Relapsing–Remitting Multiple Sclerosis Treated with Interferon Beta-1a: Immunological and Short-Term Brain Volume Changes

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Disclosures

- MG Dwyer received consulting fees from EMD Serono and Claret
- R Zivadinov received personal compensation from Teva Pharmaceuticals, Biogen Idec, EMD Serono, Novartis, Claret, and Sanofi-Genzyme for speaking and consultant fees. He also received financial support for research activities from Biogen Idec, Teva Pharmaceuticals, Claret, Sanofi-Genzyme, Novartis, and EMD Serono
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- B Hayward and F Dangond are employees of EMD Serono, Inc.*
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Introduction

Relapsing–remitting multiple sclerosis (RRMS) is associated with loss in brain tissue volume (atrophy) over time[1]

- These changes in volume are usually thought to reflect underlying tissue damage or destruction

Pseudoatrophy[2] is the term used to describe short-term brain volume decreases in patients with RRMS following shortly after initiation of anti-inflammatory therapy

- Pseudoatrophy is attributed to therapy-related resolution of inflammation-related hydrodynamic changes, rather than true atrophy

Immunological biomarkers can provide insights into the mechanisms underlying brain volume changes in patients undergoing treatment for RRMS

- Responses to RRMS therapy may be influenced by the pre-existing immunological status of patients before and during treatment

1. Rudick RA, *et al. Neurology* 1999;53:1698–704. **2.** Zivadinov R, *et al. Neurology* 2008;71:136–44.

Objectives of Study

To measure global (whole brain) and tissue-specific (gray matter [GM] and white matter [WM]) percent brain volume change (PBVC) in patients with RRMS (n=23) treated over 6 months with interferon beta-1a 44 mcg given subcutaneously three times weekly (IFN β-1a SC tiw) and to compare with healthy controls (HCs; n=15)

To analyze correlations between immunological markers and short-term brain volume changes in treated patients

Methods

Study details

- The Advanced MRI and Immunology Pilot Study (NCT01085318) was an open-label study of 15 HCs and 23 patients with RRMS treated with IFN β-1a SC tiw for 6 months[1]
- Enrolled patients were 18–65 years old with a diagnosis of RRMS (2010 McDonald criteria revision)
- Patients received 6 months of treatment with IFN β-1a SC tiw titrated up to 44 mcg over the first 4 weeks

1. Zivadinov R, *et al. PLoS One* 2014;9:e91098.

Magnetic resonance imaging (MRI)

MRI brain exams were performed on a 3T GE Signa LX Excite 12.0 scanner at baseline (0 months) and at 3- and 6-month follow-up visits

Changes in whole brain and GM and WM volumes were measured:

- From baseline to 3 months; from 3 months to 6 months; from baseline to 6 months
- Using Structural Image Evaluation using Normalization of Atrophy (SIENA) and SIENAX Multi Time Point 3-component (SX-MTP3)

SIENA

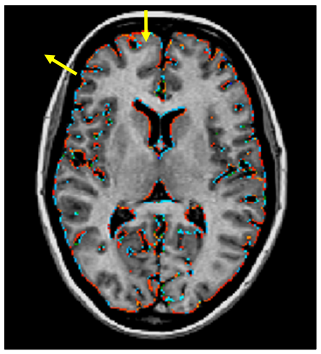
PBVC measures tissue volume change between scans of the same subject acquired on different dates (baseline to 24 weeks)

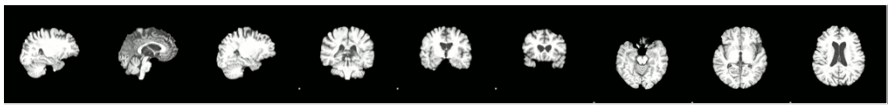
Serial scans are co-registered and relative edge motions are detected between scans (blue points represent the expansion of the brain, and red ones the contraction of the brain)

Edge motion information can be used to extrapolate PBVC

Coefficient of variation (COV) is 0.2%

SIENA measures are improved by inpainting, nonuniformity correction, and intensity standardization





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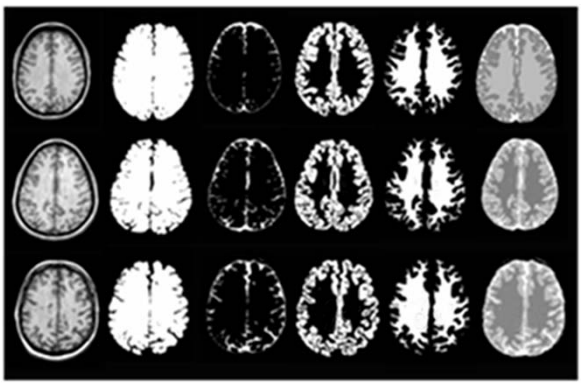
SIENAX

Segments cross-sectional brain image into GM/WM/ cerebrospinal fluid (CSF) using Hidden Markov Random Field (HMRF) model

Normalized to standard atlas space to correct for variations in head size

MTP modifications allow 4D GM/WM segmentation with improved accuracy



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Immunology

Immunological measures at baseline (HCs and patients) and 6 months (patients only) were performed

- Blood for immunological samples was collected at baseline and post-IFN β-1a SC tiw treatment at 6 months
- **Protein expression**
  - Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll density gradient, and $CD4^+$ T cells and $CD8^+$ monocytes were isolated by magnetic bead separation
  - Cells were stained with fluorescein-conjugated antibodies against cytokines and growth factors
  - Cytokine expression was measured in fixed, permeabilized CD4+ and CD8+ T cells using a BD FACSCalibur™ Flow Cytometer and CellQuest software
- **Gene expression**
  - RNA was harvested from separated $CD4^+$ T cells and analyzed for gene expression
  - Relative gene expression, normalized against 18S rRNA, was measured by quantitative real-time polymerase chain reaction (qRT-PCR)

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Markers of Interest

Immunology markers of interest

Known pro-inflammatory action	Known anti-inflammatory action
Cytokines and cytokine receptors	
IFN γ, IL-1α,* IL-1β,* IL-1R1, IL-12, IL-17A, IL-17F, IL-21, IL-21R,* IL-22, IL-23*	IL-4, IL-10, IL-27Rα,* TGF-β*
Toll-like receptors	
TLR3,* -7,* -9*	-
Transcription factors	
AHR,* IRF4,* RORc,* T-bet*	GATA3,* Foxp3*

AHR, aryl hydrocarbon receptor; IL, interleukin, IFN γ, interferon gamma; IRF4, interferon regulatory factor 4; RORc, retinoic acid receptor (RAR)-related orphan receptor C; TGF-β, transforming growth factor-beta; TLR, toll-like receptor
*Markers measured by RT-PCR only.

Neurotrophic factors of interest

- Brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF)

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Statistics

Wilcoxon rank-sum test

- Differences in PBVC between patients and HCs during each timeframe (from baseline to 3 months [to measure short-term volume changes], from 3 months to 6 months [to measure changes occurring in the second half of the treatment period], and over the longer term of baseline to 6 months)

Wilcoxon signed-rank test

- Within-group differences in PBVC from baseline to 3 months, from 3 months to 6 months, and from baseline to 6 months

Holm–Bonferroni was applied to correct for multiple comparisons

Spearman's rank test

- Correlations between immunological parameters and PBVC over 3 and 6 months of treatment in patients

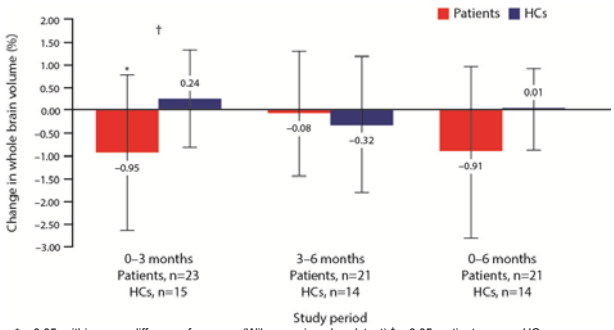
Baseline Characteristics

Characteristic	Patients (n=23)	Healthy controls (n=15)
Age, years, mean (SD)	39.9 (10.17)	36.7 (10.31)
Female, n (%)	14 (61)	8 (53)
Race, n(%) White African American Other: Indian	 20 (87) 3 (13) 0	 14[a] (93) 0 1 (7)
Weight, kg, mean (SD)	79.9 (22.25)	87.0 (18.35)
Height, cm, mean (SD)	171.0 (8.48)	168.5 (6.99)
BMI, kg/m^2, mean (SD)	27.2 (6.90)	30.5 (5.37)
Multiple sclerosis history		
Years since multiple sclerosis diagnosis, mean (SD), range	6.6 (5.95), 0-20	–
Years since most recent relapse, mean (SD), range	1.0 (1.14), 0.1-5.0	–
Number of relapses in past 12 month,[b] mean (SD) 0, n (%) 1, n (%) 2, n (%) 4, n (%)	1.3 (1.18) 7 (30) 7 (30) 7 (30) 2 (9)	 –
EDSS score, median (range)	2.5 (1.0-5.5)	–
Ambulation distance, meters, mean (SD)	475 (94.2)	–

BMI, body mass index; EDSS, Expanded disability scale; HC, healthy control; SD, standard deviation
[a] Includes one of Hispanic ethnicity, all others were not Hispanic
B Patients reported the same number of relapses for the past 24 months

Results: Brain Volume Analysis with MRI

Mean Percent Change in Whole Brain Volume in Patients and HCs



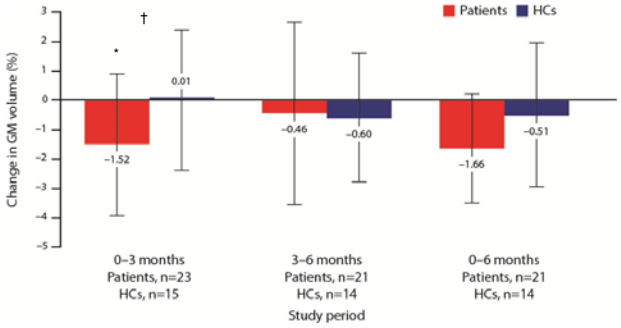
*p<0.05, within-group difference from zero (Wilcoxon signed-rank test) †p<0.05, patients versus HCs (Wilcoxon rank-sum test).

Baseline to 3 months: PBVC decreased significantly in patients; –0.95% (standard deviation [SD], 1.71%); p=0.030

3 to 6 months: PBVC in patients was not significant

No significant PBVCs were noted over any time period in the HC group

Mean Percent Change in GM Volume in Patients and HCs

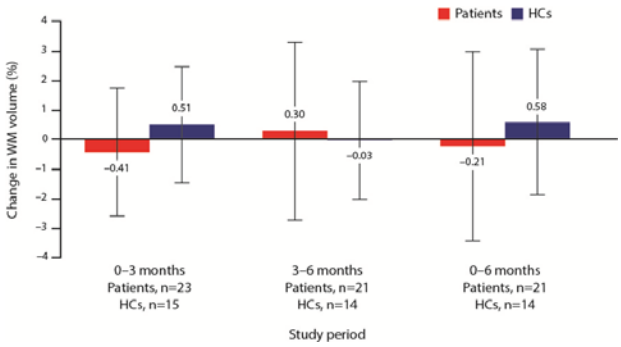


*p<0.004, within-group difference from zero (Wilcoxon signed-rank test) †p<0.05, patients versus HCs (Wilcoxon rank-sum test).

In patients, GM volume decreased significantly from baseline to 3 months (p=0.004)

The significant decrease remained evident over the baseline to 6 months time period (p<0.001)

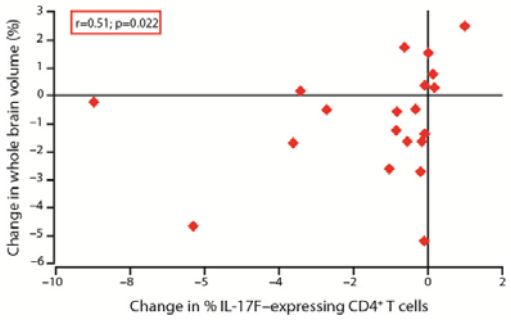
Mean Percent Change in WM Volume in Patients and HCs



There were non-significant numerical changes to WM volume following treatment

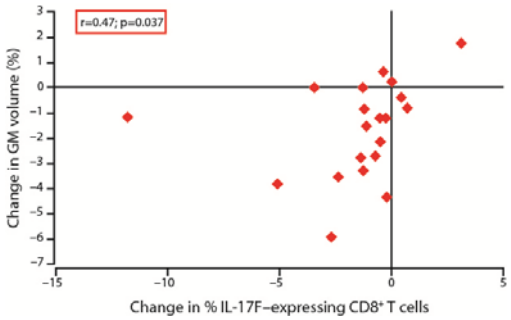
Results: Correlations between Change in Brain Volume and Immunological Markers in RRMS patients treated with Interferon Beta-1a

Percentage of CD4+ T Cells Expressing IL-17F: Correlation with Whole Brain Volume



Decreased whole brain volume from baseline to 6 months was associated with a decreased percentage of IL-17F–expressing $CD4^+$ T cells from baseline to 6 months in patients with RRMS following IFN β-1a treatment (n=20)

Percentage of CD8⁺ T Cells Expressing IL-17F: Correlation with GM Volume



Decreased GM volume from baseline to 6 months was associated with a decreased percentage of IL-17F–expressing CD8⁺ T cells from baseline to 6 months in patients with RRMS following IFN β-1a treatment (n=20)

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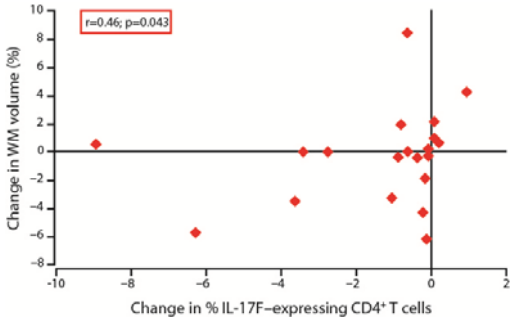
Percentage of CD4⁺ T Cells Expressing IL-17F: Correlation with WM Volume



Decreased WM volume from baseline to 6 months was associated with a decreased percentage of IL-17F–expressing CD4⁺ T cells from baseline to 6 months in patients with RRMS following IFN β-1a treatment (n=20)

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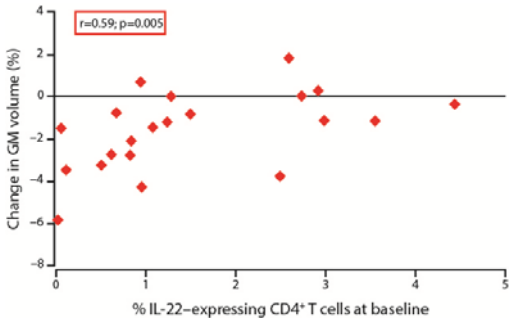
Percentage of CD4⁺ T Cells Expressing IL-22: Correlation with GM Volume



Patients with fewer IL-22–expressing CD4⁺ T cells at baseline had greater decreases in GM volume from baseline to 6 months following IFN β-1a treatment than patients with more IL-22-expressing CD4⁺ T cells (n=21)

Brain Volume and Other Markers

Other markers of interest that were analyzed for correlations with brain volume changes were:

- Cytokines IL-1α, IL-1β, IL-4, IL-10, IL-17A, Il-21, IL-23, TGF-β, IFN γ
- Cytokine and toll-like receptors IL-1R, Il-21R, IL-27Rα, TLRs-3, -7, -9
- Transcription factors AHR, IRF4, RORc, T-bet, GATA3, Foxp3
- The neurotrophic factors BDNF, NGF

However, no significant correlations were found between percent change in whole brain volume and the percentages of CD4⁺ and CD8⁺ T cells expressing any of these biomarkers

Conclusions

Treatment with IFN β-1a SC 44 mcg tiw is associated with reduced brain volume during the 3-month period following treatment initiation, but not over the subsequent 3 to 6 months, a result consistent with an acute treatment-induced pseudoatrophy effect

Correlation between decreased percentage of $CD4^+$ T cells producing pro-inflammatory IL-17F and reductions in whole brain, GM, and WM tissue volumes is supportive of an early and anatomically extensive anti-inflammatory therapeutic effect of IFN β-1a SC tiw

A higher percentage of IL-22–producing $CD4^+$ T cells at baseline correlated with less GM atrophy following treatment initiation. The relevance of this finding is unclear and requires further investigation

The results of this study should be taken into account in the design of future neuroprotective trials