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Vedolizumab (MLN0002)  
Clinical Study Report C13012 Synopsis

C13012 FINAL CLINICAL STUDY REPORT SYNOPSIS

Study Title: A Phase 1 Single-Arm Study to Evaluate the Effects of a Single Intravenous Dose of Vedolizumab (MLN0002) on the CD4+:CD8+ Lymphocyte Ratio in the Cerebrospinal Fluid of Healthy Subjects

Study Center(s): The study was conducted at a single center: PRA International Clinical Pharmacology Center, 9755 Ridge Dr, Lenexa, KS 66219.


Phase: 1

Initiation Date (first subject enrolled): 03 November 2010

Completion Date (last subject completed): 10 May 2011

Study Objectives:

Primary:

- To evaluate the change in cerebrospinal fluid (CSF) CD4+:CD8+ lymphocyte ratio before and after a single 450-mg intravenous (IV) dose of vedolizumab

Secondary:

- To determine if reversal of the normal CSF CD4+:CD8+ lymphocyte ratio to < 1 occurs after a single 450-mg IV dose of vedolizumab
- To assess the safety and tolerability of a single 450-mg IV dose of vedolizumab

METHODS

Design: This was a phase 1, single-arm, open-label study designed to investigate the effect of a single 450-mg IV dose of vedolizumab on the CD4+:CD8+ T-lymphocyte ratio in CSF in healthy subjects. Fourteen subjects were enrolled in the study. A subject was considered to be enrolled when he/she had received any amount of study drug. Cerebrospinal fluid was obtained by lumbar puncture (LP) prior to and 5 weeks after administration of a single 450-mg IV dose of vedolizumab.

A staggered approach to enrollment was taken to ensure that obtaining adequate CSF volume and CSF immunophenotyping were technically feasible. The first 5 subjects screened comprised a “technical feasibility” cohort; these subjects underwent a baseline LP to determine the feasibility of obtaining approximately 20 mL of CSF in a single LP, as well as an adequate number of lymphocytes for definitive evaluation of CD4+ and CD8+ lymphocyte subsets by flow cytometry. Once technical feasibility was confirmed based on this initial cohort, enrollment of subjects was initiated.
The study design included a screening period (Days -28 to -1), a treatment period (Day 1), and an observation/sampling period (Day 1 through Week 16). Subjects were followed poststudy by telephone contact at 6 months after the date of dosing (ie, Day 1) to determine if progressive multifocal leukoencephalopathy (PML) or malignancies had been diagnosed in any subject.

Eligibility for the study was based on the results of assessments obtained during screening; subjects who appeared eligible based on all screening requirements except for LP then underwent a baseline LP to determine if the CSF sample provided evaluable data for immunophenotyping (ie, the baseline LP was the last screening procedure performed before the Day 1 predose assessments). If the screening CSF sample met study criteria for eligibility (inclusion criterion #8), and all Day 1 predose assessments showed no clinically significant abnormalities, then the subject was enrolled in the study and administered the single dose of vedolizumab within 2 to 10 days of the screening LP (this window was not applicable to the first 5 subjects who completed the baseline LP procedure during the study’s technical feasibility assessment). Because the “screening” LP was considered the baseline LP for enrolled subjects, the terms “screening” and “baseline” are used interchangeably in this report.

The second CSF sample was obtained by LP 5 weeks after the dose of vedolizumab. Each subject was his/her own control with regard to statistical analyses of the CD4⁺ and CD8⁺ lymphocyte counts in the CSF.

Before each LP, subjects underwent a physical examination, including funduscopic examination, and it was confirmed that there were no clinically significant laboratory findings. The LP procedure was conducted in a controlled manner to minimize potential risk to subject safety or comfort. All LPs were performed in a standardized manner by a single neurologist. Each subject received a local anesthetic to numb the skin and underlying tissues, and could have received a short-acting anxiolytic medication if deemed necessary by the subject and investigator. After the LP, subjects laid down for at least 1 hour and then were assessed for adverse sequelae from the procedure, including, but not limited to headache, pain at the insertion site, leakage of CSF, and bleeding. Subjects were allowed to leave the clinic after evaluation and clearance from the principal investigator or designee.

Adverse events (AEs) associated with the LP (eg, headache, leakage of CSF) were treated according to standard medical practice.

Safety was evaluated based on data collected during the study through the end of the sampling/observation period, including AEs, serious adverse events (SAEs), vital signs, and results of the PML checklists and clinical laboratory tests.

Peripheral blood samples for serum vedolizumab concentrations and pharmacodynamic (PD) markers were obtained at prespecified time points to confirm vedolizumab exposure and $\alpha_4\beta_7$ receptor saturation, respectively, especially at the timing of the endpoints. Vedolizumab concentration also was analyzed in the postdose CSF sample. Peripheral blood CD4⁺ and CD8⁺ lymphocytes were measured in parallel with CSF samples before and after dosing to determine if changes in peripheral blood may have affected lymphocyte counts in the CSF. Testing for human antihuman antibody (HAHA) to study drug was done to determine if neutralization of drug effect occurred in order to exclude such subjects from the primary analyses.

**Number of Subjects (planned and analyzed):** Up to 22 subjects were planned for enrollment to obtain 14 evaluable subjects, the sample size required to provide 85% and 97% power to reject the null hypotheses for the primary and secondary endpoints, respectively. Fourteen subjects were included in the Safety Population. One subject with detectable HAHA was excluded from the CSF CD4⁺:CD8⁺ Evaluable Population (also referred to as the CD4⁺:CD8⁺ Evaluable Population), Pharmacodynamics Population, and Serum Pharmacokinetics Population (thus, the sample size of these analysis populations was 13 subjects). One subject was lost to follow-up. Therefore, 12 subjects were included in the CSF CD4⁺:CD8⁺ Evaluable Population who completed the study evaluation.
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through Week 16 (also referred to as the CD4⁺:CD8⁺ Evaluable Population with Week 16 Assessments) for the additional sensitivity analyses.

**Diagnosis and Main Criteria for Inclusion:** Healthy male and female subjects between the ages of 18 and 45 years with body mass index (BMI) between 18 and 32 kg/m², inclusive, who had no significant medical problems and did not use concomitant immunosuppressive medications, were eligible for enrollment.

**Test Product, Dose and Mode of Administration, Batch Number:** Vedolizumab lyophilized powder for reconstitution, 60 mg/mL reconstituted solution; 450 mg IV; Lot No. IC014LA01

**Duration of Treatment:** Single dose, with observation for up to 16 weeks postdose

**Reference Therapy, Dose and Mode of Administration, Batch Number:** Not applicable

**Pharmacokinetic Assessments:** Blood samples were collected for the determination of serum vedolizumab concentrations on Day 1 (predose and postdose), and at Weeks 5 and 16. Vedolizumab concentration in CSF was determined from CSF samples obtained at baseline and at Week 5.

**Pharmacodynamic Assessments:** Blood samples were collected for the determination of MAdCAM-1-Fc binding (MAdCAM-1-Fc is a fusion of the α₄β₇ ligand human mucosal addressin cell adhesion molecule-1 with the heavy and light chain Fc of a mouse monoclonal antibody), indicative of the extent of α₄β₇ receptor saturation by vedolizumab in order to determine if adequate target saturation was achieved at the time of endpoint analyses (Week 5). Blood samples were collected on Day 1 (predose) and at Week 5.

**Immunophenotyping Assessments:** Cerebrospinal fluid was collected during screening and at Week 5 for measurement of T lymphocytes expressing CD4⁺ and CD8⁺ and the ratio of CD4⁺ to CD8⁺ lymphocytes.

In parallel with CSF assessments, a peripheral blood sample was obtained during screening and at Weeks 2, 5, and 16 to evaluate the effect of vedolizumab on peripheral cell populations. As vedolizumab targets the α₄β₇-expressing CD4⁺ and CD8⁺ T-cell population, these peripheral blood cell populations were specifically evaluated.

**Other Assessments:** Immunogenicity (HAHA and Neutralizing HAHA) Assessments

Blood samples for the assessment of HAHA were collected at prespecified time points. On days when both HAHA and pharmacokinetic (PK) sampling were required, the sample collections were timed as close to each other as feasible. The sample collected for HAHA analysis could also be assessed for neutralizing HAHA if HAHA was detected.

**Safety Assessments:** Safety was assessed by monitoring AEs and SAEs, vital signs, clinical laboratory tests, physical and neurological examinations, and results of PML checklists.

**Statistical Methods:** The CSF CD4⁺:CD8⁺ Evaluable Population was used to test the primary, secondary, and exploratory CSF endpoints. A sample size of approximately 14 evaluable subjects was planned in order to provide adequate power for testing the primary (85% power) and secondary (97% power) endpoints. Formal hypothesis testing was used for the primary and secondary CSF endpoints.

To test the primary endpoint of change in CD4⁺:CD8⁺ ratio after vedolizumab exposure, the lower bound of the 90% confidence interval (CI) for the ratio change, derived from a paired t-test, was used. If the lower bound was ≥ -1.67, then the null hypothesis that there is a statistically significant reduction in the ratio was rejected. To test the secondary endpoint that the postdose CD4⁺:CD8⁺ ratio is < 1, a 1-sample t-test was used at α = 0.05 (1-sided). In order to control for the overall type I error rate at the 5% level, a closed sequential testing procedure was used for the hypotheses testing.
If the primary endpoint was significant, the secondary endpoint was to be tested at a 5% level. If the primary endpoint was not significant, then the secondary endpoint analysis was to be regarded as exploratory.

Descriptive summary statistics were provided for all exploratory endpoints and for safety assessments. These included CD4⁺ and CD8⁺ lymphocyte levels in blood and CSF, other blood immunophenotyping assessments, serum and CSF PK, PD, and HAHA. The respective sample populations for each of these assessments are described in Section 9.7.2 of the clinical study report (Populations for Analysis).

Two sets of sensitivity analyses were added for the primary and secondary endpoint analyses: 1 set using the Safety Population (N = 14) and 1 set using all subjects in the CSF CD4⁺:CD8⁺ Evaluable Population who had completed all study assessments (ie, had Week 16 assessments; N = 12).

RESULTS

Disposition and Demographic Results: Disposition of subjects is summarized in the table below. All 14 enrolled subjects received vedolizumab and were included in the Safety Population. One of these subjects (Subject 030) tested positive for HAHA at Weeks 5 and 16 and hence was excluded from the CD4⁺:CD8⁺ Evaluable Population (also referred to as the CSF CD4⁺:CD8⁺ Evaluable Population), Pharmacodynamics Population, and Serum Pharmacokinetics Population but was included in the CSF Pharmacokinetics Population. One subject (Subject 039) was lost to follow-up after Week 8, and 13 (93%) subjects completed the study. Twelve subjects (86%) were included in the CD4⁺:CD8⁺ Evaluable Population with Week 16 Assessments (completed the study) for the additional sensitivity analyses. It is noted that the Disposition of Subjects summary table (below) shows 2 subjects (Subjects 037 and 039) lost to follow-up. This was due to a data entry error that was not detected prior to database lock. Subject 037 completed the study.
Disposition of Subjects

<table>
<thead>
<tr>
<th></th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safety Population(^a)</td>
<td>14 (100)</td>
</tr>
<tr>
<td>Have not received a complete dose of 450 -mg vedolizumab(^b)</td>
<td>0</td>
</tr>
<tr>
<td>No postdose evaluable CSF samples(^c)</td>
<td>0</td>
</tr>
<tr>
<td>Tested positive for HAHA at any time point postdose</td>
<td>1 (7)</td>
</tr>
<tr>
<td>CD4(^+):CD8(^+) Evaluable Population(^d)</td>
<td>13 (93)</td>
</tr>
<tr>
<td>CSF Pharmacokinetics Population(^d)</td>
<td>14 (100)</td>
</tr>
<tr>
<td>Pharmacodynamics Population(^d)</td>
<td>13 (93)</td>
</tr>
<tr>
<td>Serum Pharmacokinetics Population(^d)</td>
<td>13 (93)</td>
</tr>
<tr>
<td>Subjects Completing Study</td>
<td>12 (86)</td>
</tr>
<tr>
<td>Subjects Completing Study (corrected)(^e)</td>
<td>13 (93)</td>
</tr>
<tr>
<td>Subjects Not Completing Study, Primary Reason</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Subjects Not Completing Study, Primary Reason (corrected)(^f)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Lost to Follow-up</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Lost to Follow-up (corrected)(^e)</td>
<td>1 (7)</td>
</tr>
</tbody>
</table>

Source: Clinical study report Table 14.1.1.1.

Abbreviations: CSF = cerebrospinal fluid; HAHA = human antihuman antibody; RBC = red blood cell; WBC = white blood cell.

\(^a\) Safety Population is defined as subjects who receive any amount of vedolizumab. Percents are based on safety population.

\(^b\) Complete is defined as receiving at least 90% of 450-mg vedolizumab.

\(^c\) Evaluable CSF samples i) WBC and cell types, glucose, and protein must be within the normal range as specified by the local analyzing laboratory; ii) the RBC count in the sample of CSF to be analyzed for WBC markers (immunophenotyping) must be ≤ 10 RBCs/µL; iii) the cell culture result must be negative; and iv) the CSF sample must be evaluable for immunophenotyping.

\(^d\) CD4\(^+\):CD8\(^+\) Evaluable Population (also referred to as CSF CD4\(^+\):CD8\(^+\) Evaluable Population), Serum Pharmacokinetics Population, CSF Pharmacokinetics Population and Pharmacodynamics Population are defined in the Clinical Study Report Section 9.7.2. Subject 030 developed detectable HAHA at Week 5 and was excluded from the CSF CD4\(^+\):CD8\(^+\) Evaluable Population, Serum Pharmacokinetics Population, and Pharmacodynamics Population but was included in the CSF Pharmacokinetics Population.

\(^e\) Twelve subjects are shown as completing the study and 2 subjects are shown as lost to follow-up in Table 14.1.1.1. Subject 039 was lost to follow-up. Subject 037, who completed the study, was categorized as lost to follow-up due to a data entry error that was not detected prior to database lock. Thus, only 1 subject was lost to follow-up and 13 subjects completed the study. See the Note to Trial Master File and Clinical Study Report.

Of the 14 enrolled subjects 10 (71%) were white and male. Subjects ranged in age from 19 to 41 years (median: 24 years), and BMI ranged from 20.2 to 30.9 mg/m\(^2\) (median: 25.6 mg/m\(^2\)).

**Pharmacokinetic Results:** At 5 minutes after the end of the 30-minute IV infusion of vedolizumab 450 mg, the median peak serum vedolizumab concentration value was 187 µg/mL, which declined to a median serum vedolizumab concentration of 32.5 µg/mL at Week 5, the time point of the endpoint assessment. This median Week 5 concentration was higher than the projected steady-state vedolizumab trough concentration (approximately 24 µg/mL) for the dosing regimen of 300 mg administered once every 4 weeks and currently under evaluation in the phase 3 clinical development program. The individual subject serum concentration data also show that in this single-dose study,
all subjects in the CD4⁺:CD8⁺ Evaluable Population had Week 5 serum vedolizumab concentrations at or above the projected phase 3 median steady-state trough concentrations of vedolizumab.

In the single subject (Subject 030) who developed detectable HAHA at Week 5 and was excluded from the Serum Pharmacokinetics Population, the serum concentration of vedolizumab at Week 5 was approximately 0.4 µg/mL, which was approximately 1.23% of the median Week 5 vedolizumab concentration (32.5 µg/mL) among subjects who did not have detectable HAHA. This subject did not have measurable concentrations of vedolizumab at Week 16.

Analysis of vedolizumab in CSF was performed on samples obtained prior to and at 5 weeks after the infusion of vedolizumab. None of these samples had detectable vedolizumab (detection limit of vedolizumab in this assay was 0.125 µg/mL) in the CSF. These data indicate that no measurable vedolizumab was distributed into the CSF despite its presence in the serum.

**Pharmacodynamic Results:** The PD marker, MAdCAM-1-Fc, was used to evaluate the extent of α4β7 receptor saturation by vedolizumab. The relationship between the serum concentration of vedolizumab at Week 5 and the percent decrease from baseline in MAdCAM binding for CD4⁺ and CD8⁺ cell population (the decrease in binding is a marker of α4β7 receptor saturation) was assessed. Over the serum concentration range of vedolizumab observed, there was a high degree of saturation (> 90%) of the α4β7 receptor at Week 5 in most subjects as shown by the low percentage of MAdCAM-Fc binding. Overall, these data confirmed the adequacy of vedolizumab exposure at the Week 5 endpoint in effectively saturating α4β7 receptors as measured by the MAdCAM-Fc assay.

**Immunophenotyping Results:**

**Cerebrospinal Fluid Immunophenotyping:** One subject was excluded from the CSF CD4⁺:CD8⁺ Evaluable Population because HAHA was detectable at Weeks 5 and 16; thus, N = 13 for the population for analyses of CSF endpoints. For the primary endpoint, change from baseline in the CSF CD4⁺:CD8⁺ lymphocyte ratio before and after vedolizumab dosing (mean of 3.59 and 3.61, respectively). The mean of the difference in ratios before and after vedolizumab dosing was 0.013 (90% CI: -0.337, 0.363).

For the secondary endpoint, reduction in the postdose CSF CD4⁺:CD8⁺ ratio to < 1, the p value (p < 0.0001; 1-sided, 1-sample t-test) was highly significant for rejecting the null hypothesis of reduction in the postdose CSF CD4⁺:CD8⁺ ratio to < 1.

There were no significant changes in mean and median absolute cell counts and essentially no change in the mean percentages of CD4⁺- and CD8⁺-expressing T lymphocytes in the CSF from baseline to Week 5. The mean change in percent CD4⁺ and CD8⁺ cells in the CSF after a single dose of vedolizumab was < 1%.

**Blood Immunophenotyping:** There were essentially no changes in the mean percentages of CD4⁺- and CD8⁺-expressing T lymphocytes in blood from baseline to Week 5 (mean change 0.87 and -1.05, respectively) after a single 450-mg dose of vedolizumab. There also was little change in the mean percentages of CD4⁺- and CD8⁺-expressing memory T lymphocytes at baseline and Week 5 (mean of 27.8 vs 27.1, respectively, for CD4⁺ and mean of 11.2 vs 10.8, respectively, for CD8⁺). These results demonstrate that there were no peripheral blood changes that could have influenced the overall CD4⁺:CD8⁺ ratio in the CSF. There also was no evidence of lymphocytosis or elevation of CD34⁺ hematopoietic progenitor cells. This is consistent with results of previous vedolizumab studies.

**Immunogenicity:** One of the 14 subjects in the Safety Population had detectable HAHA (Subject 030). This subject had a HAHA titer of 1:125 at Weeks 5 and 16, and rapidly cleared vedolizumab from the serum (as noted previously, the Week 5 serum concentration for this subject
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was 0.4 µg/mL, approximately 1.23% of the median concentration of 32.5 µg/mL observed for subjects who did not test positive for HAHA). Due to the effect of the HAHA on the overall PK in the HAHA-positive subject, the HAHA was deemed to be neutralizing and no further neutralization testing was performed.

Safety Results: Ten of the 14 subjects (71%) experienced at least 1 treatment-emergent AE. The only treatment-emergent AEs reported by more than 1 subject were headache (6 subjects [43%]) and dizziness (2 subjects [14%]). None of the treatment-emergent AEs was considered by the investigator to be related to the study drug; almost all appeared to be related to the LP procedure. Only 1 subject experienced an AE (headache on the day of the Week 5 LP) that was assessed by the investigator as moderate in intensity; all other subjects had AEs assessed as mild.

There were no reported SAEs, deaths, AEs resulting in study drug discontinuation, or AEs assessed as severe in intensity.

There were no clinically notable changes from baseline at the scheduled assessment time of Week 2, Week 5, or Week 16 for hematology parameters or at Week 5 or Week 16 for clinical chemistry parameters. One subject experienced an elevated aspartate aminotransferase (AST) level (2.3 × upper limit of the normal range) that was reported as an AE. The event was reported at the Week 16 visit, almost 4 months after the dose of vedolizumab was administered. The event was assessed by the investigator as mild in intensity and not related to study drug.

None of the subjects had positive subjective or objective findings on the PML symptom checklist prior to enrollment or at any time during the study.

No clinically notable changes in vital signs were observed.

CONCLUSIONS

In conclusion, the study data demonstrate that vedolizumab does not affect CD4⁺ cell counts, CD8⁺ cell counts, or CD4⁺:CD8⁺ ratio in the CSF of humans. These findings support the hypothesis that vedolizumab is unlikely to lead to impairment of the immune system in the central nervous system that can increase the risk of opportunistic infections such as PML.

Date of Report: 12 December 2011