Effect of plasma exchange in accelerating natalizumab clearance and restoring leukocyte function

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ABSTRACT

Background: Accelerating the clearance of therapeutic monoclonal antibodies (mAbs) from the body may be useful to address uncommon but serious complications from treatment, such as progressive multifocal leukoencephalopathy (PML). Treatment of PML requires immune reconstitution. Plasma exchange (PLEX) may accelerate mAb clearance, restoring the function of inhibited proteins and increasing the number or function of leukocytes entering the CNS. We evaluated the efficacy of PLEX in accelerating natalizumab (a therapy for multiple sclerosis [MS] and Crohn disease) clearance and α4-integrin desaturation. Restoration of leukocyte transmigratory capacity was evaluated using an in vitro blood–brain barrier (ivBBB).

Methods: Twelve patients with MS receiving natalizumab underwent three 1.5-volume PLEX sessions over 5 or 8 days. Natalizumab concentrations and α4-integrin saturation were assessed daily throughout PLEX and three times over the subsequent 2 weeks, comparing results with the same patients the previous month. Peripheral blood mononuclear cell (PBMC) migration (induced by the chemokine CCL2) across an ivBBB was assessed in a subset of six patients with and without PLEX.

Results: Serum natalizumab concentrations were reduced by a mean of 92% from baseline to 1 week after three PLEX sessions ($p < 0.001$). Although average α4-integrin saturation was not reduced after PLEX, it was reduced to less than 50% when natalizumab concentrations were below 1 μg/mL. PBMC transmigratory capacity increased 2.2-fold after PLEX ($p < 0.006$).

Conclusions: Plasma exchange (PLEX) accelerated clearance of natalizumab, and at natalizumab concentrations below 1 μg/mL, desaturation of α4-integrin was observed. Also, CCL2-induced leukocyte transmigration across an in vitro blood–brain barrier was increased after PLEX. Therefore, PLEX may be effective in restoring immune effector function in natalizumab-treated patients. ***Neurology*® 2009;72:402–409**

GLOSSARY

AE = adverse event; **BBB** = blood–brain barrier; **BW** = body weight; **EDSS** = Expanded Disability Status Scale; **hIgG4-PE** = human IgG4 monoclonal antibody conjugated with phycoerythrin; **Ig** = immunoglobulin; **ivBBB** = in vitro blood–brain barrier; **mAb** = monoclonal antibody; **MFI** = mean fluorescence intensity; **MS** = multiple sclerosis; **PBMC** = peripheral blood mononuclear cell; **PLEX** = plasma exchange; **PML** = progressive multifocal leukoencephalopathy; **TDL** = total drug load; **V1** = volume of distribution of the central compartment; **Vd** = volume of distribution.

Several monoclonal antibodies (mAbs), including natalizumab, rituximab, daclizumab, and alemtuzumab, target proteins expressed on circulating blood cells.[1-3] Rare but serious complications have been associated with a number of these therapies.[4-10] The pharmacokinetic half-life of mAbs is typically only 10 to 30 days, but the pharmacodynamic half-life can be significantly longer. For example, the pharmacokinetic half-life of natalizumab in patients with multiple

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sclerosis (MS) is approximately 11 ± 4 days; however, mean α4-integrin saturation levels remain greater than 70% at 4 weeks after infusion. In addition, natalizumab is detectable in the circulation for up to 12 weeks,[11,12] and CSF cell counts are significantly reduced for up to 6 months.[13] Accelerated removal of these mAbs, along with increased availability of their ligands, may improve clinical outcomes of some therapy-associated complications.

Natalizumab is an effective therapy for the treatment of relapsing forms of MS and Crohn disease.[14-16] However, natalizumab is associated with a risk of progressive multifocal leukoencephalopathy (PML, which is caused by JC virus), with an estimated incidence of 1:1,000 after a median of 18 months of treatment.[17] The original natalizumab PML reports were of patients also receiving other immunomodulating therapies, but PML has now been reported with natalizumab monotherapy.[18] The α4-integrin is an adhesion molecule involved in the entry of leukocytes into tissues, including the CNS.[1] The mechanism by which PML develops in the setting of natalizumab therapy is not well understood.[19] However, it is clear that immune effector responses to CNS JC viral infection require lymphocyte migration across the blood–brain barrier (BBB), a function suppressed by natalizumab. Accelerated removal of natalizumab from the body may lead to reduced α4-integrin saturation, thereby allowing lymphocytes to adhere to vascular endothelium and traffic into the CNS. This could restore immune function, potentially improving the clinical outcome from PML.[20,21] Immune reconstitution is the only intervention with demonstrated efficacy for PML, including patients with HIV infection taking highly active antiretroviral therapy[22,23] and in transplant patients after reduction in immunosuppressant medications.[24,25]

Little is known about how to remove therapeutic proteins from the body and whether their removal will restore the native function of the endogenous targets. We evaluated the efficacy of plasma exchange (PLEX) in accelerating the clearance of natalizumab and the subsequent decrease in saturation of α4-integrin by comparing these measures after natalizumab infusion both with and without PLEX. Restoration of the transmigratory capacity of circulating leukocytes was ascertained using an in vitro BBB model.

METHODS

Patients. Patients with MS were recruited to receive three courses of PLEX. All patients gave written informed consent. Eligible patients were aged 18–50 years, had a diagnosis of relapsing MS, were treated with natalizumab consistent with product labeling, and were free of signs and symptoms suggestive of immune compromise or serious opportunistic infection, based on medical history, physical examination, or laboratory testing. Patients were excluded from the study if they tested positive for anti-natalizumab antibodies. At the time of PLEX, all patients must have received three or more doses of natalizumab so that natalizumab concentrations would be at stable levels before PLEX.

Study design. This was an open-label, single-arm, time-series longitudinal study conducted at two sites. Only the patients from site 2 were enrolled in the in vitro BBB substudy. Pharmacokinetic and pharmacodynamic data were compared before and after PLEX as well as with data from a historic natalizumab-treated control group[26,27] (Biogen Idec and Elan Pharmaceuticals, unpublished data).

PLEX was started 10–14 days after natalizumab infusion. Patients underwent three separate exchanges of 1.5 plasma volumes.[28,29] Two PLEX schedules were followed: a Monday–Thursday–Monday schedule at site 1 and a Monday–Wednesday–Friday schedule at site 2. Each PLEX occurred over approximately 2.5 to 3 hours, using continuous-flow systems. Vascular access was achieved by a radial artery catheter placed daily or a large-bore, double-lumen catheter placed via the internal jugular vein.[30] Figure e-1 on the *Neurology*® Web site at www.neurology.org depicts the study flow.

Two methods were used to calculate plasma volume: a weight-only–based formula (site 1, and three patients from site 2): volume exchanged = 1.5 × 0.05 × weight (kg); and a weight-, height-, sex-, and hematocrit-based formula (three patients from site 2): volume exchanged = 1.5 × blood volume (L) × (1 − hematocrit). Blood volume (mL) was estimated as follows: men, (367 × height [m]^3) + (32.2 × weight [kg]) + 604; women, (356 × height [m]^3) + (33.1 × weight [kg]) + 183. Because both methods yielded similar pharmacokinetic results, combined data are reported here.

Serum natalizumab concentration and α4-integrin saturation. Natalizumab concentrations (Charles River Laboratories, Sennevile, Quebec, Canada) and α4-integrin saturation (Esoterix Clinical Trial Services, Brentwood, TN) were determined by independent laboratories. Serum natalizumab concentration was measured using a sandwich ELISA. Briefly, serum samples were incubated at room temperature for 90 ± 15 minutes in microtiter plates coated with anti-natalizumab antibody. After washing, mouse anti–human immunoglobulin (Ig) G4 alkaline phosphatase conjugate was added to detect bound natalizumab. Para-nitrophenyl phosphate was added to detect the antibody, and absorbance was measured at 405 nm. The concentration of natalizumab was determined by interpolation from a standard curve. Saturation of α4-integrin was measured by a flow cytometry assay designed to directly measure natalizumab bound to the surface of peripheral blood mononuclear cells (PBMCs).

Briefly, 100 μL whole-blood aliquots were incubated with or without saturating natalizumab (10 μg/mL) for 20 minutes at room temperature. After incubation, red blood cells were lysed, and the remaining leukocytes resuspended with phosphate-buffered saline–normal calf serum. Bound natalizumab was subsequently detected by a fluorescently labeled anti–human IgG4 monoclonal antibody conjugated with phycoerythrin (hIgG4-PE). Leukocytes were measured by flow cytometer, collecting 100,000 total nucleated events. Percent natalizumab saturation was calculated from the mean fluorescence intensity (MFI) in each sample by the following formula: MFI – hIgG4-PE signal (without natalizumab)/MFI – hIgG4-PE signal (with natalizumab) × 100. The performance of the serum natalizumab and α4-integrin saturation assays is shown in table e-1.

Calculation of total drug load. Total drug load (TDL) was estimated for each patient based on a mean volume of distribution (Vd) value of 84.1 mL/kg derived from previous studies (Biogen Idec and Elan Pharmaceuticals, unpublished data). The volume of distribution of the central compartment (V1) was estimated based on body weight (BW) in kilograms: $V1 = 3.97 \times (BW/70)^{0.539}$ (Biogen Idec and Elan Pharmaceuticals, unpublished data). The amount of drug removed was calculated by multiplying the difference in plasma natalizumab concentration immediately before and after each PLEX procedure by V1. The total amount of natalizumab removed was estimated using pharmacokinetic volumes of distribution (V1 and Vd) and the measured natalizumab concentrations just before initiating PLEX, as well as at the beginning and end of each PLEX session to correct for possible underestimates arising from variations in sample collection.

Modeling of a PLEX protocol. Population pharmacokinetic modeling was performed using serum natalizumab concentration data from this study and data from 245 patients who participated in a natalizumab phase 3 clinical trial (AFFIRM)[14] to develop a model PLEX schedule that would maximize the speed of immune reconstitution. A two-compartment model with adjustments for body weight and volume of distribution terms was considered the best model for natalizumab. An additive factor was included with the clearance term that represented the impact of PLEX based on the volume of plasma exchanged and the rate of plasma exchange. The α4-integrin binding was then modeled based on a direct E_{max} response relationship determined from previous measurements (Biogen Idec and Elan Pharmaceuticals, unpublished data).

Leukocyte transmigration. An in vitro BBB model was used to assay leukocyte transmigratory capacity.[31,32] The in vitro BBB model consisted of SV40 T-antigen–immortalized human brain microvascular endothelial cells cultured to confluence in transwell inserts and stimulated with tumor necrosis factor α (10 U/mL) and interferon γ (20 U/mL) for 24 hours.[32] PBMCs were isolated using Ficoll cushions and labeled with AM calcein. Immediately ex vivo, 10^6 PBMCs/well were introduced into the upper compartment, with or without the chemokine CCL2 in the lower compartment, which induces α4-integrin–dependent transmigration.[33] Differences in PBMC transmigration between basal and CCL2-stimulated conditions were assessed using fluorometry to quantify the transmigrated PBMCs.[31] Patients were evaluated approximately 2 and 4 weeks after natalizumab infusion without PLEX and 2.5 and 4.5 weeks after natalizumab infusion with PLEX. Controls were patients with MS not receiving any long-term immunomodulating MS therapy (n = 8) and healthy patients (n = 7). Appropriate positive controls (natalizumab, which blocks CCL2-induced migration) and negative controls (IgG) were performed with each sample. The difference in cell migration between basal and CCL2-stimulated conditions reflects the functional capacity of α4-integrins to mediate leukocyte transmigration in this assay.[32]

Table Patient demographics and baseline plasma exchange characteristics (n = 12)

Age, mean ± SD, y	40.8 ± 8.1
Weight, mean ± SD, kg	82.83 ± 18.65
Height, mean ± SD, cm	168.8 ± 12.07
Sex, % female	58
Race, % white	92
Time from previous natalizumab infusion to PLEX, median (min, max), days	13.0 (10, 14)
Volume of PLEX per session, mean ± SD, L	5.65 ± 1.40

PLEX = plasma exchange.

Safety. Safety assessments included clinical examination with Expanded Disability Status Scale (EDSS),[34] routine laboratory tests, and adverse event (AE) and concomitant therapy monitoring. All patients resumed natalizumab 2 to 2.5 weeks after completion of PLEX. A safety follow-up telephone call was made 12 weeks after the last PLEX.

The study protocol was approved by local ethics committees and was overseen by an independent safety monitor, in accordance with NIH guidelines.[35]

Statistical analysis. The preplanned, protocol-defined primary outcome was serum concentration of natalizumab after plasma exchange. Changes in natalizumab concentration and α4-integrin saturation were assessed using summary statistics, and changes after PLEX were evaluated using a paired *t* test. Comparisons with historic controls were made using the Satterthwaite *t* test. Changes in leukocyte transmigration were analyzed using analysis of variance.

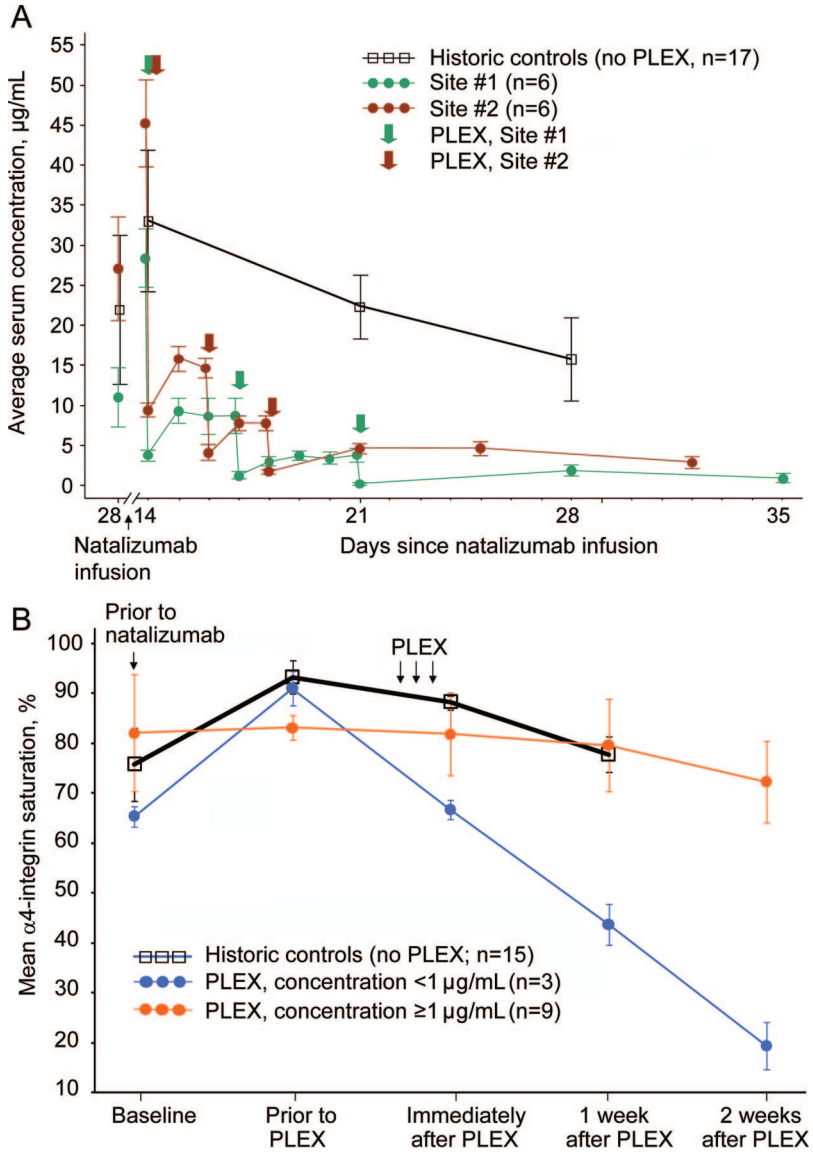
Trial registration. This trial is registered at the ClinicalTrials.gov Web site with the following identifier: NCT00424788.

RESULTS

Patients. Thirteen patients with relapsing MS were enrolled. One patient developed anti-natalizumab antibodies and was excluded before initiation of PLEX. All remaining 12 patients completed the three planned PLEX sessions and subsequent follow-up. The table shows the demographic and baseline characteristics of the patients who received PLEX.

Serum natalizumab concentration. Each PLEX session reduced serum natalizumab concentrations (figure 1A). After a single session of PLEX, natalizumab concentrations for all patients decreased by a mean of 82 ± 8.1%. Natalizumab concentrations re-equilibrated within 24 hours of the first PLEX to a mean reduction of 65 ± 8.3%. One week after the final PLEX, the mean serum natalizumab concentration was 3.2 ± 2.4 μg/mL, representing a mean reduction of 92% (range 84%–100%) compared with before PLEX. Compar-

Figure 1 Effects of plasma exchange on serum concentration of natalizumab and α4-integrin saturation



Effects of plasma exchange (PLEX) on serum concentration of natalizumab (A) and α4-integrin saturation (B). Historic data were obtained from a separate group of patients with multiple sclerosis after six monthly doses of natalizumab, with no PLEX.[28,29] For α4-integrin saturation (B), PLEX patients were divided into two groups: those with sustained natalizumab concentration of less than 1 µg/mL after PLEX and those with natalizumab concentration of 1 µg/mL or greater after PLEX.

ing natalizumab concentrations in the same patients with and without PLEX, PLEX led to a 75 ± 28% reduction in natalizumab concentrations ($p = 0.002$) 4 weeks after natalizumab infusion. Comparison with historic controls also showed a similar result ($p = 0.003$).

TDL and amount of natalizumab removed. The mean (±standard deviation) TDL before PLEX was 256 ± 127 mg. The three PLEX sessions removed a mean total of 191 ± 82 mg of natalizumab, which was 75% of the initial TDL.

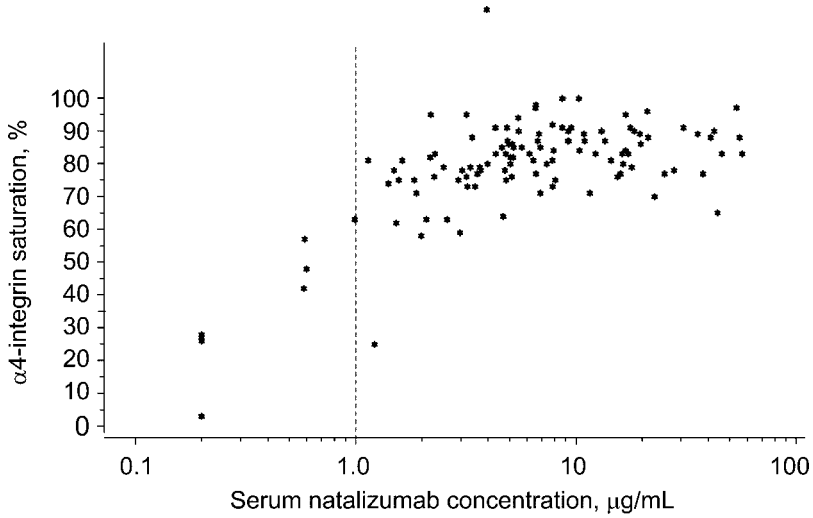
α4-Integrin saturation. PLEX had a variable effect on α4-integrin saturation (figure 1B). Average α4-integrin saturation was not decreased by PLEX. However, in the three patients in whom natalizumab concentration was sustained below 1 µg/mL, receptor saturation declined immediately after PLEX and continued to decline over the following 2 weeks to less than 50%. In the patients who had natalizumab levels greater than 1 µg/mL, receptor saturation showed no consistent change. Figure 2 illustrates the dependence of α4-integrin saturation on natalizumab concentration. At concentrations less than 1 µg/mL, receptor saturation was generally below 50%.

Leukocyte transmigration. Patients with MS receiving natalizumab displayed significant reductions in CCL2-induced transmigration (figure 3). Mean CCL2-induced leukocyte transmigration in patients at 2 and 4 weeks after natalizumab infusion (without PLEX) was 29.3% of that observed in eight MS controls not receiving MS therapy ($p = 0.03$) and 37.6% of that observed in seven healthy controls ($p < 0.01$). At 18 days after PLEX (corresponding to 4.5 weeks after natalizumab), CCL2-induced transmigration was increased an average of 2.2-fold, with all patients demonstrating increased CCL2-induced transmigration ($p < 0.006$). At that time, mean CCL2-induced leukocyte transmigration was 64.1% of that observed in MS controls ($p = 0.27$) and 82.4% of that observed in healthy controls ($p > 0.4$).

Modeling of a PLEX protocol. Assuming that PLEX would be initiated approximately 1 week after administration of the last natalizumab dose (i.e., a higher initial TDL than in the present study), the model predicts that five PLEX sessions of 1.5 plasma volumes each (calculated by the weight-only formula above) would be required for more than 95% of patients to reach a serum natalizumab concentration less than 1 µg/mL (figure 4). Extrapolation of the historic pharmacokinetic data suggests that it would take approximately 97 days to achieve a serum natalizumab concentration less than 1 µg/mL without PLEX.

Safety. PLEX was generally well tolerated, with no relapses or other disease activity and no evidence of a rebound in disease activity. AEs were generally mild or moderate with no resultant discontinuations. The most common AE was hypotension (n = 4), one case of which was serious, because the patient required overnight hospitalization for observation. Other AEs considered by the investigator to be possibly related to PLEX included fatigue, catheter pain, knee pain, diaphoresis, dry mouth, anxiety, and emesis (all n = 1). One patient developed auditory hallucinations after the unanticipated removal of his antipsychotic medication by PLEX. No AEs were considered related to natalizumab. All patients returned to natalizumab treatment at the conclusion of the study

Figure 2 **Relationship between serum natalizumab concentration and α4-integrin saturation**



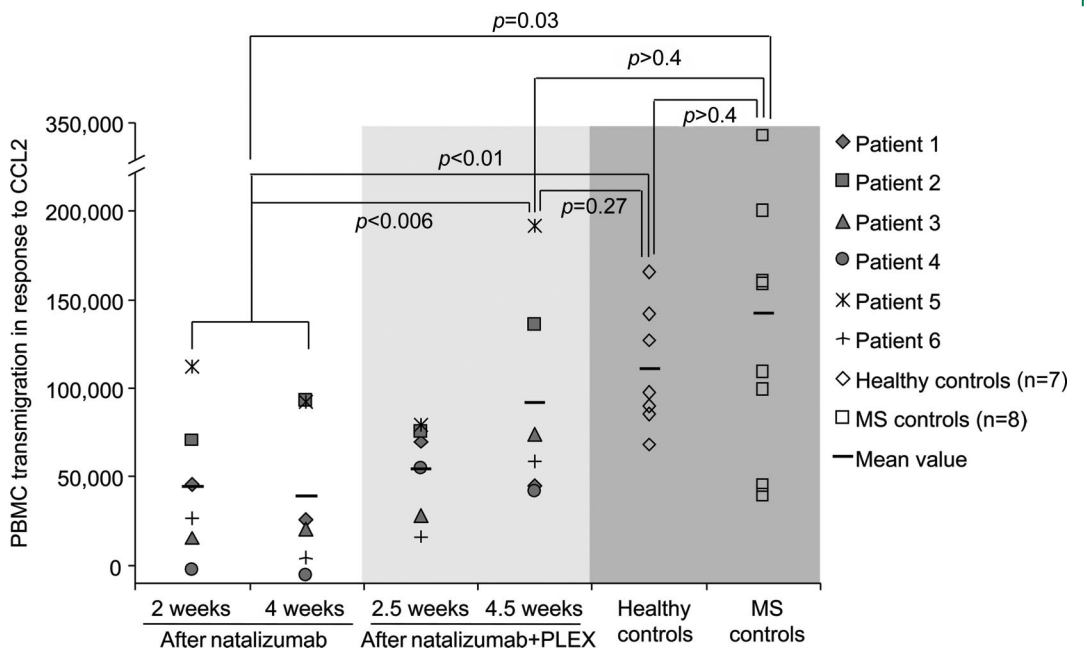
The steady-state correlation between serum natalizumab concentration (log scale) and α4-integrin saturation in plasma exchange (PLEX) patients is shown. At serum natalizumab concentrations below 1.0 μg/mL, there is reliable α4-integrin desaturation. To allow for re-equilibration, only data points ≥24 hours after each PLEX session are shown.

without incident. All patients had stable or improving EDSS. Telephone follow-up 12 weeks after PLEX revealed no new AEs.

DISCUSSION Clinically effective mAbs may cause rare complications for which expedited removal of the therapeutic entity from the body would be desirable. Given the efficacy of plasmapheresis in removing serum proteins, it is not surprising that PLEX accelerated the clearance of natalizumab in this study, reducing mean serum natalizumab concentrations by an average of 92% from baseline to 1 week after the final PLEX session. Using the same patients as their own controls, PLEX reduced natalizumab concentration 75% compared with the same time after natalizumab without PLEX (figure 1A). Comparison with historic pharmacokinetic data shows similar results.

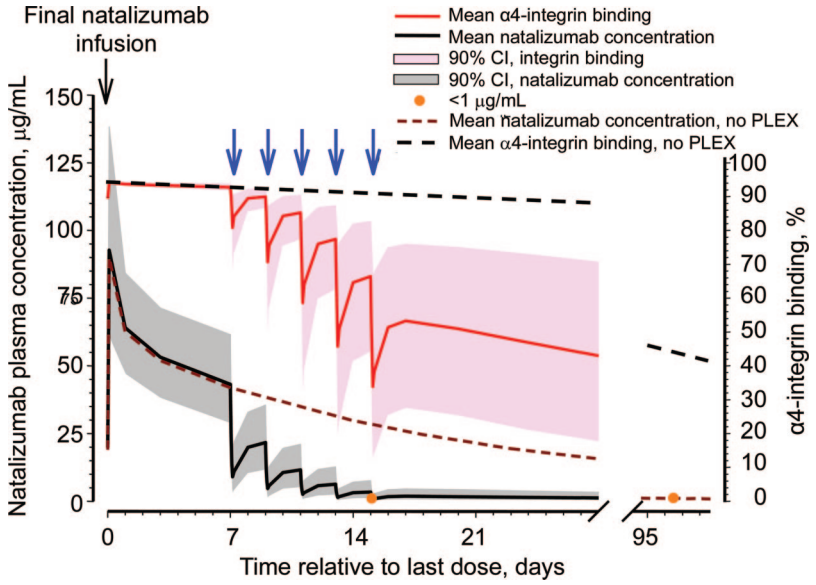
The clinical efficacy of natalizumab in MS is thought to be mediated via the blockade of the α4-integrin, thereby decreasing leukocyte transmigration across the BBB or blood–CSF barrier into the CNS.[26,36] Accordingly, decreased α4-integrin saturation is a desired target to restore trafficking of immune cells into the CNS, which would be desired in the case of a CNS-based infection such as PML. Clinical data from phase 3 clinical trials suggest that saturation levels greater than 70% are associated with continued therapeutic efficacy (Biogen Idec, data on file). Although average α4-integrin saturation was not decreased after PLEX, we observed a reduction of α4-integrin saturation to less than 50% when natalizumab concentration was below 1 μg/mL (figure 2). Factors that likely influence the efficacy of natalizumab removal by PLEX are initial TDL and total plasma volume exchanged. After three PLEX sessions, only 25% of the initial TDL remained.

Figure 3 **Absolute peripheral blood mononuclear cell transmigration to chemokine CCL2 in natalizumab-treated patients**



Induced transmigration (i.e., difference in transmigrating peripheral blood mononuclear cells across an in vitro blood–brain barrier in the presence and absence of CCL2) is shown at two time points before plasma exchange (PLEX; no shading) and after PLEX (lighter shading). Black bars represent the mean. For comparison, values for eight patients with multiple sclerosis (MS) not receiving any MS treatment or PLEX and values for seven healthy patients also are shown (darker shading).

Figure 4 **Population pharmacokinetic and pharmacodynamic modeling of plasma exchange**



Through population modeling of five alternating-day plasma exchange (PLEX) sessions (blue arrows) starting 7 days after natalizumab dosing, PLEX reduces natalizumab concentration to less than 1 μg/mL by day 15. Without PLEX, the same concentration would be expected by day 97.

In a model based on results from this study and pharmacokinetic data from a phase 3 clinical trial, five PLEX sessions, each of 1.5 plasma volumes 2 days apart, would reduce serum natalizumab concentrations to less than 1 μg/mL and α4-integrin saturation levels to less than 50% in more than 95% of patients. Fewer PLEX sessions may be needed in patients with lower initial TDL (e.g., in those who have a greater time interval between the last dose of natalizumab and the start of PLEX), whereas an additional session may be required in patients with a higher initial TDL, such as those who start PLEX less than 1 week after natalizumab infusion. Similar protocols have been shown to be safe in other neurologic disorders, including MS, Guillain–Barré syndrome, chronic inflammatory demyelinating polyneuropathy, and myasthenia gravis.[35,37-40] Thus, using the protocol suggested by the model, the 1-μg/mL threshold can be reached approximately 15 days after natalizumab dosing. In the absence of PLEX, historic pharmacokinetic data indicate that the same threshold would take approximately 82 days longer.

PLEX significantly increased the ability of PBMCs from natalizumab recipients to transmigrate across an in vitro BBB in response to CCL2. We previously reported that addition of exogenous natalizumab to the in vitro BBB assay consistently abolished induction of leukocyte transmigration by chemokines, including CCL2, confirming that this assay is a valid tool to assess the efficiency of α4-integrin inhibition of cell trafficking.[32] Somewhat surprisingly, even in patients with greater than 70% receptor saturation, we consistently observed increased CCL2-induced transmigration after PLEX. Even though gross changes in receptor saturation were not observed, increased receptor-mediated transmigratory capacity was demonstrated in many patients. We speculate that restored transmigratory capacity despite persistently high α4-integrin saturation may be attributable to sensitivity of the functional transmigration assay to small changes in receptor saturation.

In the present study, three sessions of PLEX accelerated the clearance of natalizumab, restored CCL2-induced leukocyte transmigration across the in vitro BBB, and led to decreased α4-integrin saturation when the serum natalizumab concentration reached levels below approximately 1 μg/mL. The validity of our results is supported by comparison both with the same patients without PLEX and with an external historic control group. The results of this study suggest that PLEX may be effective in rapidly restoring CNS immune effector responses in natalizumab-treated patients, which may benefit patients with serious opportunistic infections such as PML. However, none of the patients who underwent PLEX had PML, and the utility of this procedure in such cases is unknown. Similarly, the long-term effect of PLEX on clinical relapses and disability in the present setting are unknown.

To our knowledge, this is the only study to date to demonstrate the efficacy of PLEX in reducing serum concentrations and receptor saturation of any mAb. Because mAbs differ in their pharmacokinetic and pharmacodynamic profiles, the efficacy of PLEX in accelerating the clearance of other protein-based therapeutic agents is unknown.

AUTHOR CONTRIBUTIONS

The main study protocol was written by G.G. and B.O.K.; the in vitro BBB substudy protocol was written by R.J.F., S.M., and R.M.R.; the manuscript was written by R.J.F. and B.O.K. The other authors provided input to each of these documents. Pharmacokinetic and pharmacodynamic data were held and analyzed by the study sponsor; in vitro BBB data were held and analyzed by the Cleveland Clinic. The authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. Statistical analyses were performed by F.L., J.W., and J.-C.L.

ROLE OF MEDICAL WRITER OR EDITOR

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DISCLOSURE

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Editor's Note to Authors and Readers: Levels of Evidence coming to *Neurology*®

Effective January 15, 2009, authors submitting Articles or Clinical/Scientific Notes to *Neurology*® that report on clinical therapeutic studies must state the study type, the primary research question(s), and the classification of level of evidence assigned to each question based on the classification scheme requirements shown below (left). While the authors will initially assign a level of evidence, the final level will be adjudicated by an independent team prior to publication. Ultimately, these levels can be translated into classes of recommendations for clinical care, as shown below (right). For more information, please access the articles and the editorial on the use of classification of levels of evidence published in *Neurology*.[1-3]

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Classification scheme requirements for therapeutic questions

Class I. A randomized, controlled clinical trial of the intervention of interest with masked or objective outcome assessment, in a representative population. Relevant baseline characteristics are presented and substantially equivalent among treatment groups or there is appropriate statistical adjustment for differences.

Class II. A randomized, controlled clinical trial of the intervention of interest in a representative population with masked or objective outcome assessment that lacks one criterion a-e in Class I or a prospective matched cohort study with masked or objective outcome assessment in a representative population that meets b-e in Class I. Relevant baseline characteristics are presented and substantially equivalent among treatment groups or there is appropriate statistical adjustment for differences.

Class III. All other controlled trials (including well-defined natural history controls or patients serving as their own controls) in a representative population, where outcome is independently assessed, or independently derived by objective outcome measurements.

Class IV. Studies not meeting Class I, II, or III criteria including consensus or expert opinion.

AAN classification of recommendations

A = Established as effective, ineffective, or harmful (or established as useful/predictive or not useful/predictive) for the given condition in the specified population. (Level A rating requires at least two consistent Class I studies.)

B = Probably effective, ineffective, or harmful (or probably useful/predictive or not useful/predictive) for the given condition in the specified population. (Level B rating requires at least one Class I study or two consistent Class II studies.)

C = Possibly effective, ineffective, or harmful (or possibly useful/predictive or not useful/predictive) for the given condition in the specified population. (Level C rating requires at least one Class II study or two consistent Class III studies.)

U = Data inadequate or conflicting; given current knowledge, treatment (test, predictor) is unproven.