016 Utility of miRomics for Identification of Circulating Pharmacodynamic Biomarkers of IFNβ-1a Biologics

Mai Mehanna[1], Lakshmi Manasa S. Chekka[1], Deepti Samarth[1], Erica Decker[1], Esraa Mohamed[1], Yan Guo[1], James L. Weaver[1], Sarah S. Schrieber[2], Jeffry Florian[1], Yow-Ming Wang[3], David G. Strauss[1], Paula L. Hyland[1]

[1]Division of Applied Regulatory Sciences (DARS), Office of Clinical Pharmacology (OCP) / Office of Translational Sciences (OTS) / Center for Drug Evaluation and Research (CDER), U.S. FDA. [2]Office of Biologics and Biosimilars, Office of New Drugs (OND), CDER, U.S. FDA. [3]Therapeutic Biologics Protein Team, OCP/OTS/CDER, U.S. FDA.

Abstract

Background: The U.S. Food and Drug Administration is conducting research to identify pharmacodynamic (PD) biomarkers to support the demonstration of biosimilarity. These PD biomarkers can streamline development programs by negating the need for comparative clinical studies with efficacy endpoint(s).

Purpose: To evaluate the utility of miRNA profiling (miRomics) and develop an analytical framework for identifying potential circulating PD biomarkers of interferon beta-1a (IFNβ-1a) and pegIFNβ-1a products.

Methodology: A pilot study was conducted using plasma samples from 36 healthy subjects from a placebo-controlled randomized single dose clinical study with IFNβ-1a and pegIFNβ-1a. Using miRNA-sequencing, we measured miRNAs at baseline/pre-treatment in all subjects, and at 9 timepoints over 6 days in the IFNβ-1a group (n=11 [30μg]), and at 11 timepoints over 13 days in the pegIFNβ-1a group (n=11[125μg]) and placebo-specific groups (n=6 each). We identified 108 mature miRNAs (with a minimum of 10 read counts in at least 50% of samples). We conducted linear-mixed effect models regressing the normalized count changes from baseline with treatment*time interaction. miRNAs with false discovery rate (FDR)-corrected p-values<0.1 were considered differentially expressed. Analysis was conducted in R (v4.1.2). DIANA-miRPath v3.0 was used for functional characterization of miRNA biomarkers.

Results: We identified 11 and 13 differentially expressed miRNAs over treatment and time by IFNβ-1a and pegIFNβ-1a, respectively, compared to placebo. hsa-miR-223-3p and hsa-miR-21-5p were common for both products. Importantly, hsa-miR-223-3p regulates Mx1 and STAT1 which are proposed individual candidate PD biomarkers for IFNβ-1a and pegIFNβ-1a and are also involved in IFNβ-1a signaling. Functional analysis of top miRNAs identified 24 overlapping pathways for both products including Hepatitis B and Hippo signaling.

Conclusion: Using miRomics, we identified plasma miRNAs as potential PD biomarkers of IFN-β1a biologics for further investigation to support biosimilar development programs.

Introduction

- Comparative clinical studies required to approve biosimilars can be costly and time consuming.
- Novel omics technologies, including miRNA-sequencing (miROmics) may have the potential to identify PD biomarkers, which can support biosimilarity assessment without the need for clinical efficacy endpoints.
- IFNβ-1a biologics used to treat multiple sclerosis have limited well-characterized PD biomarkers and complex mechanisms of action.

- **Aim:** Evaluate the utility of miROmics and provide an analytical framework to identify potential circulating PD biomarkers of INFβ-1a and pegINFβ-1a biologic products.

Funding: This study was supported by the US Food and Drug Administration

Materials and Methods

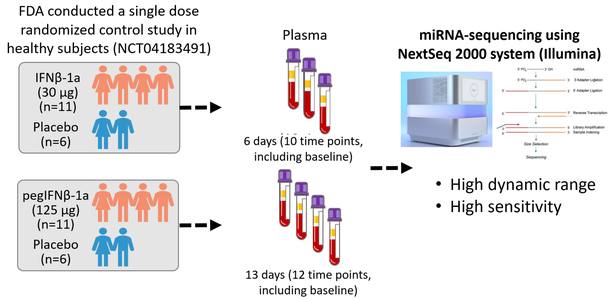


Figure 1. Diagram showing the study design

- MiRNAs measured at baseline and all other timepoints were normalized using R package DESeq2 v1.34.0 and filtered for those with ≥ 10 read counts in at least 50% of samples.
- MiRNAs differentially expressed by treatment were identified by regressing log2 normalized fold change (FC) values with treatment group, time point, and their interaction using linear mixed effects regression (lmer) models, adjusted for categorical age, sex, BMI, race, and batch effect. MiRNAs with a Type-3 ANOVA F-test FDR-corrected $P < 0.1$ were considered differentially expressed.
- Differentially expressed miRNAs with a significantly different response from placebo (≥20% difference in peak FC and ttest $P < 0.05$) were prioritized.
- DIANA-miRPath v3.0 was used for functional characterization of the prioritized candidate miRNA PD biomarkers.

Results and Discussion

Table 1. Characteristics of the study participants

Characteristic	Placebo (n=12)	INFβ-1a (n=11)*	pegINFβ-1a (n=11)*	*P*-value
Age, years (IQR)	34.0 (26.8 – 40.8)	37.0 (30.0 – 49.5)	32.0 (30.5 – 34.5)	0.55
Women, n (%)	4 (33.3%)	3 (27.3%)	5 (45.5%)	0.74
Race White, n (%)	6 (50.0%)	4 (36.4%)	6 (54.5%)	0.76
BMI, kg/m² (IQR)	27.4 (26.2 – 28.9)	27.6 (26.5 – 29.6)	25.4 (24.5 – 27.3)	0.09

Continuous variables are presented as median with interquartile ranges (IQR). Categorical variables are summarized as N (percentage). *one study participant from the high-dose INFβ-1a group, and one study participant from the high-dose pegINFβ-1a group were discontinued from the study. Abbreviations: IQR, interquartile range; BMI, body mass index.

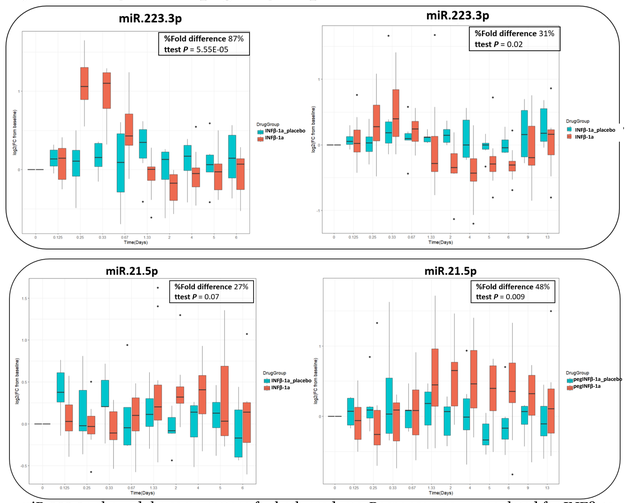
Results and Discussion

Table 2. Prioritizing Discovery miRNA PD Candidates

MiRNA PD Candidates	INFβ-1a	pegINFβ-1a
Discovery (high dose) candidates	11	13
Prioritized candidates	4	2
Upregulated	7	6
Downregulated	3	5
Biphasic	1	2

Prioritized miRNA PD candidates based on ≥20% difference in peak FC and ttest $P<0.05$ between drug and placebo. MiRNAs with a biphasic response shows ≥10% up-regulation and ≥10% down-regulation at two time points.

Figure 2. Common top differentially expressed miRNAs impacted by both INFβ-1a and pegINFβ-1a products



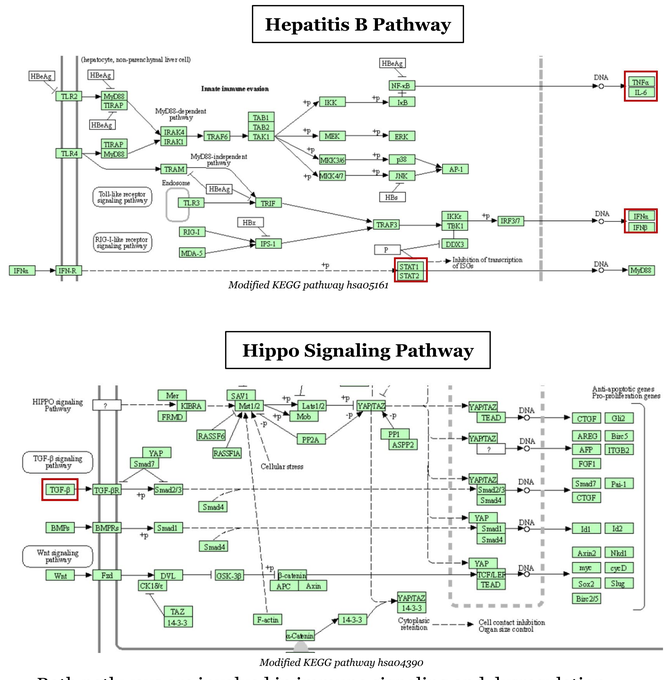
miR.223.3p showed the same pattern for both products. Response was up-regulated for INFβ-1a and peaked at 0.33 days, while response was biphasic for pegINFβ-1a and peaked at 4 days. miR.223.3p regulates Mx1 and STAT1, which are proposed PD biomarkers for INFβ-1a biologics and are also involved in INFβ-1a signaling.

miR.21.5p was up-regulated in response to both products with a peak response at 4 vs 2 days in response to INFβ-1a and pegINFβ-1a, respectively.

- miR.150.5p was significantly differentially expressed in response to INFβ-1a (FDR-corrected $P= 0.0019$), and nominally differentially expressed in response to pegINFβ-1a (FDR-corrected $P= 0.19$).
- miR.150.5p was down-regulated and peaked at 0.33 days in response to both INFβ-1a and pegINFβ-1a. miR.150.5p regulates EPHB2 and CD48, which are proposed PD biomarkers for pegINFβ-1a.

Using the DIANA-MiRPath tool (dianalab.e-ce.uth.gr), we identified 24 overlapping pathways for both products including Hepatitis B and Hippo signaling as the top two.

Figure 3. Functional analysis of top miRNAs



Both pathways are involved in immune signaling and dysregulation, which is related to the action of IFNβ-1a as an immunomodulator

Conclusion

- We identified three candidate PD miRNAs of INFβ-1a biologics in plasma for future investigation and replication.
- Our findings demonstrate the utility of miRomics for the identification of PD biomarkers that might be used to support biosimilars development programs in the future.

Acknowledgment

Division of Applied Regulatory Sciences Omics Team, OCP Management, Biosimilar Guidance & PD biomarker working groups.