Vectorized Interferons Provide a Robust and Sustained Transcriptional Response Over Traditional Non-Vectorized Recombinant Cytokine Therapies in Human High-Grade Glioma Cells

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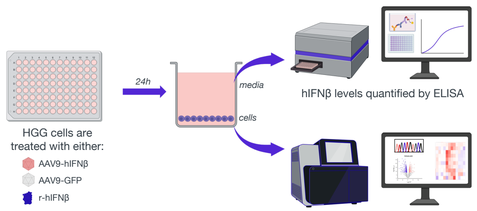
INTRODUCTION

Interferons are pleiotropic immunomodulatory cytokines that drive anti-tumor functions[1]. However, their short half-lives limit their utility as a recombinant protein therapy for oncology as they are unable to mount sustained anti-tumor responses[2]. Siren Biotechnology has developed an AAV immuno-gene therapy platform that delivers vectorized engineered interferons to overcome half-life challenges seen with traditional cytokine therapies. This new modality is designed to target multiple cancer types, with our first program in high-grade gliomas (HGGs), aggressive brain cancers with no effective therapeutic options. Here, we use an *in vitro* model to demonstrate increased cytokine durability and sustained transcriptional responses induced by an AAV immuno-gene therapy in human HGG cells.

APPROACH

Transduction of HGG cells with AAV9-hIFNβ



Two human HGG cell lines (GBM6-FL and U251-FL) were treated with either an AAV carrying an engineered human IFN-beta (AAV9-hIFNβ), AAV9-GFP, recombinant hIFNβ cytokine protein (r-hIFNβ), or media alone. 50% media washouts were performed every 5h for the first 20h post-treatment in the r-hIFNβ cytokine samples to mimic hIFNβ cytokine clearance *in vivo* (half-life = 4-5h)[2]. Full media exchanges were performed for all wells at 24, 48, 72, and 96h post-treatment to ensure measured hIFNβ levels were timepoint-specific and not representative of accumulation. Cell culture media and cells were collected 24, 48, 72, and 96h post-treatment for quantification of hIFNβ protein and bulk transcriptomic profiling, respectively. Quantification of hIFNβ protein was performed by ELISA. Bulk transcriptomic profiling was performed by mRNA-sequencing on an Illumina NovaSeq 6000 with 150bp paired-end reads. Reads were aligned to the human genome (GRCh38.p14) using STAR v2.7.11b and counted using subread v2.0.6. Differentially-expressed genes (DEGs) were identified relative to media alone and were considered significant if *pAdj<0.01*.

References

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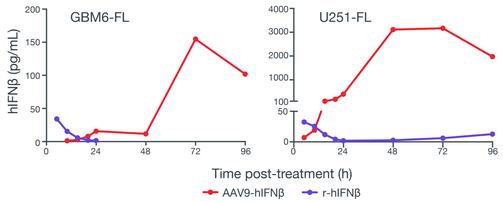
2. Wills, R. J. Clinical pharmacokinetics of interferons. *Clinical Pharmacokinetics* 19, 390–399 (1990).
3. Liberzon, A. *et al.* The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst* 1, 417–425 (2015).

RESULTS

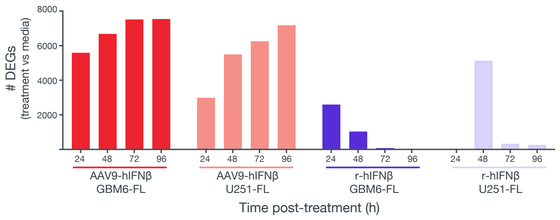
Vectorization of interferons overcomes limitations of protein clearance and induces sustained, biologically relevant transcriptional changes

AAV9-hIFNβ induces sustained expression of hIFNβ protein in HGG cells



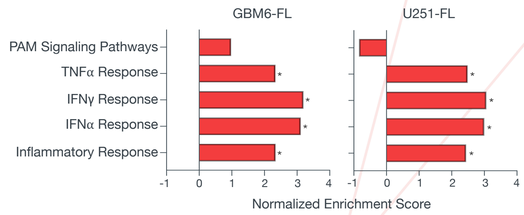
Supernatant hIFNβ levels in GBM6-FL or U251-FL HGG cells treated with either vectorized AAV9-hIFNβ or unvectorized r-hIFNβ cytokine were measured by hIFNβ ELISA 0, 5, 10, 15, 20, 24, 48, 72, and 96h post-treatment.

AAV9-hIFNβ, but not r-hIFNβ, induces a sustained and increasing transcriptomic response in HGG cells



DEGs identified by differential gene expression analysis of GBM6-FL and U251-FL HGG cells treated with AAV9-hIFNβ or r-hIFNβ cytokine vs their respective media-only treated controls. Performed in technical triplicates.

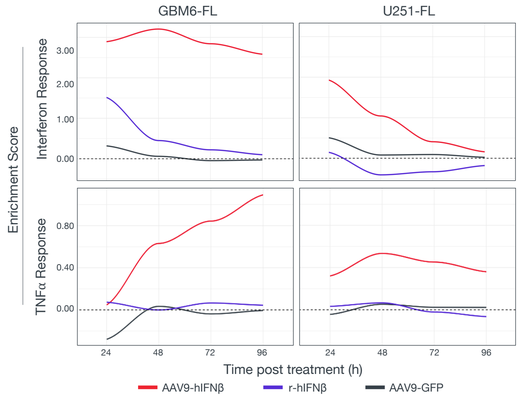
Gene ontology of AAV9-hIFNβ induced transcriptomic changes supports an IFN-specific pro-inflammatory response



*Example gene ontology terms[3] enriched in DEGs from GBM6-FL and U251-FL HGG cells 48h after treatment with AAV9-hIFNβ vs their respective media-only treated controls. *pAdj<0.01.*

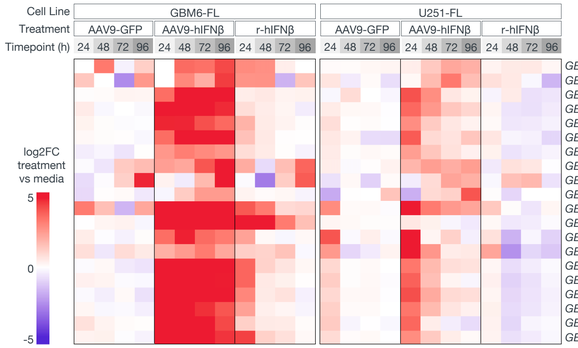
Sustained hIFNβ protein expression stimulates a durable pro-inflammatory transcriptional response in HGG tumor cells

Only vectorized hIFNβ induces durable enrichment of interferon and TNFα responses in HGG cells



Time course of IFN and TNFα Response Enrichment Scores (identified by IFNα and TNFα response gene ontology terms[3]) from GBM6-FL and U251-FL HGG cells after treatment with AAV9-hIFNβ, r-hIFNβ cytokine, or AAV9-GFP vs their respective media-only treated controls.

Gene expression analysis identifies 20 relevant hIFNβ-response genes across both vectorized and recombinant hIFNβ treatments in HGG cells



Heatmap of the top hIFNβ-treatment response genes involved in combined IFN and TNFα responses (identified by IFNα, IFNβ, IFNγ, and TNFα response gene ontology terms[3]) in GBM6-FL or U251-FL HGG cells at 24, 48, 72, and 96h post-treatment with either AAV9-hIFNβ, r-hIFNβ cytokine, or AAV9-GFP. Colors represent log2 fold change (log2FC) of either AAV9-hIFNβ, r-hIFNβ cytokine, or AAV9-GFP treatment vs media control.

CONCLUSIONS

AAV vectorized hIFNβ, but not recombinant hIFNβ cytokine protein, induces sustained hIFNβ expression and a durable IFN-specific, pro-inflammatory transcriptomic response in HGG cells. This supports cytokine vectorization in AAVs as a powerful new modality class that overcomes the half-life challenges seen with traditional cytokine immunotherapy approaches. Our data also define a hIFNβ transcriptional response signature that can be used to inform future mechanism of action and discovery studies.

Our findings have already translated to significant *in vivo* efficacy when vectorized cytokines were administered to mice bearing human HGG tumors, and we are rapidly advancing our first program with this improved AAV immuno-gene therapy platform to the clinic.

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