Analysis and Integration of Machine Learning for Biomarker Assessments and Their Association With Treatment Response: Insights from the ENLIVEN Phase 2b Trial of Pegozafermin

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INTRODUCTION

- Pegozafermin (PGZ) is a long-acting glycoPEGylated analog of fibroblast growth factor 21 (FGF21) in development for the treatment of metabolic dysfunction-associated steatohepatitis (MASH) and severe hypertriglyceridemia (SHTG).
- While histological assessment is required by international regulatory agencies to assess disease grade and stage in MASH, it has recognized limitations, including variability of pathologist readings, liver tissue heterogenicity, and potential complications with the procedure.
- The use of non-invasive biomarkers provides an important alternative to assess treatment response in MASH, for which there is a growing body of evidence.
- Machine learning (ML) has become increasingly essential in discovering patterns of data, and by integrating both unsupervised and supervised learning, it allows for a more comprehensive analysis.

AIM

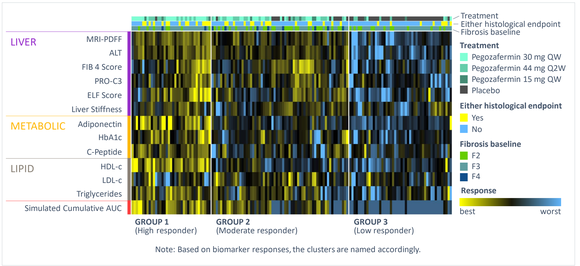
- To correlate PGZ-induced changes between a pre-defined panel of non-invasive biomarkers and Week 24 histological response. This analysis also aimed to generate a predictive model for treatment success.
- To establish a non-invasive biomarker panel at Week 12 that is predictive for subsequent PGZ treatment response at Week 24.

METHODS

- Data source: ENLIVEN study, a randomized, double-blind, placebo-controlled phase 2b study that evaluated the efficacy, safety, and tolerability of PGZ administered subcutaneously in subjects with biopsy-confirmed MASH and F2-F4 fibrosis. Data and results from the ENLIVEN study were recently published (Loomba, 2023).
  - Study arms: PGZ 15 mg QW, PGZ 30 mg QW, PGZ 44 mg Q2W, or Placebo
  - Histological endpoint for this analysis was for subjects to meet either (1) resolution of MASH with no worsening of fibrosis (MASH Endpoint), OR (2) ≥1-stage fibrosis improvement with no worsening of MASH (Fibrosis Endpoint) at Week 24
- Biomarkers included in the analysis were grouped into 3 categories:
  - Liver fibrosis
  - Metabolic Regulation
  - Lipid metabolism
- Biomarkers were measured at Weeks 12 and 24. Consistency in clustering using Week 12 and Week 24 data was also compared, and an agreement statistic was calculated.
- K-means cluster analysis, an unsupervised ML algorithm, was used to cluster subjects into different groups based on biomarker responses excluding histological outcome. These data were visualized using heatmaps, along with their treatment and endpoint data.
- Week 12 and Week 24 data used:
  - Data (% change from baseline) for each biomarker were normalized (scaled to mean 0 and standard deviation 1)
  - Removed subjects without complete biomarker data (due to requirements of the k-means clustering algorithm)
  - Descriptive statistics were calculated for each cluster
- Logistic regression, a supervised ML technique, was conducted using the averaged value from all normalized biomarkers of each subject against the corresponding outcome (i.e., meeting either of the 2 histological endpoints).

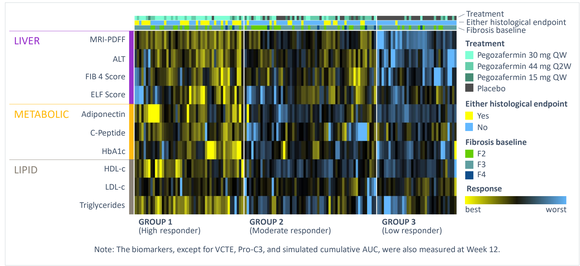
RESULTS

Figure 1. Unsupervised Clustering of Biomarkers at Week 24



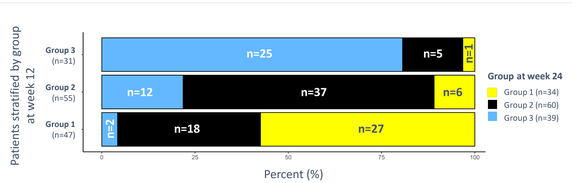
Note: Based on biomarker responses, the clusters are named accordingly.

Figure 3. Heatmap of Biomarker Distribution at Week 12



Note: The biomarkers, except for VCTE, Pro-C3, and simulated cumulative AUC, were also measured at Week 12.

Figure 5. Generally Consistent Subject Clustering Over Time (Weeks 12 to 24)



67% of patients don't change group

Table 1. Proportion of Patients in Each Response Clustering Group at Week 24

	Group 1 (High responder)	Group 2 (Moderate responder)	Group 3 (Low responder)
Proportion of subjects; n (%)*	37 (25%)	64 (43%)	48 (32%)
Proportion of group that met either endpoint	41%	25%	17%
Proportion of group that is placebo	3%	20%	79%
Proportion of all 15 mg patients in group	30%	50%	20%
Proportion of all 30 mg patients in group	44%	50%	6%
Proportion of all 44 mg patients in group	31%	56%	13%

*note sample size drop due to missing data

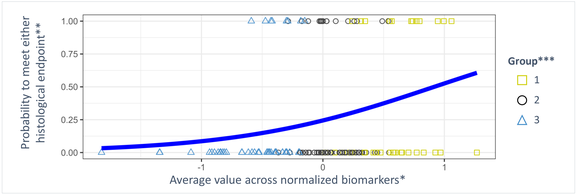
- Based on unsupervised clustering of non-invasive biomarker data, 3 clusters were identified.
- Clustering was consistent with likelihood of histological response and PGZ exposure:
  - Group 1: High likelihood of histological response (predominantly PGZ-treated patients)
  - Group 2: Moderate likelihood of histological response (mostly PGZ-treated patients)
  - Group 3: Low likelihood of histological response (mostly PBO-treated patients)

Table 2. Proportion of Patients in Each Response Clustering Group at Week 12

	Group 1 (High responder)	Group 2 (Moderate responder)	Group 3 (Low responder)
Proportion of subjects; n (%)*	48 (34%)	58 (41%)	35 (25%)
Proportion of group that met either endpoint	48%	24%	3%
Proportion of group that is placebo	2%	31%	77%
Proportion of all 15 mg patients in group	30%	60%	10%
Proportion of all 30 mg patients in group	69%	27%	4%
Proportion of all 44 mg patients in group	32%	55%	12%

*note sample size drop due to missing data

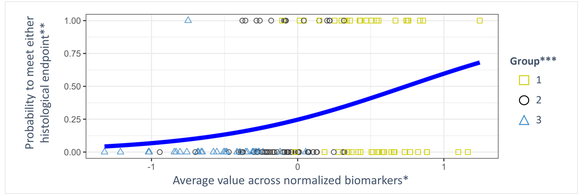
Figure 2. Subjects With Improvements in Biomarkers at Week 24 Were More Likely to Meet One of the Histological Endpoints



*Higher value indicates better biomarker response; **Histological endpoints defined as Resolution of MASH with no worsening of fibrosis (MASH Endpoint), OR ≥1-stage fibrosis improvement with no worsening of MASH (Fibrosis Endpoint) at Week 24; ***As defined by unsupervised clustering, p-value of logistic regression: 0.0029

- Logistic regression demonstrated a greater concordance of biomarker improvement (higher average values) correlating with greater probability of meeting histological endpoints.
- Logistic regression aligns with and further validates the effectiveness of cluster analysis.

Figure 4. Subjects With Improvements in Biomarkers at Week 12 Were More Likely to Meet One of the Histological Endpoints



p-value of logistic regression: 0.0019

- Trends in biomarker change at Week 12 correlate with subsequent histological response at Week 24.

CONCLUSIONS

- In the ENLIVEN study, heatmap/cluster analysis based on non-invasive biomarkers resulted in the clustering of subjects into 3 groups that aligned with likelihood of attaining a histological response.
- Logistic regression analysis, a supervised ML technique, showed consistency with and further validated the unsupervised cluster analysis.
- The consistency of results and clustering at Weeks 12 and 24 indicated that Week 12 biomarker data may potentially predict subsequent histological response at Week 24. These data may therefore support the future development of predictive tools to enable early triage of PGZ treatment response.
- Future independent validation in larger cohorts, as well as health outcome data, are required to validate the current findings.

REFERENCE:

Looma R, Sanyal AJ, Kowdley, et al. Randomized, controlled trial of the FGF21 analogue pegozafermin in NASH. *N Engl J Med.* 2023;389(11):998-1008.

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