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The effect of interferon beta-1b on size of short-lived enhancing lesions in patients with multiple sclerosis

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Abstract

Background—Contrast enhancing lesions (CELs) in MRI represent inflammatory events in multiple sclerosis (MS). IFN- β -1b decreases the formation of CELs. However, the ability of IFN- β -1b to reduce the size of CELs arising during therapy has not been extensively investigated.

Methods—Thirty patients with relapsing-remitting (RR) MS were followed for a 3-month pre-therapy phase then for a 6-month therapy phase during which treatment with IFN- β -1b at a dosage of 250 μ g subcutaneously injected every other day was employed. Each patient underwent monthly clinical and MRI examinations. For all patients, CELs were identified on postcontrast T1-weighted MRIs. CEL number, size, and volume were computed using Medx software.

Results—The average number and total lesion volume of CELs visible during the therapy phase were significantly lower than the number and total lesion volume of CELs observed in the pre-therapy phase. However, there was no significant reduction between pre-therapy and therapy phases in the mean size of individual lesions arising during the respective phases.

Conclusions—Since size of CELs has been related to severity of tissue damage, the lack of size decrease during therapy suggested a limited therapeutic effect of IFN- β -1b if a blood–brain barrier breakdown has occurred.

Keywords

contrast enhancing lesions; interferon beta 1b; lesion size; magnetic resonance imaging; multiple sclerosis

1. Introduction

In patients with multiple sclerosis (MS), monitoring the occurrence of contrast-enhancing lesions (CELs) on T1-weighted (T1W) magnetic resonance imaging (MRI) provides a reliable indication about the inflammatory damage occurring at the level of the blood–brain barrier (BBB) [1-6]. The decrease of CEL number reflects the ability of a given drug to prevent BBB breakdown, ultimately reducing the incidence of new inflammatory flares [7]. The occurrence and evolution of CELs serve as important measures in daily clinical practice and are outcome factors for assessing the efficacy of drugs in MS clinical trials.

IFN- β is one of the most widely used drugs for preventing BBB leakage and promoting its recovery over time [8]. IFN- β s are naturally occurring cytokines that have both immunomodulatory and antiviral effects [9]. In MS, IFN- β is thought to be a regulatory agent, acting at different stages of the inflammatory process, and ultimately downregulating the abnormally-mediated immunologic activity [10].

The effect of IFN- β on BBB disruption is quantified by measuring the number and volume of CELs before and after therapy. Previous reports showed an on-treatment reduction of CEL number and total volume ranging from 33.3 to 85.6% in patients with relapsing remitting (RR) or secondary progressive (SP) MS [11-16].

Complementary information on the effect of IFN- β in reducing inflammatory activity comes from measuring changes in individual CEL's size persisting during therapy. Measuring the size of CELs provides information on the quality of CELs arising during therapy.

The clinical importance of this depends on the fact that the size of a given CEL is known to indirectly indicate underlying disease pathology. Larger CELs are more likely to evolve into T1W black holes [17] which represent areas of severe tissue destruction [18,19]. Furthermore, larger CELs exhibit lower magnetization transfer ratios (MTRs), indicative of significant myelin or axonal loss [20]. Therefore, decreased CEL size effected by a given drug can be considered to be a good indicator that the severity of residual CELs is attenuated during therapy phase.

Previous literature indicated an inverse relationship between the effectiveness of IFN- β and the degree of BBB damage present in CELs. Both IFN- β -1a and IFN- β -1b prevented the occurrence of smaller CELs more often than larger CELs during therapy. [13,14]. Such observations indicate that the ability of IFN- β to prevent CELs is non-homogeneous with respect to CEL size. However, the specific question as to whether IFN- β reduced CELs size once formed or persisting during the therapy phase has rarely been investigated. In previous work by our group, it was shown that IFN- β -1b effectively reduced the maximum lesion size of larger lesions enhancing multiple times over the course of several monthly observations, rather than short-lived lesions enhancing only once [21]. Although potential biases due to outlier cases were considered and corrected in this work, the results suffered from the small number of patients examined and warranted investigation in larger study cohorts. Subsequently, Di Rezze [22] and colleagues explored the effect of IFN- β -1a in reducing CELs in a larger cohort of MS patients and pointed to the possible dose-dependency of IFN- β -1a in reducing CEL size during therapy. However, this study used low-field-strength MRI (i.e., 0.5 Tesla) and a very low dose of IFN- β -1a in one group of patients, and there was no statistical analysis on monthly MRIs.

To provide more insight into this issue and as a continuation of previous studies, we investigated the ability of a standard dose of IFN- β -1b to decrease CELs in a retrospective open-label study. The volumes of individual CELs were measured on multiple monthly

MRIs in a relatively large cohort of MS patients, during both pre-therapy and therapy phases.

2. Patients and methods

2.1 Patients and study design

The current investigation took place at the National Institutes of Health in Bethesda, MD, USA. The review board of the National Institute of Neurological Disorders and Stroke approved the study and all participants gave written informed consent. The study relied on the retrospective analysis of monthly MRIs of 30 patients with RRMS according to Poser's criteria [23] consecutively enrolled in an observational study at NIH. At the time of the study's first MRI, none of the patients had been treated with any other immunomodulatory therapy. Additionally, no patients underwent steroid therapy within the month before the first examined scan. For each patient, monthly MRIs were acquired during the 3-month pre-therapy phase (i.e., months -2 , -1 and 0 or baseline) and the following 6 months of therapy phase (i.e., months $+1/+6$). Monthly clinical assessments were quantified by the Expanded Disability Status Scale (EDSS) [24]. Alterations in EDSS score were defined as when patients with an EDSS of ≥ 5.5 changed by ≥ 0.5 , or patients with an EDSS ≤ 5.0 or less had an EDSS score that changed by ≥ 1.0 [25,26]. During the therapy phase, patients were administered IFN- β -1b at a dosage of 250 micrograms subcutaneously every other day.

Table 1 describes MRI characteristics, clinical measures and demographics of patients at the time of enrollment in the study.

2.2 Image acquisition

A 1.5 Tesla MRI with a standard head coil (General Electric Medical Systems) was used for imaging. Details of the adopted MRI protocol have been described previously [21]. Contrast-enhanced images were collected within 15 min after gadolinium dimegumine (Gd) (Magnevist, Berlex Labs. J) injection at 0.1 mmol/kg of body weight (adjacent axial slices of either 3- or 5-mm thickness with 24 cm of field of view were acquired for all MRIs).

2.3 Imaging analyses

The scripting language, ImageScript, and software package MEDx 3.42 for a PC LINUX platform were used to track CELs over time and to measure CEL number and volume using a semiautomated procedure (Medical Numerics, Inc.) [27]. Details of the methodology are described elsewhere [21]. Briefly, the linear registration tool FLIRT 3.1 [28] was used to register each patient's monthly MRIs onto the first pre-therapy phase MRI. CELs were first identified on hard copies of films by an expert neurologist (F Bagnato) and radiologist (N Richert) and then manually traced on postcontrast T1W visualized on the computer by one observer (D Gaindh). In order to see a given lesion with the utmost clarity, thresholds were specified in the program. Threshold values were fixed for all lesions in a specific patient to ensure that all lesion volumes were calculated uniformly.

Lesion volume of CELs identified in five randomly selected patients was also measured by a second investigator and an agreement based upon $\leq 5\%$ variability was achieved.

2.4 Statistical analysis

Of interest were differences in i) number; ii) total lesion volume; and iii) average CEL size during the pre- and therapy phases. The average monthly number of CELs was calculated for each subject during the pre-therapy (months -2 to 0) and therapy (months $+1$ to $+6$) phases. These paired responses were then analyzed by a Wilcoxon signed rank test after using the square root of these values. The square root transformation reduced the effect of

extremely large counts and simplified graphical presentation; statistically, p-values remained highly significant ($p < 0.0001$) regardless of whether the transformation was or was not used. The same analysis was used to examine the change in total lesion volume.

The Wilcoxon analysis of average CEL size was restricted to individuals who had CELs in both the pre-therapy and therapy phases since we did not want to include 0 as a valid CEL size. This was done to better address the question about CEL size change during the pre-therapy phase. Due to this restriction, only 21 of the initial 30 MS subjects contributed to this data analysis. Additionally, the analysis was restricted only to emergent lesions. Specifically because treatment with IFN- β -1b may have changed the characteristics of lesions initially appearing in the pre-therapy phase and persisting over the therapy phase, this analysis was restricted to comparing the size of CELs that disappeared before treatment started with the size of a similar set of short-lived lesions after treatment was initiated.

Since the pre-therapy phase consisted of three monthly MRIs, the analysis was restricted to i) lesions discovered in month -2 and disappearing by the baseline scan (i.e., observed no more than two times); ii) lesions discovered in month -1 and disappearing by the baseline scan (i.e., observed 1 month); and iii) lesions developing during any month of the therapy phase and lasting less than 2 months. To ensure the exact duration of each lesion, each pre-therapy phase CEL that was present at the first pre-therapy-phase MRI or month -2 was tracked backward in time in previous MRIs until the origin was identified. Only CELs whose origin was identified at months -1 or -2 were considered. A similar process was employed for CELs visible during the therapy phase. Specifically, any therapy-phase CEL that was present at month +6 of the therapy phase was tracked onward in time in the following MRIs and kept in the analysis if, while still originating up to months +5 or +6, it was observed no more than twice. The latter included a small number of CELs which ceased enhancing at month +7.

Once the lesions were identified, a linear mixed model was applied separately to the lesions with only one measurement (i.e., those lasting less than 1 month) and those with two measurements (those lasting less than 2 months but more than 1 month). These models have the flexibility of analysis of variance (ANOVA) or regression models with the additional ability to account for within-person correlation (when looking at the lesions with one measurement) and within-lesion correlation (when looking at the lesions with two measurements). In each model, the log of the lesion size was used as the dependent variable and the period (pre-therapy phase or therapy phase) was treated as an independent factor. For the model with two measurements, an additional variable indicating whether the measurement corresponded to the first or second month was included as another independent variable. For each model, a compound symmetry correlation structure was implemented that corrected the potential correlation as described above (other, more complicated, correlation models were tried but results were consistent with this more conservative approach). In each model, the question of interest was whether there was a difference in the pre and therapy phases in modeling the average log lesion size. The logarithmic transformation better adhered to the assumptions of normally distributed error terms.

3. Results

3.1 Clinical data

By the end of the therapy phase, three patients showed a sustained 1-point decrease in the EDSS score and four showed a sustained increase by 0.5. A total of four relapses were observed during the pre-therapy phase. Eight clinical flares occurred in eight patients during

the pre-therapy phase (four during the first 3 months and three during the second trimester of therapy).

3.2 MRI outcome of patients during the study period

3.2.1 Incidence of CELs—600 CELs were identified. Of those, 391 (198 out of the 391 in the pre-therapy phase and 193 in the therapy phase) met the restricted criteria required by the proposed statistical approach employed and were thus included in the analyses. Of the 391 lesions, 198 (50.6%) were observed in the pre-therapy phase. Of these, 19 were lesions with two observations (9.6%). The corresponding figures for the therapy phase were 193 lesions (49.4%) of which 26 (13.5%) were lesions with two measurements.

3.2.2 IFN- β -1b effect in decreasing the number, total lesion volume and size of CELs—The mean number of CELs decreased with the onset of IFN- β -1b therapy ($p < 0.0001$) (Figure 1). Similarly, the average total lesion volume of CELs decreased from pre-therapy phase to therapy phase ($p < 0.0001$) (Figure 2). Figure 3 shows the log transformed lesion volumes for the subset of lesions in the pre-therapy phase that were unaffected by therapy and a set of similarly short-lived lesions in the therapy phase. The results for the model using only those lesions with one observation showed no difference between the phases with respect to lesion size. The pre-therapy phase had a slightly higher estimated average value (0.08 units on a log scale) but the p-value was insignificant ($p = 0.41$).

The model, using only those lesions with two observations, provided some interesting results. The explanatory variables (a factor indicating first or second month and a factor indicating pre-therapy or therapy phase) had no significant interaction ($p = 0.32$) but each had marginally significant effects when used together in an additive, non-interacting, manner. The first month/second month indicator estimated that the second month tended to be smaller (0.33 units on a log scale, p -value = 0.046) and the indicator for pre-therapy/therapy phase indicated that lesions during the pre-therapy phase were 0.63 units smaller ($p = 0.01$) than in the therapy phase.

4. Discussion

In the present study, we investigated further whether IFN- β -1b reduced CEL size once formed by analyzing CEL evolution on monthly MRIs of 21 patients imaged three times over the course of a pre-therapy phase and for six consecutive months during the therapy phase with IFN- β -1b. We reasoned that comparing size between all CELs observed during the pre or therapy phases could raise some comparability issues. In particular, the lesions observed within the first month of the therapy phase would probably not be representative of the treatment effect because some of these CELs arose during the pre-therapy phase. To the extent that therapy reduced the incidence of new CELs, the measurements for month 1 and perhaps month 2 may be artificially high relative to later months because persisting lesions from the pre-therapy phase were still present that therapy may have otherwise repressed. Consequently, the lesion load observed during the therapy phase (particularly during the early months of therapy) may overestimate to some extent what might be observed after a longer period of therapy.

To clarify the potential of such bias, an analysis addressing how therapy might change the course of only emergent lesions was performed. For this analysis, only CELs whose appearance was confirmed to have occurred during the pre-therapy phase and that ceased enhancement before therapy were considered. A similar CEL selection was employed for lesions emerging during the therapy phase. We recognize that our results are limited only to CELs lasting up to 2 months. However, it is well known that, as seen in our study cohort, those CELs represent the majority of active CELs in patients with MS [6,17,21,29].

Moreover, although restricted by those limitations, the analysis preserved some comparability between those lesions detected during the pre and therapy phases.

With such an analysis, the inability of IFN- β -1b to decrease CEL size during the therapy phase was confirmed. Lack of efficacy was seen for CELs arising during any of the 6 months of the therapy phase, suggesting that no changes in IFN- β -1b effect on lesions size were seen as time progressed, at least for the first 6 months of therapy.

The results may raise questions regarding the drug's ability to attenuate the severity of the inflammatory process once it is initiated and do not parallel the previous report from Di Rezze and colleagues [22]. Substantial methodological differences may account for this discrepancy and prevent robust inter-study comparisons. First, in this previous report [22] the authors examined only CELs not persisting over sequential MRIs obtained every 3 months (i.e., up to four observations). The latter resulted in pooling of CELs with different durations in time of their enhancement within the 4 months and provided a data set of lesions likely more heterogeneous than the one considered in our present study. More specifically, CELs lasting longer and potentially being larger were included, thus introducing some comparability issues with our results. In this regard, the results from Di Rezze and colleagues parallel the ones reported by Gupta and colleagues on the subset of larger and longer lasting CELs, for which an effect of IFN in reducing the lesions' size is observed instead [21]. Second, Di Rezze and colleagues took only single measurements of CELs into account, which is the one corresponding to the available MRI. The latter inevitably resulted in possible bias in estimating the real size reached by a given CEL in months of appearance not included in the analysis.

Conversely, the results partially confirm our previous findings [21], specifically those regarding the subset of single-enhancing lesions, whose duration and behavior resemble the subset of CELs examined here. As a further evolution of those previous results, the present study, in a larger cohort of patients, revealed a different drug effect with respect to enhancement duration in this subset of CELs. Specifically, while no evidence for such an effect was seen for CELs lasting only one scan, surprisingly, CELs visible on two scans were larger on average during the therapy phase than in the pre-therapy phase. It is worth emphasizing that this result was only seen in lesions appearing exactly twice (19 in the pre-therapy phase and 26 in the therapy phase); there was no indication of a similar difference among those lesions appearing only once.

Taken together, the findings show that IFN- β -1b had a dramatic effect in preventing the formation of new short-lived CELs (i.e., up to 2 months) but no effect on reducing CELs size. The latter parallels the notion that IFN- β -1b effect minimally extended beyond the BBB resolution for the majority of the CELs seen in MS patients. While the efficacy of the drug in reducing the size of larger and longer lasting lesions cannot be denied, we conclude that such an effect is not apparent for the majority of CELs, which are instead known to be short-lived.

In the future, studies combining immunological and imaging data are needed to elucidate the biological basis of our observations. Furthermore, studies with larger patient populations will help to establish whether the development of larger CELs during therapy is a patient-dependent event and to what extent it is connected to the CEL type. The latter should ultimately help to identify pre-therapy features predicting its occurrence.

5. Expert opinion

In MS patients, the size of a given CEL is considered to be an indirect indicator of the underlying active inflammation. The present study investigated the role of IFN- β -1b in

reducing new short-lived CELs (i.e., up to 2 months) size. The results showed that IFN- β -1b failed to decrease the size of CELs originating during therapy, thus yielding evidence of only a minimal effect of IFN- β -1b beyond BBB resolution. It is uncertain whether this is a patient-dependent phenomenon. Questions also remain regarding the biological basis of our findings. Future studies combining immunological and imaging data on a larger cohort of patients longitudinally followed are necessary to elucidate the biological correlates of these results.

Taken together, our results do not parallel some of the previously reported findings. It is likely that differences in several aspects of the methodologies employed (i.e., statistical approach, lesion count), type and dose of IFN- β used, play a role in determining inter-studies variability. It is also possible that the effect of IFN- β is highly lesion-dependent.

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. McFarland HF, Frank JA, Albert PS, et al. Using gadolinium-enhanced magnetic resonance imaging lesions to monitor disease activity in multiple sclerosis. *Ann Neurol.* 1992; 32:758–66. [PubMed: 1471866]
2. Miller DH, Barkhof F, Nauta JJ. Gadolinium enhancement increases the sensitivity of MRI in detecting disease activity in multiple sclerosis. *Brain.* 1993; 116:1077–94. [PubMed: 8221048]
3. Bastianello S, Pozzilli C, Bernardi S, et al. Serial study of gadolinium-DTPA MRI enhancement in multiple sclerosis. *Neurology.* 1990; 40:591–5. [PubMed: 2320230]
4. Hawkins CP, Munro PM, MacKenzie F, et al. Duration and selectivity of blood-brain barrier breakdown in chronic relapsing experimental allergic encephalomyelitis studied by gadolinium-DTPA and protein markers. *Brain.* 1990; 113:365–78. [PubMed: 2328409]
5. Kermode AG, Thompson AJ, Tofts P, et al. Breakdown of the blood-brain barrier precedes symptoms and other MRI signs of new lesions in multiple sclerosis: pathogenetic and clinical implications. *Brain.* 1990; 113:1477–89. [PubMed: 2245307]
6. Harris JO, Frank JA, Patronas N, et al. Serial gadolinium-enhanced magnetic resonance imaging scans in patients with early, relapsing-remitting multiple sclerosis: implications for clinical trials and natural history. *Ann Neurol.* 1991; 29:548–55. [PubMed: 1859184]
7. Stone LA, Frank JA, Albert PS, et al. The effect of interferon- β on blood-brain barrier disruptions demonstrated by contrast-enhanced magnetic resonance imaging in relapsing-remitting multiple sclerosis. *Ann Neurol.* 1995; 37:611–9. [PubMed: 7755356]
8. Clerico M, Contessa G, Dorelli L. Interferon- β 1a for the treatment of multiple sclerosis. *Expert Opin Biol Ther.* 2007; 7:535–42. [PubMed: 17373904]
9. Bermel RA, Rudick RA. Interferon-beta treatment for multiple sclerosis. *Neurotherapeutics.* 2007; 4:633–46. [PubMed: 17920544]
10. Markowitz CE. Interferon- β : mechanism of action and dosing issues. *Neurology.* 2007; 12,68(24 suppl 4):S8–11. [PubMed: 17562848]
11. Pozzilli C, Bastianello S, Koudriavtseva T, et al. Magnetic resonance imaging changes with recombinant human interferon- β -1a: a short-term study in relapsing-remitting multiple sclerosis. *J Neurol Neurosurg Psychiatry.* 1996; 61:251–8. [PubMed: 8795595]
12. Miller DH, Molyneux PD, Barker GJ, et al. Effect of interferon- β 1b on magnetic resonance imaging outcomes in secondary progressive multiple sclerosis: results of a European multicenter,

- randomized, double-blind, placebo-controlled trial. *Ann Neurol.* 1999; 46:850–9. [PubMed: 10589537]
13. Filippi M, Rovaris M, Capra R, et al. Interferon β treatment for multiple sclerosis has a graduated effect on MRI enhancing lesions according to their size and pathology. *J Neurol Neurosurg Psychiatry.* 1999; 67:386–9. [PubMed: 10449565]
 14. Brex PA, Molyneux PD, Smiddy P, et al. The effect of IFN β -1b on the evolution of enhancing lesions in secondary progressive MS. *Neurology.* 2001; 57:2185–90. [PubMed: 11756595]
 15. Barkhof F, Scheltens P, Frequin ST, et al. Relapsing-remitting multiple sclerosis: sequential enhanced MR imaging vs clinical findings in determining disease activity. *AJR Am J Roentgenol.* 1992; 159:1041–7. [PubMed: 1414773]
 16. Secondary progressive efficacy clinical trial of recombinant interferon-beta-1a in MS (SPECTRIMS STUDY GROUP). Randomized controlled trial of interferon-beta-1a in secondary progressive MS: clinical results. *Neurology.* 2001; 56:1496–504. [PubMed: 11402106]
 17. Paolillo A, Bastianello S, Frontoni M, et al. Magnetic resonance outcome of new enhancing lesions in patients with relapsing-remitting multiple sclerosis. *Eur J Neurol.* 1999; 6:455–9. [PubMed: 10362899]
 18. Bruck W, Bitsch A, Kolenda H, et al. Inflammatory central nervous system demyelination: correlation of magnetic resonance imaging findings with lesion pathology. *Ann Neurol.* 1997; 42:783–93. A well designed study proving pathological correlates of lesions with different MRI appearance. [PubMed: 9392578]
 19. Hiehle JF, Grossman RI, Ramer KN, et al. Magnetization transfer effects in MR-detected multiple sclerosis lesions: comparison with gadolinium-enhanced spin-echo images and nonenhanced T1-weighted images. *AJNR Am J Neuroradiol.* 1995; 16:69–77. [PubMed: 7900604]
 20. Filippi M, Rocca MA, Comi G. Magnetization transfer ratios of multiple sclerosis lesions with variable durations of enhancement. *J Neurol Sci.* 1998; 159:162–5. [PubMed: 9741401]
 21. Gupta S, Solomon JM, Tasciyan TA, et al. Interferon-beta-1b effects on re-enhancing lesions in patients with multiple sclerosis. *Mult Scler.* 2005; 11:658–68. [PubMed: 16320725]
 22. Di Rezze S, Gupta S, Durastanti V, et al. An exploratory study on interferon beta dose effect in reducing size of enhancing lesions in multiple sclerosis. *Mult Scler.* 2007; 13:343–7. [PubMed: 17439903]
 23. Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol.* 1983; 13:227–31. [PubMed: 6847134]
 24. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology.* 1983; 33:1444–52. [PubMed: 6685237]
 25. Panitch H, Goodin DS, Francis G, et al. Randomized, comparative study of interferon β -1a treatment regimens in MS: The EVIDENCE Trial. *Neurology.* 2002; 59:1496–506. [PubMed: 12451188]
 26. Leary SM, Miller DH, Stevenson VL, et al. Interferon β -1a in primary progressive MS: an exploratory randomized, controlled trial. *Neurology.* 2003; 60:44–51. [PubMed: 12525716]
 27. [last accessed 3 October 2008] Medical numerics, Inc. MEDx 3.42. Available from: www.medicalnumerics.com
 28. Jenkinson M, Smith S. A global optimisation method for robust affine registration of brain images. *Med Image Anal.* 2001; 5:143–56. A milestone paper on optimized techniques for image registration. [PubMed: 11516708]
 29. van Waesberghe JH, van Walderveen MA, Castelijns JA, et al. Patterns of lesion development in multiple sclerosis: longitudinal observations with T1-weighted spin-echo and magnetization transfer MR. *AJNR Am J Neuroradiol.* 1998; 19:675–83. One of the first papers describing variability in the outcome of active lesions evolution *in vivo*. [PubMed: 9576653]

Figure 1. Square root of average monthly lesion number during pre-therapy phase and therapy phase

Each line represents the data collected from one patient. The lines consist of two points: the first point represents the average monthly lesion count over the pre-therapy phase, while the second point is the average lesion count throughout the duration of the therapy phase. Square root values were taken to moderate the effects of extremely large changes and allow the data to better fit a normal distribution. The number of lesions decreased during treatment ($p < 0.0001$).

Figure 2. Square root of averaged monthly total lesion volume during pre-therapy phase and therapy phase

Each line represents the data collected from one patient. The lines consist of two points: the first point represents the average monthly total lesion volume (Figure 2) over the pre-therapy phase, while the second point is the average lesion volume throughout the duration of the therapy phase. Square root values were taken to moderate the effects of extremely large changes and allow the data to better fit a normal distribution. The total lesion volume decreased during treatment ($p < 0.0001$).

Figure 3. Individual lesion size (log transformed) for a subset of short-duration lesions

Each dot represents the log transformed size of a lesion; some lesions were measured only once while others were measured twice. Lesions measured twice have volumes connected by a line. Lesions in the pre-therapy phase (indicated as months -2 to 0 in this figure) were chosen so they ended prior to month 3 and therefore could not have been affected by treatment and are of 1- or 2-month duration. A comparison group of lesions initially appearing in the therapy period (months $+1$ to $+6$) were chosen to have similar short duration of no more than 2 months. Only CELs whose origin was identified at month -1 or -2 were considered. Similarly, any CEL that was present at months $+5$ or $+6$ of the therapy phase was tracked onward in time in the following MRIs and kept in the analysis if they lasted no longer than three observations.

Demographic, MRI and clinical characteristics of 30 patients (18 women and 12 men) with relapsing-remitting multiple sclerosis at baseline (i.e., study entry).

Table 1

	Age	Years since onset	EDSS	CEL count	CELs volume	Mean CEL size
Mean	36.9	5.2	2.4	5.6	364.5 mm ³	77.6 mm ³
SD	8.7	4.8	1.6	9.4	537.4 mm ³	52.8 mm ³
Median	35.3	4.0	2.0	1.5	171 mm ³	58.8 mm ³
Range	23.2 – 52.3	0.1 – 16	0.0 – 6.5	0.0 – 48	0.0 – 2135 mm ³	22.1 – 232.9 mm ³

CEL: Contrast-enhancing lesion; EDSS: Expanded disability status scale; SD: Standard deviation.

Average CEL count and volume were calculated from each person's data for month 1 and averaged over 30 people. Average CEL volume is calculated by first measuring each person's month 1 average CEL volume and then taking the average over all 30 people. The latter was done to ensure that each patient had the same weight in calculating the average.