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Nefecon treatment likely modulates downstream pathways of kidney inflammation and fibrosis in IgA nephropathy

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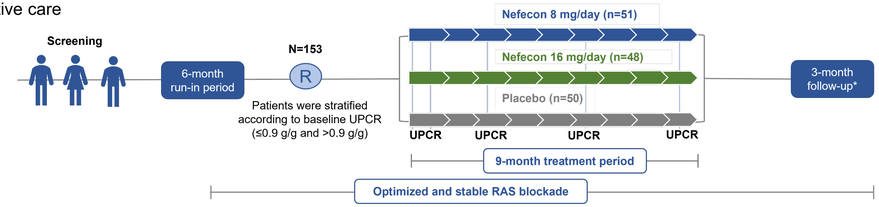
INTRODUCTION

- IgA nephropathy (IgAN) is characterized by the accumulation of immunoglobulin A1 (IgA1)-containing immune complexes (IgA1-ICs) in the renal mesangium, leading to breakdown of the glomerular filtration barrier; this allows unfiltered proteins to come into contact with cells lining the tubules, causing progressive tubulointerstitial inflammation and scarring, which is a predictor of disease progression in IgAN[1,2]
- The NEFIGAN trial (NCT01738035) tested the safety and efficacy of a novel targeted-release formulation of budesonide (Nefecon) designed to deliver budesonide to the gut-associated lymphoid tissue (GALT)-rich distal ileum in patients with IgAN in addition to optimized supportive care

Study Design



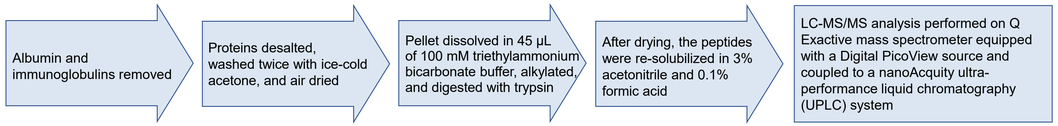
**Patients who received 16 mg/day Nefecon during months 0–9 were tapered to 8 mg/day for 2 weeks while all other patients (ie, those who received Nefecon 8 mg/day or placebo during months 0–9) received placebo to maintain masking. No further trial medication was administered after tapering*

- The headline result of the study was that Nefecon 16 mg/day, added to optimized renin-angiotensin system blockade, reduced proteinuria and stabilized estimated glomerular filtration rate in patients with IgAN. These findings have now been replicated in the NefIgArd study, which reported in 2021 and provided the basis for the recent FDA and EMA approval of Nefecon as a treatment for patients with IgAN at high risk of progressive disease. The results of the completed trial will be presented separately at this conference
- In this study, we determined the composition of urinary proteins from patients treated with placebo and 16 mg of Nefecon in the NEFIGAN trial using liquid chromatography with tandem mass spectrometry (LC-MS/MS)

METHOD

- Urine samples from 18 patients from each of the placebo and 16 mg/day arms of the NEFIGAN trial collected at start of treatment (SOT) and end of treatment (EOT) were analyzed. Patients were only included if they had received at least 8 months of treatment and the urine sample was taken up to 2 days after the completion of tapering

Preparation and LC-MS/MS analysis of urine



- For protein identification and quantification, raw data were processed with FragPipe (V16) having at least 2 peptides per protein
- The protein intensities reported in the combined_protein.txt file generated by the FragPipe were:
  1. Log_2-transformed and internally normalized against a group of peptides found in all samples
  2. The normalized protein abundance at SOT was subtracted from the abundance from the EOT for each patient
  3. A probabilistic dropout model was fitted to the data to estimate fold changes between the treatment and placebo groups at EOT
  4. The proteins were ranked using the t-statistic and a gene set enrichment analysis was performed to determine the gene sets significantly affected by the treatment compared with the placebo group

RESULTS

Gene ontology analysis revealed that treatment with 16 mg of Nefecon led to a significant enrichment of multiple pathways (n=57) involved in a number of processes previously shown to be important in the pathogenesis of kidney injury in IgAN (Tables 1-4)

Table 1: Epigenetic pathways

ID	Description	Enrichment score	qvalue
GO:0045814	negative regulation of gene expression, epigenetic	0.77822	0.000023
GO:0097549	chromatin organization involved in negative regulation of transcription	0.77822	0.000023
GO:0060968	regulation of gene silencing	0.74540	0.000132
GO:0060147	regulation of posttranscriptional gene silencing	0.76836	0.000132
GO:0060964	regulation of gene silencing by miRNA	0.76836	0.000132
GO:0060966	regulation of gene silencing by RNA	0.76836	0.000132
GO:0031047	gene silencing by RNA	0.69949	0.000173
GO:0035194	posttranscriptional gene silencing by RNA	0.71719	0.000405
GO:0016458	gene silencing	0.65049	0.000730
GO:0035195	gene silencing by miRNA	0.71011	0.001105
GO:0040029	regulation of gene expression, epigenetic	0.69539	0.001510
GO:0016441	posttranscriptional gene silencing	0.69427	0.001522
GO:0010608	posttranscriptional regulation of gene expression	0.46586	0.019032

Table 2: Microvesicle formation

ID	Description	Enrichment score	qvalue
GO:0099503	secretory vesicle	0.41340	0.002955
GO:0030312	external encapsulating structure	0.44636	0.007910
GO:0016192	vesicle-mediated transport	0.38002	0.011345

Table 3: Kidney remodeling

ID	Description	Enrichment score	qvalue
GO:0048771	tissue remodeling	0.73277	0.001004
GO:0062023	collagen-containing extracellular matrix	0.48024	0.001510
GO:0031012	extracellular matrix	0.44636	0.007910
GO:0006508	proteolysis	0.39559	0.019032
GO:0010466	negative regulation of peptidase activity	0.47351	0.022175
GO:1903035	negative regulation of response to wounding	0.60180	0.028780
GO:0010951	negative regulation of endopeptidase activity	0.47271	0.033849
GO:0009888	tissue development	0.38878	0.041148
GO:0009611	response to wounding	0.44240	0.041559

Table 4: Local immune and inflammatory responses

ID	Description	Enrichment score	qvalue
GO:0045638	negative regulation of myeloid cell differentiation	0.77415	0.000029
GO:0006953	acute-phase response	0.83084	0.000132
GO:0045596	negative regulation of cell differentiation	0.59467	0.000506
GO:0006954	inflammatory response	0.50626	0.001602
GO:0001775	cell activation	0.41658	0.001975
GO:0002682	regulation of immune system process	0.41526	0.003481
GO:0032101	regulation of response to external stimulus	0.45829	0.004380
GO:0006955	immune response	0.38373	0.006983
GO:0045321	leukocyte activation	0.40302	0.011254
GO:0034097	response to cytokine	0.43277	0.011740
GO:0002526	acute inflammatory response	0.61731	0.015653
GO:0009605	response to external stimulus	0.38349	0.015653
GO:0033554	cellular response to stress	0.42383	0.015653
GO:0002263	cell activation involved in immune response	0.40543	0.019023
GO:0009967	positive regulation of signal transduction	0.43257	0.020140
GO:0002366	leukocyte activation involved in immune response	0.40197	0.020140
GO:0080134	regulation of response to stress	0.42627	0.020417
GO:0002274	myeloid leukocyte activation	0.40534	0.021614
GO:0009986	cell surface	0.45022	0.021865
GO:0002443	leukocyte-mediated immunity	0.40019	0.021946
GO:0010647	positive regulation of cell communication	0.42567	0.022175
GO:0023056	positive regulation of signaling	0.42699	0.023965
GO:0002444	myeloid leukocyte-mediated immunity	0.40808	0.023965
GO:0043299	leukocyte degranulation	0.40453	0.028193
GO:0002252	immune effector process	0.37813	0.034174
GO:0045637	regulation of myeloid cell differentiation	0.54056	0.037771
GO:0023051	regulation of signaling	0.38341	0.038167
GO:0048584	positive regulation of response to stimulus	0.38498	0.038876
GO:0006935	chemotaxis	0.48048	0.039247
GO:0042330	taxis	0.48048	0.039247
GO:0009966	regulation of signal transduction	0.38914	0.039247
GO:0002275	myeloid cell activation involved in immune response	0.40185	0.040444

CONCLUSIONS

These urine proteomic data support the positive impact of Nefecon on downstream proinflammatory and profibrotic pathways within the kidneys. These data will be validated in biomarker analyses currently underway as part of the NefIgArd study.

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