Development of a Treatment Response Prediction Strategy for Sparsentan in Glomerular Disease

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AIM

To identify a transcriptional response profile to sparsentan that is translatable to human glomerular disease and allow interrogation of non-invasive surrogate biomarkers.

ABSTRACT

Sparsentan is a first in-class, novel, dual endothelin angiotensin receptor antagonist (DEARA) being developed for the treatment of focal segmental glomerulo-sclerosis (FSGS) and IgA nephropathy. Sparsentan is highly selective for the endothelin type A and angiotensin II type 1 receptors.

The Adriamycin-induced (ADR) nephropathy model in rats is characterized by rapid podocyte injury, proteinuria, glomerulosclerosis, tubulo-interstitial fibrosis, and lesions reflective of human FSGS.

A response profile was developed using gene expression data from the kidneys of sham, diseased, and sparsentan-treated ADR study animals which was mapped to human data.

The rat disease signature score calculated from glomerular transcriptome profiles was elevated in patients with FSGS, negatively correlated with eGFR at time of biopsy, and positively correlated with urine protein:creatinine (UPCR) in the NEPTUNE cohort

METHODS

RNA was extracted from formalin fixed paraffin embedded kidney tissue from the rat study, sequenced and aligned to Rnor genome assembly version 6.0.88. Differentially expressed genes (DEGs) were calculated with DESeq2 across different comparisons between Sham (healthy), ADR (disease model) and ADR with sparsentan (treatment model). DEGs induced in the model and suppressed by sparsentan were carried forward for human ortholog mapping (Ensembl build 104). Human transcriptional profiles were generated from microdissected glomerular and tubulointerstitial profiles from the NEPTUNE cohort. Sparsental response scores were calculated using the average profile of the aforementioned genes.

RESULTS

ADR-Rat study design

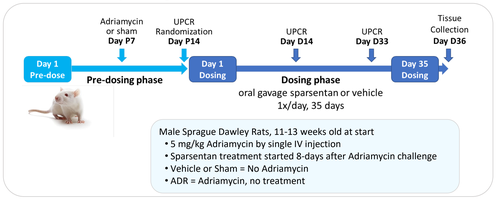
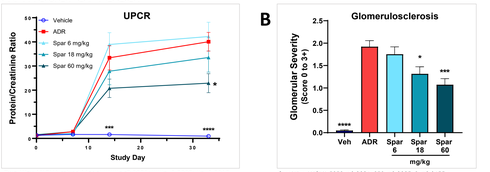


Figure 1. Study design to assess the ability of sparsentan to attenuate a FSGS-like phenotype driven by adriamycin in rats

Sparsentan attenuated measures of disease severity in the rat ADR model of FSGS.

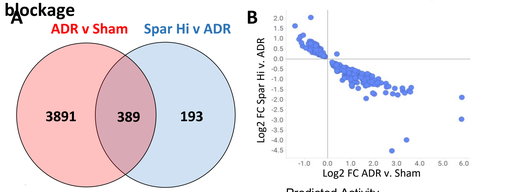


Two-Way ANOVA mixed Model ****p<0.0001; ***p=0.0005; *p=0.0215 vs. ADR.

One-Way ANOVA ****p<0.0001; ***p=0.0005; *p=0.0155 vs. ADR.

Figure 2. Proteinuria (A) and glomerulosclerosis (B) in the ADR rat model. UPCR and glomerulosclerosis were dose-dependently reduced, with significant attenuation at the high dose of 60 mg/kg.

High dose sparsentan treatment reversed directionality of many genes induced in the model, disease-associated network activities, and was consistent with angiotensin blockage



C

Upstream regulator	ADR v. Sham	Spar Hi v. ADR
TNF	11.2	-5.7
IL1B	8.2*	-4.6*
AGT	9.9	-4.5
IFNG	10.0	-3.5
JAK1	4.5*	-2.4*

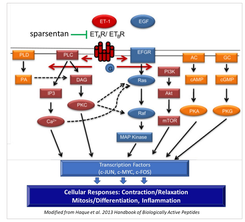
Predicted Activity

* Gene was differentially regulated in the comparison consistent with predicted activity

Figure 3. (A) A majority of significant DEGs (p-adj<0.05) in the Spar Hi v ADR comparison (389/582) were found in the ADR v Sham comparison. (B) Directionality of the 389 genes common between the two comparisons was reversed consistent with attenuation of the disease signal by sparsentan. (C) IPA upstream regulator networks identified from DEG profiles were consistent with pathways activated and implicated in human FSGS. Sparsentan attenuated predicted increases in network activities of AGT (angiotensinogen) consistent with mechanism of action.

A subset of genes reversed by sparsentan in the model were enriched in the endothelin pathway

Symbol	Entrez Gene Name	ADR v. Sham log2 FC	Spar Hi v. ADR log2 FC
ADCY5	adenylate cyclase 5	-0.5	0.4
ADCY7	adenylate cyclase 7	1.1	-0.7
HMOX1	heme oxygenase 1	1.7	-1.9
JUN	Jun proto-oncogene, AP-1 transcription factor subunit	0.7	-0.4
MYC	MYC proto-oncogene, bHLH transcription factor	1.6	-1.1
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	-0.2	0.2
PIK3CD	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta	0.9	-0.6
PIK3R5	phosphoinositide-3-kinase regulatory subunit 5	1.2	-0.7
PRKCZ	protein kinase C zeta	-0.4	0.3
PTGS2	prostaglandin-endoperoxide synthase 2	-0.7	2.1
RASD1	ras related dexamethasone induced 1	1.2	-1.1



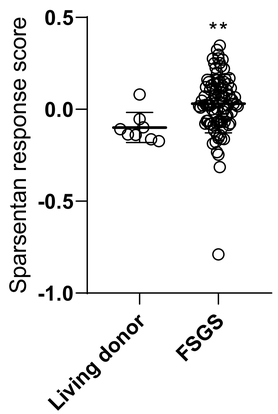
Modified from Haque et al. 2013 Handbook of Biologically Active Peptides

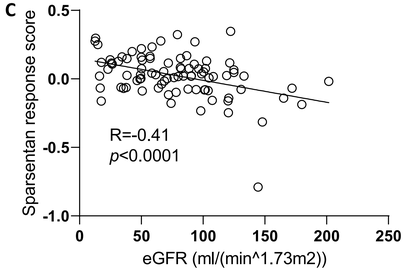
Figure 4. The 388 gene intersect between SparHi v. ADR and ADR v. Sham was enriched for endothelin pathway genes (p<0.001, Fisher's exact test). Pathway genes in both analyses that were significantly after multiple hypothesis correction are shown in the table.

Sparsentan responsive genes were associated with human FSGS and clinical measures of disease.

A

NEPTUNE cohort	FSGS
Samples with glom RNA-seq	93
Sex	59M/34F
Age (mean ± SD)	30 ± 22
eGFR at Bx (mean ± SD)	75 ± 38
UPCR (median, IQR)	2.7, 5.4





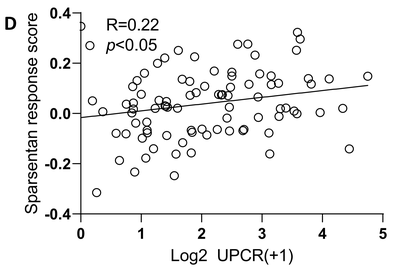


Figure 5. (A) Clinical information for samples profiled in this study. Human orthologs of genes suppressed by sparesentan in the Spar Hi vs ADR comparison (lower right quadrant, Figure 3B) were Z-transformed and the average of all genes was used to compute an intrarenal sparsentan response score from glomerular transcriptomes. The sparsentan response score was (B) elevated in patients from NEPTUNE with FSGS compared to healthy living transplant donors, was (C) negatively correlated with eGFR at time of biopsy, and (D) positively correlated with UPCR at time of biopsy.

Biomarkers were identified that helped predict intra-renal sparsentan response scores

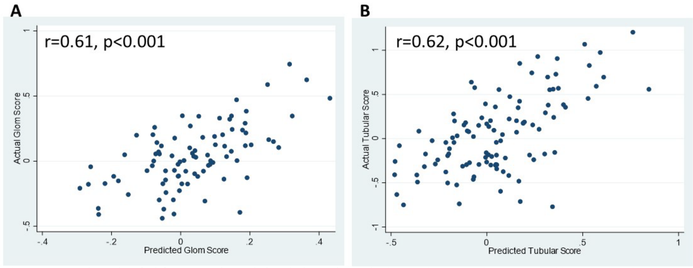


Figure 6. Biomarkers identified from urine (uA2M/Cr) and plasma (PDGFB), along with eGFR and UPCR were able to predicted sparsentan response score in patients with nephrotic syndrome. The predicted scores, correlated highly and significantly with calculated sparsentan scores from: (A) glomeruli (uA2M/Cr, PDGFB, eGFR in the predicted model), and (B) tubulointerstitium (uA2M/Cr, PDGFB, eGFR, UPCR in the predicted model) across the NEPTUNE cohort.

CONCLUSIONS

- Sparsentan treatment of an ADR rat model impacted expected target pathways (angiotensin and endothelin)
- Sparsentan treatment also impacted pathways implicated in FSGS (e.g., TNF, JAK-STAT)
- A response score was elevated in patients with FSGS from the NEPTUNE cohort and associated with routine clinical measures
- Biomarkers and clinical measures (eGFR and UPCR) were able to predict intrarenal transcriptional profiles of genes responsive to sparsentan from animal models and are being further evaluated

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DISCLOSURES

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