that glutamine antagonism might enhance immunotherapy by regulating the generation and functions of the suppressive myeloid cells

Results

4T1 tumor is resistant to immune checkpoint blockade immunotherapy

Targeting glutamine using JHU-083 inhibits growth of 4T1 tumor and leads to decreased MDSCs

Targeting glutamine increases pro-inflammatory TAMs

Glutamine antagonism enhances priming of tumor antigen

Glutamine inhibition enhances immunotherapy

Targeting glutamine using JHU-083 markedly reduces kynurenine

Fig. 1. (A) 4T1 tumor is resistant to immune checkpoint blockade (B and C) Immune checkpoint blockade does not alter increased MDSCs in the blood from 4T1 tumor bearing mice (PMN-MDSC and TAM (polymorphonuclear- MDSCs and tumor associated macrophages) F4/80/BrdU-Ly6cG+CD11b+CD11c-CD34+CD115-CD11b+Ly6cG0Ly6cG0). Fig. 2. Targeting glutamine using JHU-083 in the 4T1 tumor bearing mice showed (A) delayed 41 tumor growth (B) increased survival (C) decreased % of PMN-MDSCs and TAMs and (D) MD-MDSCs in blood (E) increased ratio of CD8+ to MDSCs and TAMs in the blood (F) increased % of CD8+ in TIL (tumor infiltrating leukocytes) (G) increased ratio of CD8+ to MDSCs and TAMs in TIL (H) reduced the relative numbers of Mo-MDSCs and (I) PMN-MDSCs and TAMs (J) decreased CSF3 in the serum (K) decreased CSF3 mRNA in TAMs

Fig. 3. LC-Mass spec of metabolites of tumors from the NT and glutamine antagonist JHU-083 groups revealed (A) two distinct metabolic clusters correlated between experimental groups (B and C) markedly reduced kynurenine in volcano plot of metabolites (D) decreased ratio of kynurenine to tryptophan

Fig. 4. (A) Targeting glutamine using JHU-083 in the 4T1 tumor does not alter the numbers of tumor-associated macrophages (TAMs) (B and C) Increased gene expressions related to lysosome and TIA signaling in TAMs (D) increased % of TINF+ TAMs (E) Increased amounts of TINF secretion in TAMs (F) Increased MHCII+ TAMs from the glutamine antagonist JHU-083 treated mice

Fig. 5. (A) LC-Mass spec of metabolites of spontaneous metastatic site (lung) from the NT and glutamine antagonist JHU-083 groups revealed two distinct metabolic clusters (B) markedly reduced kynurenine in volcano plot of metabolites, (C and D) Reduced spontaneous lung metastasis (E) Increased % of CD8+ in TIL (tumor infiltrating leukocytes) (G) Increased ratio of CD8+ to MDSCs and TAMs in cells from lung of 4T1 tumor bearing mice

Summary & Conclusion

Targeting glutamine metabolism using JHU-083 enhances immunotherapy for cancer

- Inhibition of glutamine metabolism leads to reduced MDSCs
- In part, this decrease is due to decreased CSF3 secretion from TAMs
- Inhibition of glutamine metabolism leads to markedly decreased kynurenine in both primary tumors and metastatic sites, correlating with reduced MDSCs in the lung
- Consequently, JHU-083 markedly inhibits lung metastasis
- Treatment with JHU-083 enhances inflammatory TAMs
- Treatment with JHU-083 leads to enhanced immunologic cell death resulting in enhanced tumor antigen presentation and activation
- Treatment with JHU-083 is able to enhance immunotherapy in immune checkpoint blockade resistant tumor