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Stratification of Responders and Non-responders in the REACH-1 Trial Based on Serum Proteomic Analysis

Presented at the
61st ASH Annual Meeting & Exposition
Orlando, FL, USA • December 7–10, 2019

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Abstract

Acute graft-versus-host disease (aGVHD) is a serious and potentially life-threatening condition that can develop in patients receiving hematopoietic stem cell transplantation (HSCT). Acute GVHD is characterized by increased levels of inflammatory mediators and signaling through the Janus kinases (JAKs), leading to tissue and organ damage. Ruxolitinib, an oral JAK1/JAK2 inhibitor, is the first FDA-approved treatment for steroid-refractory aGVHD. The current study evaluated proteomic signatures from participants in the REACH-1 clinical trial to identify those individuals that have an increased probability for success when treated with ruxolitinib in combination with corticosteroids.[1]

Plasma was collected for 42 participants enrolled in the REACH-1 clinical trial (NCT02953678) prior to and at designated times following treatment. Broad proteomic analysis of more than 1000 proteins was conducted using the Olink proximity extension assay. Participants were separated into responders (complete responders, very good partial responders, partial responders) (n = 36) or non-responders (mixed responders, progressive disease) (n = 6) based on their clinical response at day 28 of treatment. Baseline differences in day 28 responders versus non-responders were compared using *t* tests. Pharmacodynamic changes within responders and non-responders were assessed using paired *t* tests. Significance of statistical tests was conferred at $P < 0.05$.

Broad proteomic analysis of plasma identified a total of 145 differentially expressed proteins ($P < 0.05$) between the responders and non-responders at baseline. Thirty-five proteins were elevated and 110 proteins were lower in responders compared with non-responders. At baseline, interleukin (IL)-8, IL-6, and IL-24 were among the most significantly down-regulated, while stem cell factor (SCF/KIT ligand) was one of the most significantly up-regulated in responders compared with non-responders.

Pharmacodynamic analyses revealed 493 significantly modulated proteins in responders and 92 in non-responders. IL-2–receptor alpha (RA) was among the most significantly down-regulated from baseline to day 28 in both responders and non-responders.

This study suggests that proteomic characterization has the potential to stratify responders and non-responders to ruxolitinib and corticosteroid treatment in steroid-refractory aGVHD. Additionally, this study demonstrated robust pharmacodynamic and correlative changes in responders following treatment with ruxolitinib and corticosteroids, suggesting that defining a responder population could enrich for patients predisposed to positive therapeutic response; however, this should be prospectively validated in additional studies.

Methods

Participants

- Seventy-one steroid-refractory aGVHD patients who met the inclusion and exclusion criteria were enrolled in this study:
  - Stage II–IV GVHD
  - Refractory from corticosteroid use after 7 days, or
  - Refractory from immunosuppressive therapy

Biomarker Analyses

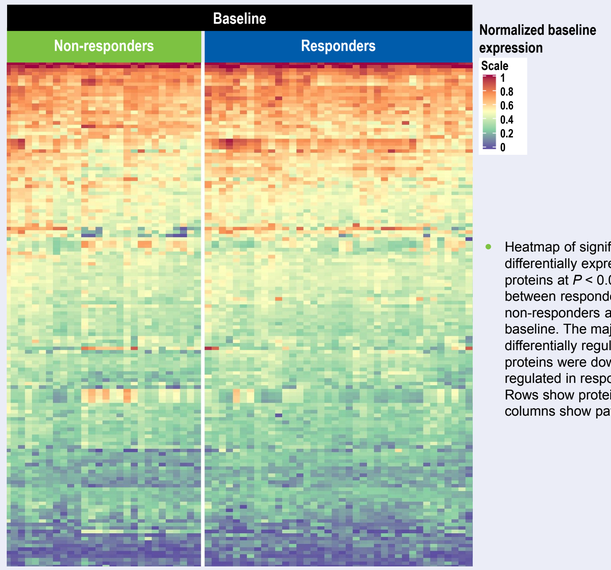
- Plasma was available for 39 responders and 28 non-responders at baseline only
- Plasma was available for 36 responders and 6 non-responders at baseline and day 28
- Proximity extension assay by Olink Proteomics Inc. (Watertown, MA) was used to conduct proteomic analysis

Statistical Analyses

- Statistical differences from baseline to day 28 within each treatment group were evaluated using paired (longitudinal) and unpaired (baseline differences) *t* tests of log2 normalized protein expression
- Different heatmaps do not represent the same sets of proteins; only significant proteins are shown for each analysis question

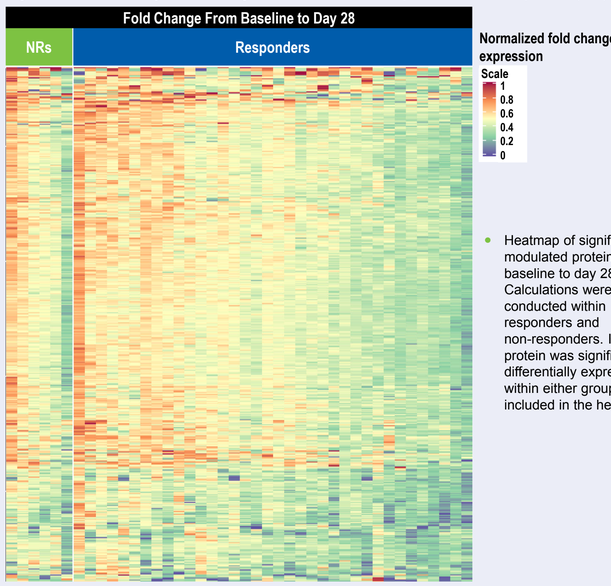
Pharmacodynamic Correlates of Response

Figure 1. Baseline Proteomic Differences in Responders and Non-responders to Ruxolitinib From Baseline to Day 28



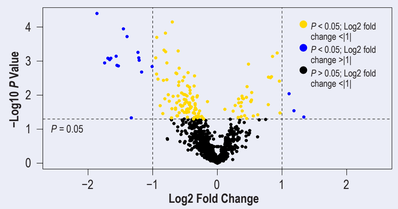
- Heatmap of significantly differentially expressed proteins at $P < 0.05$ between responders and non-responders at baseline. The majority of differentially regulated proteins were down-regulated in responders. Rows show proteins and columns show patients

Figure 2. Baseline and Day 28 Fold Change Differences Between Responders and Non-responders (NRs)



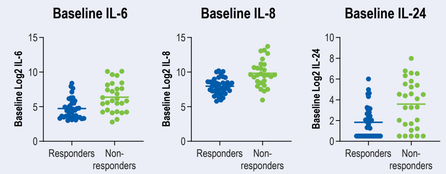
- Heatmap of significantly modulated proteins from baseline to day 28. Calculations were conducted within responders and non-responders. If a protein was significantly differentially expressed within either group, it was included in the heatmap

Figure 3. Volcano Plot Representing Significant Differences Between Responders Versus Non-responders at Baseline



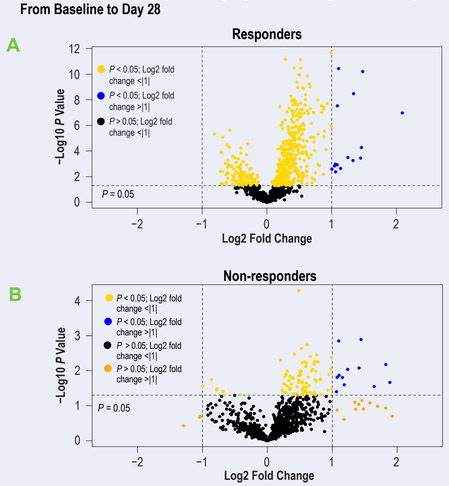
- Proteins in black did not change significantly at $P < 0.05$. Proteins in yellow changed significantly but did not yield a log2 fold change of >|1|. Proteins in blue changed significantly at a log2 fold change of >1. Log2 fold change values <0 indicate lower values in responders compared with non-responders

Figure 4. Scatterplots of Baseline Differences Between Responders and Non-responders for Select Inflammatory Mediators



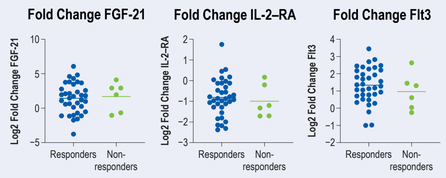
- Baseline proteins that were the most differentially expressed between responders and non-responders are shown (IL-6, $P < 0.01$; IL-8, $P < 0.0001$; IL-24, $P < 0.01$)

Figure 5. Volcano Plot Representing Significant Changes Within Responders (A) and Non-responders (B) From Baseline to Day 28



- Proteins in black did not change significantly at $P < 0.05$. Proteins in yellow changed significantly but did not yield a log2 fold change of >|1|. Proteins in blue changed significantly at a log2 fold change of >1. Proteins in orange did not change significantly at $P < 0.05$ but yielded a log2 fold change of >|1|. Proteins were generally up-regulated from baseline in both responders and non-responders. Interestingly, some proteins experienced highly significant changes, particularly those that were up-regulated

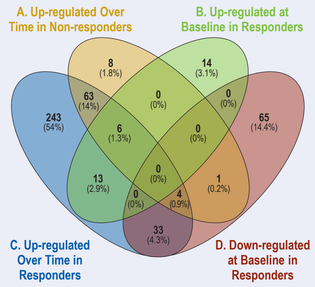
Figure 6. Scatterplots of Fold Change Differences Between Responders and Non-responders for Select Inflammatory Mediators



- FGF-21, IL-2–RA, and Flt3 are examples of differences from baseline to day 28 within responses. Proteins changed significantly in responders ($P < 0.001$). However, only IL-2–RA changed significantly in non-responders, potentially due to small numbers of non-responders

Baseline and Pharmacodynamic Overlap

Figure 7. Venn Diagram of Overlap Between Proteins Differentially Regulated at Baseline and Up-regulated From Baseline to Day 28 in Responders Versus Non-responders



- Proteins up-regulated from baseline to day 28 (**A** + **C**) are overlapped with differentially regulated proteins at baseline (**B** + **D**)
- The vast majority of proteins up-regulated from baseline to day 28 in non-responders (**A**) do not overlap significantly with proteins up-regulated in responders at baseline (**B**). Among responders, there is considerable overlap between proteins down-regulated at baseline (**D**) and proteins up-regulated from baseline to day 28 (**C**) (37/103, 36%)
- The vast majority of proteins up-regulated in responders at day 28 (**C**) were not differentially expressed at baseline between responders and non-responders

Figure 8. Venn Diagram of Overlap Between Proteins Differentially Regulated at Baseline and Down-regulated From Baseline to Day 28 in Responders Versus Non-responders

- Proteins down-regulated from baseline to day 28 (**A** + **C**) are overlapped with differentially regulated proteins at baseline (**B** + **D**)
- Proteins that decreased in responders (**C**) had almost no overlap with differentially regulated proteins at baseline (**B** + **D**)
- There is minimal overlap between proteins down-regulated with treatment (**A** + **C**) and proteins differentially expressed between responders and non-responders at baseline (**B** + **D**). This contrasts with a larger set of proteins that were low at baseline and up-regulated with treatment in responders (**Figure 7**, **C** + **D**)

Conclusions

- **Assessing differences at baseline and within responders and non-responders may offer opportunities for identifying new points of intervention in non-responders**
  - **For example, proteins that are differentially expressed at baseline but whose expression does not change with treatment may offer additional treatment strategies**
- **Both responders and non-responders showed robust changes in the expression of inflammatory mediators**
  - **Markers associated with effector T-cell (ie, IL-2–RA) activity were down-regulated from baseline to day 28**
    - **Interestingly, the expression of IL-8 and other inflammatory mediators were lower in responders compared with non-responders at baseline**
- **Few non-responders with baseline and day 28 data were available**
- **Future work should be conducted on larger cohorts and with real-world data**

Disclosures

All authors: Employment and stock ownership – *Incyte Corporation.*

Acknowledgments

The authors wish to thank the participants, investigators, and site personnel who participated in this study. Editorial, graphics, and printing support was provided by Envision Pharma Group (Philadelphia, PA), funded by Incyte Corporation.

Reference

1. Jagasia M, et al. *Biol Blood Marrow Transplant*. 2019;25:S52.

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