Fingolimod is the first orally administered drug which has been approved for the treatment of patients with relapsing and remitting forms of multiple sclerosis (RRMS).1,2 Fingolimod is the first sphingosine-1-phosphate (S1P) receptor modulator. Fingolimod needs to be phosphorylated to the active moity, S-enantiomer fingolimod-P.3 Fingolimod-P is a functional antagonist of the S1P receptor, which reduces the peripheral lymphocyte count by down-modulation of the S1P1 receptor on lymphocytes.4 This results in a reduced egress of lymphocytes from the lymph nodes; in particular, autoaggressive T-cells that perform a central role in the MS inflammatory disease process are prevented from recirculating to the central nervous system. Fingolimod-P is reversibly dephosphorylated back to the inactive form, fingolimod. At steady state (SS), fingolimod and fingolimod-P are in a dynamic equilibrium.

Due to the presence of S1P receptors in multiple tissues, fingolimod manifests a number of other biological effects in addition to the reduction in circulating lymphocytes. These include a transient reduction in heart rate and atrioventricular conduction on treatment initiation, a dose-dependent mild increase in airway resistance, macular edema, a mild increase in blood pressure, and asymptomatic elevation in serum levels of hepatic transaminases.5–7

Fingolimod at both dose groups (0.5 and 1.25 mg q.d.) showed superior efficacy in reducing the frequency of relapse relative to placebo in a 2-year study in patients with RRMS, and also demonstrated superior efficacy over a current standard of care, interferon β-1a (Avonex) in a 1-year study with little difference between the two dose groups.1 The low dose (0.5 mg q.d.) was proposed and approved for the treatment of RRMS.1

Although fingolimod has demonstrated superior efficacy over both placebo and an active control, there was a dose-dependent increase in several adverse events such as increase in alanine transaminase, macular edema, and bradycardia (Figure 1). As a result, it was reasonable to raise the question whether doses even lower than 0.5 mg would provide sufficient efficacy with less safety concerns. Therefore, our analysis focused on estimating the efficacy at doses lower than 0.5 mg based on analysis of the exposure (fingolimod concentration)–response (relapse rate reduction) relationship.

A typical efficacy analysis focuses on group comparison using a statistical method such as analysis of variance or analysis of covariance, and a decision is made based on the $P$ value. In the exposure–response analysis, we use individual concentration levels in the blood instead of the assigned dose because patients at the same dose could have different concentration levels. The concentration level is related to the response based on certain assumptions on the structure of the relationship. A maximum effect ($E_{\text{max}}$) model is a commonly used model for pharmacodynamic or clinical endpoints, and the hill coefficient parameter ($h$) governs the shape of the relationship. Figure 2 illustrates the role of the hill coefficient parameter in the shape of the exposure–response relationship for an $E_{\text{max}}$ model.

Under certain circumstances, a biomarker can provide valuable information. For example, the key pharmacodynamic effect of fingolimod is a dose-dependent reduction of the peripheral lymphocyte count mediated by down-modulation of the S1P1 receptor on lymphocyte counts, which is believed to be the mechanism to produce clinical benefit in terms of reduction in relapse rate. This served as the major rationale for the sequential linkage among exposure, biomarker, and the clinical endpoint.

As the ultimate goal is to predict the clinical endpoint (annualized relapse rate (ARR) which was computed as the total number of confirmed relapses for all the patients divided by the total number of days on the study for all the patients, multiplied by 365.25) at exposure levels of unstudied doses, confidence in the model structure is crucial. The main challenge
in analyzing the relationship between the fingolimod exposure and the clinical endpoint was that the hill coefficient in the model could not be estimated successfully due to the lack of data at lower exposure levels (declining part of the relationship in Figure 2). As a result, it was not possible to predict ARR precisely at doses lower than 0.5 mg. In order to bridge this gap, pharmacodynamic biomarker information was used to link ARR and drug exposure. Specifically, the average lymphocyte count at the SS was used to link drug exposure to ARR rather than directly modeling the relationship between drug exposure and ARR. By doing this it was expected that the fingolimod exposure–ARR relationship would be quantified more precisely given the availability of lymphocyte count data and the presumed relationship between lymphocyte counts and ARR.

In this article, we aim to show how biomarker data can be used as a bridge in exposure–response analysis to estimate clinical endpoint response at certain dose levels when the direct relationship between exposure and the clinical endpoint can not be quantified reliably.

RESULTS

The data of all patients with evaluable pharmacokinetic measurements were pooled and used in the exposure–response analysis. The active metabolite, fingolimod phosphate (fingolimod-p), concentration at SS was used as the exposure measure.

As our goal was to predict ARR at an unstudied dose of 0.25 mg, the potential range of exposure at 0.25 mg was examined. As fingolimod-p showed dose proportionality, the concentration at a dose of 0.25 mg would be half the concentration at the dose of 0.5 mg. The between-patient variability of exposure at 0.25 mg was assumed to be the same as that for 0.5 mg.

Although the fingolimod-p concentration–ARR relationship could not be characterized, fingolimod-p concentration was successfully related to the biomarker level (lymphocyte counts). In addition, it is reasonable to assume that lymphocyte counts should correlate with the clinical endpoint, ARR, due to the mechanism of action for fingolimod.

The time course of lymphocyte counts showed that the number of lymphocyte counts appeared to be at SS by month 2 (data not shown). Therefore, the lymphocyte counts after month 2 (\(N = 3–6\) per patient) were used to compute average SS lymphocyte counts for each patient. Figure 3 presents the relationship between lymphocyte counts at SS and fingolimod-p concentration, which shows that our model describes the observed data reasonably well.

As the next step, lymphocyte count was linked to the clinical endpoint, ARR, and the result is shown in Figure 4. The lymphocyte count–ARR relationship suggests that a mean
A lymphocyte count below $10^9/l$ would be necessary to achieve a mean ARR of ~0.2 and further lower lymphocyte counts do not provide additional ARR benefit.

The predicted lymphocyte counts were used as a bridge to link exposure to ARR. This was done in two steps using the PRED statement in PROC NLMIXED in SAS 9.2 (SAS Institute, Cary, NC): (i) the lymphocyte counts were predicted over the observed concentration range from the lymphocyte concentration model and (ii) the predicted lymphocyte counts were used to predict ARR from the ARR-lymphocyte counts model. Figure 5 shows the results for the concentration–ARR relationship using lymphocyte counts as a bridge. The shape of the concentration–ARR relationship was well quantified with reasonable precision.

The final result is presented in Table 1. The model-predicted average ARR is 0.26 (95% confidence interval: 0.22–0.30) at 0.25 mg, which could be almost as effective as 0.5 mg (0.21, 95% confidence interval: 0.19–0.24).

**DISCUSSION**

Modeling and simulation has become an increasingly important component in drug development. Especially, exposure–response analyses for both efficacy and safety have been applied to guide dose selection.8–11 The quantitative relationship between drug exposure and certain responses is critical in justifying a dosing regimen to balance risk and benefit. The challenge in exposure–response analysis for fingolimod dose selection was that the model structure could not be identified due to the lack of data at a lower level of exposure. Our analyses showed that an intermediate biomarker, such as lymphocyte counts, could play an important role in quantifying the exposure–clinical endpoint relationship as the reduction in lymphocyte counts is believed to be the mechanism of action for fingolimod to reduce ARR. The additional information contained in the exposure–biomarker relationship and the assumed biomarker–clinical endpoint relationship served as the bridge to link the drug exposure to the clinical endpoint. This approach allowed us to construct the structure of the relationship which was not possible by modeling the relationship between concentration and clinical endpoint directly. Indeed, ARR at 0.25 mg was predicted with reasonable precision by our approach. The improved precision was the outcome of using additional biomarker data and making additional assumptions about the relationships among drug exposure, biomarker response, and clinical endpoint. Despite the improved precision, additional confirmation was considered necessary given the extrapolative nature of our inferences about doses below the lowest dose studied in the trial. As a result, our analyses served as the justification for the sponsor to conduct post-marketing studies to explore the effectiveness of a lower dose such as 0.25 mg.12

**METHODS**

*Data.* Data from two phase III studies were included in the analyses: study 1 was a 24-month, double-blind, multicenter, parallel-group study comparing the efficacy and safety of 0.5 and 1.25 mg fingolimod q.d. vs. placebo; study 2 was a 12-month,
double-blind, randomized, multicenter, parallel-group study comparing the efficacy and safety of 0.5 and 1.25 mg fingolimod q.d. vs. interferon β-1a (Avonex; Biogen Idec, Cambridge, MA) administered intramuscularly once weekly in patients with RRMS. Blood samples were taken at pre-dose in all the patients at multiple visits. Approximately 2,500 patients between 18 and 55 years of age, who got diagnosed as a RRMS, were included in the analyses.12

Model. An inhibitory E\textsubscript{max} model was applied to describe the exposure–response relationship for both clinical endpoint (ARR) and biomarker (lymphocyte counts). Fingolimod-p concentration at SS was used as an exposure measure.

A normal distribution was applied to lymphocyte counts to quantify the relationship between lymphocyte counts and fingolimod-p (Model 1). A negative binomial distribution was assumed for the number of confirmed relapses with each patient’s on-study days as an offset variable to account for different number of study days in Model 2. The predicted lymphocyte counts from Model 1 were used to link fingolimod-p to the clinical endpoint, ARR. For the final prediction of ARR within such an exposure range. The between-subject variability for exposure and biomarker response was taken into consideration in this procedure to avoid using a median or mean exposure under 0.25 mg as the exposure of interest for ARR prediction for a group of patients who are known to have different drug exposures and different biomarker responses under 0.25 mg. This extra step of incorporating the between-subject variability on exposure and biomarker may not be necessary if the exposure–response relationship is linear. However, given the nonlinear relationship and the exposure of interest being close to the plateau range of the exposure–response relationship, it was necessary to incorporate the between-subject variability on exposure and biomarker in ARR prediction in addition to including the parameter uncertainties.

The final models for each component used for the analysis are displayed below.

Model 1: Fingolimod-p concentration-lymphocyte counts at SS relationship

\[ Y_i \sim \mathcal{N}(\mu_i, \sigma^2) \]

\[ \mu_i = \beta_0 \times (1 - f(\text{concs}) \text{if placebo}) \]

\[ f(\text{concs}) = \frac{E_{\text{max}} \times \text{lymphocytes}^n}{E_{50} + \text{lymphocytes}^n} \text{if fingolimod} \]

\[ f(\text{concs}) = \beta_i, \text{if interferon} \]

Where

\[ f(\text{concs}) = 0, \text{if placebo} \]

\[ f(\text{concs}) = \frac{E_{\text{max}} \times \text{lymphocytes}^n}{E_{50} + \text{lymphocytes}^n}, \text{if fingolimod} \]

\[ f(\text{concs}) = \beta_i, \text{if interferon} \]

\[ Y_i \text{ represents the peripheral lymphocyte counts at SS for patient i, which is assumed to follow a normal distribution with mean } \mu_i \text{ and variance } \sigma^2. \]

Where

\[ E_{\text{max}} \text{ and } E_{50} \text{ (half-maximal effective concentration) parameters are defined as the maximum response and the concentration level to reach 50% of the maximum response, and } h \text{ is the hill coefficient which reflects the steepness of model structure. } E_{50} \text{ and } E_{\text{max}} \text{ were transformed to ensure } E_{50} \text{ to be positive (log transformation) and } E_{\text{max}} \text{ to be between 0 and 1 (logit transformation).} \]

Model 2: Lymphocyte counts at SS–ARR relationship

\[ Y_i \sim \text{NB}(\mu_i, k) \]

\[ \log(\mu_i) = \beta_0 \times (1 - f(\text{lymphocytes})) + \log(t) \]

Where

\[ f(\text{lymphocytes}) = \frac{E_{\text{max}} \times \text{lymphocytes}^n}{E_{50} + \text{lymphocytes}^n} \]

\[ Y_i \text{ represents the number of confirmed relapses for each patient i, which follows a negative binomial distribution with mean } \mu_i \text{ and dispersion parameter } k. \]

\[ \log(t) \text{ is the offset variable to account for a patient’s different study period, which was calculated as on study day/365.25 to annualize relapse rate. } E_{50} \text{ parameters are defined as the lymphocyte counts level to reach 50% of the maximum response.} \]

The parameter estimates from each model are presented in Table 2 and the code for the analysis is provided in Supplementary Data online.

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| Table 2 The parameter estimates from two models |
|------------------------------------------------|
| **Model 1** | **Model 2** |
| **Parameters** | **Estimate (SE)** | **Parameters** | **Estimate (SE)** |
| Mean lymphocyte counts for placebo (\(\beta_0\)) | 1.76 (0.18) | ARR at lymphocyte count = 0 | 0.20 (0.05) |
| Mean lymphocyte counts for interferon β-1a (\(\beta_i\)) | 0.04 (0.01) | EL\(_{50}\) | 0.9 (0.09) |
| EC\(_{50}\) | 0.28 (0.17) | E\(_{\text{max}}\) | 0.5 (0.04) |
| E\(_{\text{max}}\) | 1.38 (0.14) | Hill coefficient (h) | 16.6 (14.9) |
| Hill coefficient (h) | 1.64 (0.72) | Dispersion parameter (k) | 0.7 (0.11) |
| \(\sigma^2\) | 0.35 (0.01) | | |

ARR, annualized relapse rate; EC\(_{50}\), half-maximal effective concentration; EL\(_{50}\), lymphocyte counts level to reach 50% of the maximum response; E\(_{\text{max}}\), maximum effect.
Author contributions. J.-Y.L. and Y.W. wrote the manuscript and analyzed the data.

Conflict of interest. The authors declared no conflict of interest.

Study Highlights

**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**

✓ An exposure–response analysis has been used as a useful tool for finding an optimal dose. However, the relationship between fingolimod exposure and response could not be quantified successfully due to the lack of data at lower exposure levels.

**WHAT QUESTION THIS STUDY ADDRESSED?**

✓ Our work was able to answer the question if the lower dose would be as effective as the two studied doses.

**WHAT THIS STUDY ADDS TO OUR KNOWLEDGE**

✓ Biomarker data can be used as a bridge in exposure–response analysis to estimate the response when the direct relationship between exposure and response cannot be quantified reliably.

**HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS**

✓ Biomarker data can be used to optimize dosing regimen for late phase clinical trials.

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