BRIEF COMMUNICATION

CD4 cell response to interval therapy with natalizumab

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Abstract

Natalizumab treatment alters peripheral CD4 cells counts in multiple sclerosis (MS) patients, providing a way to monitor the pharmacodynamic effects of the drug. The study was undertaken to assess whether CD4 cell counts correlate with different phases of natalizumab treatment of relapsing MS patients, including during a 12-week planned treatment interruption, and whether that might provide insights on lymphocyte trafficking. Clinical outcomes, MRI data, and CD4 cell counts were assessed at baseline prior to initiating natalizumab, while on regular dosing, at the end of the 12-week extended dosing interval, and at the time of reinitiation of natalizumab. The 12-week interruption was well tolerated and not associated with return of MS activity, disability progression, or new or worsened MRI data. Observed significant shifts in CD4 counts – dramatically increasing from the baseline while on treatment and decreasing back to the baseline level off treatment, then rising in a similar manner on natalizumab reinitiation, suggest that these measurements may aid in monitoring modulation of lymphocyte trafficking and cell redistribution.

Introduction

Natalizumab, a humanized monoclonal antibody used for relapsing forms of multiple sclerosis (MS) binds to α4 integrins and inhibits connection of lymphocytes to vascular cell adhesion molecule on activated endothelium[1] thus limiting lymphocytes trafficking through the blood–brain barrier.

Continuous administration of natalizumab leads to increased risk of progressive multifocal leukoencephalopathy (PML). While anti-JCV-antibody index may help to mitigate risks of PML,[2] specific changes to the immune landscape associated with continuous administration of natalizumab also deserve attention. Previous studies found that CD4 cell counts correlated with survival in AIDS patients with PML.[3] However, the pathogenesis of PML in AIDS patient is very different; therefore, the number of CD4 cells in peripheral blood of MS population has to be interpreted differently. Systematic examination of CD4 cell counts associated with natalizumab is important for understanding of its systemic immune mechanisms.

We wished to determine whether interruption of natalizumab treatment for 12 weeks may be well tolerated and safe, and whether it may lead to a meaningful shift in CD4 cell counts suggesting CD4 cells redistribution (this study cannot answer the question on whether 12-week natalizumar interruption may decrease incidence of PML). This publication is the first report on CD4 lymphocyte dynamics in different phases of natalizumab treatment.

Patients and Methods

The data were collected prospectively for 84 weeks from 56 MS patients who elected natalizumab treatment. The mean age of patients was 47.1 years; 36 females and 20 males; racial composition was 38 Whites, six Blacks, four Hispanics, two Asians, and six others (Table 1).

Natalizumab was infused every 4 weeks for the first 48 weeks, followed by a drug-free interval of 12 weeks, after which natalizumab treatment was reinstated. Patients were informed that it is unknown whether this approach may bear any protective value. The vast majority of patients were anti-JCV-antibody positive with unknown index.

Clinical evaluations, including MS relapse assessment, changes to the baseline Expanded Disability Status Scale (EDSS), and absolute CD4 cell counts were measured at four different phases:
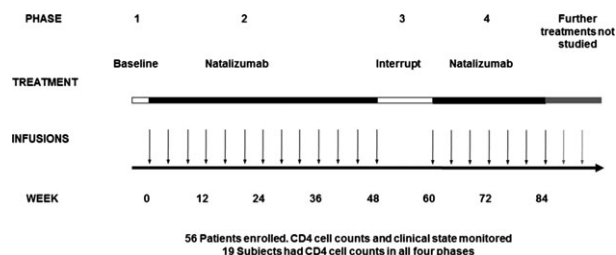
Table 1. Baseline demographic information and clinical outcomes.

Age (N = 56)	47.1 ± 9.1
Gender (N = 56)	
Male	20 (35.71%)
Female	36 (64.29%)
Race (N = 56)	
Caucasians	38 (67.86%)
African American	6 (10.71%)
Asians	2 (3.57%)
Hispanics	4 (7.14%)
Other	6 (10.71%)
Patients with documented MS relapses throughout the study period (N = 56)	0 (0%)
Patients with documented increased EDSS at any time point throughout the study period – as compared to the baseline EDSS (N = 56)	0 (0%)
Patients with new, active, or enlarged brain and spinal cord MRI lesions as compared to the baseline MRI (N = 56)	0 (0%)
Patients who developed Natalizumab NAbs at any time point throughout the study (N = 56)	1 (1.79%)[1]

[1]The only incidence was noted – on natalizumab reinstating.

1 Baseline (week 0),
2 While being administered natalizumab – 24 and 48 weeks after the treatment initiation,
3 At the end of the drug-free interval (week 60),
4 In 24 weeks after resumption of natalizumab treatment (week 84) (Fig. 1).

Brain and spine MRIs were done at the baseline and at the end of the drug-free interval. The absolute numbers of CD4-expressing cells were analyzed by flow cytometry. The CD4+ analyses were performed with a two platform approach utilizing a four-color, two-laser Becton Dickinson FACSCalibur Flow Cytometer, (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) and a Siemens Advia 2120 or Sysmex XM3000 Hematology Analyzer. A 100 μL aliquot of EDTA(Ethylene Diamine Tetra Acetic Acid)-anticoagulated peripheral whole blood was vortexed, reacted with antibodies for 15 min at room temperature, lysed with 2 mL of FACSLyse (Becton Dickinson Immunocytometry Systems, San Jose, CA), incubated for 15 min at room temperature, centrifuged for 2 min in a Clay Adams Serofuge and washed twice with PBS(Phosphate-Buffered Saline. Conjugated antibodies used include CD3-FITC and CD19-PE (BDIS, San Jose, CA) in addition to CD45-PC5 (Immunotech, Somerset, NJ). The sample was acquired on the FACSCalibur and gated on the CD45 bright + lymphocytes. Absolute CD4+ T-cells were calculated from the total WBC count, % lymphocytes, and the percentage CD4+/CD3+ lymphocytes after quality control parameters were met. The laboratory where the tests are performed is CAP accredited, CLIA certified, NIAID DAIDS Quality Assessment Program certified, and participates in the CAP Proficiency Testing Program. Neutralizing antibodies against natalizumab (Athena) and adverse events were also assessed.

Two different methods of statistical analysis were used. Data from the subset of patients with a full set of data encompassing all of the time points were analyzed using repeated-measures analysis of variance (rmANOVA), with the repeated measure being time, to determine changes from baseline (SPSS version 20; IBM Corp., Armonk, NY); only one-third of patients were able to achieve this due to various reasons (missing Labs, going to external laboratory and having orders followed incorrectly) For this analysis, data were aggregated within the same preplanned study week (±7 days).

As a sensitivity analysis, all available data from the 56 patients were used for the mixed-effect model analysis (SAS 9.4 mixed procedure; SAS Institute Inc., Cary, NC) to test the trajectory patterns. The trajectory patterns for two major phases of drug administration, that is, initial natalizumab administration phase versus natalizumab resumption phase were tested separately. In this analysis, data were aggregated within the same month of clinical visit. In all analyses, a significance level of $P < 0.05$ was used.

Results

Twelve-week-long interruption in treatment was well tolerated; no MS relapses, EDSS progression or serious side effects were observed throughout the study; no new, newly enlarged or active MRI lesions were reported. One patient experienced a mild hypersensitivity reaction after natalizumab reinitiation, and was found to have natalizumab-neutralizing antibodies.

Of the 56 patients included in this study, 19 had a full set of laboratory data, with CD4 cell counts measured during every treatment phase from the baseline through phase 4. In these patients, after natalizumab was started, the CD4 counts increased from 991 ± 460 (baseline) to 1332 ± 500 (phase 2, week 24) and remained at 1416 ± 640 (phase 2,

Figure 1. Study design.

Table 2. Clinical outcome by treatment phases.

	Baseline	On natalizumab	Off natalizumab	Natalizumab resumption
MS relapses (N = 56)	0	0	0	0
Increased EDSS as compared to the baseline (N = 56)		0	0	0
NAbs (N = 56)	0	0	0	1
CD4 (N = 19)	991 ± 460	1332 ± 500 (week 24)/1416 ± 640 (week 48)	872 ± 218	1452 ± 718
Brain and Spinal cord MRI – new, active or enlarged lesions as compared to the baseline (N = 56)	0	0	0	0

MS, multiple sclerosis; EDSS, Expanded Disability Status Scale.

week 48); the CD4 counts decreased by the end of the dose extension interval or phase 3 (week 60) to 872 ± 218; after natalizumab reinitiation – phase 4 – the CD4 numbers increased again to 1452 ± 718 (week 84) (Table 2). Thus, the mean CD4 cell counts increased with treatment and remained stable while on treatment, returned to baseline when treatment was interrupted, and again increased when treatment was resumed (Fig. 2A). Although the magnitude of the change varied for individual patients, the direction of change (increase vs. decrease) between time points was the same for all 19 (Fig. 2B). The results of the rmANOVA revealed that the within-subject effect (comparing different states of natalizumab use) was highly significant ($F = 38.665$, $P < 0.001$). Post hoc testing using paired *t*-tests indicated that the CD4 counts at baseline level differed from those at both time points during natalizumab treatment ($P < 0.001$ in both cases), but did not differ from the counts when treatment was withheld ($P = 0.281$). The counts during the two treatment periods also did not differ from each other ($P = 0.820$).

The random effect model included data from all 56 subjects (Fig. 3). The CD4 counts increased during both drug administration periods ($P < 0.0001$ in both cases). The CD4 counts during the period when treatment was withheld did not differ from baseline (phase 1), and the counts during the two treatment phases did not differ from each other. The model also revealed a deceleration in the increases during the first treatment phase, suggesting a plateau effect (Fig. 2).

Discussion

This observational study is addressing the question of CD4 cell shifts during described four phases of natalizumab treatment. Natalizumab treatment was associated with raised CD4 counts in the peripheral blood in comparison to the documented baseline levels and the

increase remained stable for the duration of treatment; this corresponds well with the known pharmacodynamics of natalizumab.[1,4] We found that withholding the treatment for 12 weeks returned the CD4 cell counts to the baseline levels, suggesting that the cells indeed redistribute. With resumption of natalizumab dosing after the interruption, the CD4 cell counts increase to the levels induced by the initial treatment phase, suggesting that the effect was similar, and that the interruption in treatment did not change the pharmacodynamics of the drug (Figures. 2A, 2B and 3). The fact that these almost identical results were seen in two different statistical analyses suggests that these observations are robust.

Discussions of previous publications[4–6] led to concerns that prolonged interruption or discontinuation of natalizumab administration may provoke MS reactivation. Study with the 24-week natalizumab treatment interruption,[7] and longitudinal analyses of natalizumab pivotal MS trials[8] demonstrated no evidence that rebound occurs, although disease activity could return to pretreatment level during long interruption. It is known that the elimination half-life of natalizumab is 11 days; it is cleared from circulation 2 months after the last dose[1]; MS activity resumes in a time frame consistent with natalizumab elimination kinetics.[8] When treatment interruptions varied from 3 to 11 months, the mean time to relapse was found to be 3.1 months[4]; this corresponds with the data on mean α4-integrin blockade dropping from 90% to 30% by week 12 of dosing interruption and further to 10% at weeks 16–24.[7] For our study, we chose to implement a shorter drug-free interval of 12 weeks; and our observations compare favorably with previous data.[9] It should be underscored, that neither our, nor previously referenced studies[4,7,8,10–12] were designed to evaluated the effect of dosage-interruption upon the risk of developing PML. Still, it should be noted that there were no cases of PML in our study. The risk of contracting PML may be
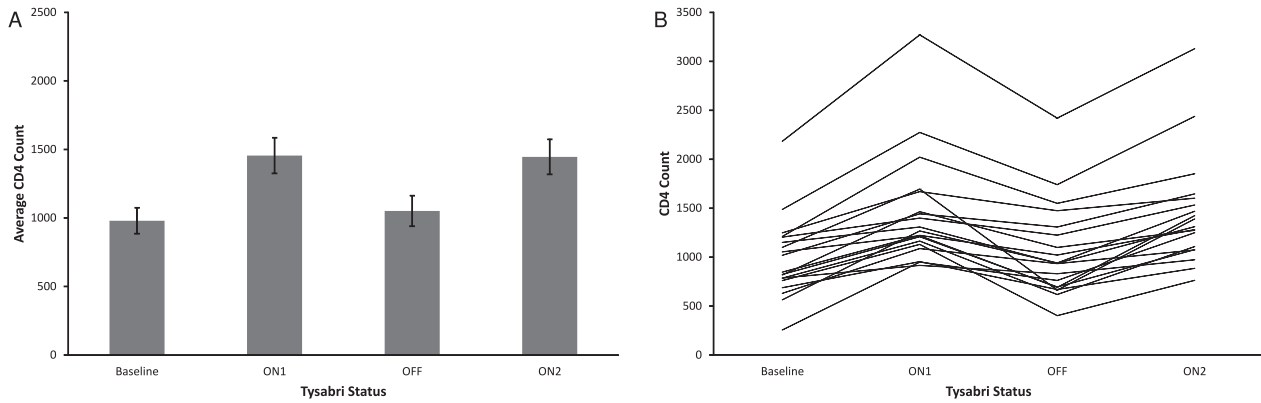
Figure 2. CD4 cell counts for patients with multiple sclerosis in each of four phases of treatment with natulizamab: before initial treatment (Baseline), during the first 48-week-long treatment phase (ON1), during a subsequent 12-week drug-free interval (OFF), and after reinitiation of treatment (ON2). Results are displayed as mean of 19 patients with error bars (A) showing standard error of the mean (SEM). Cell counts during the treatment phases (ON1 and ON2) differ from Baseline ($P < 0.001$), whereas counts during the drug-free interval do not ($P = 0.281$) (A, B).
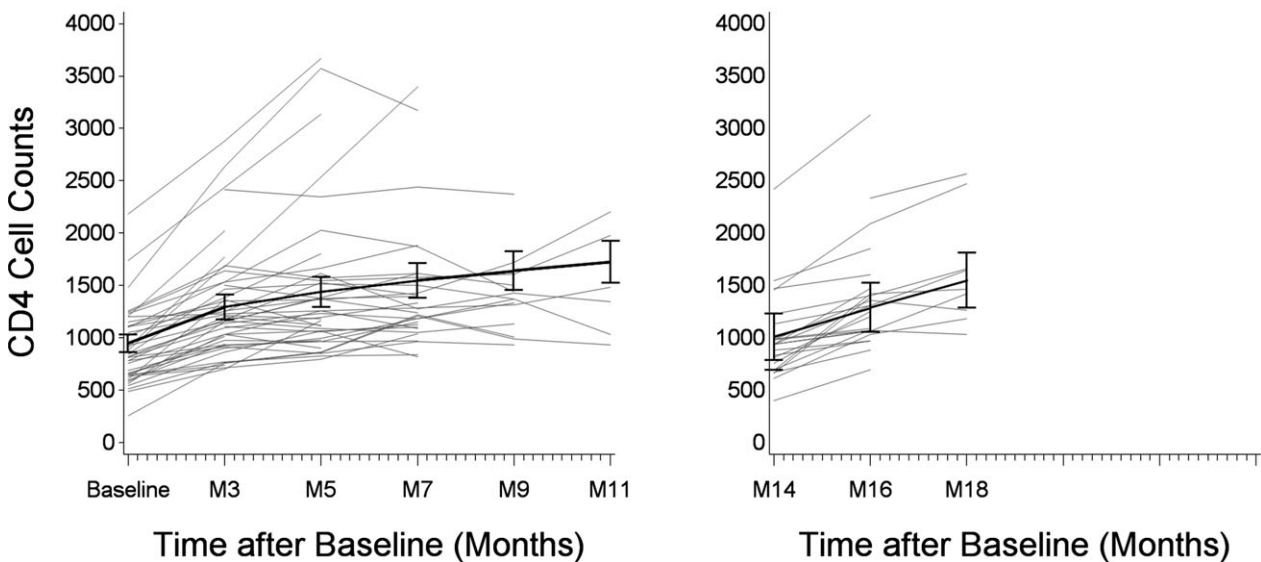

Figure 3. Trajectory of CD4 cell counts in patients with multiple sclerosis treated with natalizumab. During the first drug administration period, CD4 cell counts increased with a trend of square root of time. The trajectory of CD4 counts after reinitiation of natalizumab is close to linear.

unchanged by a temporary cessation of natalizumab; some may argue that it might be increased, because CD34+ cells and CD19+ cells, potentially harboring JCV,[13] gain entry to the central nervous system (CNS); in addition, the risk of an adverse outcome from MS may be increased as encephalitogenic cells may regain entry into the CNS.

Cell numbers in the cerebrospinal fluid (CSF) were not measured in this study, and the authors could not find sup-

porting information from the literature that would suggest that normalization of CD4 counts in periphery is associated with normalization of immunosurveillance in the intrathecal compartment. There are no supporting data from the intrathecal compartment though Stuve and colleagues studied the CNS parenchyma with immunohistochemistry.[14]

In light of the authors' observations indicating suboptimal response of previously stable natalizumab patients who were switched to other disease-modifying therapies,

corresponding with published data,[10] the presented data may be useful for those circumstances when MS patients and their neurologists for various reasons elect to continue on natalizumab therapy – in spite of patient's positive anti-JCV-antibody status or previous prolonged natalizumab exposure, although the optimal strategy for those cases still remains unknown.

Conclusion

In this cohort, the interruption in treatment was well tolerated and not associated with exacerbations, disability, or MRI progression. Twelve-week interval appears to be sufficiently long to induce significant shifts in CD4 counts, suggesting that these measurements help monitoring modulation of lymphocyte trafficking and cell redistribution in MS patients, temporarily discontinuing natalizumab therapy. Future studies on the subtypes of CD4 cells in different tissues and fluids as affected by different immunomodulating therapies and possible clinical relevance of such data are clearly needed.

Conflict of Interest

Dr. Berkovich served as a consultant for Acorda, Avanir, Bayer, Biogen Idec, Genzyme, Novartis and Teva.

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