041 Identification of Circulating Pharmacodynamic Biomarkers of IL-5 Inhibitors using a Proteomics Approach

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Abstract

Background: Proteomics can identify pharmacodynamic (PD) biomarkers to support clinical pharmacology studies for biosimilar drugs development and approval. Mepolizumab and reslizumab are two interleukin 5 (IL-5) inhibitors approved for the treatment of severe asthma with an eosinophilic phenotype. Peripheral blood eosinophil count is used for dose selection in the development programs and has been discussed as a PD biomarker for biosimilar development, though variability may limit its utility.

Purpose: The aim of the study is to assess the utility of plasma proteomics for the identification of additional circulating PD biomarkers of IL-5 inhibitors.

Methodology: A discovery pilot was conducted in 266 plasma samples from 32 healthy subjects from a placebo-controlled randomized single dose clinical study with IL-5 inhibitors by the FDA. Using the SOMAscan® assay (SomaLogic, v4.1), 7288 analytes were measured at 11 timepoints over 123 days in mepolizumab (n=8 [24 mg]), reslizumab (n=8 [0.8 mg/kg]), and placebo groups (n=8). ANOVA was conducted on linear-mixed effect models regressing protein level changes with treatment, time and their interaction. Analytes with p-values< 6.82E-06 (Bonferroni-adjusted alpha) for the interaction term were considered differentially expressed. Proteins were further prioritized based on biological relevance, peak change, and area under the effect curve (AUEC) for both products.

Results: Three candidate proteins, pappalysin (PAPPA) for mepolizumab, and proteoglycan-3 (PRG-3) and follicular dendritic cell secreted peptide (FDCSP) for reslizumab were identified as differentially expressed upon treatment. PAPPA was also associated with response to reslizumab (p= 7.16E-05) and PRG-3 with mepolizumab (p= 7.41E-06), but at a lower significance threshold. Further analysis of FDCSP response to reslizumab showed that the original association was driven by variance in the placebo group over the study time. A significant difference in AUEC of PAPPA compared to placebo was observed for mepolizumab (t-test p=1.98E-02) and reslizumab (p=1.39E-04) as well as AUEC of PRG-3 for reslizumab (p=8.6E-04), but not mepolizumab (t-test p=0.19) compared to placebo.

Conclusion: Using proteomics and a discovery cohort, we identified PAPPA and PRG-3 as potential PD biomarkers of IL-5 inhibitors for future investigation.

Introduction

IL-5 dysregulation is associated with several allergic diseases including Type 2/eosinophilic asthma which characterizes eosinophilic inflammation. IL-5 is a cytokine that plays a key role in differentiation, mobilization, survival and recruitment of eosinophils. IL-5 inhibitors bind to IL-5 preventing its binding to the IL-5α receptor on eosinophils which results in reduced inflammation. Approved IL-5 antagonists mepolizumab and reslizumab are humanized IgG monoclonal antibodies with a high affinity for IL-5. The current and best primary PD biomarker of eosinophilic asthma is changes in peripheral blood eosinophils. Other exploratory candidates are eosinophil cationic protein and and eosinophil-derived neurotoxin. However, these candidates have limited data and show high variation in levels. **The present study presents the preliminary results of the evaluation of plasma proteomics for the identification of new PD biomarkers for IL-5 inhibitor products**

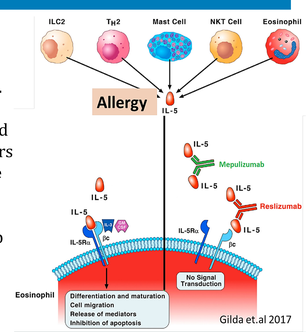


Figure 1. IL-5 Inhibitors mode of action. Adapted from: Gilda et.al. 2017[1]

Methods

A discovery pilot was conducted in 266 plasma samples from 32 healthy subjects from a placebo-controlled randomized single dose clinical study with IL-5 inhibitors by the FDA. Using the SOMAscan® assay (SomaLogic, v4.1)[2] 7288 analytes were measured at 11 timepoints over 123 days in mepolizumab (n=8 [24 mg]), reslizumab (n=8 [0.8 mg/kg]), and placebo groups (n=8). ANOVA was conducted on linear-mixed effect models regressing protein level changes with treatment, time and their interaction. Analytes with p-values< 6.82E-06 (Bonferroni-adjusted alpha) for the interaction term were considered differentially expressed. Proteins were further prioritized based on biological relevance, peak change, and area under the effect curve (AUEC) for both products. Plasma protein levels were confirmed for newly identified candidate biomarkers using replication cohort (24 healthy subjects).

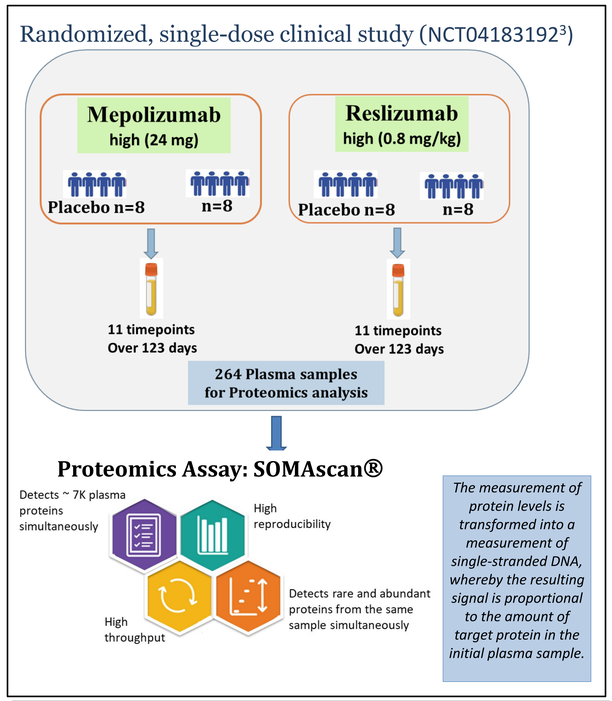
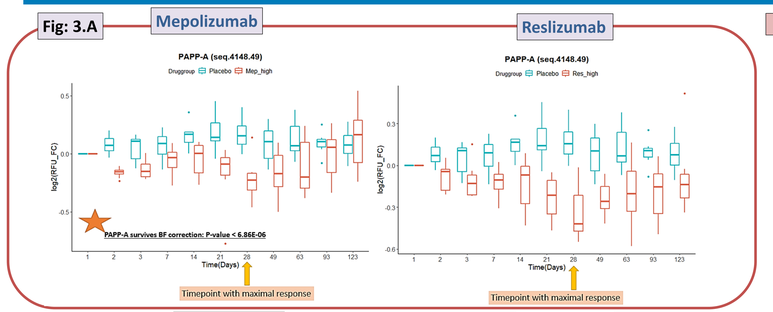


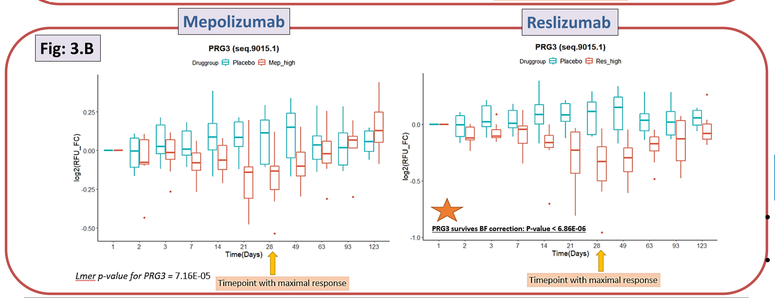
Figure 2. Experimental workflow

Results

- PAPPA for mepolizumab, and PRG-3 and FDCSP for reslizumab were identified as significantly differentially expressed upon treatment (p-values< 6.82E-06 ;Bonferroni-adjusted alpha).
- PAPPA was also associated with response to reslizumab (p= 7.16E-05) and PRG-3 with mepolizumab (p= 7.41E-06), but at a lower significance threshold.
- PAPP-A and PRG3 were independently replicated in high-intermediate dose plasma samples. A significant difference in AUEC of PAPPA compared to placebo was observed for mepolizumab (t-test p=1.98E-02) and reslizumab (p=1.39E-04) as well as AUEC of PRG-3 for reslizumab (p=8.6E-04), but not mepolizumab (t-test p=0.19) compared to placebo.

Results and Discussion





Impact of IL-5 Inhibitors on PAPP-A (Figure 3.A) and PRG3 (Figure 3.B)

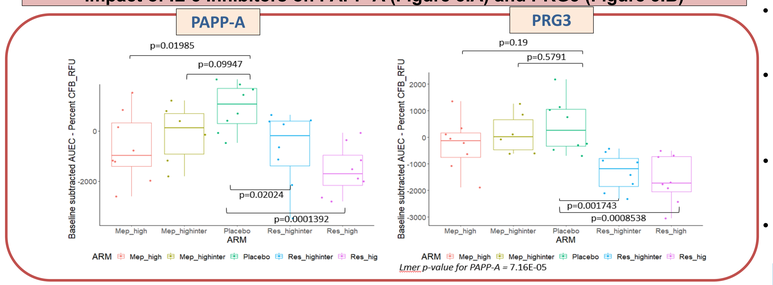


Figure 4. Area under the effect curve of PAPP-A and PRG3 show a dose response to IL-5 inhibitors

FDCSP as PD Candidate for Reslizumab

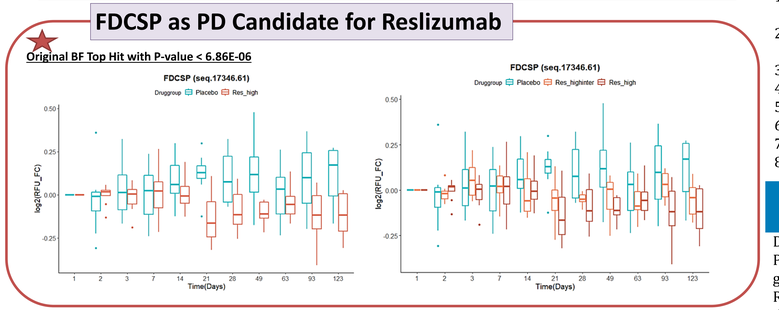


Figure 5: FDCSP Dose Response for Reslizumab

Functions of PAPP-A & PRG3

PAPP-A is linked to severe allergic astl

- Higher serum PAPP-A levels are reported in patients with asthma as compared to healthy adults. Also, PAPP-A levels are decreased following anti asthma drugs[4,5].
- It has been suggested that PAPP-A protein expression could represent a novel biomarker for Type-2 (T2) asthma severity phenotypes[5,6].

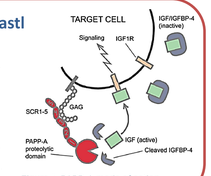
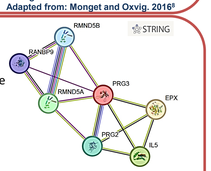


Figure 7. PAPP-A mode of action. Adapted from: Monget and Oxvig. 2016[8]

Functions of PRG3

- PRG3 is also called eosinophil major basic prote (MBP) homolog.
- MBPs are strongly implicated as mediator of bronchial asthma[7].
- PRG3 localizes to the eosinophil secondary granule in the cytoplasm



IL-5 is a predicted functional partner of PRG3

Figure 8. functional protein association networks showing IL-5 and PRG3 association

Conclusion

- PAPP-A and PRG3 proteins were identified as potential candidate PD biomarkers for mepolizumab and reslizumab.
- Both proteins have reported mechanistic links with allergic asthma and/or eosinophilia, and PRG3 is predicted to functionally interact with IL-5.
- FDCSP was identified as a potential candidate for reslizumab, but the response curve/pattern indicates this results is likely driven by variance in the placebo group over the study time.
- Because the biologics are inhibitors, their impact on IL-5 levels and/or downstream proteins responses is dependent on the basal expression of IL-5 (which in healthy subjects is low), and the dose of the drug.
- Future analyses will assess the variability of each of the candidate PD biomarker proteins and correlation with each other.
- Correlations between the candidate PD biomarkers and eosinophil counts from the same subjects will also be assessed.

References

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