ORIGINAL ARTICLE

A Population Approach to Characterize Interferon Beta-1b Effect on Contrast Enhancing Lesions in Patients With Relapsing Remitting Multiple Sclerosis

A Gulati[1,2], F Bagnato[3], P Villoslada[4] and N Velez de Mendizabal[1,2*]

In patients with relapsing-remitting multiple sclerosis (RRMS), interferon beta-1b (IFNβ-1b) reduces the occurrence of contrast enhancing lesions (CELs) on magnetic resonance imaging (MRI). Questions remain on the stability of IFNβ-1b effect over time and its action beyond the reduction of CELs. In this study, we described the IFNβ-1b effect by a mixed effects model, quantifying the interpatient variability associated with its parameters. Using a negative binomial distribution model as a natural history model, the effect of IFNβ-1b was evaluated using different mathematical functions of time. IFNβ-1b produced a decrease in the expected CEL numbers, inhibiting the formation of new CELs but did not promote the resolution of the already-formed ones. Based on the final selected model, simulations were carried out to optimize the combined IFNβ-1b-corticosteroid therapy as a proof-of-concept. In summary, we provide evidence on the dynamics of CELs under IFNβ-1b treatment that can be used to monitor the effects of therapies in MS.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC? ☑ IFNβ-1b is first-line treatment for patients with MS. MS activity is monitored through active and new lesions on MRI. Dynamics of these lesions is complex and there is high variability in the IFNβ-1b response between and within individuals. Corticosteroids resolve existing lesions but do not prevent the development of new ones. • WHAT QUESTION DID THIS STUDY ADDRESS? ☑ This study characterizes the IFNβ-1b effect on MRI activity in patients with MS. Various combination schemes of IFNβ-1b and corticosteroids as proof-of-concept for any combination paradigm are also simulated. • WHAT THIS STUDY ADDS TO OUR KNOWLEDGE ☑ This analysis suggests that IFNβ-1b reduces the formation of new CELs but does not promote disappearance of already formed ones. Simulations suggest that more frequent dosing of either IFNβ-1b or corticosteroids given alone may be sufficient to lower accumulated CELs through different mechanisms. • HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS ☑ Combination therapies with the administration of IFNβ-1b and/or corticosteroids at different dosing frequency can be designed. By acting differently, drugs in combination might affect inflammation in individual patients more effectively than alone.

Multiple sclerosis (MS) is a chronic disease of the central nervous system that leads to myelin and axons destruction to varying degrees.[1] More than 250,000 patients suffer from MS in the United States[2] and 50% of these patients may not be fully ambulatory within 15 years after the onset of the disease if they do not receive therapy.[3] Relapsing-remitting MS (RRMS) is the most common disease type affecting about 85% of patients with MS. RRMS is characterized by exacerbations of symptoms followed by periods of remission. Relapse occurrence in MS is highly variable among patients and within the same patient over time. The dynamics of relapse occurrence is unpredictable and not very well understood. Previous modeling studies from our group suggest that relapse dynamics is an inherent property of the immune system design.[4] A significantly different response to MS-specific therapies in terms of relapse frequency may be observed among patients and even within

the same patient over time. Such a variability is only due, in part, to changes in patient age, disease duration, and evolution into secondary progressive stage.[5] There is still not full understanding of the biological basis explaining differences in treatment responses.

Magnetic resonance imaging (MRI) is a fundamental tool for diagnosing and monitoring disease activity in MS.[6] The presence of an acute exacerbation is thought to be associated with the presence of acute inflammatory contrast enhancing lesions (CELs) on MRI. On average, one acute clinical exacerbation occurs every 10 CELs.[7] As a result, the presence of CELs, quantified as a CEL count, is considered a highly sensitive marker of disease activity in the RRMS phase. The size of CELs is also an additional imaging metric of disease. Larger CELs are more likely to evolve into black holes[8] (which represent areas of severe tissue destruction)[9] and have magnetization transfer ratios

indicative of significant myelin or axonal loss.[10] Most likely because it is a more objective identification, changes in CEL counts, more than size, are used as a primary endpoint to assess the efficacy of treatments in phase II and phase III clinical trials[11–13] and also in daily clinical practice. Similar to clinical exacerbations, the dynamics of CEL counts are known to be unpredictable and are characterized by high intrasubject and intersubject variability, especially during the natural history phase of the disease. Previous work from our group and other investigators as well has shown that CEL dynamics in MS are best described by a negative binomial (NB) distribution.[12,14]

Once they occur, acute clinical relapses of MS are usually treated with brief courses of corticosteroids, such as intravenous methylprednisolone (3–7 days) or dexamethasone (up to a few weeks).[15–17] Several treatments are instead available to prevent their occurrence.[16] Among these therapies, interferon beta-1b (IFNβ-1b) is the most common first-line agent.[18,19] Although the partial effectiveness of IFNβ-1b in MS is firmly established, several questions remain on the actual mode of action of IFNβ-1b. Clinicians lack the information as to whether IFNβ-1b is effective beyond the blood brain barrier breakdown. It remains unclear if the medication not only reduces the quantity of inflammation but also affects the quality of CELs once formed by promoting a better and faster resolution. It also remains unknown (1) how to predict which patient is destined to a better (or worse) outcome while on treatment and (2) the stability of the IFNβ-1b effect over time. Clinicians focus on tailoring the treatment to individual patients and tend to change therapy if a drug is poorly tolerated or ineffective. In addition, clinical trials do not provide enough information that is applicable to an individual patient. Personalized use of these treatment options is currently based on clinical judgments and expert opinions. However, there is a need to precisely understand the treatment effect and to quantify the variability between individuals.

We have recently reported that the NB distribution model best describes the monthly CEL count during the natural history of RRMS i.e., in the absence of any treatment but in the setting of corticosteroids administration for clinical relapses.[14] The model was found to adequately characterize the observed CEL dynamics in the studied patient population and had a good predictive ability. This analysis revealed that the corticosteroids helped in the resolution of existing CELs but did not have any effect in preventing the formation of new CELs. As logical continuation of our previous work, we aimed to develop a population model for IFNβ-1b effect in order to describe and quantify the drug effect and the associated variability. We used a dataset derived from patients with RRMS treated with IFNβ-1b for three years and imaged monthly for 42 months. Our analysis aimed to characterize the effect and the associated variability of IFNβ-1b in preventing CEL formation and promoting CEL resolution over an extensive period of time. Based on the model simulations and as a proof-of-concept, other treatment schemes, alternating periods of times with IFNβ-1b, and corticosteroids were explored.

METHODS
Study design
The population analysis was carried out using a combined dataset from the two separate studies, as detailed below (**Figure 1**). Both studies were performed at the National Institutes of Health, Bethesda, MD. The studies were approved by the Intramural Research Board of the National Institute of Neurological Disorders and Stroke.

Study I. Nine patients with RRMS were sequentially enrolled and imaged monthly for four years during a natural history phase. That is, none of the patients was on any treatment except intravenous methylprednisolone at 1 g/day for three to five days, or oral prednisone for the treatment of acute clinical relapses. The dose of oral prednisone was variable among patients and was dependent upon the severity of the symptoms. A total of 48 precontrast and postcontrast T1-weighted and T2-weighted MRIs were obtained in each subject using the imaging protocol previously described.[20,21] Clinical and imaging details of this patient cohort are described elsewhere.[20] At each monthly MRI, the numbers of CELs were identified by a radiologist (**Figure 1a**).

Study II. Monthly MRIs of 15 patients with RRMS from a six-month pretherapy phase followed by a 36-month therapy phase.[22] Forty-two consecutive precontrast and postcontrast T1-weighted and T2-weighted MRIs were obtained from each patient as previously described.[21] No patients were treated with any immunomodulatory or immunosuppressive therapy, except for steroids given for acute clinical relapses. During the therzapy phase (36 months), patients received a 250-μg dose of subcutaneous IFNβ-1b every other day. The total number of CELs was identified on each monthly MRI (**Figure 1b**).

Data analysis
All analyses were performed using NONMEM version 7.2 (Icon Development Solutions, Hanover, MD). The Laplacian numerical estimation method was used for parameter estimation. Between-subject variability was modeled using exponential functions and was expressed as coefficient of variation (%).

Natural history model. An NB model was recently shown to have the best predictive ability to characterize the observed CEL dynamics in patients in study I (no treatment; Eq. 1).[14] More information on these models has been published previously.[23,24] The NB model has two parameters λ and overdispersion parameter (OVDP) that represent the mean number of counts in a given time period, expected number of CELs in this case, and the degree of overdispersion between the observed mean and variance.

$$P(Y_i = n) = \left[\frac{\Gamma\left(n + \frac{1}{OVDP}\right)}{n! \times \Gamma\left(\frac{1}{OVDP}\right)}\right] \times \left(\frac{1}{1 + OVDP \times \lambda}\right)^{\frac{1}{OVDP}} \times \left(\frac{\lambda}{\frac{1}{OVDP} + \lambda}\right)^n$$

(1)

Here, $\lambda(t)$ is a function of the baseline expected number of CELs [$\lambda_0(t)$], the observation in the previous month DV_{t-1} (previous dependent variable [PDV]) and the observation
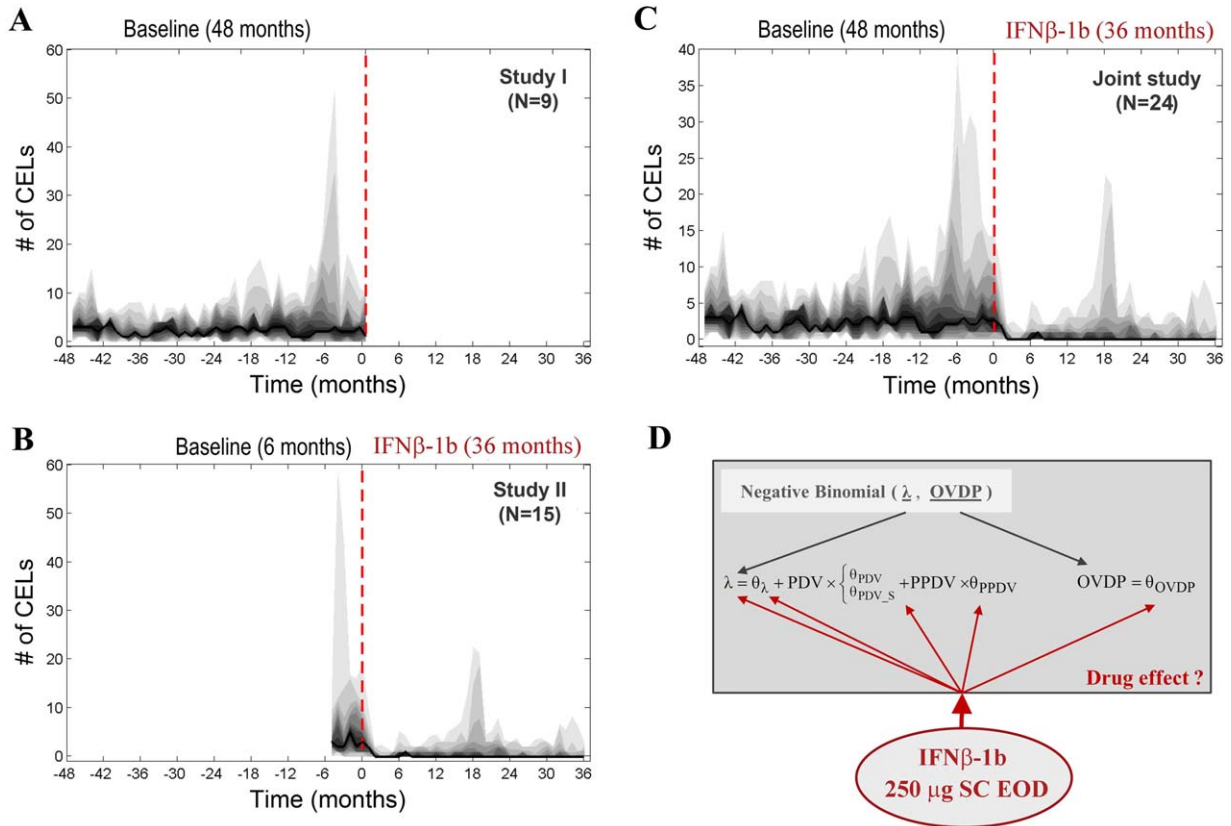
Figure 1 Datasets and the model. (**a**) Dataset from study I in which nine untreated patients, except for intravenous methylprednisolone or oral prednisone for the treatment of acute clinical relapses, for a 48 month time period. (**b**) Dataset from study II with 15 patients, which consisted of a six-month pretherapy phase followed by a 36-month therapy phase. Therapy consisted of subcutaneous administration of 250 μg interferon beta-1b (IFNβ-1b) every other day. (**c**) Data used for this analysis was a combination of data from both studies (I and II). Numbers of contrast enhancing lesions (CELs) are represented on the y-axis and time (in months) of treatment on the x-axis. Negative and positive numbers on the x-axis represent the pre-IFNβ-1b and the post-IFNβ-1b treatment periods, respectively. Different intervals were calculated from the observed data with a decreasing step of 10 starting from the 90% interval. Darker grey colors represent smaller intervals. Solid black line shows the observed median for the CELs. Red dashed line indicates the beginning of the treatment period. (**d**) Model used to study the effect of IFNβ-1b in patients with multiple sclerosis (MS). Effect of IFNβ-1b was evaluated on all the parameters of the model: λ_0, λ, overdispersion parameter (OVDP), θ_{PDV} and θ_{PPDV}. EOD, every other day; PDV, previous dependent variable; PPDV, previous previous dependent variable (PPDV); SC, subcutaneous.

two months before DV_{t-2} (previous previous dependent variable [PPDV]; Eq. 2).

$$\lambda(t) = \lambda_0(t) + PDV \times \theta_{PDV} + PPDV \times \theta_{PPDV} \quad (2)$$

This disease progression model defines the baseline expected number of CELs [$\lambda_0(t)$] as a constant $\theta_{\lambda 0}$ (**Table 1**; model M0).[14]

IFNβ-1b effect model. Using the model previously described for the natural history of the disease,[14] the effect of IFNβ-1b was evaluated on all the model parts $\lambda(t)$, $\lambda_0(t)$, OVDP, θ_{PDV}, and θ_{PPDV} using different functions of time. The time functions that were used to describe the inhibitory effect of IFNβ-1b on the parameter $\lambda_0(t)$ have been summarized in the **Supplementary Material S1**. However, all of them can easily be applicable to the rest of the parameters (i.e., $\lambda(t)$, OVDP, θ_{PDV}, and θ_{PPDV}). **Supplementary Material S1** shows the representative kinetics of inhibitory functions that were explored: M1, M2, M3, M4, and M5.

Model selection and evaluation. Selection between models was based on several factors: (i) visual inspection of goodness-of-fit plots for several descriptors of CEL profiles; (ii) the objective function value; and (iii) the precision of the parameter estimates. The minimum objective function value provided by NONMEM $-2\times$log[likelihood] ($-2LL$) served as a guide for model comparison. Statistical significance was set at $P < 0.01$. A decrease in $-2LL$ of 6.63 points for one additional parameter, was regarded as a significant model improvement corresponding to a *P* value of 0.01 for nested models. Akaike information criterion was calculated for selection among the non-nested models. This was calculated as equal to $-2LL + 2 \times n_p$ where n_p is the number of parameters in the model.[25] Model parameter estimates from the final model are presented with the corresponding relative standard error (RSE%), as a measure of parameter imprecision, which were computed from the results obtained from bootstrap analysis. Precision of parameter estimates expressed as 5th, 50th, and 95th percentiles were

Table 1 Summary of the discrete-distribution models evaluated with and without IFNβ-1b effect

Models			Parameters											−2 × log likelihood (Δ model)
Model M0 (Baseline)	$\theta_{\lambda 0}$ 0.472	θ_{OVDP} 0.563	θ_{PDV} 0.568	θ_{PDV_S} 0.328	θ_{PPDV} 0.123							$\omega_{\lambda 0}$ 0.89	ω_{PDV} 0.0365		3378.956 (−)
Model M1b[c]	$\theta_{\lambda 0}$ 1.81	θ_{OVDP} 0.223	θ_{PDV} 0.389	θ_{PDV_S} 0.23	θ_{PPDV} 0.0614	$\theta_{\lambda 0\_IFN\beta\text{-}1b}$ 0.119						$\omega_{\lambda 0}$ 0.944	ω_{PDV} 0.174	$\omega_{\lambda 0\_IFN\beta\text{-}1b}$ 2.12	3025.852[a] (−353.10 model M0)
Model M1a	$\theta_{\lambda 0}$ 1.82	θ_{OVDP} 0.219	θ_{PDV} 0.381	θ_{PDV_S} 0.221	θ_{PPDV} 0.0641	$\theta_{\lambda 0\_IFN\beta\text{-}1b}$ 0.0679	θ_{slp} 0.0264					$\omega_{\lambda 0}$ 0.946	ω_{PDV} 0.186	$\omega_{\lambda 0\_IFN\beta\text{-}1b}$ 2.33	3024.247 (−1.61 model M1b)
Model M2	$\theta_{\lambda 0}$ 1.81	θ_{OVDP} 0.245	θ_{PDV} 0.401	θ_{PDV_S} 0.267	θ_{PPDV} 0.011	$\theta_{\lambda 0\_IFN\beta\text{-}1b}$ 0.115		θ_{kout} 4.22				$\omega_{\lambda 0}$ 0.851	ω_{PDV} 0.192	$\omega_{\lambda 0\_IFN\beta\text{-}1b}$ 2.39	3028.709 (+2.86 model M1b)
Model M3b	$\theta_{\lambda 0}$ 1.8	θ_{OVDP} 0.219	θ_{PDV} 0.384	θ_{PDV_S} 0.212	θ_{PPDV} 0.0726	$\theta_{\lambda 0\_IFN\beta\text{-}1b}$ 0.148			θ_{k50} 5.39			$\omega_{\lambda 0}$ 0.948	ω_{PDV} 0.181	$\omega_{\lambda 0\_IFN\beta\text{-}1b}$ 2.6	3020.478 (−5.38 model M1b)
Model M3c	$\theta_{\lambda 0}$ 1.84	θ_{OVDP} 0.216	θ_{PDV} 0.377	θ_{PDV_S} 0.211	θ_{PPDV} 0.0716	$\theta_{\lambda 0\_IFN\beta\text{-}1b}$ 0.119			θ_{k50} 3.8	h 8.66		$\omega_{\lambda 0}$ 0.95	ω_{PDV} 0.186	$\omega_{\lambda 0\_IFN\beta\text{-}1b}$ 2.63	3010.562[b] (−15.29 model M1b)
Model M4c	$\theta_{\lambda 0}$ 1.82	θ_{OVDP} 0.215	θ_{PDV} 0.372	θ_{PDV_S} 0.207	θ_{PPDV} 0.0709	$\theta_{\lambda 0\_IFN\beta\text{-}1b}$ 0.12			θ_{k50} 4.1	h 77.2	θ_{min} 0.104	$\omega_{\lambda 0}$ 0.951	ω_{PDV} 0.194	$\omega_{\lambda 0\_IFN\beta\text{-}1b}$ 2.65	3009.133 (−16.72 model M1b)
Model M5c	$\theta_{\lambda 0}$ 1.81	θ_{OVDP} 0.218	θ_{PDV} 0.379	θ_{PDV_S} 0.212	θ_{PPDV} 0.0717	$\theta_{\lambda 0\_IFN\beta\text{-}1b}$ 0.17	θ_{slp} 0.0104		θ_{k50} 4	h 8.31		$\omega_{\lambda 0}$ 0.95	ω_{PDV} 0.181	$\omega_{\lambda 0\_IFN\beta\text{-}1b}$ 2.59	3009.464 (−16.39 model M1b)

Parameters λ and OVDP represent the mean number of counts in a given time period and the degree of overdispersion, respectively. Terms PDV and PPDV refer to covariates that took the values of the previous dependent variables. The parameter λ was modified by these first (PDV) and second (PPDV) order Markovian components. Values between parentheses are the changes in the objective function value relative to the specified reference model.
IFNβ-1b, interferon beta-1b; OVDP, overdispersion parameter; PDV, previous dependent variable; PDV_S, effect of corticosteroids on that parameter; PPDV, previous previous dependent variable.
[a]Significant improvement ($P < 0.001$). [b]Significant improvement ($P < 0.01$). [c]Selected model.

computed from the analysis of 500 nonparametric bootstrap datasets (sampling with replacement) performed using Perl-speaks-NONMEM.[26,27]

Models were evaluated based on: (i) visual numerical predictive checks (VNPC) and (ii) predicted intervals of VNPCs.

VNPC. The following dynamic descriptors were calculated for the observed data as well as for the simulated datasets (one per model): (1) probability of having of 0, 1, or ≥2 CELs at each month during the three-year treatment period; (2) maximum elapsed time (in months) without lesions during the three years of treatment; (3) mean elapsed time (in months) without lesions during the three-year treatment period; and (4) the number of cumulative CELs during the first, second, and third year of treatment. For each descriptor, the increasing percentiles from 5th to 95th were calculated. The results for the observed data and prediction intervals derived from the simulated data from different models were plotted and compared graphically.

Predicted interval of VNPC. One thousand studies were simulated using the selected model. The same dynamic descriptors that were described for the VNPC were used here. For every descriptor, the increasing percentiles from the 10th to 90th were calculated (one value per simulated study). Then, the 95% prediction interval was calculated and overlapped with the data.

More exhaustive evaluations of the selected model were then performed.

Probability distribution of CEL. Observed data was compared to the probability distribution of simulated data generated by the selected model.

Predicted interval for variance vs. mean of number of CELs. One thousand individuals were simulated with the selected model. The individual mean CEL counts and the individual variance for every patient were computed from the observed data. Similar computations were then carried out for each simulated individual and year for a total of 1,000 individuals. The results were divided into 20 intervals for the mean of CELs, with each interval containing 50 simulated individuals. For each interval, variances were binned and the median and 5th to 95th percentiles were calculated. Finally, the overall median and percentiles were represented graphically together with those corresponding to the observed data.

Model simulations for new treatments. Based on the selected model, simulations were carried out to explore the combined IFNβ-1b/corticosteroid therapies during six years (72 months). PDVs and PPDVs were initialized to zero for the simulations. The first 12 simulated months were then discarded in order to avoid any possible bias produced by the initialization of the PDVs and PPDVs. All the simulated treatment combinations were based on months with/without treatment, always assuming (i) the same IFNβ-1b dose, 250 μg IFNβ-1b given every other day during one month, and (ii) a binary variable if corticosteroids were administered to the simulated patient during the corresponding month. Four different IFNβ-1b schemes were simulated: (i) every month (the patient was under the IFNβ-1b treatment every month without interruptions), which equals a total of 60 months with IFNβ-1b; (ii) one month on, one month off, which equals a total of 30 months with IFNβ-1b; (iii) one month on and two months off, which equals a total of 20 months with IFNβ-1b; (iv) one month on and three months off, equals a total of 15 months with IFNβ-1b. Each
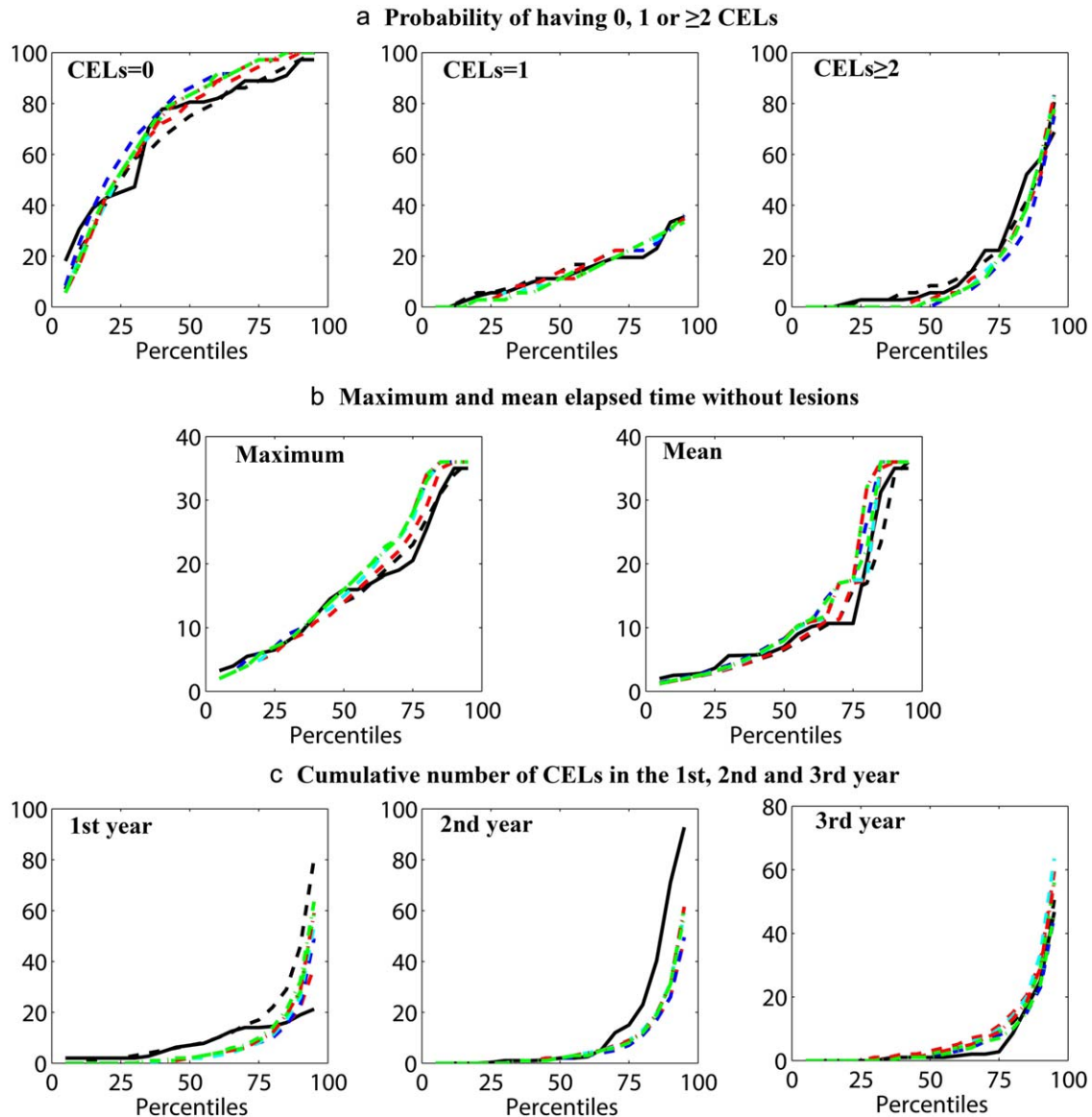
Figure 2 Visual numerical predictive check (VNPC) of the number of new contrast enhancing lesions (CELs). Different dynamic descriptors were calculated for the observed data (black solid line) and the simulated data from the models – M1b (black dashed line), M1a (red dashed line), M2 (blue dashed line), M3b (cyan dashed line), M3c (green dashed line), M4c (red dash dotted line), and M5c (green dash dotted line). The descriptors were evaluated at different percentiles from 5th to 95th with an increasing step of five. (**a**) Probability of having 0, 1, or ≥2 CELs at each month during the three-year treatment period (y-axis) vs. the percentiles on the x-axis. (**b**) Maximum and mean elapsed time without lesions (y-axis) during the three-year treatment period. Percentiles are shown on the x-axis. (**c**) Cumulative number of CELs (y-axis) in the first, second, and third year of the treatment period. Percentiles are shown on the x-axis.

of the IFNβ-1b schemes was combined with each of the four different corticosteroid schemes: (i) no corticosteroids; (ii) one month on and two months off, which equals a total of 20 months in which the patient was dosed with steroids; (iii) one month on and five months off, which equals a total of 10 months in which the patient was dosed with steroids; and (iv) one month on and eleven months off, which equals a total of five months in which the patient was dosed with steroids. Therefore, 16 combinations of treatments were simulated. Accumulated CELs were then calculated from the simulations and plotted as surface plots. The surfaces

for the first, second, and third years were calculated for 5th, 50th, and 95th percentiles. Simulations were carried out in NONMEM version 7.2 and the plots were created in MAT-LAB R2013a.

RESULTS

We analyzed the dynamics of CELs in 24 patients with RRMS (**Figure 1c**) where 15 of them were treated with IFNβ-1b during 36 months, with six-month pretherapy

Table 2 Parameter estimates from the final selected model M1b

| Parameters | Estimate (RSE%) | BSV (RSE%) | Bootstrap analysis 50th (5th−95th percentiles) | |
			Estimate	BSV (%)
$\theta_{\lambda 0}$	1.81 (24.1)	97.2 (41.7)	1.84 (1.21–2.69)	93.0 (56.0–125)
θ_{OVDP}	0.223 (21.7)		0.219 (0.151–0.300)	
θ_{PDV}	0.389 (12.3)	41.7 (39.7)	0.387 (0.314–0.474)	40.3 (24.4–53.4)
θ_{PDV_S}	0.230 (41.9)		0.211 (0.074–0.361)	
θ_{PPDV}	0.0614 (50.2)		0.0624 (0.0115–0.117)	
$\theta_{\lambda 0\_IFN\beta\text{-}1b}$	0.119 (43.3)	146 (52.0)	0.114 (0.0502–0.227)	136 (82.5–199)

Parameters λ and OVDP represent the mean number of counts in a given time period and the degree of overdispersion, respectively. Terms PDV and PPDV refer to covariates that took the values of the previous dependent variables. The parameter λ was modified by these first (PDV) and second (PPDV) order Markovian components. The RSE is the standard error calculated from the bootstrap analysis, from the bootstrap standard error and bootstrap mean of the bootstrap empirical distribution and displayed as a percentage.
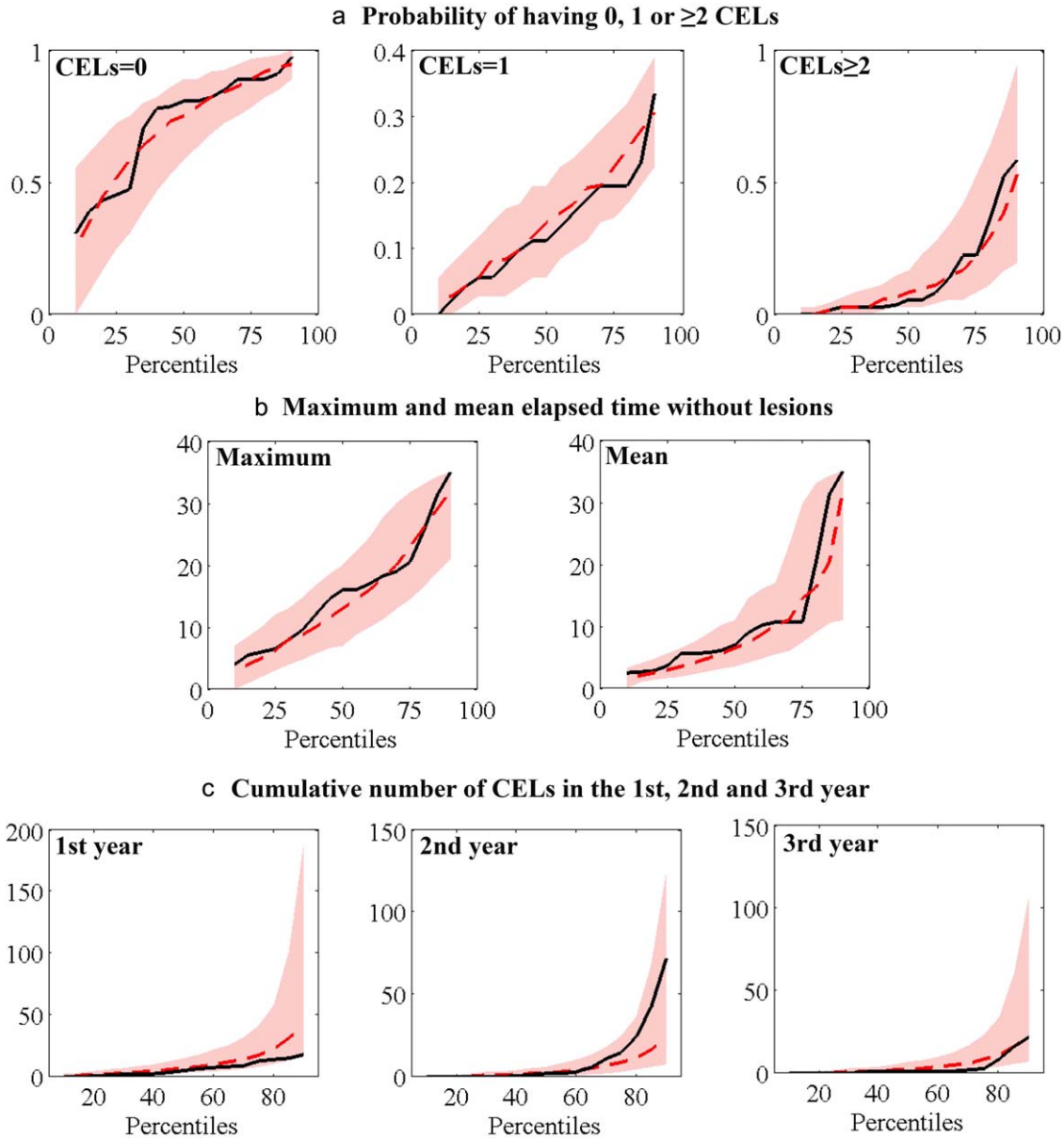BSV, between-subject variability; IFNβ-1b, interferon beta-1b; OVDP, overdispersion parameter; PDV, previous dependent variable; PDV_S, effect of corticosteroids on that parameter; PPDV, previous previous dependent variable; RSE, relative standard error.

(**Figure 1b**). The rest of the patients were imaged monthly for four years during a natural history phase (**Figure 1a**). Using the previously published NB model with first and second order Markovian parameters as the disease progression model,[14] we evaluated different mathematical functions of time (**Supplementary Material S1**) to best describe the effect of IFNβ-1b in patients with RRMS. Drug effect was evaluated on all the disease progression model parts (**Figure 1d**): the expected number of CELs (λ(t)), the baseline expected number of CELs (λ_0(t)), the overdispersion ($\theta_{OVDP} \times$ OVDP), the first order Markovian effect ($\theta_{PDV} \times$ PDV), and the second order Markovian effect ($\theta_{PPDV} \times$ PPDV; **Figure 1d**). An inhibitory effect on the baseline expected number of CELs (λ_0(t)) best described the data. No inhibitory effects of IFNβ-1b on the rest of the model parameters were found to be significant (P value > 0.01). This included the Markovian component ($\theta_{PDV} \times$ PDV), suggesting that IFNβ-1b does not contribute to resolution of already existing CELs. To characterize the drug effect, several types of inhibitory functions of time on the baseline expected number of CELs (λ_0(t)) were used (see Methods and **Supplementary Material S1**), including effects that: (1) may increase, stay constant, or decrease with time; (2) imitate an exponential decay in the effect; (3) may change with time following a sigmoid function with varying slopes; and (4) imitate a combination of a sigmoid and a linear model. **Table 1** summarizes the parameter estimates and the changes in objective function values observed among all the models that were evaluated. Several dynamic descriptors were calculated and compared for the observations and the simulated data (**Figure 2**). Models M1b and M3c were the two models that better described the data. This suggests that IFNβ-1b effect can be described as either an instant effect on λ_0(t) that stays constant with time or an effect on λ_0(t) that changes in a sigmoid shape with time and with a slope greater than one. **Supplementary Material S3** shows the results for VNPCs for models M1b and M3c for all the dynamic descriptors. Based on all the dynamic descriptors that were evaluated, model M1b performed slightly better with two less degrees of freedom. Therefore, based on the number of model parameters, objective function values (**Table 1**), and the precision of the parameter estimates, the NB distribution model with a constant effect (model M1b) was the selected model: λ_0(t)$= \theta_{\lambda 0\_IFN\beta\text{-}1b}$.

This change on λ_0(t) produces an instant effect on λ(t), that is, on the expected number of CELs. However, because of the Markovian effects, the drug effect takes approximately six months to reach the steady-state (see **Supplementary Material S2**). As soon as the patients start the IFNβ-1b treatment, a decrease in the number of CELs is observed, reaching the minimum value for CELs after six months. The selected drug model also implies that on a population level, the inhibitory effect of IFNβ-1b persisted over time. The parameter that defines the λ_0(t) function during the treatment, $\theta_{\lambda 0\_IFN\beta\text{-}1b}$, was 93.4% smaller than the one without treatment $\theta_{\lambda 0}$. **Table 2** summarizes the parameter estimates along with their RSE% and percentiles from the bootstrap. The bootstrap medians were very similar to the final estimates. The bootstrap confidence intervals did not include any zero. In general, fixed and random effect parameters were adequately estimated. No bias was detected. NONMEM code for the final selected model can be found as part of the **Supplementary Material S4**.

To better evaluate the predictive ability of the selected model M1b, 95% predicted intervals for the dynamic descriptors described above were calculated based on simulated data (**Figure 3**). The model captures the observed percentiles of all the descriptors reasonably well. To evaluate the model, the CEL count distributions for the observed and simulated data from the selected model M1b were also compared (**Supplementary Material S5**). **Figure 4** shows the 95% predicted interval for variance vs. mean number of CELs with the patient data. The model was able to capture the relationship between the mean number of CELs and the variance of these counts. Based on these model evaluation methods, it was concluded that model M1b adequately describes the observed data and their dispersion. This therapeutic effect was maintained during the 36-month treatment period. That is, there is neither a decrement nor an increment in the IFNβ-1b effect with time.

Based on the final selected model M1b, simulations were carried out to assess the combined effects of IFNβ-1b and corticosteroids for MS. This was a proof-of-concept for modeling any ideal combination-therapy approach. **Figure 5** shows the surface plots describing the accumulated CELs for the first, second, and third years during the simulated treatment period. It can be seen that both corticosteroid and IFNβ-1b treatments result in lowering of the

a Probability of having 0, 1 or ≥2 CELs

b Maximum and mean elapsed time without lesions

## c Cumulative number of CELs in the 1st, 2nd and 3rd year

Figure 3 Predicted interval of visual numerical predictive check of the number of new contrast enhancing lesions (CELs). Different dynamic descriptors were compared for the observed and the simulated data based on the final selected model M1b. For each of the descriptors, 10th to 90th percentiles were calculated with an increasing step size of five. Solid black line shows the observed median. The 95% predicted interval is represented by the red area and the simulated median is represented by the dashed red line. (**a**) Probability of having 0, 1, or ≥2 CELs at each month during the three-year treatment period (y-axis) vs. the percentiles on the x-axis. (**b**) Maximum and mean elapsed time without lesions (y-axis) during the three-year treatment period. Percentiles are shown on the x-axis. (**c**) Cumulative number of CELs (y-axis) in the first, second, and third year of the treatment period. Percentiles are shown on the x-axis.

accumulated CELs. Higher frequency of either of them might be sufficient without the other being administered.

DISCUSSION

We applied a population analysis approach to describe CEL dynamics during IFNβ-1b treatment in patients with RRMS imaged monthly. We used a previously published model for the natural history of the disease. As it was previously published,[14] the need of Markovian factors are attributable to the fact that the CEL counts noted every month were the total number of CELs, and, thus, older lesions observed in previous months might persist in the current one. This result suggested that although the symptoms that appear during episodic acute periods in patients with MS usually last less than a month, the active inflammatory event might persist for a longer period of time.
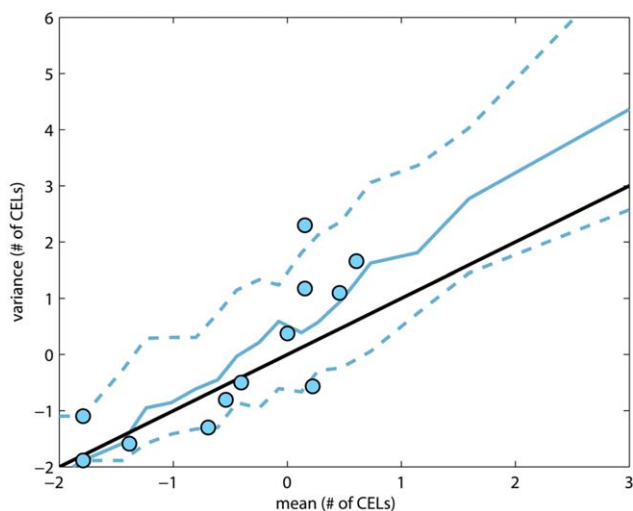
Figure 4 Predicted interval for variance vs. mean of number of contrast enhancing lesions (CELs). Variance and mean of number of CELs in each patient (observed-simulated) were calculated and represented in natural logarithmic scale. Solid line in black corresponds to the identity line. Blue circles are the observations. Blue dashed lines correspond to the 5th and 95th quartiles of simulated data and solid blue line corresponds to the median of simulated data.

These focal inflammatory events in the central nervous system enclose very complex dynamics that are a result of multiple feedbacks among different immune line cells and cytokines.[4]

We evaluated several temporal functions to best describe the effect of IFNβ-1b over time in patients suffering from RRMS. The model that best described the effect of IFNβ-1b was the NB distribution model with an immediate effect on $\lambda_0(t)$. This model indicated that the steady-state drug effect was reached after six months and was maintained during the 36-month treatment period. That is, on a population level, there is neither a decrement nor an increment in the IFNβ-1b effect with time. This should, however, only be interpreted in terms of this dataset and the associated analysis. For this set of patients and the duration of treatment (36 months), there was no loss of effect observed. Had the data been collected for a longer duration, there is a possibility that the loss of IFNβ-1b effect may have been observed. Care also needs to be taken in comparing our findings with the ones existing in literature. On a population basis, this finding is certainly in alignment with all large clinical trials. On an individual (i.e., patient) level, however, previous results indicate that when adopting a 60% reduction in the number of CELs as criterion of being a responder, only two-thirds of the patients achieve and maintain a constant response to the drug over a three-year therapy phase.[22]

In this article, we incorporated the drug action to a disease progression model that describes the natural history of the CELs. As mentioned by Holford,[28] the drug effect can be categorized as symptomatic effect and/or disease-modifying effect. The distinction between the two of them can be very difficult, depending on the study designed too.

In the present analysis, IFNβ-1b effect can be defined as an additive effect: $\lambda_0(t) = \theta_{\lambda 0}\text{-}E_D$, where $\theta_{\lambda 0\_IFN\beta\text{-}1b} = \theta_{\lambda 0}\text{-}E_D$. Therefore, the effect of IFNβ-1b might be considered symptomatic. Similarly, the steroid effect might also be a symptomatic drug effect, because it can be described as an additive effect on the impact of previous CELs. However, in the specific case of RRMS and using the number of CELs as a biomarker for disease progression, we discussed the applicability of such definitions (because the CEL count does not increase with time). The population analysis performed here identified the inhibitory effect on the baseline expected number of CELs ($\lambda_0(t)$). This implies that the reduction in the number of CELs is produced by the inhibition of the formation of new CELs. The effect of IFNβ-1b on the rest of the model components was also explored but found not significant. The lack of effect on the Markovian component suggests that IFNβ-1b does not promote the resolution of CELs that have already been formed. The effect of IFNβ-1b beyond the blood brain barrier breakdown is, at the moment, poorly understood. It is known that the drug reduces the occurrence of black holes,[29] a pathologically more advanced lesion type that may originate from up to 40% of CELs.[30] Such a reduction, however, may be the indirect effect of a reduction in CEL count operated by IFNβ-1b, not necessarily the effect of the medication in promoting the formation of CELs with less severe inflammation or ameliorating the outcome of the newly formed ones. There is little evidence in support of the fact that IFNβ-1b has poor effect on the resolution of inflammation. A subset of patients included in this study (i.e., 6 patients) was imaged monthly for 72 months[31] (i.e., 36 months before therapy and 36 months during IFNβ-1b therapy). In this cohort of patients, it was found that although the absolute count of black holes was reduced during treatment, the proportion of black holes originating from CELs was not reduced by the medication.[31] Because the conversion of CELs into black holes is an indirect sign of more aggressive pathology, it was concluded that IFNβ-1b was not able to affect the severity of CELs once these were formed. In a larger cohort of 30 patients with RRMS treated with IFNβ-1b at the same dosage and regimen, it was found that although the count of CELs was dramatically reduced during treatment, the average size of each CEL was not affected.[32] The results of the current analysis provide an additional demonstration that IFNβ-1b, on a population level, may affect the count of CELs, but once a CEL is formed the medication is not effective in promoting lesion resolution. Interestingly, it was previously suggested by our group that the use of steroids would contribute to the inflammatory resolution of persistent CELs but not affecting generation of the new CELs.[14] From a pharmacological perspective, the findings imply that although IFNβ-1b successfully decreases the formation of new lesions, it has no effect in promoting a better or faster resolution of existing CELs once these have been formed. These results reflect the utility of this modeling approach for drug effect evaluation, providing a quantitative framework that can support the informed design of future longitudinal studies and other clinical trials. Various simulations were carried out using the final selected model to optimize the combined IFNβ-1b and
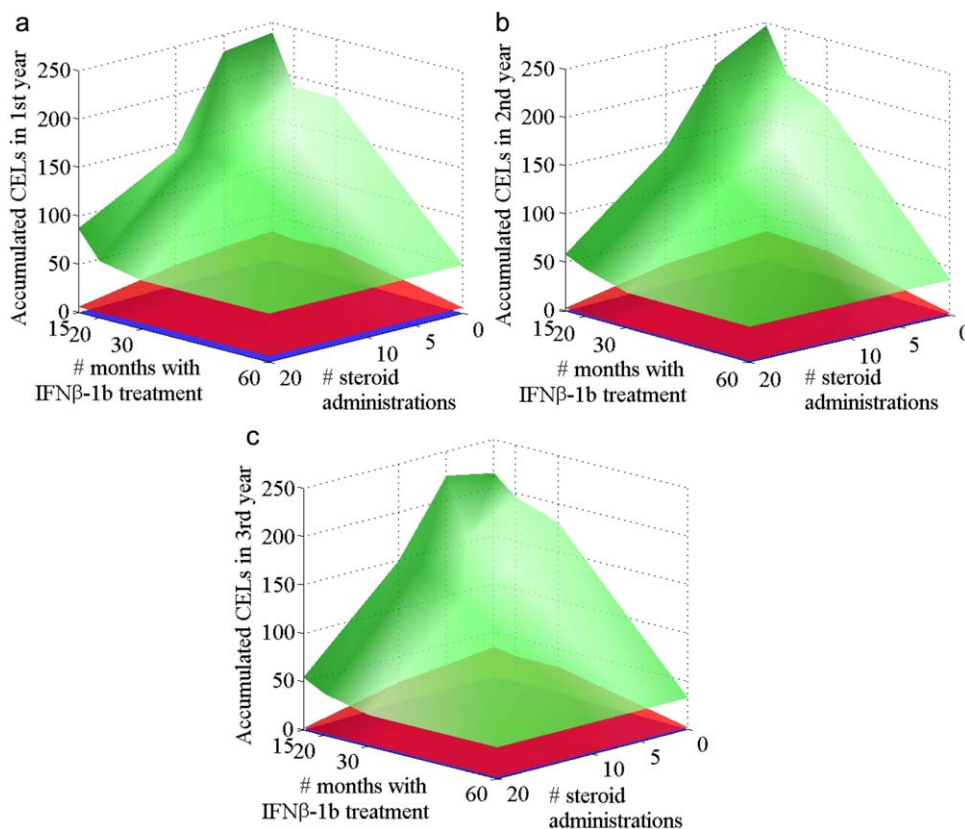
Figure 5 Simulated interferon beta-1b (IFNβ-1b) and corticosteroid treatments in patients with multiple sclerosis (MS). Number of accumulated contrast enhancing lesions (CELs) for the first (**a**), second (**b**), and third years (**c**) of the treatment period were simulated using the final model M1b and are shown as surface plots. The blue, red, and green surfaces represent the 5th, 50th, and 95th percentiles, respectively. Number of months of corticosteroid administrations and IFNβ-1b treatment are shown on the x-axis and y-axis, respectively. Numbers of simulated accumulated CELs are represented on the z-axis.

corticosteroid therapy. These simulations were not intended to be translated at the clinical level because of the well-known adverse effects of chronic use of corticosteroids as well as the notion that numerous additional treatments are available today to be used in combination with IFNβ-1b. The aim of the simulations was to provide proof-of-concept that our modeling approach can be used for proposing new combination treatments in MS. According to the US Food and Drug Administration[33] and European Medicines Agency[34] guidelines, recommended doses of IFNβ-1b are fixed. For the simulations, doses of IFNβ-1b as well as corticosteroids were kept constant and their frequencies of administration were altered, and their effect on the accumulated CELs was simulated for a five-year (60 months) therapy period. It was found that both corticosteroid and IFNβ-1b treatments resulted in lowering of the accumulated CELs but by different mechanisms of action as they affect different parameters in the model. Based on the simulations, more frequent dosing of either one given alone may be sufficient. However, considering that IFNβ-1b and corticosteroids act via different biological mechanisms, it is the concomitant administration of both drugs that increases the probability of a successful therapeutic outcome in individual patients. IFNβ-1b and corticosteroid combinations might be optimized for a better clinical outcome while improving toler-

ability and compliance. No data after INFβ-1b treatment were available in this analysis. Clinical trials, including disease progression recovery, would be useful and informative but difficult or impossible to apply because of ethical issues. Because this analysis was performed based on the available data, clinical conclusions derived from the simulations performed as proof-of-concept to show different treatment scenarios have to be taken with precaution.

1. Goldenberg, M.M. Multiple sclerosis review. *P. T.* **37**, 175–184 (2012).
2. Singh, V.K., Mehrotra, S. & Agarwal, S.S. The paradigm of Th1 and Th2 cytokines: its relevance to autoimmunity and allergy. *Immunol. Res.* **20**, 147–161 (1999).
3. Navikas, V. & Link, H. Review: cytokines and the pathogenesis of multiple sclerosis. *J. Neurosci. Res.* **45**, 322–333 (1996).
4. Vélez de Mendizábal, N. *et al.* Modeling the effector – regulatory T cell cross-regulation reveals the intrinsic character of relapses in multiple sclerosis. *BMC Syst. Biol.* **5**, 114 (2011).
5. Rudick, R.A. & Polman, C.H. Current approaches to the identification and management of breakthrough disease in patients with multiple sclerosis. *Lancet Neurol.* **8**, 545–559 (2009).
6. Calabresi, P.A. Diagnosis and management of multiple sclerosis. *Am. Fam. Physician* **70**, 1935–1944 (2004).
7. Harris, J.O., Frank, J.A., Patronas, N., McFarlin, D.E. & McFarland, H.F. Serial gadolinium-enhanced magnetic resonance imaging scans in patients with early, relapsing-remitting multiple sclerosis: implications for clinical trials and natural history. *Ann. Neurol.* **29**, 548–555 (1991).
8. Ciccarelli, O. *et al.* Magnetic resonance outcome of new enhancing lesions in patients with relapsing-remitting multiple sclerosis. *Eur. J. Neurol.* **6**, 455–459 (1999).
9. Brück, W., Bitsch, A., Kolenda, H., Brück, Y., Stiefel, M. & Lassmann, H. Inflammatory central nervous system demyelination: correlation of magnetic resonance imaging findings with lesion pathology. *Ann. Neurol.* **42**, 783–793 (1997).
10. Filippi, M., Rocca, M.A. & Comi, G. Magnetization transfer ratios of multiple sclerosis lesions with variable durations of enhancement. *J. Neurol. Sci.* **159**, 162–165 (1998).
11. Bielekova, B. & Martin, R. Development of biomarkers in multiple sclerosis. *Brain* **127**(Pt 7), 1463–1478 (2004).
12. Sormani, M.P., Bruzzi, P., Miller, D.H., Gasperini, C., Barkhof, F. & Filippi, M. Modelling MRI enhancing lesion counts in multiple sclerosis using a negative binomial model: implications for clinical trials. *J. Neurol. Sci.* **163**, 74–80 (1999).
13. Ziemann, U., Wahl, M., Hattingen, E. & Tumani, H. Development of biomarkers for multiple sclerosis as a neurodegenerative disorder. *Prog. Neurobiol.* **95**, 670–685 (2011).
14. Velez de Mendizabal, N. *et al.* Predicting relapsing-remitting dynamics in multiple sclerosis using discrete distribution models: a population approach. *PLoS One* **8**, e73361 (2013).
15. Beck, R.W. *et al.* The effect of corticosteroids for acute optic neuritis on the subsequent development of multiple sclerosis. The Optic Neuritis Study Group. *N. Engl. J. Med.* **329**, 1764–1769 (1993).
16. Cree, B.A.C. Multiple sclerosis. In *Current Diagnosis and Treatment in Neurology* (ed. Brust, J.C.M.). (New York, New York, Lange Medical Books/McGraw-Hill Medical, 2007).
17. La Mantia, L., Eoli, M., Milanese, C., Salmaggi, A., Dufour, A. & Torri, V. Double-blind trial of dexamethasone versus methylprednisolone in multiple sclerosis acute relapses. *Eur. Neurol.* **34**, 199–203 (1994).
18. Prisms Study Group and the University of British Columbia MS/MRI Analysis Group. PRISMS-4: long-term efficacy of interferon-beta-1a in relapsing MS. *Neurology* **56**, 1628–1636 (2001).
19. Paty, D.W. & Li, D.K. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. UBC MS/MRI Study Group and the IFNB Multiple Sclerosis Study Group. *Neurology* **43**, 662–667 (1993).
20. Bagnato, F. *et al.* Evolution of T1 black holes in patients with multiple sclerosis imaged monthly for 4 years. *Brain* **126**(Pt 8), 1782–1789 (2003).
21. Chiu, A.W. *et al.* A case study on the effect of neutralizing antibodies to interferon beta 1b in multiple sclerosis patients followed for 3 years with monthly imaging. *Clin. Exp. Immunol.* **150**, 61–67 (2007).
22. Chiu, A.W. *et al.* Heterogeneity in response to interferon beta in patients with multiple sclerosis: a 3-year monthly imaging study. *Arch. Neurol.* **66**, 39–43 (2009).
23. Plan, E.L., Maloney, A., Trocóniz, I.F. & Karlsson, M.O. Performance in population models for count data, part I: maximum likelihood approximations. *J. Pharmacokinet. Pharmacodyn.* **36**, 353–366 (2009).
24. Plan, E.L. Modeling and simulation of count data. *CPT Pharmacometrics Syst. Pharmacol.* **3**, e129 (2014).
25. Ludden, T.M., Beal, S.L. & Sheiner, L.B. Comparison of the Akaike Information Criterion, the Schwarz criterion and the F test as guides to model selection. *J. Pharmacokinet. Biopharm.* **22**, 431–445 (1994).
26. Lindbom, L., Pihlgren, P. & Jonsson, E.N. PsN-Toolkit–a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. *Comput. Methods Programs Biomed.* **79**, 241–257 (2005).
27. Lindbom, L., Ribbing, J. & Jonsson E.N. Perl-speaks-NONMEM (PsN)–a Perl module for NONMEM related programming. *Comput. Methods Programs Biomed.* **75**, 85–94 (2004).
28. Holford, N. Clinical pharmacology = disease progression + drug action. *Br. J. Clin. Pharmacol.* **79**, 18–27 (2015).
29. van Waesberghe, J.H. *et al.* Patterns of lesion development in multiple sclerosis: longitudinal observations with T1-weighted spin-echo and magnetization transfer MR. *AJNR Am. J. Neuroradiol.* **19**, 675–683 (1998).
30. Bagnato, F. Application of interferon beta-1b in multiple sclerosis. *US Neurol.* **7**, 46–54 (2011).
31. Bagnato, F. *et al.* Effects of interferon beta-1b on black holes in multiple sclerosis over a 6-year period with monthly evaluations. *Arch. Neurol.* **62**, 1684–1688 (2005).
32. Gaindh, D. *et al.* The effect of interferon beta-1b on size of short-lived enhancing lesions in patients with multiple sclerosis. *Expert Opin. Biol. Ther.* **8**, 1823–1829 (2008).
33. US Food and Drug Administration. Interferon beta-1b, Betaseron. <http://www.accessdata.fda.gov/drugsatfda_docs/label/2003/ifnbchi031403lb.pdf> (2003). Accessed 15 September 2014.
34. European Medicines Agency. Extavia (Interferon Beta-1b). <http://0077ww.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Summary_for_the_public/human/000933/WC500034702.pdf> (2012). Accessed 15 September 2014.

Supplementary information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website (http://www.wileyonlinelibrary.com/psp4)