WNT INHIBITION IMPROVES EFFICACY OF IMMUNE CHECKPOINT BLOCKADE IN Glioblastoma

Background Glioblastoma (GBM) is a fatal malignancy with a median overall survival of <2 years with the current standard of care.\(^1\) Wnt signaling fuels GBM progression by promoting proliferation, stemness and chemo-resistance.\(^2\) Importantly, Wnt is enriched in glioma stem cells (GSCs), that confer resistance to chemotherapy and anti-tumor immunity.\(^3\) This may partly explain why immune checkpoint blockers (ICBs) have failed in Phase III clinical trials in GBM.\(^4\) Thus, Wnt signaling is an appealing therapeutic target to overcome resistance of ICBs in GBM. Here, we tested the hypothesis that Wnt inhibition potentiates anti-PD1 antibody (aPD1) treatment by reprogramming the GBM tumor microenvironment (TME) from immuno-suppressive to immune-stimulatory in an orthotopic mouse model rich in GSCs.

Methods 005GSC-GFP cells were orthotopically implanted in immunocompetent C7BL6 mice bearing cranial windows. Tumor growth was tracked with ultrasound-based imaging and later randomized into treatment groups of control, WNT974 (a porcupine inhibitor of Wnt signaling; 5 mg/kg, oral gavage, daily), aPD1 (250 ug i.p., once every three days), and WNT974+aPD1. GBM samples were later collected for fluorescence activated cell sorting (FACS) to compare immune cell subpopulations among different treatment groups. Western blot, qPCR, and immunohistochemistry (IHC) analysis was conducted on mouse and human GBM samples to analyze the expression level of Wnt signaling molecules.

Results The combination of WNT974 and aPD1 prolonged the median survival of 005GSC-GFP bearing mice compared to the non-treated control group, from 25 days to 59 days (figure 1A). FACS analysis revealed that the WNT974+aPD1 treated group had an increased frequency of DC3-like dendritic cells expressing CCR7, CD80, and CD40+, decreased frequency of CD45+CD11b+Ly6G+Ly6C+ granulocytic myeloid-derived suppressor cells (gMDSCs), and increased frequency of CD45+CD11b+Ly6G+Ly6C+ monocyctic MDCs (mMDSCs) compared to the untreated or aPD1 monotherapy mice. The combination therapy did not affect the CD4+ and CD8+ T cells in TME (figure 1C). Molecular and IHC analysis revealed a decreased expression level of Wnt signaling molecules in the WNT974+aPD1 group as compared to that in the non-treated group (figure 1B).

Conclusions Wnt inhibition improved the efficacy of ICB therapy in a stem cell rich GBM mouse model by reprogramming the myeloid cell subpopulation in the TME. This combination showed a heterogeneous response making some animals poor responders. We are currently investigating the resistance mechanisms to this combination therapy, and building our previous findings on the role of Wnt signaling on the interaction between the GBM vasculature with GSCs.\(^5\)

Acknowledgements We thank Carolyn Smith for outstanding technical support of the immunohistochemistry studies. We thank Sampurna Chatterjee and William Ho for guidance with the flow acquisition of the DC and MDSC data. We thank Nilesh Talele for the quantification of Iba1+ staining. This work was supported by the National Foundation for Cancer Research; the Ludwig Center at Harvard; the Jane’s Trust Foundation; the Advanced Medical Research Foundation; and the NIH grants P01-CA080124, R35-CA197743 and U01-CA224348 (to Rakesh K. Jain) and R01-CA208205 (to Dai Fukumura and Rakesh K. Jain). Zohreh Amoozgar was supported by Aid for Cancer Research Award, Tosteson Fellowship, and Cancer Center Excellence Award from Massachusetts General Hospital. Shammaraganar Krishnan was supported by DoD fellowship W81XWH-19-1-0723. JP was supported by NIH Training Grants (grant no. T32HL007627 and T32CA251062). MD was supported by NIH/NCI K22CA258410.

REFERENCES
7. Filer AC, Henriquez M, Dey M. Recurrent glioma clinical trial, CheckMate-143: the game is not over yet. Oncotarget. 2017;8:19779.
Abstract 897-F Figure 1 (A) 005GSC-GFP cells were orthotopically implanted and randomized into control, WNT974, αPD1 and WNT974+αPD1 and survival was monitored. The median survival for each arm. *p<0.05 using Log Rank test for survival study. (B) IHC for Wnt7b Protein (dark) and immunofluorescence for beta-catenin (red) detection were performed on the tumor tissue collected from time-matched manner post treatment with Control, anti-PD1, and the combination (n=5–7 per group). (C) FACS analysis on the tumor tissue collected from different treatment groups to analyze the subpopulation of DC3-like dendritic cell (% from CD45+CD11C+ cells) and myeloid-derived suppressor cells (% from CD45+CD11b+ cells). Error bars show ±SEM. * p<0.05, ** p<0.01. One-Way ANOVA followed by test for multiple comparison of means.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0897-F