Identification of Pharmacologically Relevant Species for IND-Enabling Toxicology Studies by Deep Comparative Transcriptomic Profiling of PBMCs Treated with Recombinant Human Payloads

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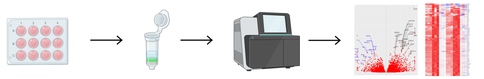
SIREN BIOTECHNOLOGY

INTRODUCTION

Identifying pharmacologically relevant animal species for toxicology assessments ensures study findings translate into safe clinical trials. Siren Biotechnology has developed vectorized cytokines—AAV immuno-gene therapies—as an improved modality for treating solid tumors. As cytokine payloads are predicted to exhibit species specificity, we developed an assay to identify pharmacologically relevant species for IND-enabling toxicology assessments using readily available peripheral blood mononuclear cells (PBMCs). Here, we describe the methodologies and assay findings when implemented for an engineered human interferon beta (hIFNβ) transgene.

APPROACH

Perform bulk transcriptomics of PBMCs from multiple species



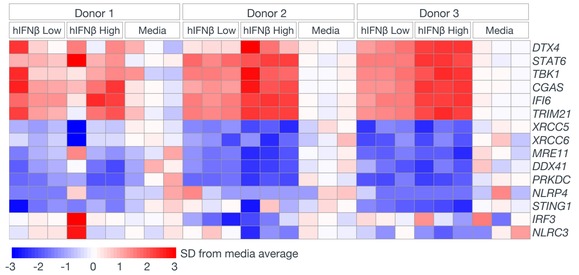
Quickly and robustly identify the relevant species for hIFNβ pharmacologics

Bulk transcriptomics were performed on PBMCs from human, cynomolgus macaque (cyno), Göttingen minipig, canine, guinea pig, mouse, and rat donors following treatment with recombinant hIFNβ protein. Mouse PBMCs were used as a negative control species for the assay because they have an IFNAR1 receptor (the receptor for IFNβ) known *not* to be cross-reactive to hIFNβ[1]. Cyno PBMCs were used as a positive control because they are a gold-standard toxicology species and have been used in preclinical safety packages for approved hIFNβ drugs[2]. RNA was purified from PBMCs treated in triplicate with either 1,000 IU/mL (high dose) of hIFNβ, 100 IU/mL (low dose), or media alone for 24h. Bulk transcriptomic profiling was performed by RNA-sequencing on an Illumina NovaSeq 6000 with 150 bp paired-end reads. Reads were aligned to the host species' genome 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*. Each species was classified as responsive/non-responsive based on both the relative degree of differentially expressed genes and modulation of IFN-specific pathways.

RESULTS

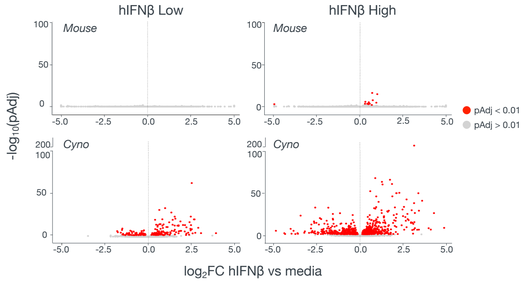
Transcriptomic analysis of PBMCs from varying species can be used to identify cross-species responsiveness to a human cytokine

Human PBMCs dosed with hIFNβ define a target hIFNβ response signature



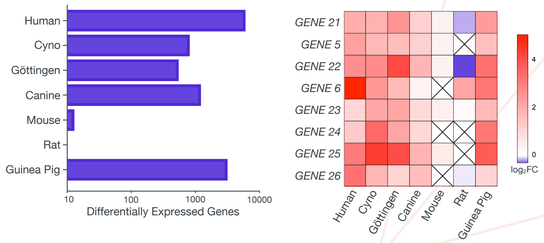
Heatmap of selected genes within the Sting Mediated Immune Response Pathway[3] in human PBMCs treated with either hIFNβ or media. Mean read count of each gene in media treated controls was determined on a per donor basis. Colors represent the number of standard deviations (SD) the individual samples' mean read count is away from the media mean read count.

Assay successfully identifies non-responders and responders in negative and positive control species



Differential gene expression analysis of control species PBMCs treated with hIFNβ vs their respective media-only treated controls. N=3 cyno donors, N=3 pooled mouse lots; tested in technical triplicates.

Transcriptomic profiling identifies cyno, göttingen, canine, and guinea pigs as responsive species to hIFNβ treatment

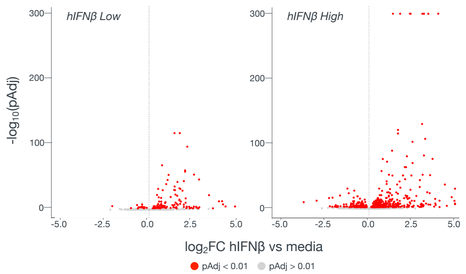


Left: Total # DEGs induced by hIFNβ treatment in human, cyno, Göttingen, canine, mouse, rat, and guinea pig PBMCs. DEGs were defined as genes with significantly up- or down-regulated expression (pAdj<0.01) in hIFNβ-treated PBMCs vs media-treated controls.

Right: Differential expression levels (log2FC) of hIFNβ signaling-specific genes in tested species.

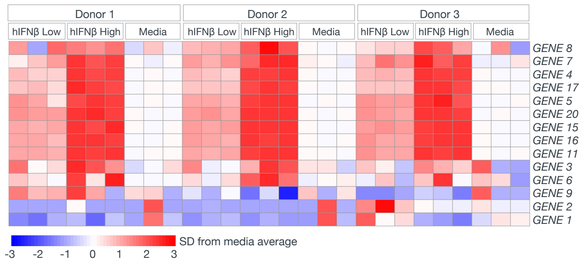
Göttingen minipigs, our selected toxicology species, activate hIFNβ signaling response pathways in response to treatment with hIFNβ

Number of hIFNβ-induced DEGs is dose responsive in Göttingen minipigs



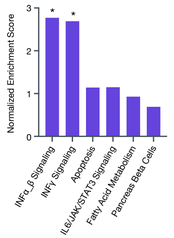
Differential gene expression analysis of Göttingen PBMCs treated with hIFNβ vs their media-only treated controls. N=3 donors; technical triplicates.

Known cancer-relevant interferon response genes are induced with hIFNβ treatment



Heatmap of selected cancer-relevant interferon-response genes (identified in Poster #713) in Göttingen PBMCs treated with either hIFNβ or media control. Colors represent the standard deviation (SD) from average media mean read count as described above.

Gene ontology supports an IFN-specific response



*Example gene ontology terms[4,5] enriched in differentially expressed genes in Göttingen PBMCs treated with hIFNβ vs media control *pAdj<0.01.*

References

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Acknowledgements

Siren's work would not be possible without investor, partner, & grant support. We would like to thank:

Partners & Collaborators: Recombinetics Inc., WhiteLab Genomics, Catalent Inc.

Grant Funding: CIRM, Merck Pharma/Millipore-Sigma, GenScript, Beckman Coulter

Investors: Founders Fund, Lux Capital, ARTIS Ventures, KMAK Capital, GRIDS Capital, Tau Capital, Civilization Ventures, Innovation Endeavors, Kirimil Family Charitable Foundation, Avila Ventures, Carnrite Ventures, Women's Equity Lab, Plum Alley Ventures, Gaingels, West Coast Angel Network

CONCLUSIONS Siren demonstrated that bulk transcriptomic profiling of cytokine-treated PBMCs can be used to quickly and robustly identify pharmacologically relevant toxicology species while bypassing development of costly species-specific reagents, enabling investigators to propose cost-effective and easier to source species alternatives to primates for IND-enabling studies. This approach shows promising applications to guide species selection for other immunotherapeutic modalities or to justify the use of species-specific drug surrogates if a relevant species cannot be found. In the case of hIFNβ, pigs were identified as a pharmacologically relevant species, and safety evaluations for vectorized hIFNβ (SRN-101) are underway.