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MO264

Selective Endothelin A Receptor Antagonist Atrasentan Attenuates Mesangial Cell Injury, Proteinuria and Intra-Renal Proliferative, Inflammatory and Fibrotic Transcriptional Networks in a Rat Model of Mesangio-proliferative Glomerulonephritis

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Mesangial Cell Activation is the Initiating Intra-Renal Response to Glomerular IgA Immune Complex Deposition in IgA Nephropathy

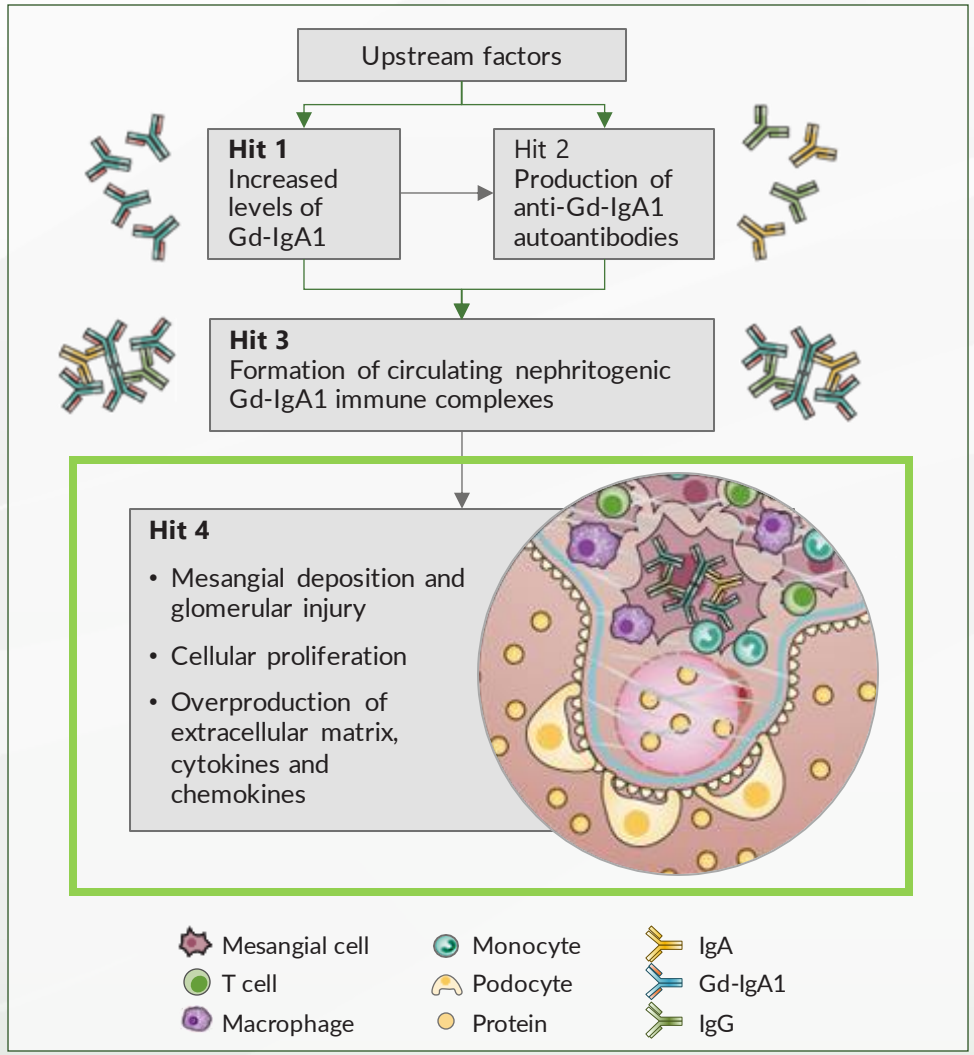
Mesangial cell (MC) activation is characterized by:

- Cellular proliferation
- Overproduction of extracellular matrix and inflammatory cytokines and chemokines

Cellular crosstalk results in podocyte injury and proteinuria, the strongest predictor of IgAN progression

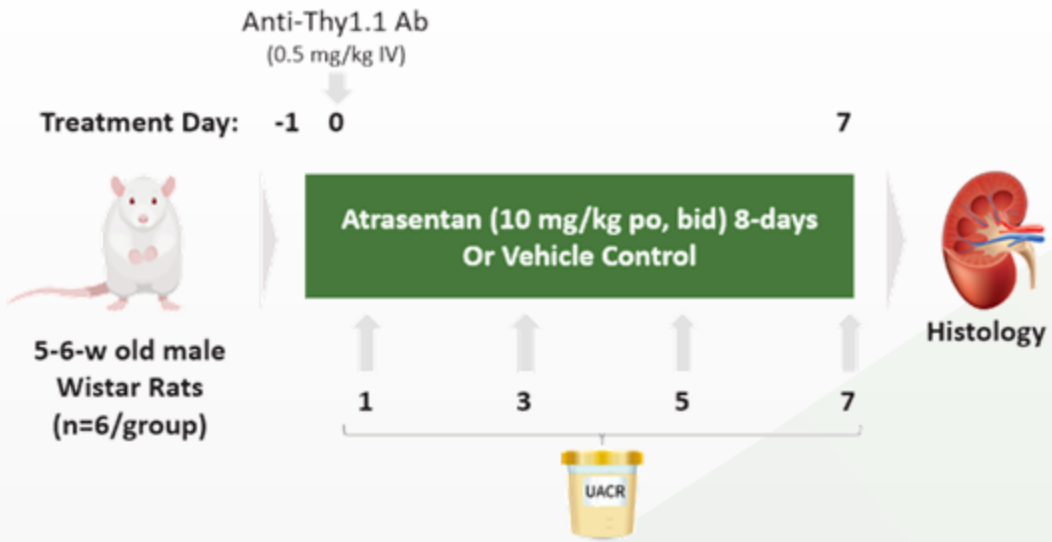
Subsequent tubulointerstitial inflammation and fibrosis leads to progressive kidney function loss

The **molecular pathways** responsible for MC activation and subsequent podocyte injury/proteinuria following glomerular IgA-complex deposition have **not been well-defined**



Methods

Anti-Thy1.1 Mesangioproliferative GN



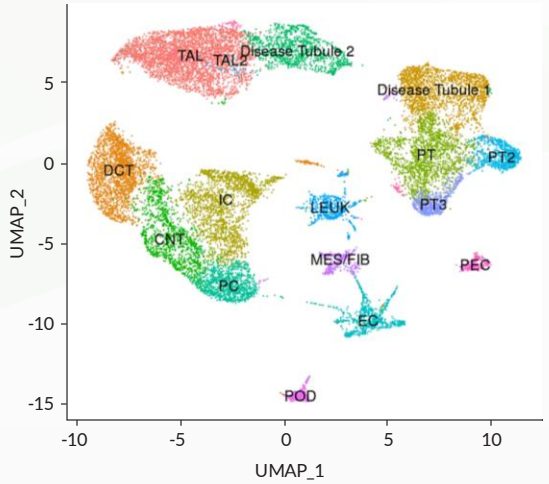
- Urinary protein and creatinine measured by standard methods
- Kidney histology: fixed (10% neutral buffered formalin), paraffin embedded, sectioned at 4-6 microns, stained (H&E and PSR) and evaluated by light microscopy using a semi-quantitative grading scale by blinded pathologist
- Recapitulates key mechanistic aspects of the mesangial cell response in IgAN, representing a surrogate model

RNA sequencing and data analysis

RNA-seq was performed on flash-frozen kidney cortex and analyzed using DESeq2 to identify differentially expressed genes followed by gene set enrichment analysis to identify dysregulated transcriptional networks which were cross-validated to the glomerular transcriptome of kidney biopsy samples from IgAN patients (GSE104066).

Samples were scored for a "Failed Repair" signature derived from Kirita et al.[1]

Cell type-specific signatures were derived from a scRNA-seq dataset (GSE171314) from IgAN patients.



A cluster of cells (Disease Tubule 1) was identified with the following properties

- Cells were highly expanded in IgAN vs healthy kidney
- Cells had high expression of a TNF activation signature

The top 75 differentially expressed genes in this cluster compared to all other cells were used to derive an IgAN Disease Tubule signature.

1. *PNAS* 2020 Jul 7;117(27):15874-15883.

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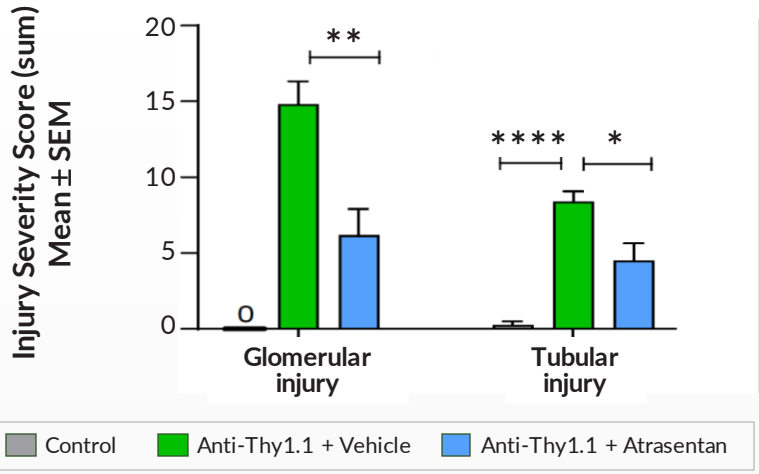
Effect of Atrasentan in a Rat Model of Mesangio-Proliferative Glomerulonephritis

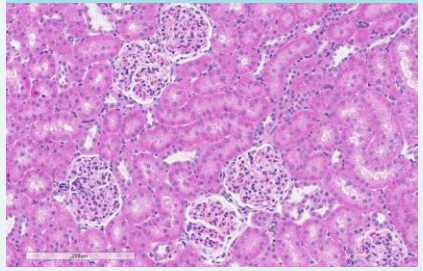


Glomerular injury score includes mesangial hypercellularity and matrix expansion, adhesions, segmental mesangiolysis and glomerulosclerosis

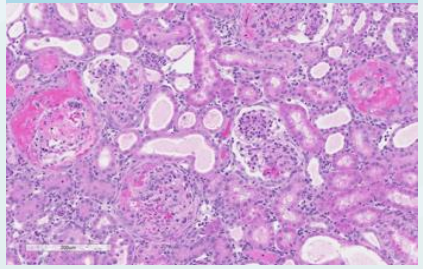
Tubulointerstitial injury score includes protein casts, tubular degeneration, tubular dilation and interstitial fibrosis

Atrasentan attenuated mesangial cell response, glomerular injury and secondary tubulointerstitial injury

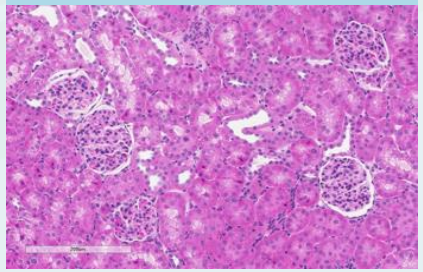
Vehicle + Vehicle



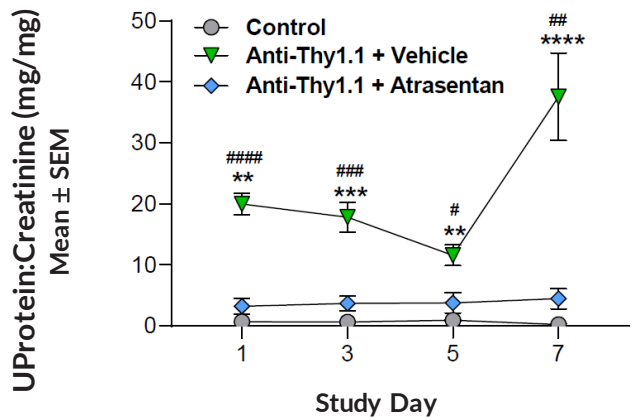
Anti-Thy1.1 + Vehicle



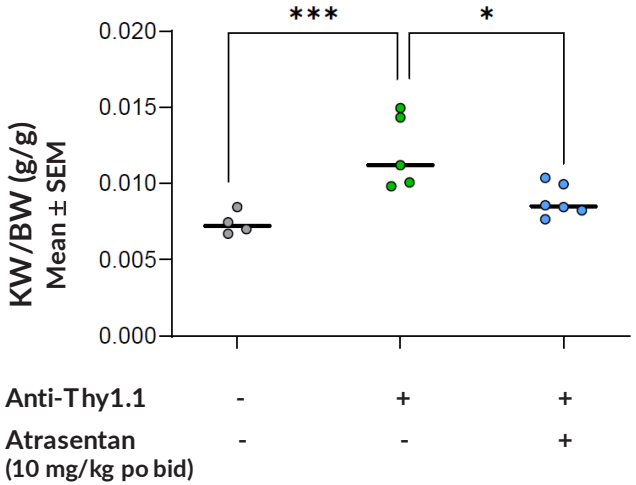
Anti-Thy1.1 + Atrasentan



Atrasentan reduced anti-Thy1.1 induced proteinuria



Atrasentan reduced anti-Thy1.1 induced increase in kidney weight



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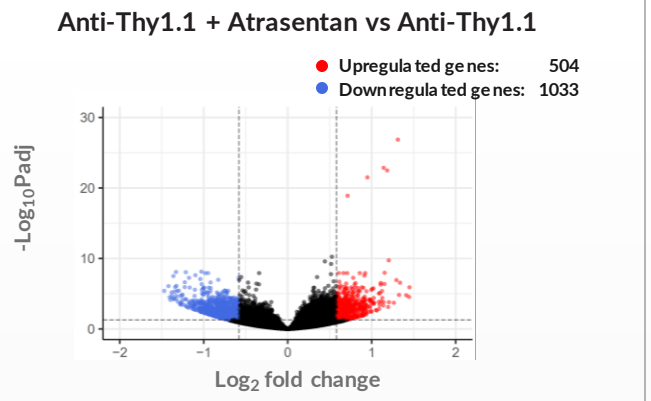
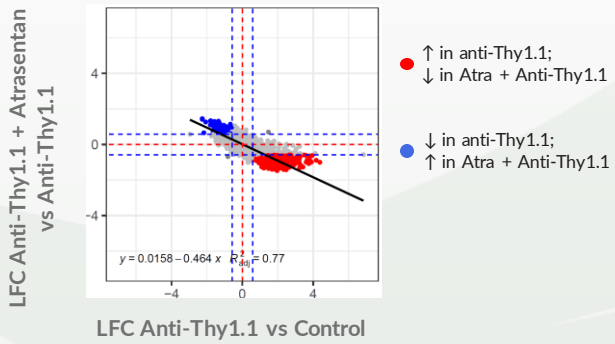
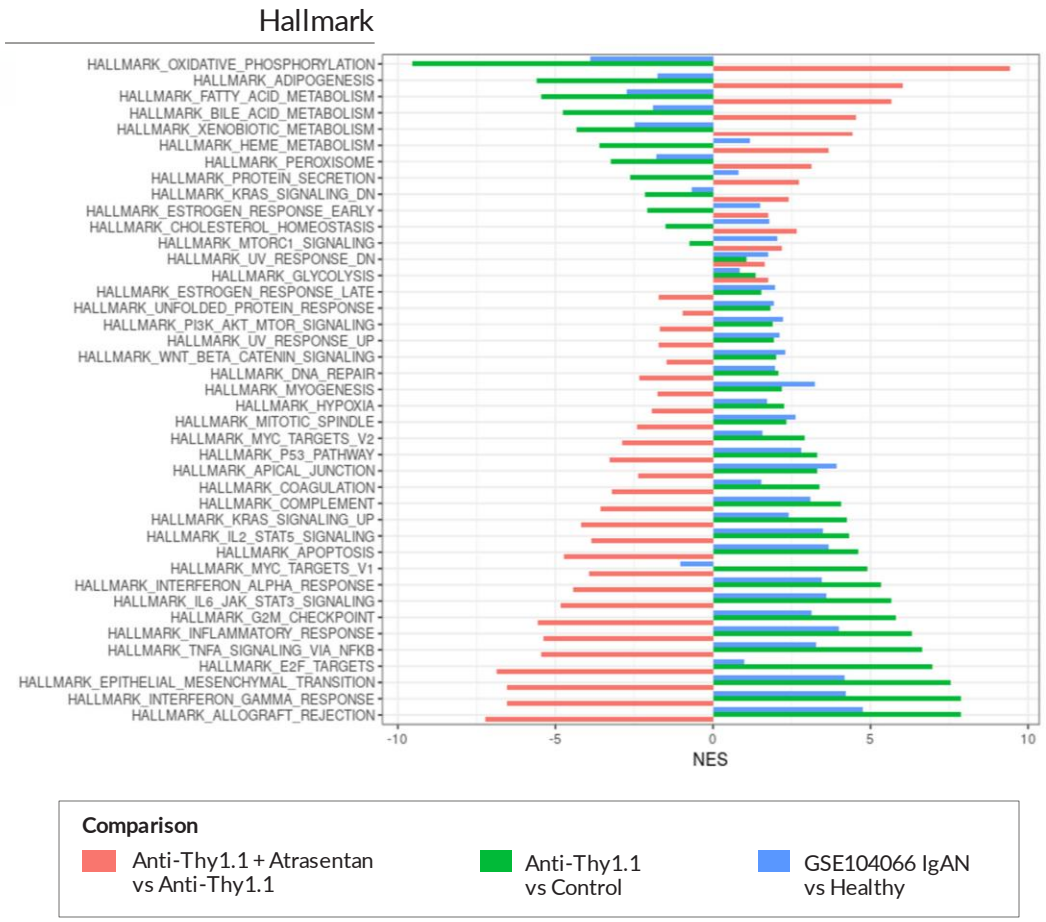
Atrasentan Reverses Transcriptomic Changes Induced in Anti-Thy1.1 Model



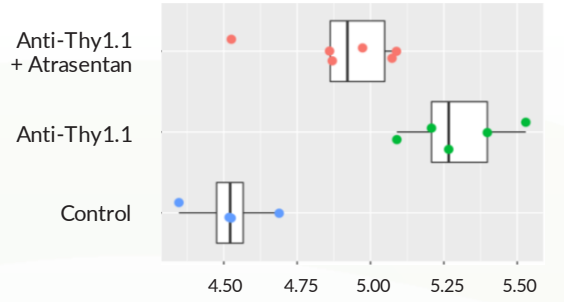
1,537 are differentially expressed (log fold-change > 0.58 or < -0.58 and padj <= 0.05) in anti-thy1.1 treated rats following atrasentan treatment



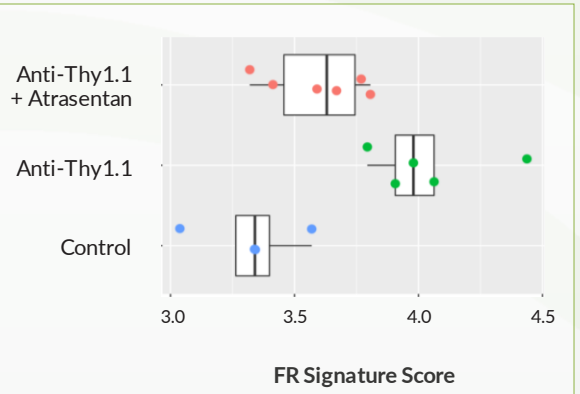
Atrasentan tends to reverse gene expression changes induced by anti-Thy1.1. 1,132 genes increased by anti-Thy1.1 are downregulated by atrasentan and 514 genes decreased by anti-Thy1.1 are upregulated by atrasentan



Atrasentan reverses Hallmark gene set expression enrichments induced in the anti-Thy1.1 rat model of mesangioproliferative GN, most of which are also observed in the glomerular transcriptome of IgAN patients in the NEPTUNE cohort (GSE104066)



A signature derived from disease-associated tubules in IgAN is increased in anti-Thy1.1 rats and is decreased by atrasentan



A "Failed Repair" (FR) gene expression signature is increased in anti-Thy1.1 rats and is decreased by atrasentan

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Summary and Conclusions

Atrasentan attenuated mesangial cell response, glomerular injury and secondary tubulointerstitial injury and reversed the increase in proteinuria and kidney weight caused by anti-Thy1.1 treatment

Induction of MPGN in rats was transcriptionally characterized by a down-regulation of metabolism gene networks and up-regulation of networks associated with proliferation, inflammation and fibrosis, consistent with the hallmark gene sets dysregulated in the glomeruli of IgAN patients

Atrasentan down-regulated these intra-renal proliferative, inflammatory and fibrotic transcriptional networks and restored metabolism networks

Gene signatures derived from "failed repair" cells and IgAN disease tubule cluster were increased following anti-Thy1.1 treatment and atrasentan decreased the expression of these signatures

This study suggests an important role of the ETA receptor in MC activation, subsequent proteinuria and activation of pathogenic proliferative, inflammatory and fibrotic intra-renal transcriptional networks in MPGN

This further supports the therapeutic potential of atrasentan, a selective ETA receptor antagonist, to attenuate mesangial cell activation, proteinuria and pathogenic intra-renal signaling in MPGNs such as IgAN

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