Serum proteomic biomarker analysis of the interleukin-2 receptor pathway agonist rezpegaldesleukin in patients with atopic dermatitis

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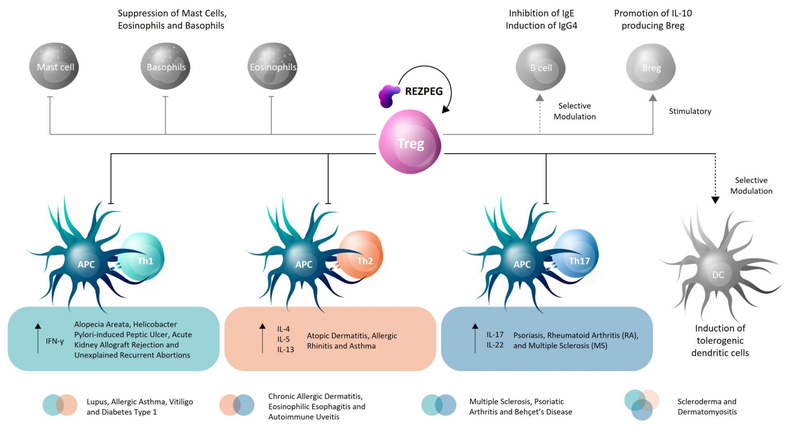
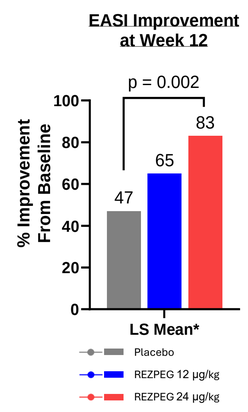
INTRODUCTION

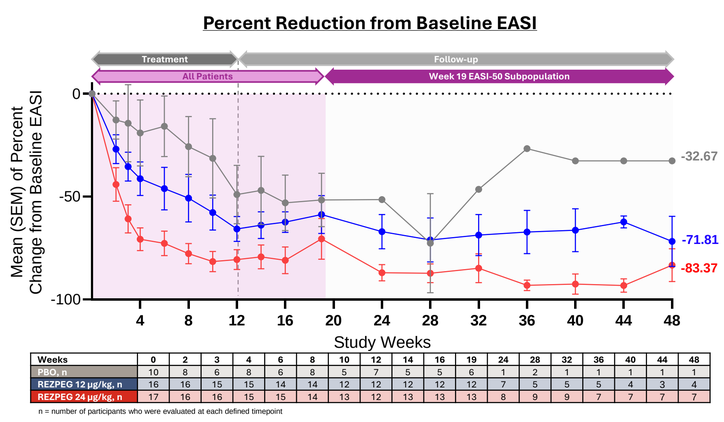
Rezpegaldesleukin (REZPEG) is an interleukin-2 receptor (IL-2R) pathway agonist that has been shown to stimulate the expansion and function of regulatory T cells (Tregs) that are impaired in inflammatory cutaneous conditions including atopic dermatitis (AD). REZPEG has demonstrated clinical activity in AD[1] and has the potential to orchestrate immune homeostasis through the restoration of the Treg compartment. Proteomic analyses have been shown to be useful for identifying biomarkers related to AD pathogenesis and for monitoring therapeutic responses.[2] While Tregs are the primary cellular target of REZPEG,[3,4] here we sought to use proteomic analysis to characterize REZPEG's disease modifying agonistic mechanisms of action and dynamics at the biomolecular level.



Rezpegaldesleukin (REZPEG)

- Polymer conjugated recombinant human Interleukin-2 (rhIL-2)[3]
- Administered as an active drug, with pegylation conferring high selectivity for Tregs without activation of effector T-cells (Tcons)[3,4]
- Enhancing Treg function is a novel therapeutic strategy for restoring immunological homeostasis[3,4]
- Nearly 600 healthy volunteers and patients have been administered REZPEG to-date across 9 studies
- REZPEG results in dose-dependent, selective, and up-to 17-fold increase in CD25^{bright} Tregs over baseline that is sustained for 20–30 days[4]



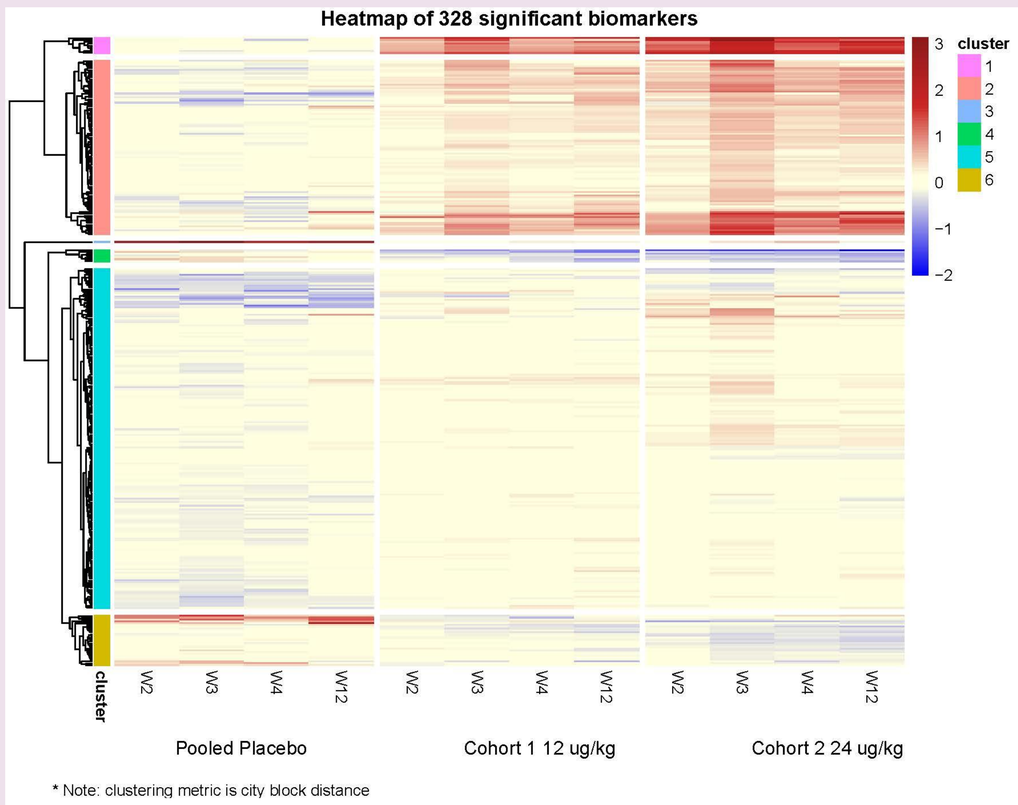


Weeks	0	2	3	4	6	8	10	12	14	16	19	24	28	32	36	40	44	48
PBO, n	10	8	6	8	6	8	5	7	5	5	6	1	2	1	1	1	1	1
REZPEG 12 µg/kg, n	16	16	15	15	14	14	12	12	12	12	12	7	5	5	5	4	3	4
REZPEG 24 µg/kg, n	17	16	16	15	15	14	13	12	13	13	13	8	9	9	7	7	7	7

n = number of participants who were evaluated at each defined timepoint

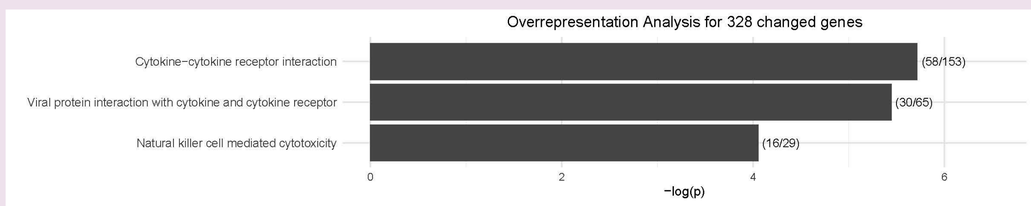
EASI: eczema area and severity index; SEM: Standard error of the mean; continuous endpoint using observed data; *EASI Improvement results are least squares (LS) mean percent change from baseline obtained from Mixed Model for Repeated Measures (MMRM) as specified in the statistical analysis plan (SAP) defined in the protocol (generated by independent statistical audit firm)

RESULTS

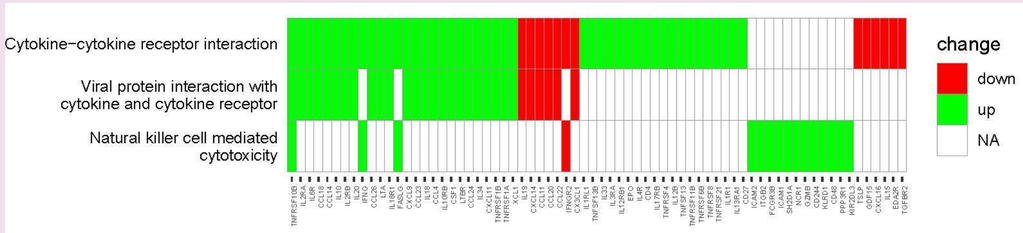


* Note: clustering metric is city block distance

Proteins that are grouped within the same cluster share more similar patterns over time than those grouped in other clusters.



* Note: For each KEGG pathway, in the annotation (x/n), x is the number of significant changed proteins to the treatment of REZPEG and n is the total number of proteins in a pathway. In Olink data analysis for 1460 proteins, 328 proteins were significantly elevated or decreased in response to REZPEG treatment relative to baseline and compared to placebo. IL2 was also significantly increased but was removed from the results due to it being a component of REZPEG. Benjamini–Hochberg method is used to correct for multiple testing.

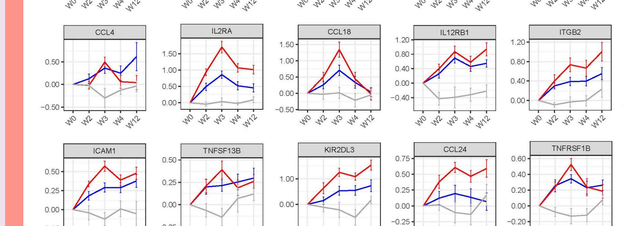


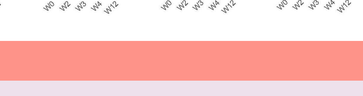
* Note: colored by direction of log2(FC) at week 3

The 3 enriched pathways overrepresented by the 328 differentially expressed proteins are visualized with log2(fold-change) at week 3 from baseline in patients treated with 24 µg/kg REZPEG.

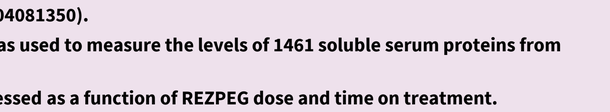
Enrichment analysis was run within each cluster to identify the KEGG pathway(s) overrepresented by the proteins in the cluster.[5-7] The same pathway could be overrepresented in different clusters. For each bar in the bar plots, the pathway name is presented at the left side; the number of proteins in the cluster and the pathway over the number of analyzed proteins in the pathway are presented at the right side. Line plots are provided for the overrepresented proteins in each cluster represented in one of the 3 enriched pathways (cytokine-cytokine receptor interaction, viral protein interaction with cytokine and cytokine receptor, natural killer cell mediated cytotoxicity).

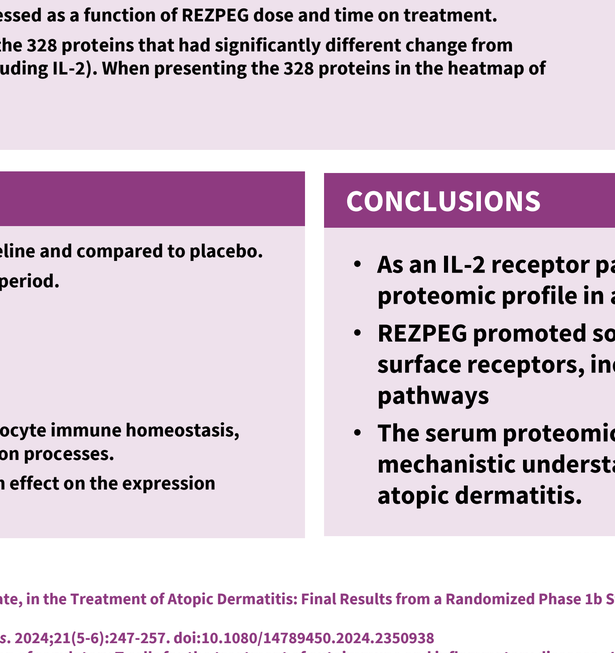
Cluster 1





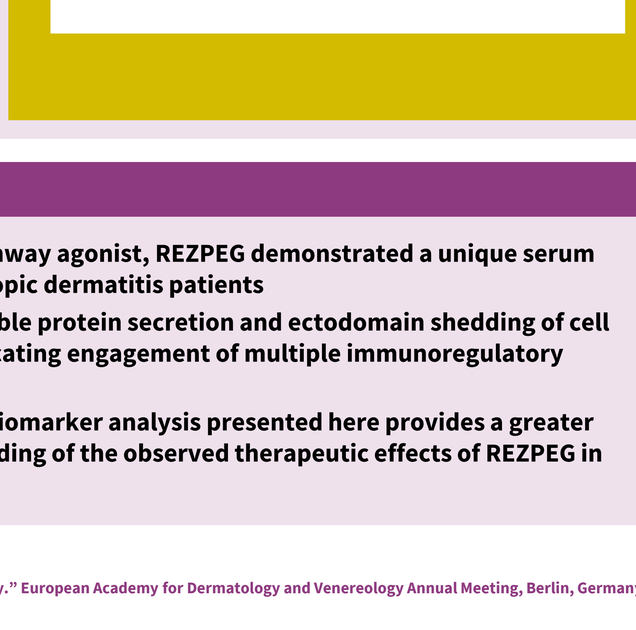
Cluster 2





Clusters 3 and 4

Cluster 5



For cluster 6, no enriched pathway was detected.

METHODS

- **Patients with moderate-to-severe AD received 12 or 24 µg/kg REZPEG or placebo once every 2 weeks for 12 weeks (NCT04081350).**
- **The Olink proteomics platform with the Explore 384 cardiometabolic, inflammation, neurology, and oncology panels was used to measure the levels of 1461 soluble serum proteins from patients at baseline and throughout the induction period at weeks 2, 3, 4, and 12.**
- **Results were analyzed using a longitudinal linear mixed effects model to identify proteins that were differentially expressed as a function of REZPEG dose and time on treatment.**
- **Pathway enrichment analysis was performed with a hypergeometric test to identify the pathways overrepresented by the 328 proteins that had significantly different change from baseline between REZPEG groups and placebo while considering all the 1460 analyzed proteins as the background (excluding IL-2). When presenting the 328 proteins in the heatmap of Log2(FC), the hierarchical clustering method using City Block (also referred as Manhattan) distance was applied.**

SUMMARY

- **There were 328 proteins that were significantly elevated or decreased in response to REZPEG treatment relative to baseline and compared to placebo.**
- **The expression profiles of these biomarkers exhibited REZPEG dose- and time-dependency over the 12-week induction period.**
- **The serum protein profiles that differed between REZPEG treatment and placebo included those that:**
  - **Increased across all timepoints through week 12,**
  - **Increased over the first month of treatment then normalized toward baseline by week 12, and**
  - **Decreased across all timepoints through week 12.**
- **Consistent with its biological activity as an IL-2R agonist, REZPEG modulated Treg pathways and those involving lymphocyte immune homeostasis, MHC expression and regulation, ectodomain shedding of cell surface receptors, as well as cellular migration and adhesion processes.**
- **REZPEG reduced the expression of serum proteins known to be elevated in patients with AD as well as demonstrating an effect on the expression levels of known targets for current AD therapy**

CONCLUSIONS

- **As an IL-2 receptor pathway agonist, REZPEG demonstrated a unique serum proteomic profile in atopic dermatitis patients**
- **REZPEG promoted soluble protein secretion and ectodomain shedding of cell surface receptors, indicating engagement of multiple immunoregulatory pathways**
- **The serum proteomic biomarker analysis presented here provides a greater mechanistic understanding of the observed therapeutic effects of REZPEG in atopic dermatitis.**

REFERENCES

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