Study Title: Pharmacodynamic Biomarkers to Support Biosimilar Development: Clinical Study 3 (IFN-β Products – Avonex and Plegridy)

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Statistical Analysis Plan

SCR-008: Pharmacodynamic Biomarkers to Support Biosimilar Development: Clinical Study 3 (IFN-β Products – Avonex and Plegridy)

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Table of Abbreviation

Abbreviation	Definition
$AUC_{0\text{-}inf}$	area under the concentration-time curve from time zero to infinity
$AUC_{0\text{-}t}$	area under the concentration-time curve from time zero to last on-study sample
$AUEC_{0\text{-}t}$	area under the effect curve from time zero to last on-study sample
AUEC	area under the effect curve
$pAUEC_{0\text{-}t}$	percentage area under the effect curve from time zero to last on-study sample
CL/F	apparent clearance
C_{max}	maximum observed concentration
EC_{50}	the exposure that gives-half maximal response, model parameter
ED_{50}	the dose that gives-half maximal response, model parameter
$E_{max,exp}$	maximum PD response, model parameter – concentration analysis
$E_{max,dose}$	maximum PD response, model parameter – dose analysis
FDA	Food and Drug Administration
IFN β-1a	interferon beta-1a (Avonex®)
K_{el}	elimination rate
mg	milligram
$\Delta MxA_{dec,\,max}$	maximum increase from baseline in MxA protein
$\Delta Neopterin_{dec,\,max}$	maximum increase from baseline in neopterin
PD	pharmacodynamic
pegIFN β-1a	pegylated interferon beta-1a (Plegridy®)
PK	pharmacokinetic
RNA	ribonucleic acid
SC	subcutaneously
SD	standard deviation
T_{max}	time of maximum concentration (C_{max})
$t_{1/2}$	terminal half-life
V/F	apparent volume of distribution

Change Log

Version	Section	Changes
2	5	Added that 'Subjects who did not complete their treatment (referred to as early termination subjects) will not be included in PK and PD parameter summary calculations.'
2	7.3.1, 7.3.2	The descriptive summary for PD parameters is changed from geometric mean to mean. Changes in PD parameter from baseline for placebo and low doses may have negative values and would not allow for calculation of geometric mean. Text describing the primary, secondary, and exploratory PD parameter was updated for clarity.
2	General	Typographical changes included throughout

1. Introduction

This document outlines the proposed statistical methods for data analysis on data collected from Protocol 'SCR-008: Pharmacodynamic Biomarkers to Support Biosimilar Development: Clinical Study 3 (IFN-β Products – Avonex and Plegridy)'.

2. Study Objectives

2.1. Primary Objective

The primary objective of this study is to report clinical trial operating characteristics for future clinical pharmacology pharmacokinetics (PK) and pharmacodynamics (PD) similarity studies using the different biomarker-based approaches.

2.2. Secondary Objectives

The secondary objectives of this study are:

1. To determine the values and variability of PK and PD parameters at three dose levels (i.e., low, intermediate, and high doses) for interferon beta-1a (Avonex®, IFN β-1a) and pegylated interferon beta-1a (Plegridy®, pegIFN β-1a).
2. Explore PK/PD relationships using appropriate models for IFN β-1a and pegIFN β-1a.

2.3. Exploratory Objective

The exploratory objectives of this study are:

1. To evaluate the utility of circulating proteins and small RNAs as potential PD biomarkers.
2. To inform analytical approaches and experimental designs needed for identifying exploratory proteomic- and small RNA-based PD biomarkers in plasma.

3. Study Overview

3.1. Study Design

This is a randomized, double-blind, placebo-controlled, single-dose, parallel arm, pilot study. Healthy subjects will be randomized to one of three dose groups (low, intermediate, and high) for each drug (IFN β-1a and pegIFN β-1a) or placebo (See Table 1). The study will be conducted at one center in the United States (Spaulding Clinical Research unit in West Bend, Wisconsin).

Subjects randomized to Treatment Groups A, B, and C will stay in-house for the duration of the study and will be discharged on Day 7. Subjects randomized to Treatment Groups D, E, F, and G will stay-in house until Day 10 and return for the Day 14 assessment. Each treatment group should include equal representation of male and female subjects.

Table 1: Study Treatment Groups

Subjects (n)	Treatment Group	Drug
12	A	Interferon beta-1a (Avonex®) low (7.5 µg)
12	B	Interferon beta-1a (Avonex®) intermediate (15 µg)
12	C	Interferon beta-1a (Avonex®) high (30 µg)
12	D	Peginterferon beta-1a (Plegridy®) low (31.25 µg)
12	E	Peginterferon beta-1a (Plegridy®) intermediate (62.5 µg)
12	F	Peginterferon beta-1a (Plegridy®) high (125 µg)
12	G	Placebo

3.2. Sample size

Up to 98 healthy subjects will be enrolled (84 subjects planned for treatment and up to 14 potential replacement subjects). Subjects will be randomized to one of 6 different active treatment arms (i.e. 12 per treatment arm) or placebo. This study did not have any formal sample size or power calculations. Doses expected to characterize the dynamic range of the primary PD biomarker (neopterin) were selected. A total of 12 subjects are planned per dose, which is slightly higher than a typical sample size for estimating values and variability of PK and PD measures in single ascending dose trials.

4. Study Endpoints

4.1. Primary Endpoints

- Neopterin area under the effect curve from time zero to last on-study timepoint ($AUEC_{0\text{-}t}$, referred to as AUEC for brevity).
- Maximum increase from baseline in neopterin ($\Delta Neopterin_{dec,\ max}$)

4.2. Secondary Endpoints

- Area under the curve (AUC) of IFN β-1a and pegIFN β-1a from time zero to infinity ($AUC_{0\text{-}inf}$)
- Maximum concentration (C_{max}) of IFN β-1a and pegIFN β-1a

- $AUEC_{0-t}$ of MxA protein.
- Maximum increase from baseline in MxA protein ($\Delta MxA_{dec, max}$)
- Model parameter estimates for IFN β-1a and pegIFN β-1a exposure-response models (exposure parameter versus $AUEC_{0-t}$ or ΔNeopterin-C_{min})

4.3. Exploratory Endpoints

- Additional pharmacokinetic parameters for IFN β-1a and pegIFN β-1a, including time of maximum concentration (T_{max}), elimination rate constant (K_{el}), area under the curve from time 0 to end of study (AUC_{0-t}), half-life ($t_{1/2}$), apparent clearance (CL/F), and apparent volume of distribution (V/F).
- Time course profiles, maximum increase/decrease from baseline, and AUEC for plasma proteomics and small RNA transcriptomics (set of markers be determined)

5. Analysis Populations

- For all analyses, subjects will be analyzed according to the dose and treatment received, not the dose and treatment to which subjects were randomized.
- Subjects who discontinued from the study before their assigned end of study day (referred to as early termination subjects) will be excluded from pharmacokinetic and pharmacodynamic parameter summary calculations.
- The PD population will include all subjects who received study drug and have at least 1 estimable PD parameter after dosing. For baseline-adjusted analyses, subjects must also have at least one valid baseline sample between screening to dose administration on day 1. Subjects without a valid baseline sample will be excluded from PD assessments where the derived metric is baseline adjusted.
- The PK population will include all subjects who received study drug and have at least 1 estimable PK parameter after dosing. Subjects with all samples below the lower limit of quantification will not be included in PK population summaries.
- The PK/PD population (exposure-response population) will be used for the model-based exposure-response analysis. This population includes all subjects from the PK and PD populations, including those who received placebo treatment, as well as subjects with PK samples below the lower limit of quantification.

- The safety population will include all subjects who received at least 1 dose of any of the study drugs,

6. Data Screening and Acceptance

6.1. Handling of Missing and Incomplete Data

The following imputation of missing values will be done:

- Pharmacokinetic measurements below the quantification limits will be considered equal to zero for all analyses.
- Non-pharmacokinetic measurements (e.g., neopterin, MxA) below the quantification limits will be considered equal to the lower limit of quantification for all analyses unless explicitly noted otherwise.
- Missing pharmacokinetic or pharmacodynamic data (e.g., skipped outpatient visit) will not be imputed.
- For baseline adjusted measures, samples from Day 1, time 0 will be used for calculating this derived metric. In cases where the Day 1, time 0 sample is missing or invalid, the sample collected at check-in (Day -1) will be used, followed by the screening sample. If none of these samples are available or valid, then no baseline value will be calculated for the subject.

7. General Statistical Considerations

All data will be presented in data listings. Data from subjects excluded from the analysis population will be presented in the data listings, but not included in the calculation of summary statistics.

7.1. Subject Disposition

The number of subjects who enroll in the study and the number and percentage of subjects who complete each assessment will be presented. The frequency and percentage of subjects who withdraw or discontinue from the study and the reason for withdrawal or discontinuation will be summarized. In addition, significant known protocol deviations will be noted for individual subjects.

7.2. Demographics and Baseline Characteristics

Descriptive statistics will be used to summarize demographic and baseline subject characteristics for age, sex, weight, body mass index, race, and ethnicity. For continuous variables, the mean,

median, standard deviation (SD), minimum, and maximum values will be reported. For categorical (nominal) variables, the number and percentage of subjects (or observations) will be reported.

7.3. Pharmacodynamic Analysis

7.3.1 Neopterin

Individual neopterin versus time plots will be presented for each participant. For neopterin, AUEC and maximum increase from baseline will be calculated for each participant using all on treatment timepoints. Calculations will be performed using non-compartmental analysis packages available in statistical software. The primary analysis with neopterin will use baseline-adjusted AUEC and maximum increase from baseline, while other derived measures (AUEC, pAUEC) may be calculated to evaluate how derived PD metrics impact trial design. PD parameters of IFN β-1a and pegIFN β-1a, and placebo groups will be listed and summarized using descriptive statistics (n, mean, SD, coefficient of variation, minimum, median, interquartile range, and maximum) for each treatment arm.

7.3.2 MxA

Individual MxA versus time plots will be presented for each participant. For MxA, baseline-adjusted AUEC and maximum increase from baseline will be calculated for each participant using all on treatment timepoints. Calculations will be performed using non-compartmental analysis packages available in a statistical software. The secondary analysis with MxA will use baseline-adjusted AUEC and maximum increase from baseline. Other derived measures (AUEC, pAUEC) for MxA may be calculated to evaluate how derived PD metrics impact trial design. PD parameters of IFN β-1a and pegIFN β-1a, and placebo groups will be listed and summarized using descriptive statistics (n, mean, SD, coefficient of variation, minimum, median, interquartile range, and maximum) for each treatment arm.

7.4. Pharmacokinetic Analysis – IFN β-1a and pegIFN β-1a

Individual serum concentration versus time plots for IFN β-1a and pegIFN β-1a will be presented for each participant. $AUC_{0\text{-}inf}$ and C_{max} will be calculated for each participant using all on treatment timepoints as part of the secondary analysis. In addition, the following PK parameters will be calculated for each individual as exploratory analyses: $AUC_{0\text{-}t}$, T_{max}, apparent clearance (CL/F), apparent volume of distribution (V/F), elimination rate (Kel), and terminal half-life ($t_{1/2}$). PK

parameters will be calculated for each participant using all on treatment timepoints. All parameters will be summarized using descriptive statistics (n, geometric mean, coefficient of variation, minimum, median, interquartile range, and maximum) for each IFN β-1a and pegIFN β-1a treatment arm.

Calculations will be performed using non-compartmental analysis packages available in a statistical software. Serum concentrations below the limits of quantification will be set to zero for the purpose of this analysis. Subjects with all samples below the limit of quantification will be excluded from PK summaries. $AUC_{0\text{-}inf}$, $t_{1/2}$, and Kel for subjects will only be included for subjects with 3 or more concentration values on the terminal portion of the pharmacokinetic curve and with an adjusted coefficient of determinations (R^2) greater than 0.80.

7.5. PK/PD Analyses

The dose- and exposure-response relationship between baseline-adjusted AUEC for neopterin and $\Delta Neopterin_{dec,\ max}$ and IFN β-1a and pegIFN β-1a will be explored graphically (separate assessments for both response measures). Based on these observations, model-based analyses using statistical software will be conducted separately for each drug with respect to each of the above-mentioned PD measures.

The model-based analysis will explore both dose and exposure (i.e., $AUC_{0\text{-}inf}$, referred to as AUC below) as the dependent variable. Data for all dose levels for a drug, as well as placebo, will be combined for the analysis. Model selection will be based on the initial graphical assessment and will be selected from one of the following structures – linear, E_{max}, and sigmoidal. Multiple models may be evaluated based on the initial graphical analysis, in which case model selection will be based on a combination of goodness of fit plots, parameter uncertainty (i.e., parameter confidence intervals including zero), and Akaike's information criterion (AIC). General representations for each of the model structures and parameterizations is shown below:

Dose-relationship

Linear: $Response \sim E_0 + Slope * Dose$

E_{max}: $Response \sim E_0 + E_{max,dose} * Dose / (Dose + ED_{50})$

Sigmoidal: $Response \sim E_0 + E_{max,dose} * Dose^{\gamma} / (Dose^{\gamma} + ED_{50}^{\gamma})$

Exposure-relationship

Linear: Response ~ E_0 + Slope * AUC

E_{max}: Response ~ $E_0 + E_{max,exp} * AUC / (AUC + EC_{50})$

Sigmoidal: Response ~ $E_0 + E_{max,exp} * AUC^{\gamma} / (AUC^{\gamma} + EC_{50}^{\gamma})$

Model evaluation will include residual variability error term but will not include any random effects or covariate evaluation on fixed effect.

7.6. Exploratory Omics Analysis

Various proteomics, transcriptomics, and genomic analyses may be performed on collected data for biomarker exploration. Additional details regarding the statistical methods for these analyses will be described in a separate plan.

7.7. Safety Analyses

7.7.1 Adverse Events

All AEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities. The incidence of TEAEs, organized by system organ class and frequency, will be summarized by seriousness, severity, relationship to treatment, and by treatment at onset of the TEAE. A detailed listing of serious AEs and TEAEs leading to withdrawal will also be provided.

7.7.2 Clinical Laboratory Tests

Clinical laboratory results (hematology, serum chemistry, and urinalysis) will be summarized using descriptive statistics (number of subjects, mean, SD, minimum, median, and maximum). Clinical laboratory results will be classified as normal or abnormal, according to the reference ranges of the individual parameter. No statistical testing will be performed on clinical laboratory data.

7.7.3 Vital Sign Measurements

Vital sign measurements and changes from Baseline will be summarized using descriptive statistics (number of subjects, mean, SD, minimum, median, and maximum) by treatment and time point.

7.7.4 Safety 12-lead Electrocardiograms

The incidence of pathological ECG interpretive statements at Baseline and during treatment will be assessed among the treatments.

7.7.5 Physical Examinations

Physical examination findings will be presented in a data listing, and abnormal physical examination findings will be recorded as AEs.

7.7.6 Other Safety Data

All concomitant medication usage and medications that changed in daily dose, frequency, or both since the subject provided informed consent will be summarized for each subject.

8. Data Quality Assurance

Completed eCRFs are required for each subject randomized to study drug. Electronic data entry will be accomplished through the ClinSpark® remote electronic data capture system, which allows for on-site data entry and data management. This system provides immediate, direct data transfer to the database, as well as immediate detection of discrepancies, enabling site coordinators to resolve and manage discrepancies in a timely manner. Each person involved with the study will have an individual identification code and password that allows for record traceability. Thus, the system, and subsequently any investigative reviews, can identify coordinators, investigators, and individuals who have entered or modified records.

Furthermore, the investigator retains full responsibility for the accuracy and authenticity of all data entered into the electronic data capture system.

Attachment A. Pharmacokinetic, Pharmacodynamic, and Biomarker Sample Collection Schedule

Pharmacokinetic Sample Collection

Pharmacokinetic blood samples (5 mL) for determination of IFN β-1a and pegIFN β-1a concentration will be collected at the following time points:

- Day 1: 0 (pre-dose), 1, 3, 6, 8, 16 h
- Day 2: 24, 32, 40 h
- Day 3, 4, 5, 6, 7
- Day 8, 9, 10, and 14 (Arms D, E, F, and G, only)

Blood samples will be collected by direct venipuncture or by inserting an IV catheter into the subject's forearm region. Each blood sample will be labeled with subject number, study number, study day, time point, event, and a barcode that matches that belonging to the subject.

Pharmacodynamic Sample Collection

Blood samples for primary pharmacodynamic biomarker (neopterin, 5 mL) and secondary pharmacodynamic biomarker (MxA, 1 mL) assessments will be collected at the following time points:

- Screening (Day -21 to -2)
- Day 1: 0 (pre-dose), 1, 3, 6, 8, 16 h
- Day 2: 24, 32, 40 h
- Day 3, 4, 5, 6, 7
- Day 8, 9, 10, and 14 (Arms D, E, F, and G, only)

Blood samples will be collected by direct venipuncture or by inserting an IV catheter into the subject's forearm region. Each blood sample will be labeled with subject number, study number, study day, time point, event, and a barcode that matches that belonging to the subject.

Exploratory PD Biomarkers

Exploratory PD biomarkers will be evaluated using plasma proteomics and small RNA transcriptomics. Whole blood samples (5 mL) will be collected and processed for plasma at the following time points:

- Day 1: 0 (pre-dose), 1, 3, 6, 8, 16, 24 h
- Day 2: 24, 32, 40 h

- Day 3, 4, 5, 6, 7
- Day 8, 9, 10, and 14 (Arms D, E, F, and G, only)

Blood samples will be collected by direct venipuncture or by inserting an IV catheter into the subject's forearm region. Each blood sample will be labeled with subject number, study number, study day, time point, event, and a barcode that matches that belonging to the subject. All blood samples will be processed for preparation of plasma.

Attachment B. Randomization Schedule

Subjects will enter the study clinic for check-in procedures the day before study drug administration of the first period (Day -1). IFN β-1a will be administered intramuscularly and pegIFN β-1a will be administered subcutaneously. Placebo will be administered either subcutaneously or intramuscularly to match study drug. On Day 1, subjects will receive their assigned treatment according to the randomization schedule generated by the randomization biostatistician (unblinded and unaffiliated with study data analysis) and provided to designated unblinded recipients (i.e., pharmacist) at the clinical site.

Treatment Group	Drug
A	Interferon beta-1a (Avonex®) low (7.5 µg)
B	Interferon beta-1a (Avonex®) intermediate (15 µg)
C	Interferon beta-1a (Avonex®) high (30 µg)
D	Peginterferon beta-1a (Plegridy®) low (31.25 µg)
E	Peginterferon beta-1a (Plegridy®) intermediate (62.5 µg)
F	Peginterferon beta-1a (Plegridy®) high (125 µg)
G	Placebo