Pharmacodynamic effects of daclizumab in the intrathecal compartment

Mika Komori1,a, Peter Kosa1, Jason Stein1, Vivian Zhao2, Andrew Blake1, Jamie Cherup1, James Sheridan2, Tianxia Wu3 & Bibiana Bielekova1,4

1Neuroimmunological Diseases Unit, National Institute of Neurological Disorders and Stroke (NINDS), Bethesda, Maryland
2AbbVie Biotherapeutics, Redwood City, California
3Clinical Trial Unit, NINDS, Bethesda, Maryland
4NIH Center for Human Immunology (CHI), The National Institute of Health (NIH), Bethesda, Maryland

Correspondence
Bibiana Bielekova, Neuroimmunological Diseases Unit (NDU), National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health (NIH), Bethesda, MD 20892. Tel: (301) 496-1801; Fax: (301) 402-0373; E-mail: Bibi.Bielekova@nih.gov

Funding Information
The study was supported by the intramural research program of the National Institute of Neurological Disorders and Stroke (NINDS) and collaborative agreement (CRADA) between NINDS and AbbVie/Biogen.

Received: 19 December 2016; Revised: 18 April 2017; Accepted: 19 April 2017

Annals of Clinical and Translational Neurology 2017; 4(7): 478–490
doi: 10.1002/acn3.427

4M. K. contributed to this work as a former employee of NINDS, and the opinions expressed in this work do not represent her current affiliation – Eli Lilly Japan K.K., Kobe, Japan.

Abstract

Objective: It was previously demonstrated that daclizumab therapy normalizes cellular cerebrospinal fluid (CSF) abnormalities typical of multiple sclerosis (MS) in the majority of treated patients. However, CSF cells represent only the mobile portion of intrathecal immune responses. Therefore, we asked whether daclizumab also reverses compartmentalized inflammation and if not, whether residual inflammation correlates with clinical response to the drug. Methods: Forty MS patients treated with an intravenous or subcutaneous injection of daclizumab were followed for up to 16 years in two open-label clinical trials. MRI contrast-enhancing lesions (CELs), clinical scales, and CSF biomarkers quantified residual disease. Results: Rapid decreases in CELs, sustained throughout the observation period, were observed with daclizumab treatment. Daclizumab therapy induced modest but statistically significant (P < 0.0001) decreases in CSF levels of T-cell activation marker CD27 and IgG index. Interleukin 2 (IL-2) CSF levels increased from baseline levels during treatment, consistent with reduced IL-2 consumption by T cells, as a consequence of daclizumab’s saturation of high-affinity IL-2 receptors. CSF levels of IL-12p40, chitinase-3-like protein-1 (CHI3L1), chemokine C-X-C motif ligand 13, and neurofilament light chain (NFL) were also significantly reduced by daclizumab. Among them, inhibition of CHI3L1 correlated with inhibition of NFL and with lack of disease progression. Interpretation: These observations confirm daclizumab’s direct pharmacodynamics effects on immune cells within central nervous system tissues and identify inhibition of CSF biomarkers of myeloid lineage as a stronger determinant of reduction in clinical MS activity than inhibition of biomarkers of adaptive immunity.

Introduction

Application of the “No evidence of disease activity” (NEDA) outcome to Phase III clinical trials or longitudinal multiple sclerosis (MS) cohorts demonstrated remarkably prevalent (e.g., 78% in 2-year trial of daclizumab or >90% in 7-year natural history cohort) residual MS disease activity on current disease-modifying therapies (DMTs). The mechanism(s) for this residual activity are currently unknown. Based on generally strong effects of DMTs on magnetic resonance imaging (MRI) contrast-enhancing lesions (CELs), which are considered a biomarker of MS-related inflammation, the prevailing hypothesis implies neurodegeneration, rather than subclinical inflammation as the cause of residual MS activity.

However, we recently demonstrated that intrathecal inflammation, as measured by cerebrospinal fluid (CSF) biomarkers specific for cells of adaptive immunity (i.e., T and B cells and plasma cells) and innate immunity (e.g., microglia/macrophages), is as prevalent in patients who lack CELs (including primary progressive MS [PPMS] patients) as it is in relapsing–remitting MS (RRMS).4 This observation prompted a hypothesis that subclinical MS disease activity may be mediated by residual inflammation
compartmentalized to the central nervous system (CNS) and therefore mostly inaccessible to current DMTs. To test this hypothesis, we investigated a group of patients with sustained clinical response to daclizumab (Zinbryta), a humanized monoclonal antibody that selectively binds to the high-affinity interleukin-2 (IL-2) receptor subunit (CD25), and has been recently approved for the treatment of relapsing forms of MS.

## Methods

### Subjects

All subjects (Table 1) participated in one of the two NIH open-label, baseline versus treatment Phase I/II trials of daclizumab: ZAP-MS trial (intravenous [IV] daclizumab [Zenapax] 1 mg/kg q 4 weeks; ClinicalTrials.gov identifier NCT0071838; n = 16) or daclizumab High Yield Process (DAC-HYP) trial (subcutaneous [SC] daclizumab [Zinbryta] 150 mg q 4 weeks; ClinicalTrials.gov identifier NCT01143441; n = 32). (Note: Zinbryta™ has a different form and structure than the earlier form of daclizumab – Zenapax). The DAC-HYP trial consisted of two groups of subjects: Cohort A (subjects switched from Zenapax [either as part of or outside of ZAP-MS trial]) and Cohort B (de novo initiation of Zinbryta). We used all CSF samples we collected in these protocols (Fig. 1).

The study was approved by the NIH Institutional Review Board, and all patients provided written informed consent.

### CSF collection and processing

CSF was collected during pretreatment baseline (month 0 [Mo0]), and at different time points after initiation of treatment as outlined in the CONSORT diagram (Fig. 1).

To assess long-term effects of daclizumab therapy, we collected CSF from all subjects who received daclizumab for more than 12 months (>1 year–11 years; average 5.4 years) and called this time point long-term treatment (LTT). To assess stability of such LTT we also collected CSF sample 1 year later (LTT + 1y).

CSF was processed according to written standard operating procedures (SOP). A portion of CSF was sent to measure IgG index. Research CSF aliquots were assigned alpha-numeric codes, centrifuged at 335g for 10 min at 4°C within 15 min of collection, and stored in polypropylene tubes at −80°C until blinded analyses.

### Measurement of daclizumab concentration and other biomarkers

Electrochemiluminescent assays were developed or optimized to quantify the concentrations of serum and CSF biomarkers using the Meso Scale Discovery® platform (MSD; Meso Scale Diagnostics, Rockville, MD, https://www.mesoscale.com/) as described previously. The concentration of interleukin 12p40 (IL-12p40) was measured by MSD V-plex using the manufacturer’s protocol. The assays for CSF soluble CD21 (sCD21), sCD27, sCD14, sCD163, chitinase-3-like protein 1 (CHI3L1), and chemokine C-X-C motif ligand 13 (CXCL13) and serum/CSF daclizumab were developed through de novo. Neurofilament light protein (NFL) was measured using a UmanDiagnostics ELISA (UmanDiagnostics AB Sweden). The IL-2 was measured by Singulex® IL-2 kit (Singulex, Alameda, CA), following the manufacturer’s protocol.

All samples excluding IL-12p40 and NFL were run in duplicate. Each assay contained a minimum of two additional reference samples per plate to evaluate intra- and interassay reliability, which are depicted in Table S1.

Threshold for abnormal values was based on mean ± 2 standard deviation (SD) of healthy donors’ data, yielding 56 different CSF samples as some HD contributed two samples 1 year apart; Table S2). The HD samples were collected by the same group of investigators using identical SOP and prospectively assigned the alphanumeric code that does not allow laboratory personnel to differentiate MS from HD samples, resulting in random distribution of MS and HD samples on assay plates.

After observing no differences between Zenapax- and Zinbryta-treated subjects in a pharmacokinetic marker (CSF daclizumab levels) and pharmacodynamic marker (CSF IL-2), we merged these cohorts for all further analyses, combining data from Mo6.5 in ZAP-MS trial with Mo12 of DAC-HYP trial as short-term treatment (STT).
Combinatorial weight-adjusted disability score (CombiWISE)

CombiWISE is a sensitive, continuous composite scale developed and optimized by machine learning that ranges from 0 to 100 and combines EDSS, Scripps NRS, 25 foot walk, and nondominant hand data for nine hole peg test. CombiWISE slope was calculated from linear regression equations for patients with more than 3 CombiWISE data points.

MRI

Routine spin-echo and gradient-echo T1-weighted images were collected following intravenous administration of 0.1 mmol/kg gadopentetate dimeglumine as described previously. CELs were quantified according to the consensus of two neurologists with neuroimmunology/MS subspecialty training (B. B. and M. K.) based on precontrast T1- and T2-weighted images.

Statistical analyses

Repeated measures analysis of variance (ANOVA; SAS 9.3) was used to evaluate the change in biomarker and clinical score variables from baseline (Mo0) to the follow-up visits. Dunnett’s method was applied to each biomarker and each group to adjust for multiple comparisons with the baseline, but not for multiple biomarkers. The median changes in the results were computed only from patients who have baseline and LTT visit points. We also considered mixed effects model with time as a continuous variable and intercept and slope as random variables. However, because of nonlinear relationships between time and biomarkers, we resolved to treat time as categorical variable (with combining some points) to assess...
M. Komori et al.

Intrathecal Effects of Daclizumab

We observed that serum IL-2 levels significantly increased after the initiation of daclizumab treatment (median 0.71 pg/mL in Zinbryta only cohort at Mo12 [STT], median 0.77 pg/mL in Zenapax only/and Zinbryta cohort at Mo1.5, Fig. 3A–B) and remained elevated till the end of the study. CSF IL-2 levels also increased at Mo1.5 (median 0.42 pg/mL in Zenapax only/and Zinbryta cohort and at Mo12 (STT, median 0.34 pg/mL) in Zinbryta only cohort and remained elevated till LTT.

Finally, there was no correlation between CSF and serum IL-2 levels (Fig 3C).

**Effects of daclizumab on CSF biomarkers of innate immunity: daclizumab inhibits biomarkers secreted by activated myeloid cells without altering M1 versus M2 phenotypic markers**

Because measured pharmacokinetic and pharmacodynamic data were comparable, we combined data from Mo6.5 on Zenapax and data from Mo12 on Zinbryta into a single STT time point. We also calculated the proportion of patients with abnormally high values of biomarkers compared to normal range (mean ± 2 standard deviation of HD, Table S2), shown in Figure 4 as pie charts with dark fill representing proportion of patients with abnormally elevated biomarkers.

We investigated the effect of daclizumab on CSF biomarkers previously validated as biomarkers of MS-related inflammation. First, we focused on biomarkers of myeloid lineage (Fig. 4A–D): proposed phenotypical surface markers of M1 (sCD14; expressed on macrophages activated by proinflammatory stimuli) versus M2 macrophages (sCD163; expressed on alternatively activated macrophages), even though we recognize that M1/M2 classification, as well as the ability of these surface markers to reliably reflect M1 versus M2 phenotypes, are oversimplifications. Daclizumab marginally and transiently decreased sCD163 levels, but otherwise did not significantly affect markers of M1 versus M2 lineage (note that the proportion of patients with abnormal values of these biomarkers shifted between STT and LTT, but this apparent shift is due to contribution of patients from different cohorts, see CONSORT diagram).

Next, we measured biomarkers secreted by activated cells of myeloid lineage (macrophages, microglia, and myeloid dendritic cells [DCs]): cytokine IL-12p40 and CHI3L1, a secreted glycoprotein linked to inflammation, tissue remodeling, and fibrosis. Both of these myeloid activation markers decreased significantly (IL-12p40 [median −58.8%, *P* = 0.0001 at LTT] and CHI3L1 [median −16.9%, *P* = 0.0007 at LTT]), during daclizumab therapy and remained in the normal range for the majority of treated patients.

We also measured levels of CHI3L1 in matching Mo0, STT, and LTT serum samples (data not shown), revealing no statistically significant change between baseline and differences on CSF biomarkers induced by short-term versus long-term daclizumab therapy. Box–Cox transformation was applied to biomarker and clinical score variables.

**Results**

**Pharmacokinetic and pharmacodynamic data**

(A) Pharmacokinetic marker: Selected dosing leads to measurable CSF concentrations of daclizumab, representing approximately 0.1% of serum levels

Intravenously administered daclizumab could be detected intrathecally already at Mo1.5 after the first dose (median 15.1 ng/mL in CSF, median 9.97 μg/mL in serum; Fig. 2A–B, left). Even though this cohort changed the injection method from IV administered version of daclizumab (i.e., Zenapax, 1 mg/kg IV q 4 weeks at Mo1.5, Mo6.5 [STT]) to subcutaneously administered newer version of the drug (i.e., Zinbryta, 150 mg SQ q 4 weeks at LTT and LTT+1y), the median concentrations of serum and CSF daclizumab did not change significantly in this small cohort.

The cohort of subjects treated only with Zinbryta also increased serum and CSF daclizumab concentrations to similar levels at the first follow-up time point (Mo12 [STT]) and kept the level throughout the treatment course (median 15.1 ng/mL in CSF, median 17.8 μg/mL in serum at STT; Fig. 2A–B, right). CSF and serum daclizumab concentrations correlated strongly (Fig. 2C), supporting the notion that approximately 0.1% of serum daclizumab crosses the blood–CSF barrier.

(B) Pharmacodynamic marker: daclizumab therapy significantly increases serum and CSF levels of IL-2 without any correlation between the two compartments

As daclizumab limits consumption of IL-2 by T cells [14] without limiting the ability of activated T cells or dendritic cells to secrete IL-2, [15] we predicted that daclizumab therapy should lead to increased IL-2 levels in biological fluids. However, this prediction was difficult to prove because the levels of IL-2 were below the detection limits of standard immunoassays. Here, we employed a “digital ELISA” (i.e., Singulex: single molecule counting technology; https://www.singulex.com/) that measures low abundance analytes in femtoliter-sized wells with a theoretical detection limit of single molecules.

We observed that serum IL-2 levels significantly increased after the initiation of daclizumab treatment (median 0.71 pg/mL in Zinbryta only cohort at Mo12 [STT], median 0.77 pg/mL in Zenapax only/and Zinbryta
Figure 2. Daclizumab (DAC) concentrations in serum and CSF are comparable between intravenous and subcutaneous regimens. Daclizumab level in serum (A) and CSF (B) by intravenous (Zenapax) and subcutaneous (Zinbryta) injection. Red lines show median values of biomarkers at each follow-up visit. Black brackets represent statistical significance ($P < 0.05$) based on adjusted $P$-value (Dunnett’s method). Concentration of DAC in serum and CSF was compared to baseline (visit Mo0) and each follow-up visit (Zenapax only/and Zinbryta cohort: Mo1.5, Mo6.5 [STT], long-term treatment [LTT; >1–11 years], and 1 year after [LTT+1y]; Zinbryta only cohort: Mo12 [STT], LTT, and LTT+1y). (C) Pearson’s correlation between CSF and serum DAC concentrations.
Figure 3. Cerebrospinal fluid (CSF) IL-2 is independently produced intrathecally and its levels are elevated following daclizumab therapy. IL-2 level in serum (A) and CSF (B) by intravenous (Zenapax) and subcutaneous (Zinbryta) injection. Red lines show median values of biomarkers at each follow-up visit. Black brackets represent statistical significance (P < 0.05) based on adjusted F-value (Dunnett’s method). Concentration of IL-2 in serum and CSF was compared to baseline (visit Mo0) and each follow-up visit (Zenapax only/and Zinbryta cohort: Mo1.5, Mo6.5 [STT], long-term treatment [LTT; >1–11 years], and 1 year after [LTT+1y]; Zinbryta only cohort: Mo12 [STT], LTT, and LTT+1y). (C) Pearson’s correlation between CSF and serum IL-2 concentrations. Note that even though the graph does not display the highest measured IL-2 level (1.75 in Fig. 3B), the correlation coefficient and P-value include this outlier.

STT (P = 0.2922) or baseline and LTT (P = 0.2645). Furthermore, the correlation between serum and CSF levels of CHI3L1 for all measured samples was weak and did not reach statistical significance (Pearson’s r = 0.2066, P = 0.0659).

**CSF biomarkers of adaptive immunity are decreased, but remain above healthy donor range in majority of treated patients**

To assess effect of daclizumab on cells of adaptive immunity, we measured surface molecules released specifically from B cells (sCD21) and activated T cells (sCD27, shed from activated more than resting T cells and from CD8 T cells more than CD4 T cells). While sCD21 is a specific B-cell marker, it is highly expressed on naïve cells and its expression diminishes as B cells mature. Thus, class-switched memory B cells express very little, if any, sCD21 and therefore may not secrete this biomarker. Consequently, we also measured IgG index, which quantifies secretion of immunoglobulins by plasma cells and plasmablasts.

While sCD21 was not affected by daclizumab therapy, we observed significant inhibition of sCD27 through the observation period (maximum reduction at LTT median −37.5%, P < 0.0001; Fig 4E–F). However, the majority of treated patients had persistent elevations of CSF sCD27 levels above normal range. Finally, the level of IgG index decreased after daclizumab treatment (median −26.3%, P < 0.0001 at LTT; data not shown as this finding was partially presented previously).

**MS-related biomarkers CXCL13 and NFL are normalized in majority, but not all treated patients**

CXCL13 is a chemokine typically elevated in MS CSF that is secreted by follicular DCs in lymphatic tissue, including in tertiary lymphoid follicles, but also by activated T and B cells. CSF levels of CXCL13 declined posttreatment (median −70.0%, P = 0.0527 at LTT; Fig. 4G), although this change did not reach statistical significance.

The axonal damage marker, NFL, significantly decreased (median −59.3%, P < 0.0001 at LTT; Fig. 4H) and remained in normal range for the majority (but not all) of treated patients throughout the duration of follow-up.

**Linking changes in CSF biomarkers to clinical efficacy of daclizumab therapy**

Consistent with strong efficacy on CELs (P < 0.0001, median 1.5 at Mo0, median 0.0 at LTT), we observed no significant disability progression, when measured either by EDSS, or more sensitive CombiWISE scale (Fig. 5A–B, Table S3). However, these measurements exclude subjects who withdrew from this long-term study, some for progression of disability (Fig. 1).

When investigating the relationship between CSF biomarkers and disability progression, we observed that only CHI3L1 showed biologically meaningful correlations with the change in clinical disability measures (Fig. 5C–D). CHI3L1 levels correlated with NFL (r = 0.5052, P = 0.0061) during the observation period (Fig. =0.50525E) although change in CHI3L1 was a better predictor of clinical response than change in NFL (Fig. 5C–D). Even higher and highly statistically significant correlation exists between change in NFL and CHI3L1 (r = 0.6769, P < 0.0001, Fig. 4F). Intriguingly, pretreatment CSF levels of CHI3L1 also correlated with observed clinical response measured as CombiWISE change, although this correlation was not very strong (Fig. 5E).

**Discussion**

The current study demonstrates that approximately 0.1% of systemically administered daclizumab reaches the intrathecal compartment, consistent with other monoclonal antibodies such as rituximab. The observed rise in serum and CSF IL-2 levels provides evidence for the ability of daclizumab to inhibit consumption of IL-2 by activated T cells in both systemic and intrathecal compartments. Relatively high median CSF concentrations of IL-2 (0.34–0.42 pg/mL) in comparison to measured serum IL-2 concentrations (0.71–0.77 pg/mL) suggest that the source of IL-2 is intrathecal, rather than blood derived. Assuming that IL-2 and IgG use similar passive transport across the blood–brain barrier, based on their molecular
weights (~15.5 kDa for IL-2 vs. 150 kDa for IgG) we could estimate that up to 1% of serum IL-2 concentrations gain access to the intrathecal compartment, which represents 0.008 pg/mL, or less than 3% of IL-2 levels we measured in the CSF. Our conclusion that measured increase in CSF IL-2 levels is likely derived from the intrathecal compartment is also supported by the observed lack of correlation between blood and CSF IL-2 levels. The source of this intrathecal IL-2 is not known, but may represent activated T cells or dendritic cells. The interesting question is how low measured intrathecal concentration of daclizumab (i.e., 15.1 ng/mL) can inhibit consumption of IL-2 by intrathecal T cells. The answer resides in the previously published study that in contrast to progressive MS, where majority of patients have inflammation compartmentalized to CNS tissue with little turnover between blood and intrathecal compartment, majority of RRMS patients have communicating inflammation, where immune cells are constantly accessing intrathecal compartment from the blood, as already activated T or B cells. Consequently, the CD25 on these activated immune cells is blocked in the blood, where the concentration of daclizumab is saturating. Furthermore, daclizumab potently inhibits intrathecal immunity but does not normalize biomarkers of adaptive immunity, demonstrating the presence of residual T and B cells/plasma cells in the intrathecal compartment even in clinically stable patients that do not form new MS lesions on MRI imaging for years. Such residual inflammation reflected by T and B cell/plasma cell biomarkers did not correlate with clinical efficacy. The most successful CSF biomarker in this regard was CHI3L1, a marker of activated myeloid cells, previously implicated in tissue remodeling and fibrosis.

The consistency of results between Cohorts A and B (Fig. 1) suggests that the mechanism of action (MOA) of daclizumab is comparable between the two tested daclizumab preparations.

We acknowledge the following limitations: (1) Potential biases introduced by patient dropouts. The purpose of the NIH clinical trial “Investigating mechanism of action of DAC-HYP in the treatment of high-inflammatory MS” (NCT01143441) was to define the MOA of the new generation of SC daclizumab (Zinbryta) while allowing patients who experienced strong clinical response to earlier generation of IV daclizumab (Zenapax) before it was taken off the market (Cohort A) to transition to Zinbryta prior to its commercial availability. In recognition that Cohort A was self-selected for high efficacy of daclizumab, we recruited Cohort B of de novo dosed patients to mitigate potential bias. The original protocol to which all patients consented included only CSF examination for Cohort B before and 12 months (STT time point) after initiation of Zinbryta. When it became clear that Zinbryta will not reach regulatory approval before completion of the protocol, in view of patients’ requests to continue Zinbryta, we extended the protocol until Zinbryta gained regulatory approval. This extension included two additional LPS (LTT and LTT+1y) for patients from both cohorts (A and B). Patients with persistent formation of new lesions during extension were switched to alternative FDA-approved therapies, even though all of them demonstrated therapeutic efficacy of daclizumab when compared to pretreatment (-Mo0) data. Because we do not observe significant differences in any measured biomarkers between STT, LTT, and LTT+1y time points, or between Cohorts A and B, we do not believe that dropouts have a significant bearing on our conclusions. The only results possibly affected by dropouts and small samples size are correlations between CSF biomarkers and therapeutic efficacy, which we therefore consider preliminary and necessitating validation in an independent cohort. (2) Potential for biases due to investigators’ conflicts of interests (COI) in the open-label study. In response, we implemented safeguards: CSF samples were processed only by investigators who lacked COI using SOP and prospective coding of samples. Clinical and MRI data were generated by different group of investigators who likewise lacked COI. All analyses of CSF biomarkers were blinded; investigators
### Table A

| Visit months | Zenapax only/and Zinbryta (number, median, range) | Zinbryta only (number, median, range) |
|--------------|--------------------------------------------------|--------------------------------------|
| Mo -3        | n=16 2.0 (0.0 – 3.5)                             | n=13 2.5 (1.0 – 6.5)                 |
| Mo 0         | n=16 1.5 (1.0 – 6.5)                             | n=13 2.5 (1.0 – 6.5)                 |
| Mo 1.5       | n=16 1.5 (1.0 – 3.5)                             |                                      |
| Mo 6/6.5     | n=15 1.5 (0.0 – 4.0)                             | n=13 2.5 (1.0 – 6.5)                 |
| Mo 12        | n=13 2.5 (1.0 – 6.5)                             | n=13 2.5 (1.0 – 6.5)                 |
| Mo 18        | n=12 2.3 (1.0 – 6.5)                             | n=12 2.3 (1.0 – 6.5)                 |
| Mo 24        | n=8 2.0 (1.0 – 6.5)                              | n=8 2.0 (1.0 – 6.5)                  |
| LTT          | n=14 1.5 (1.0 – 3.0)                             | n=9 2.5 (1.5 – 6.0)                  |
| LTT+0.5y     | n=14 1.5 (0.0 – 3.0)                             | n=9 2.5 (1.0 – 6.0)                  |
| LTT+1y       | n=14 1.5 (0.0 – 3.0)                             | n=6 2.5 (1.0 – 6.0)                  |
| LTT+1.5y     | n=14 1.5 (0.0 – 3.5)                             | n=6 2.5 (1.0 – 6.0)                  |
| LTT+2y       | n=14 1.5 (0.0 – 3.5)                             | n=5 2.5 (2.5 – 6.5)                  |
| LTT+2.5y     | n=14 1.5 (0.0 – 3.5)                             | n=6 2.5 (1.0 – 6.5)                  |

### Figure B

- **Graph B1** shows the relationship between CEL number and visit months.
- **Graph B2** illustrates the correlation between CEL number and EDSS change.

### Figure C

- **Graph C** displays the correlation between change in EDSS and change in CHI3L1 (pg/ml).
- The correlation is significant with r=0.5260 and p=0.0048.

### Figure D

- **Graph D** depicts the correlation between change in NFL (ng/ml) and change in EDSS.
- The correlation is significant with r=0.4923 and p=0.0091.

### Figure E

- **Graph E** shows the correlation between change in NFL (ng/ml) and CHI3L1 (pg/ml).
- The correlation is significant with r=0.5052 and p=0.0061.

### Figure F

- **Graph F** illustrates the correlation between change in CHI3L1 (pg/ml) and change in NFL (ng/ml).
- The correlation is significant with r=0.6769 and p<0.0001.

### Figure G

- **Graph G** shows the correlation between first visit CHI3L1 (pg/ml) and change in CombiWISE.
- The correlation is significant with r=0.4323 and p=0.0217.
who generated CSF data had no access to clinical or imaging data. We included every CSF sample we have ever collected on any daclizumab-treated subject, including those who later dropped out. Finally, data were analyzed by an independent statistician. (3) *Relatively small sample size.* Based on previous CSF studies, we estimated that 15 patients analyzed before and after therapy provide sufficient power to reproducibly identify 20–30% effect sizes. High statistical significance, reproducibility of results between STT, LTT, and LTT +1y time points and between Cohorts A and B support our conclusion that the study is powered to detect biologically meaningful effect sizes. However, small sample sizes could have influenced the power of detecting reproducible relationships between CSF biomarkers and clinical data; thus, we consider these data preliminary and necessitating future validation in an independent cohort.

Notwithstanding these limitations, we conclude that the previously noted normalization of cellular abnormalities in the CSF of MS patients by daclizumab is not associated with normalization of markers of adaptive immunity in the CNS tissue. This expands the existing knowledge of persistent presence of plasma cells/plasmablasts (reflected by elevated IgG index) in treated MS patients by providing evidence for persistence of T and B cells in the intrathecal compartment of successfully treated MS patients, reflected by elevated CSF levels of sCD27 and sCD21, respectively. The lack of correlation between these residual markers of adaptive immunity and clinical outcomes (or NFL levels) suggests that mere presence of these cells in the intrathecal compartment does not lead to progressive accumulation of disability associated with axonal damage.

Because sCD27 is released by all T cells (activated > resting and CD8 > CD4), elevated sCD27 levels may reflect intrathecal accumulation of regulatory T cells (Tregs). However, the most studied population of FoxP3 + Tregs do not secrete IL-2 and therefore cannot contribute to daclizumab-induced rise in CSF IL-2 levels. Furthermore, daclizumab actually inhibits FoxP3 + Treg activation, proliferation, and numbers. Therefore, the daclizumab-induced increase in CSF IL-2 levels indicate that at least part of the residual intrathecal T-cell population includes effector T cells, or regulatory T cells other than FoxP3 + Treg, such as Tr1 cells. Remaining CNS T cells may hypothetically retain some advantage over treatments that abrogate CNS immunosurveillance from the standpoint of patient safety and by permitting “beneficial autoimmunity,” necessary for the maintenance of adult neurogenesis and for neural repair and remyelination. This hypothesis requires future comparative analyses of long-term safety and efficacy outcomes.

In contrast to modest inhibitory effects of daclizumab on intrathecal adaptive immunity, the markers of activation of myeloid lineage (e.g., IL-12p40, CHI3L1, and CXCL13) were normalized in the majority of treated subjects. Furthermore, pretreatment levels of CHI3L1 correlated with subsequent clinical efficacy measured by the sensitive disability scale CombiWISE. Finally, treatment-induced reduction of CHI3L1 levels in CSF also correlated with inhibition of axonal damage measured by CSF NFL and with clinical efficacy measured by both EDSS and CombiWISE. CHI3L1 has been reported as a prognostic biomarker in clinically isolated syndromes. It is expressed by macrophages/microglia (and also by reactive astrocytes) in actively demyelinating MS lesions and by CD14+ monocytes in the CSF. While our dynamic observations suggest pathogenic role of activated members of myeloid lineage (e.g., macrophages, microglia, and myeloid DCs) in axonal damage associated with MS, correlation cannot prove causation. Indeed, activation of myeloid lineage may also be secondary to danger-associated molecular patterns released upon demyelination.

This interpretation is supported by the fact that we observed only trend, which did not reach statistical significance, for systemic (i.e., serum) inhibition of CHI3L1 levels upon initiation of daclizumab therapy, with no significant correlations between two compartments (data not shown). On the other hand, daclizumab may exert inhibitory effects on activated macrophages, which express CD25 during activation, and it also limits the ability of myeloid DCs to present antigens in immunostimulatory manner. Consequently, whether the activated myeloid lineage drives MS pathology or is an epiphenomenon cannot be determined without therapy that strongly inhibits either myeloid lineage or demyelination and ability to measure its efficacy in vivo.

In conclusion, this study provides evidence for the intrathecal pharmacodynamic effect of daclizumab. CSF biomarker studies support broad inhibitory effects of daclizumab on intrathecal immunity, but show residual presence of adaptive immunity in the majority of
treated patients. Nevertheless, the inflammation represented by aberrant activation of myeloid lineage is severely limited by daclizumab, to the extent that this therapy normalizes the biomarker of axonal damage and inhibits progression of disability in this long-term cohort. The emerging hypothesis of pathogenicity of activated myeloid cells in axonal damage represents a rationale for testing treatments that inhibit myeloid lineage in trials targeting residual accumulation of disability on current DMTs.

Acknowledgments

The study was supported by the intramural research program of the National Institute of Neurological Disorders and Stroke (NINDS) and collaborative agreement (CRADA) between NINDS and AbbVie/Biogen. We thank clinician Alison Wichman, MD, research nurse Jenifer Dwyer, regulatory nurse Rosalind Hayden, and patient schedulers Anne Mayfield and Kewounie Pumphrey for processing of CSF samples. Finally, we would like to thank the patients and their caregivers without whom this study would not be possible.

Author Contributions

BB designed and supervised the study. JC participated in the evaluation of patients. MK, PK, VZ, JS, AB, and JS acquired data. MK analyzed data and created figures. TW did the statistical analysis. MK and BB did literature search and interpreted data. BB acquired funding. MK and BB wrote the manuscript and all authors reviewed the final version.

Conflict of Interest

Dr. Bielekova is a co-inventor on NIH patent related to daclizumab therapy and as such has received patent royalty payments. Drs. Zhao and Sheridan were employed by AbbVie Biotherapeutics or its predecessors, receiving royalty payments. Drs. Zhao and Sheridan were employed by AbbVie Biotherapeutics or its predecessors, receiving royalty payments. Dr. Bielekova is a co-inventor on NIH patent related to daclizumab therapy and as such has received patent royalty payments.

References

1. Nixon R, Bergvall N, Tomic D, et al. No evidence of disease activity: indirect comparisons of oral therapies for the treatment of relapsing-remitting multiple sclerosis. Adv Ther 2014;31:1134–1154.
2. Kappos L, Wiendl H, Selmaj K, et al. Daclizumab HYP versus Interferon Beta-1a in Relapsing Multiple Sclerosis. N Engl J Med 2015;373:1418–1428.
3. Rotstein DL, Healy BC, Malik MT, et al. Evaluation of no evidence of disease activity in a 7-year longitudinal multiple sclerosis cohort. JAMA Neurol. 2013;72:152–158.
4. Komori M, Blake A, Greenwood M, et al. CSF markers reveal intrathecal inflammation in progressive multiple sclerosis. Ann Neurol 2015;78:3–20.
5. Bielekova B. Daclizumab therapy for multiple sclerosis. Neurotherapeutics 2013;10:55–67.
6. Gross CC, Schulte-Mecklenbeck A, Runzi A, et al. Impaired NK-mediated regulation of T-cell activity in multiple sclerosis is reconstituted by IL-2 receptor modulation. Proc Natl Acad Sci USA 2016;113:E2973–E2982.
7. Tran JQ, Othman AA, Mikulskis A, et al. Pharmacokinetics of daclizumab high-yield process with repeated administration of the clinical subcutaneous regimen in patients with relapsing-remitting multiple sclerosis. Clin Pharmacol 2016;89–13.
8. Lin YC, Winokur P, Blake A, et al. Daclizumab reverses intrathecal immune cell abnormalities in multiple sclerosis. Ann Clin Transl Neurol 2015;2:445–455.
9. Han S, Lin YC, Wu T, et al. Comprehensive Immunophenotyping of Cerebrospinal Fluid Cells in Patients with Neuroimmunological Diseases. J Immunol 2014;7.
10. Kosa P, Ghazali D, Tanigawa M, et al. Development of a sensitive outcome for economical drug screening for progressive multiple sclerosis treatment. Front Neurol 2016;7.
11. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33:1444–1452.
12. Sipe JC, Knobler RL, Braheny SL, et al. A neurologic rating scale (NRS) for use in multiple sclerosis. Neurology 1984;34:1368–1372.
13. Bielekova B, Richert N, Howard T, et al. Humanized anti-CD25 (daclizumab) inhibits disease activity in multiple sclerosis patients failing to respond to interferon beta. Proc Natl Acad Sci USA 2004;101:8705–8708.
14. Martin JF, Perry JS, Jakhete NR, et al. An IL-2 paradox: blocking CD25 on T cells induces IL-2-driven activation of CD56(bright) NK cells. J Immunol 2010;185:1311–1320.
15. Wuest SC, Edwan JH, Martin JF, et al. A role for interleukin-2 trans-presentation in dendritic cell-mediated T cell activation in humans, as revealed by daclizumab therapy. Nat Med 2011;17:604–609.
16. Perry JS, Han S, Xu Q, et al. Inhibition of LTI Cell Development by CD25 Blockade Is Associated with Decreased Intrathecal Inflammation in Multiple Sclerosis. Sci Transl Med 2012;4:145ra06.
17. Rubenstein JL, Fridlyand J, Abrey L, et al. Phase I study of intraventricular administration of rituximab in patients with recurrent CNS and intraocular lymphoma. J Clin Oncol 2007;25:1350–1356.
18. Komori M, Lin YC, Cortese I, et al. Insufficient disease inhibition by intrathecal rituximab in progressive multiple sclerosis. Ann Clin Transl Neurol 2016;3:166–179.

19. Bielekova B, Richert N, Herman ML, et al. Intrathecal effects of daclizumab treatment of multiple sclerosis. Neurology 2011;77:1877–1886.

20. Komori M, Blake A, Greenwood M, et al. CSF markers reveal intrathecal inflammation in progressive multiple sclerosis. Ann Neurol 2015;78:3–20.

21. Stuve O, Marra CM, Bar-Or A, et al. Altered CD4+/CD8+ T-cell ratios in cerebrospinal fluid of natalizumab-treated patients with multiple sclerosis. Arch Neurol 2006;63:1383–1387.

22. Astier AL, Meiffren G, Freeman S, Hafler DA. Alterations in CD46-mediated Tr1 regulatory T cells in patients with multiple sclerosis. J Clin Invest 2006;116:3252–3257.

23. Ziv Y, Avidan H, Pluchino S, et al. Synergy between immune cells and adult neural stem/progenitor cells promotes functional recovery from spinal cord injury. Proc Natl Acad Sci U S A 2006;103:13174–13179.

24. Bieber AJ, Kerr S, Rodriguez M. Efficient central nervous system remyelination requires T cells. Ann Neurol 2003;53:680–684.

25. Canto E, Tintore M, Villar LM, et al. Chitinase 3-like 1: prognostic biomarker in clinically isolated syndromes. Brain 2015;138:918–931.

26. Bielekova B, Komori M, Xu Q, et al. Cerebrospinal Fluid IL-12p40, CXCL13 and IL-8 as a Combinatorial Biomarker of Active Intrathecal Inflammation. PLoS ONE 2012;7:e48370.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Methodological details of biomarker measurements.

Table S2. Normative data for healthy donors’ CSF biomarkers.

Table S3. Clinical scores.