THE TIGIT/CD226/CD155 AXIS AND THE EFFECTS OF COMBINING PD-1/PD-L1 BLOCKADE WITH TIGIT-TARGETING ANTIBODY THERAPY IN SYNGENEIC MURINE GLIOBLASTOMA MODELS

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Background Recently, TIGIT+PD1 blockade was shown to confer additive survival benefits in orthotopic glioblastoma and colon cancer mouse models^{1,2}—notably, this included experiments³ that used the 1G9 TIGIT monoclonal antibody (mAb) that has potentially agonistic effects.¹ Herein we investigated the TIGIT/CD226/CD155 axis and effects of combining clinically analogous PD-1/PD-L1 mAbs with TIGIT-targeting 1G9 mAb in murine glioblastoma models.

Methods The overall survival (OS) associated with TIGIT (non-depleting 1G9; 200µg every 3days for 4 doses),¹ PD-1 (8H3; initial 500µg followed by 250µg every 3days for 7 doses), PD-L1 (6A2; initial 500µg followed by 250µg every 3days for 7 doses), and/or IgG mAbs was assessed in immuno-competent C57BL/6 albino mice intracranially implanted with syngeneic GL261-luc2 or CT2A-luc.^{4,5} The roles of T cells and NK cells were examined using depletion with CD8a, CD4, or NK1.1 mAbs. Expression of TIGIT/CD226/CD155 and PD-1/PD-L1/PD-L2 by tumor and tumor-infiltrating immune cells was evaluated using flow cytometry and RT-qPCR.

Results In vitro, GL261-luc2 and CT2A-luc tumor cells moderately expressed PD-L1, PD-1, and TIGIT; but strongly expressed TIGIT's inhibitory ligand CD155.(Figure1A-B) Ex vivo, >83% of CD8+ and CD4+TILs in GL261-luc2 co-expressed TIGIT+/CD226+: ≥2x the proportions in CT2A-luc.(Figure 2A) CD155 and PD-L1 were highly co-expressed on tumor-infiltrating macrophages: greater in GL261-luc2 than CT2A-luc tumors.(Figure 2B)In GL261-luc2 mice, anti-TIGIT monotherapy displayed minimal OS improvement; whereas anti-PD-1 and anti-PD-L1 monotherapies demonstrated robust OS responses.(Figure 3A) Adding anti-TIGIT to PD-1/PD-L1 blockade demonstrated synergism with anti-PD-1. Anti-TIGIT plus anti-PD-1 displayed nominally improved OS in CT2A-luc compared to anti-PD-1 monotherapy (p=0.11).(Figure 3B). Given robust T-cell expression of TIGIT and PD-1, we examined how CD4+ or CD8+ depletion affected responses in GL261-luc2 mice: depletion completely abrogated anti-PD-1's benefits.(Figure 4) Although CD4/CD8 depletion also reduced anti-TIGIT+anti-PD1's efficacy, the resulting OS matched that of non-depleted anti-PD-1 monotherapy. Additionally, NK cell depletion had no effect on anti-TIGIT+anti-PD1's efficacy.

Conclusions Our results recapitulate published findings regarding the synergistic benefits of combining TIGIT 1G9 mAb with anti-PD-1 using the clinically-relevant 8H3 mAb in syngeneic mouse glioblastoma, and extend those findings to anti-TIGIT+anti-PDL1 combinations. TIGIT/CD226 was highly co-expressed by immuno-responsive GL261-luc2's tumor-infiltrating lymphocytes (TILs); whereas CD155/PD-L1 expression predominated in tumor-infiltrating myeloid cells. Depletion of CD8+ or CD4+ TILs modestly reduced anti-TIGIT+anti-PD1's efficacy—suggesting a mechanism that is at least

partially independent of T (and NK) cells. Our preliminary results suggest a complex interplay between TIGIT/CD226/CD155 and PD-1/PD-L1/PD-L2 axes in tumors and their microenvironmental constituents that warrants further investigation; plus, careful consideration of antibody clones' functionality is necessary for designing immunotherapy combinations.

Abstract 256 Figure 1 TIGIT/CD155 axis and PD-1/PD-L1/PD-L2 axis expression in murine glioblastoma model tumor cells. Protein and RNA expression of the TIGIT and PD-1 immune checkpoints — and their ligands CD155 and PDL-1/PD-L2 respectively — on GL261-luc2 and CT2A-luc tumor cells using (A) flow cytometry (blue=samples, red=unstained controls) and (B) RT-qPCR (grey=GL261-luc2, black=CT2A-luc). Pcd1 encodes PD-1, Cd274 encodes PD-L1, Pvr encodes CD155

Abstract 256 Figure 2 TIGIT/CD155 axis and PD-1/PD-L1/PD-L2 axis expression in murine glioblastoma model tumor-infiltrating immune cells. (A) Flow cytometry analysis of protein expression for TIGIT (y-axis) and CD226 (x-axis; a competitor of TIGIT) on CD8+, CD4+/FOXP3+ Treg, and CD4+/FOXP3- Teff tumor-infiltrating lymphocytes (TILs) from GL261-luc2 and CT2A-luc tumor-bearing mice. (B) Flow cytometry analysis of protein expression for CD155 (TIGIT's ligand; bottom) and PD-L1 (PD-1's ligand; top) on tumor-infiltrating myeloid populations from GL261-luc2 and CT2A-luc tumor-bearing mice. Mean fluorescent intensity (MFI) was compared between cell lines, *indicates statistical significance. Myeloid populations included CD45+/CD11b+/CD11c+/F4-80+ macrophages, CD11b+/CD11c+ and CD11b-/CD11c+ dendritic cells (DCs), CD11b+/CD11c-/Ly6C+/Ly6G- monocytes, CD11b+/CD11c-/Ly6Cmid/Ly6G+ granulocytes, and CD45dim/CX3CR1+ microglia. Tumors were dissociated and leukocytes were enriched for using Percoll gradient. n=5 mice per group.

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Abstract 256 Figure 3 The survival associated with TIGIT-targeting mAb therapy with/without clinically-analogous PD-1/PD-L1 blockade. (A) Kaplan-Meier estimated overall survival (measured from day of intracranial tumor implantation) of GL261-luc2 mice treated with TIGIT (1G9), PD-1 (8H3), PD-L1 (6A2), TIGIT + PD-1, TIGIT + PD-L1 mAbs, or IgG control. (B) A Kaplan-Meier estimated overall survival (measured from day of intracranial tumor implantation) of CT2A-luc mice treated with TIGIT (1G9), PD-1 (8H3), TIGIT + PD-1 mAbs, or IgG control. For both experiments, all treatments were started on day 6 following implantation, with the following dosing: anti-TIGIT was given as 200µg every 3days for 4 doses. Both anti-PD-1 and anti-PD-L1 were given as an initial 500µg dose followed by 250µg every 3days for 7 doses.⁴ The n per group and number at risk table is included underneath each graph, along with the corresponding Cox regression analysis. Treatment groups with significantly different OS from the combination TIGIT + PD-1 combination-treated reference group were highlighted in yellow. HR = hazard ratio, CI = confidence interval.

Abstract 256 Figure 4 How depletion of CD8+ T cells, CD4+ T cells, or NK cells affects survival associated with TIGIT-targeting mAb therapy with/without clinically-analogous PD-1 blockade. Kaplan-Meier estimated overall survival (measured from day of intracranial tumor implantation) of GL261-luc2 mice treated with TIGIT (1G9), PD-1 (8H3), TIGIT + PD-1 mAbs, or IgG control; and compared to groups that additionally had CD8+, CD4+, or NK1.1+ antibody-based depletion. The treatment and dosing characteristics were the same as Figure 3. The n per group and number at risk table is included underneath the graph, along with the corresponding Cox regression analysis. Treatment groups with significantly different OS from the combination TIGIT + PD-1 combination-treated reference group were highlighted in yellow. HR = hazard ratio, CI = confidence interval.

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