GLUTAMINE ANTAGONIST PRODRUG JHU083 REPROGRAMS IMMUNOSUPPRESSIVE TUMOR-ASSOCIATED MACROPHAGES TO DRIVES TUMOR IMMUNITY IN UROLOGIC CANCERS

Background Tumor metabolism is emerging as a regulator of immune mediated anti-tumor responses. Previously, we reported increased infiltration of immunosuppressive tumor associated macrophages (TAMs) with disease progression in prostate adenocarcinoma. Glutamine metabolism has been implicated in immunosuppressive TAMs as well as mCRPC and radioresistant urothelial carcinoma. To harness the potent anti-tumor effects of 6-Diazo-5-oxo-L-norleucine (DON) which targets glutamine utilizing enzymes and to mitigate known significant toxicities, we here use a novel pro-drug moiety of DON, i.e., JHU083. We hypothesize that JHU083 will enhance anti-tumor immunity by simultaneously targeting both TAMs and cancer cells.

Methods Using a scRNA-seq dataset from prostate cancer bone metastatic patients we investigated the importance of glutamine metabolism in TAMs in the tumors. We utilized a novel pro-drug JHU083 to treat two urological syngeneic immunogenic mouse tumor models in vivo; B6CaP (prostate cancer) and MB49 (bladder cancer). We studied the direct effect on tumor cells, as well as the required immune compartment for drug efficacy in vivo using antibody targeted depletions or adoptively transferred treated TAMs. Moreover, we used RNA-sequencing, multi-parameter flow cytometry and targeted LC-MS/MS metabolic profiling to characterize the effect of JHU083 on sorted TAMs transcriptional program, proteomic expression profiles and metabolite flux in vivo. Lastly, we assessed the functional phagocytic capacity of TAMs with flow cytometry and IF microscopy.

Results Enriched expression of glutamine utilizing enzymes was observed in increased TAMs in the tumor relative to bening tissue from patient samples. JHU083 showed significant tumor regression in both urologic cancer models. Using in vivo depletion of CD4 or CD8 T cells, or adoptively transferring previously in-vivo JHU083 treated TAMs we established a direct anti-tumor role of TAMs. These TAMs were inflammatory. Strikingly, in the TME we also observed a metabolic flux of glycolytic metabolites which corresponded with increased of Glut1 and Hexokinase II in their protein expression indicating metabolic reprogramming towards glycolytic phenotype. Importantly, JHU083-treated TAMs showed significantly increased phagocytic activity, providing direct evidence of functional reprogramming.

Conclusions We found that JHU083 has two distinct functions in vivo; first, it directly impairs cancer cells which are glutamine dependent and second, it reprograms TAMs from an immunosuppressive to an inflammatory state. These macrophages convert to a highly glycolytic state, have increased TNF production, and have improved phagocytic activity against tumor cells. As urologic cancers are heavily infiltrated with immunosuppressive TAMs, JHU083 is an excellent pre-clinical candidate.

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