MULTIMODAL GLIOMA IMMUNOTHERAPY COMBINING TLR9-TARGETED STAT3 ANTISENSE OLIGODEOXYNUCLEOTIDES WITH PD1-SPECIFIC IMMUNE CHECKPOINT BLOCKADE

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Background Malignant gliomas (MG) are rapidly fatal despite multimodal treatments including radiation therapy, used to treat nearly all MG patients, or even the emerging cellular immunotherapies. Therapeutic resistance in glioma is related to tolerogenic STAT3 activity in both glioma cancer stem cells (GCSs) and in the tumor-associated myeloid immune cells, such as macrophages and microglia, which dominate MG microenvironment.1,2 We previously demonstrated that STAT3 activity in GSCs and tumor-associated myeloid cells can be targeted using Toll-like Receptor-9 (TLR9)-targeted oligonucleotide therapeutics such as siRNA or antisense oligonucleotides (ASO).2,3

Methods Here, we describe development of a new TLR9-targeted and double-stranded STAT3 antisense oligonucleotide (CpG-STAT3dsASO) with optimized efficacy and safety for glioma immunotherapy.

Results Compared to our benchmark ASO oligonucleotides, the LNA-modified CpG-STAT3dsASO showed enhanced target gene knockdown in human and in mouse glioma cells and also in TLR9+ immune cells, such as macrophages and microglia. When tested against orthotopic model of human U251 glioma, intracranial injections of CpG-STAT3dsASO (1 mg/kg q2w) inhibited tumor growth and significantly extended survival of immunodeficient NSG mice compared to benchmark oligonucleotide. Next, we tested CpG-STAT3dsASO against syngeneic GL261 model in immunocompetent mice. Our results demonstrated that CpG-STAT3dsASO was more effective but also significantly better tolerated than single-stranded CpG-STAT3ASO when injected intracranially, without evidence of severe acute neural toxicities within tested dosing. All tested CpG-STAT3ASO variants induced maturation/activation of intratumoral DCs, macrophages and microglia, while reducing numbers of tumor-associated M2 macrophages and resting microglia as assessed using flow cytometry. Importantly, CpG-STAT3ASO treatments improved the ratio of intratumoral CD8 T cells to Tregs. To elucidate changes in the glioma microenvironment related to STAT3-inhibition/TLR9-activation, we performed an initial single-cell RNAseq analysis of transcriptomic profiles in immune cell subsets isolated from tumors after treatment using CpG-STAT3dsASO. Our analysis indicated the reprogramming of tumor-associated myeloid cell populations within treated glioma with an increased ratio of CD8:regulatory T cells. Our results also suggested the elevation of several immune checkpoint molecules on tumor-infiltrating T cells likely as a result of IFN signaling. Importantly, our preliminary experiments demonstrated a synergy between systemic PD1 inhibition with low-dose (0.25 mg/kg) CpG-STAT3dsASO local therapy. While, neither of treatments alone was curative, the combination anti-PD1/CpG-STAT3dsASO therapy resulted in complete rejection of orthotopic GL261 tumors in the majority of treated mice (figure 1).

Conclusions We believe that further development of CpG-STAT3dsASO will pave way to clinical translation of this strategy to immunotherapy of malignant glioma.

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REFERENCES

Abstract 887 Figure 1 Combination of CpG-STAT3dsASO with PD1 blockade in GBM model C57BL/6 mice with established orthotopic GL261 gliomas were injected twice weekly using intraperitoneal injections of 200 μg of PD1-specific or control antibodies, using intratumoral/IC injections of 0.25 mg/kg of LNA-modified CpG-STAT3dsASO or both treatments combined; shown is the Kaplan-Meier survival curve (n=6-7/group).