Title of Trial: A multinational, multicenter, single visit, exploratory pharmacogenetic trial and long-term follow-up of the PRISMS (Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis) trial

Investigational Product: Not applicable; no investigational product was administered in this trial.

Trial No.: EMR 200136-022

Study Center: 18 centers in 9 countries (Australia, Belgium, Canada, Finland, Germany, Sweden, Switzerland, the Netherlands, United Kingdom)

Trial Initiation Date: 04 March 2010

Trial Completion Date: 28 January 2011

Development Phase: Phase IV

Publication (reference): None

Study Objectives:

Primary Objective:

- To analyze the association between single nucleotide polymorphism (SNP) markers and treatment response. Treatment response is based on Expanded Disability Status Scale (EDSS) progression and relapse outcomes over the first 2 years of treatment in the PRISMS trial

Secondary Objectives:

- To assess disease evolution in subjects over the long term (15-16 years after initial randomization)

- To assess long-term immunogenicity of interferon (IFN) beta-1a

Methodology:

This was a Phase IV, non-interventional, multinational, multicenter, long-term follow-up (LTFU), single visit, exploratory pharmacogenetic (PGx) trial involving subjects who previously participated in the PRISMS trial. The first PRISMS trial (PRISMS-6789) took place 15-16 years ago and a follow-up trial (PRISMS-22930 LTFU) was performed 8 years later to assess long-term efficacy and safety. To address the current trial objectives, subjects originally randomized in the PRISMS trial (560 subjects) were identified and invited to participate either proactively or during a routine clinic visit. During the visit, medical and treatment history since the final visit of the PRISMS-6789 trial or the PRISMS-22930 LTFU trial was retrospectively collected based on history and available documents, a neurological examination (EDSS /

Kurtzke functional systems [KFS]) was performed, and a blood sample was collected for PGx analysis and immunogenicity assessment. A second visit for EDSS assessment was to be performed if the subject had suffered a relapse within 3 months prior to the primary visit.

Serious adverse events (SAEs) related to the blood sampling procedure were to be collected and followed-up; for this purpose, subjects remained on-site for an observation period of 30 minutes after the sample was drawn.

Efficacy, immunogenicity, and safety data from periods 2 (PRISMS-2), 4 (PRISMS-4 and PRISMS-2/4), 6, 7-8 (PRISMS-22930 LTFU) and 15-16 years after initial randomization – where available – were used to analyze the association between genetic markers and response to treatment in the original PRISMS treatment groups.

SNP markers from the following genes were evaluated: IFN alpha/beta (α/β) receptor 2 (IFNAR2), tumor necrosis factor superfamily member 10 (TNFSF10), 2',5'-oligoadenylate synthetase 1 (OAS1), and proteasome subunit beta type 8 (PSMB8).

Disease progression over the long-term was evaluated based on standardized EDSS assessments over the 15-16 years after initial randomization. The EDSS assessment was to be performed at least 3 months after onset of the last relapse to prevent biases from recent/concomitant relapse.

Number of Subjects (Planned and Analyzed):

A total of 560 subjects were randomized and treated in PRISMS-6789 and 291 consented to participate in the PRISMS-15 study and were enrolled. One subject was later withdrawn due to a changed disease diagnosis, so 290 subjects were analyzed. In PRISMS-6789, 100 of these 290 subjects received placebo, 95 received Rebif® 22 micrograms (mcg) subcutaneously (sc) three times weekly (tiw), and 95 received Rebif® 44 mcg sc tiw. Of the 100 subjects who received placebo in the initial 2 years of PRISMS-6789, 47 were switched to Rebif® 22 mcg and 48 were switched to Rebif® 44 mcg thereafter (5 did not continue).

Diagnosis and Main Criteria for Inclusion/Exclusion:

Diagnosis:

- Multiple sclerosis (MS)

Inclusion Criteria:

- Was randomized in the PRISMS-6789 study
- Was willing and able to comply with the protocol
- Written informed consent given before any trial-related activities were carried out

Exclusion Criteria:

- Was unwilling or unable to participate in the study

Study Treatment:

No Investigational Medicinal Product (IMP) was dispensed during this trial.

Duration of Treatment:

This trial consisted of a single visit during which subjects underwent medical examination and blood sampling. For safety reasons, subjects were kept under observation for 30 minutes after the blood sampling was performed. A second visit for EDSS assessment was to be performed if the subject had suffered a relapse within 3 months prior to the primary visit.

Criteria for Evaluation:

Primary Endpoint:

The primary endpoint was defined as the proportion of responders by group as defined by SNP markers. A responder was defined as a subject with no MS relapse and no EDSS progression during the first 24 months of treatment in PRISMS-6789.

The efficacy data used for the primary endpoint were:

- Data at Month 24 of PRISMS-6789 (Year 2) from the groups of subjects initially randomized to Rebif® or placebo

- Data at Month 48 of PRISMS-6789 (Year 4) from the group of subjects initially randomized to placebo who were re-randomized to Rebif® at Month 24 of PRISMS-6789 (Year 2)

Secondary Endpoints:

- Current course of MS: Relapsing-remitting MS (RRMS) or Secondary progressive MS (SPMS)

- Current EDSS score

- Change in EDSS since randomization

- Immunogenicity assessment

Safety Endpoints:

Safety measurements, such as adverse events (AEs) or SAEs from the PRISMS-6789 and PRISMS-22930 LTFU trials, were used to analyze potential associations between safety parameters and genetic markers.

Statistical Methods:

The sample size was determined by the available number of subjects from PRISMS-6789 who agreed to participate in this follow-up trial. No randomization or blinding was performed, as this was a long-term follow-up of a previously randomized clinical trial.

Populations for Primary Objectives:

- The PRISMS-2 population included all subjects who, during the first 2 years of the PRISMS-6789 study, were randomized and received at least one dose of IMP (placebo, Rebif® 22 mcg tiw, or Rebif® 44 mcg tiw) during PRISMS-6789. 'Early treatment' refers to subjects who received treatment with Rebif® in the first 24 months of PRISMS-6789.

- The PRISMS-4 population included all subjects initially randomized to placebo in PRISMS-6789, who were re-randomized to active treatment with Rebif® 22 mcg tiw or 44 mcg tiw after 24 months, and who received at least one dose of IMP during the second 2 years (i.e. Years 3 and 4) of the PRISMS-6789 trial. 'Late treatment' refers to subjects who received placebo during the first 24 months of PRISMS-6789, and who received treatment with Rebif® in the last 24 months (i.e. Months 24 to 48) of PRISMS-6789.

- The PRISMS-2/4 population was a combination of the PRISMS-2 and PRISMS-4 populations; this population included all subjects who were randomized to and received at least one dose of Rebif® during either PRISMS-2 or PRISMS-4. In the analyses, the data used for each subject came from the first 2-year period in which the subject received active treatment. Thus, for subjects who received either Rebif® 22 mcg tiw or Rebif® 44 mcg tiw in both PRISMS-2 and PRISMS-4, only the PRISMS-2 data related to the endpoint were used in this analysis. For subjects who received placebo in PRISMS-2, only the PRISMS-4 data related to the endpoint were used for the analyses of this population.

Population for Secondary Objectives:

The population for the analyses of the secondary objectives was the PRISMS-2 population, i.e. all subjects who were randomized into the PRISMS-6789 trial and received at least one dose of IMP in the first two years.

Additional analyses were planned to be conducted for subjects who were randomized into one of the Rebif® treatment groups of the PRISMS-6789 trial and who continued receiving the

same dose of Rebif® until the PRISMS-15 visit. However, only 2.1% (2/95) of subjects originally randomized to Rebif® 22 mcg and 11.6% (11/95) of subjects originally randomized to Rebif® 44 mcg were still receiving this dosage at the PRISMS-15 visit, so this population was too small to enable meaningful evaluation.

SNP Markers:

The SNP markers chosen for analysis in this trial were determined by the Biomarker Strategy group following an Administrative Interim Analysis (AIA). The AIA was conducted after samples had been collected from 158 subjects from the PRISMS-15 trial and 197 subjects from another PGx study, the REbif® vs. Glatiramer Acetate in Relapsing MS Disease (REGARD) 24735-PGx trial (total of 355 subjects).

Inferential Statistics:

Statistical analyses were performed using 2-sided tests with an alpha level of 5% and/or corresponding 2-sided 95% confidence intervals (CIs). The p-values given and the results of the statistical tests performed on all endpoints should be interpreted in an exploratory sense only. No adjustment of p-values for multiple testing was performed.

For 2-level SNP markers (SNP2 IFNAR2, SNP4 IFNAR2, SNP6 PSMB8), a Fisher's exact test was applied to test for associations between the genotypic data and the primary endpoint outcome, for each treatment group (SNP markers present Yes/No). This included an odds ratio as point estimate, 95% CI and the corresponding p-value.

For 3-level SNP markers (SNP3 IFNAR2, SNP1 TNFSF10, SNP5 OAS1), a logistic regression model was applied to account for all levels of the additive effect. The number of copies of the minor allele observed (i.e. 0, 1 or 2 copies) was modeled using indicator variables, with 0 copies as the reference category. Associations were assessed as for the dichotomous comparison and again included odds ratios as point estimates, 95% CIs and the corresponding p-values.

Analysis Periods:

- The first 24 months of data from PRISMS-6789 from the groups of subjects initially randomized to Rebif® or placebo such that endpoint calculations for response to two years of Rebif® treatment in the PRISMS-2 population rely on post-baseline data collected from Month 3 to Month 24. Changes from baseline were calculated as change from Month 0 (Study Day 1 [SD1]).

- The second 24 months of data from Month 24 up to Month 48 of PRISMS-6789 from the group of subjects initially randomized to placebo who were re-randomized to Rebif® at Month 24, such that endpoint calculations for response to two years of Rebif® treatment on the PRISMS-4 population rely on post-baseline data collected from Month 27 to Month 48. Changes from baseline were calculated as change from Month 24, the last subject visit on placebo.

The secondary endpoints were analyzed from baseline until each follow-up evaluation time-point (e.g., 24 months, 48 months, PRISMS-22930 LTFU, PRISMS-15).

Primary Endpoint Analysis:

The number of subjects who were responders or non-responders was summarized for each SNP marker and each treatment group. Responders were defined as subjects with no MS relapse and no 3-month confirmed EDSS progression during the first 24 months of active treatment in PRISMS-6789.

An EDSS progression was defined as an increase in the EDSS score of at least 1.0 point compared to baseline. However, for subjects with an EDSS score at baseline of 0, progression was defined as an increase of at least 1.5 points.

Secondary Endpoint Analyses:

Secondary endpoints were analyzed using the PRISMS-2 and PRISMS-4 population by originally randomized treatment group (placebo, Rebif® 22 mcg sc tiw [hereafter written as 'Rebif® 22 mcg'], or Rebif® 44 mcg sc tiw [hereafter written as 'Rebif® 44 mcg']) and by the following treatment groups: Rebif® 22 mcg (late start, from PRISMS-4), Rebif® 44 mcg (late start, from PRISMS-4), Rebif® 44 mcg (pooled, from PRISMS-2 and PRISMS-4), Rebif® 22 mcg (pooled, from PRISMS-2 and PRISMS-4), Rebif® 22 or 44 mcg (pooled, from PRISMS-2 and PRISMS-4), Rebif® 22 or 44 mcg (early start pooled, from PRISMS-2), Rebif® 22 or 44 mcg (late start pooled, from PRISMS-4).

Secondary endpoint variables were not censored at the time of treatment termination or trial withdrawal.

Safety Endpoint Analyses:

AEs during the first 2 years of PRISMS-6789 for the PRISMS-2 population were to be compared between genetic subgroups for SNP markers that showed a statistically significant association with the primary response variable. The following pre-specified AEs were considered: cytopenia, depression and suicidal ideation, flu-like syndrome, hepatic disorders, hypersensitivity reactions, injection site reactions, skin rashes, and thyroid disorders. No IMP was administered in this trial, so only SAEs related to the trial procedure per se (e.g., hematoma or bleeding at the puncture site) were collected in the context of this trial.

Results:

Subject Disposition:

Of the 560 subjects enrolled in the PRISMS-6789 trial, 291 (52.0%) were enrolled in the PRISMS-15 trial. All enrolled subjects completed the trial; however, one subject was excluded from the final analyses because it was determined that this subject did not have MS. Thus, 290 subjects comprised the PRISMS-15 population.

Demographics and Baseline Characteristics:

The mean age of the PRISMS-15 population (N=290) at the PRISMS-15 visit was 51.0 years, 70.3% were female and 99.0% were Caucasian. 61.7% of the subjects had RRMS at the PRISMS-15 visit and the remainder had SPMS. For the PRISMS-15 population as a whole, the median EDSS score was 4.00 (range: 0 to 9). For the SNPs evaluated, the genotype frequencies for subjects in the PRISMS-15 population were generally similar to those reported for a European Caucasian population.

PGx Analysis Results:

All variables related to SNP marker identification were censored during the 2-year analysis period at the first occurrence of (1) treatment discontinuation or (2) trial withdrawal without having an event. Conversely, events occurring before discontinuation or withdrawal were not censored. This censoring affected the primary endpoint response variable; it was introduced in order to provide more precision for variables related to treatment response (i.e. to remove equivocal results). Therefore, for these parameters, the numbers of subjects analyzed may be different from the number of subjects in the relevant population.

Variables related to the secondary endpoints remained uncensored as planned.

SNP3 IFNAR2 Minor Allele, Clinical and Magnetic Resonance Imaging (MRI) Parameters:

For the three SNP3 IFNAR2 genotype groups, there were no statistically significant differences in proportion of responders during the first 2 years of Rebif® treatment in the PRISMS-2, PRISMS-4 and PRISMS-2/4 populations. However, subjects in the PRISMS-2 population who were treated with Rebif® 44 mcg and who had 1 copy of the SNP3 IFNAR2 minor allele showed a tendency towards an increased response to treatment as compared to subjects with 0 copies: 40.6% (13/32) responders vs. 25.5% (12/47) responders respectively (p=0.160). Results from clinical measures of efficacy (i.e. relapses and EDSS) indicated that having 1 or 2 copies of the SNP3 IFNAR2 minor allele was associated with an increased likelihood that subjects would have no relapse over 2 years or over 15 years, regardless of whether treatment was started early (i.e. at baseline of PRISMS-6789) or 2 years later; this was statistically significant for subjects with 2 copies, and a tendency in this direction was observed for subjects with 1 copy. The protective effect of having 2 copies of the SNP3 IFNAR2 minor allele, in combination with the effect of early treatment with Rebif® 44 mcg, led to a long-lasting reduction in relapses, and this effect carried over into the pooled populations that included these subjects.

For 3-month confirmed EDSS progression, only occasional statistically significant results were observed, which suggests that relapse parameters were a more sensitive measure of differences in disease progression and treatment response among the different genotype groups.

MRI measures of efficacy reinforced the findings from clinical measures of efficacy: in the PRISMS-2 population, among Rebif® 22 mcg-treated subjects with 1 copy of SNP3 IFNAR2, a statistically significant lower proportion of subjects had new T2 lesions as compared to subjects with 0 copies (1 copy 37.8% [14/37]; 0 copies: 9.5% [4/42], p=0.005).

SNP2 IFNAR2 Homozygote Major Allele and SNP4 IFNAR2 Minor Allele, Clinical and MRI Parameters:

For all populations and all time-points evaluated, there were no statistically significant differences between subjects with absence or presence of the SNP2 IFNAR2 homozygote major allele or the SNP4 IFNAR2 minor allele with regard to the proportion of responders.

Results from clinical measures of efficacy over 15-16 years of follow-up indicated that subjects exposed to Rebif® 44 mcg in PRISMS-2 and subjects exposed to Rebif® 22 mcg in PRISMS-4 with presence of the SNP2 IFNAR2 homozygote major allele (Rebif® 44 mcg: N=79; Rebif® 22 mcg: N=36) had a greater mean number of relapses from baseline until the PRISMS-15 visit than subjects with absence of the SNP2 IFNAR2 homozygote major allele (Rebif® 44 mcg N=16; Rebif® 22 mcg: N=11). These differences were statistically significant for the PRISMS-2 Rebif® 44 mcg treatment group (p=0.005) and for the PRISMS-4 Rebif® 22 mcg treatment group (p=0.011) and carried through into the pooled populations that contained these treatment groups.

Subjects with presence of the SNP4 IFNAR2 minor allele who were exposed to Rebif® 44 mcg in PRISMS-2 (N=47) had fewer relapses from baseline until the PRISMS-15 visit than subjects with absence of the SNP4 IFNAR2 minor allele (N=48). The differences were significant at Month 48 (p=0.017), at the PRISMS-22930 LTFU visit (p=0.017) and at the PRISMS-15 visit (p<0.001), and these differences carried through into the pooled populations that contained this group.

For change in EDSS score and time to first 3-month EDSS confirmed progression, there were no statistically significant differences between subjects with absence or presence of the SNP2 IFNAR2 homozygote major allele or the SNP4 IFNAR2 minor allele, for all populations and all time-points evaluated.

Presence or absence of the SNP2 IFNAR2 homozygote major allele had no notable effect on MRI parameters, but – in contrast to the clinical parameters -- presence of the SNP4 IFNAR2 allele was associated with worse outcomes for the MRI parameters T2 lesion volume and change in brain volume.

SNP1 TNFSF10 Minor Allele, Clinical and MRI Parameters:

Among subjects in the PRISMS-2 population randomized to the Rebif® 22 mcg treatment group, a statistically significantly greater proportion of subjects with 1 copy of SNP1 TNFSF10 minor allele (29.3% [12/41]) as compared to 0 copies (10.9% [5/46]) responded to treatment during the first 2 years of treatment (p=0.037). This result was also seen in the relapse-free component of the response outcome, suggesting that relapses drive this response. The relapse results carried through to pooled populations that contained this group.

Results from clinical measures of efficacy (i.e., relapses, EDSS) suggested that, among subjects treated with placebo or Rebif® 22 mcg, those with 2 copies of SNP1 TNFSF10 minor allele had a decreased rate of disease progression in comparison to subjects with 0 or 1 copies of SNP1 TNFSF10 minor allele. However, a reverse effect was observed in Rebif®

44 mcg-treated subjects, where subjects with 0 copies of the SNP1 TNFSF10 minor allele had a lower relapse rate at the later time-points than subjects with 2 copies.

Results from certain MRI measures of efficacy (i.e. new T2 lesions and change in T2 lesion volume) supported the findings from clinical measures of efficacy: subjects with 2 copies of SNP1 TNFSF10 showed tendencies towards a decreased rate of disease progression, and the differences were more apparent at the latest time-points evaluated. However, the number of subjects with 2 copies of SNP1 TNFSF10 was small, and these differences were not statistically significant.

SNP5 OAS1 Minor Allele, Clinical and MRI Parameters:

For the SNP5 OAS1 minor allele, there were no statistically significant differences between genotype groups in the responder rates.

In the PRISMS-2 population, among Rebif® 22 mcg-treated subjects, those with 0 copies of SNP5 OAS1 minor allele (N=38) had a statistically significantly greater mean number relapses from baseline to the PRISMS-15 visit than subjects with 1 copy of SNP5 OAS1 minor allele (N=46) at Month 48 (p=0.027), at the PRISMS-22930 LTFU visit (p=0.003) and at the PRISMS-15 visit (p<0.001).

The opposite effect was observed for Rebif® 44 mcg-treated subjects in the PRISMS-2 population, where subjects with 0 copies of SNP5 OAS1 minor allele (N=49) had a statistically significantly lower mean number relapses than subjects with 1 copy of SNP5 OAS1 minor allele (N=36) at all time-points: Month 24 (p=0.005), Month 48 (p=0.003), at the PRISMS-22930 LTFU visit (p<0.001) and at the PRISMS-15 visit (p<0.001).

For other clinical parameters (e.g., other measures of relapse, EDSS parameters) and MRI parameters, there were no notable differences between the SNP5 OAS1 genotype groups.

SNP6 PSMB8 Minor Allele, Clinical and MRI Parameters:

In the PRISMS-2/4 population, Rebif® 22 mcg or 44 mcg late treatment group, a statistically significant difference in responder rate was observed during the first 2 years of treatment for the SNP6 PSMB8 genotype groups. A lower proportion of subjects with presence of SNP6 PSMB8 minor allele were responders (10.5% [2/19]) as compared to subjects with absence of the SNP6 PSMB8 minor allele (34.8% [24/69]; p=0.048).

Subjects with presence of SNP6 PSMB8 minor allele generally had a greater mean number of relapses as compared to subjects with absence of SNP6 PSMB8 minor allele.

For other clinical parameters (e.g. EDSS) and MRI parameters, there were no notable differences between the SNP6 PSMB8 minor allele genotype groups.

Immunogenicity and Safety Results:

There were no consistent patterns for neutralizing antibody (NAb) immunogenicity and the evaluated SNPs, although there were indications of some tendencies towards higher or lower levels of antibodies with certain genotypes. Similarly, there were no consistent patterns between the evaluated SNPs and the incidence of the pre-specified AEs.

Conclusion:

Any interpretation of the results of this study should take into account its exploratory nature, which creates potential uncontrolled effects: e.g., confounding factors influencing treatment response or disease activity and the retrospective collection of samples which led to the inclusion of only about 60% of the original study population. Also, for some genotypes the number of subjects was quite small, which limited the sensitivity of the statistical analyses and limited the interpretation of results. No adjustment for multiplicity was performed, so the type-1 error rate was not controlled and false positive results cannot be excluded.

Despite these caveats, this study enabled an exploration of the relationship between genetic markers and the long-term (over 15 years) progression of MS disease, with direct comparison between MS subjects who were or were not treated with standard therapy at early time-points. At present, there is a clear obligation to treat people with MS with the most effective therapies. The opportunity to investigate a population of MS patients who were not initially treated with IFN beta-1a (i.e. the PRISMS-6789 placebo group) presents a unique opportunity to evaluate the relationship between response to therapy and genetic profile. With the standardization of effective treatments for patients with MS, placebo-controlled trials may become increasingly difficult to justify, and a narrow window of opportunity has emerged that may enable understanding of the relationship between genetic profile and response to IFN beta-1a treatment. This study involves a unique population of subjects that may not be available in the future.

The results of this study suggest that two genetic markers, IFNAR2 and TNFSF10, may have a potential impact on certain indicators of disease activity and response to IFN beta-1a treatment, whereas other markers showed inconsistent results.