

## INVESTIGATING THE FUNCTIONAL ROLE OF GPNMB IN GLIOBLASTOMA AND THE TUMOR IMMUNE MICROENVIRONMENT AND ITS TARGETED ELIMINATION USING CAR-Ts

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**Background** Glioblastoma (GBM) is the most common primary malignant brain tumor in adults. Due to GBM displaying extreme heterogeneity and immune suppression, prognosis remains dismal. Glycoprotein nonmetastatic melanoma protein B (GPNMB) has previously been identified as a clinically relevant target in GBM while being absent in normal brain tissues and shown to be active in the tumor immune microenvironment.<sup>1,2</sup> Chimeric Antigen Receptor T-cells (CAR-Ts) fail clinical trials because multiple antigens will be required to eliminate all GBM subpopulations.<sup>3,4</sup> We previously proved CD133 to be an effective CAR-T target in GBM models.<sup>5</sup>

**Methods** Immunohistochemistry was performed on patient derived xenograft (PDX) brains and tissue microarrays of 16 patient matched primary/recurrent GBMs as well as 23 normal organ tissues. Whole cell proteomics was performed on 43 matched primary/recurrent GBM samples. Flow cytometry measured surface expression levels of CD133 and GPNMB to confirm CAR-T accessibility. CRISPR/Cas9 was used to eliminate expression in GBM lines to measure proliferation and mouse survival times. GPNMB knockout clones were generated in GL261 and engrafted in immunocompetent mice to examine single cell transcriptomes using scRNAseq at endpoint. A second-generation CAR-T was developed to target GPNMB-expressing populations, and efficacy was interrogated using standard *in vitro* assays and PDX models.

**Results** GPNMB detected in residual tumors of PDX brains treated with CD133 CAR-Ts revealed it as a targetable subpopulation. Tissue microarrays and proteomics found GPNMB to be upregulated in recurrent GBMs compared to primary ( $p=0.0349$  and  $p=0.0033$  respectively), particularly in macrophage populations while being absent in normal tissues. Eliminating GPNMB in cell lines decreased proliferation ( $P\leq 0.001$ ) and prolonged survival times in all mouse models ( $P\leq 0.01$ ) indicating its functional relevance. GPNMB knockout clones displayed downregulation of hallmark signalling pathways of GBM such as PDGFR, TGF-beta, Integrins and Stats, as well as decreased innate/adaptive immune activation. CAR-T cytotoxicity and activation was observed *in vitro* and *in vivo* resulting in decreased tumor burden ( $P\leq 0.001$ ) and increased survival times ( $P\leq 0.001$ ). Ultimately a CD133+ population was observed in residual tumors of GPNMB treated mice at endpoint. Surface expression of CD133 and GPNMB revealed co-expression and distinct populations.

**Conclusions** We show GPNMB influences tumor intrinsic biology of GBM and is active in the tumor immune microenvironment. By targeting GPNMB along with CD133, combinatorial therapeutic regimens could target the cancer stem cell hierarchy and its supportive niche. We therefore plan to administer both CAR-T cells to provide better cytotoxic coverage and increase efficacy.

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**Ethics Approval** Human GBM samples were obtained from consenting patients, as approved by the Hamilton Health Sciences/McMaster Health Sciences Research Ethics Board(#07-366). All animal work was conducted by Animal Research Ethics Board (AREB)-approved Protocols (AUP#19-01-01).

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