PHARMACOKINETIC DYNAMIC RELATIONSHIPS

Population PK–PD analyses of CD25 occupancy, CD56^{bright} NK cell expansion, and regulatory T cell reduction by daclizumab HYP in subjects with multiple sclerosis

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AIM

Daclizumab high yield process (HYP) is a humanized IgG1 monoclonal antibody that binds to the α-subunit of the interleukin-2 receptor and is being developed for treatment of multiple sclerosis (MS). This manuscript characterized the pharmacokinetic–pharmacodynamic (PK–PD) relationships of daclizumab HYP in subjects with MS.

METHODS

Approximately 1400 subjects and 7000 PD measurements for each of three biomarkers [CD25 occupancy, CD56^{bright} natural killer (NK) cell count, regulatory T cell (Treg) count] from four clinical trials were analyzed using non-linear mixed effects modelling. Evaluated regimens included 150 or 300 mg subcutaneous (s.c.) every 4 weeks.

RESULTS

CD25 occupancy was characterized using a sigmoidal maximum response (E_{max}) model. Upon daclizumab HYP treatment, CD25 saturation was rapid with complete saturation occurring after approximately 7 h and maintained when daclizumab HYP serum concentration was ≥5 mg l^{-1}. After the last 150 mg s.c. dose, unoccupied CD25 returned to baseline levels in approximately 24 weeks, with daclizumab HYP serum concentration approximately ≤1 mgl^{-1}1L. CD56^{bright} NK cell expansion was characterized using an indirect response model. Following daclizumab HYP 150 mg s.c. every 4 weeks, expansion plateaus approximately at week 36, at which the average maximum expansion ratio is 5.2. After the last dose, CD56^{bright} NK cells gradually declined to baseline levels within 24 weeks. Treg reduction was characterized by a sigmoidal E_{max} model. Average maximum reduction of 60% occurred approximately 4 days post 150 mg s.c. dose. After the last dose, Tregs were projected to return to baseline levels in approximately 20 weeks.

CONCLUSIONS

Robust PK–PD models of CD25 occupancy, CD56^{bright} NK cell expansion and Treg reduction by daclizumab HYP were developed to characterize its key pharmacodynamic effects in the target patient population.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Daclizumab high yield process (DAC HYP) is a humanized monoclonal antibody that selectively blocks the α-subunit (CD25) of the high affinity interleukin-2 receptors.
- Treatment with daclizumab HYP results in expansion of the CD56^{bright} NK cells and reduction of Tregs.
- In a large phase 3 trial, daclizumab HYP showed efficacy that was superior to interferon β-1a and a manageable safety profile with patient monitoring, in treatment of relapsing remitting MS (RRMS).

WHAT THIS STUDY ADDS

- This study characterized the relationships between DAC HYP serum exposure and its PD effects including CD25 occupancy on target T cells, CD56^{bright} NK cell expansion and reduction of Tregs in subjects with RRMS.
- These analyses integrate available PD data of daclizumab HYP from its phase 2 and 3 clinical trials in subjects with RRMS.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system, characterized by focal areas of demyelination, astrogliosis, the relative preservation of axons and varying degrees of inflammation [1].

The interleukin-2 (IL-2) pathway plays a central role in the activation and regulation of immune responses and therefore might be important in the pathogenesis of MS. The IL-2 receptor exists as both an intermediate-affinity receptor and a high-affinity receptor [2, 3]. Both receptor complexes utilize identical signalling molecules CD122 and CD132, but the high affinity IL-2 receptor includes an accessory α-subunit, CD25, which does not contain a signalling domain but does increase the affinity of the receptor for IL-2. CD25 is expressed at low levels on resting T cells but is rapidly upregulated after T cell activation, which enhances high affinity interleukin-2 signalling [2, 3].

Daclizumab high yield process (DAC HYP) is a humanized IgG1 monoclonal antibody that binds to the α subunit of the human high affinity IL-2 receptor (CD25) expressed on the surface of activated T cells, thereby inhibiting high affinity but not intermediate affinity IL-2 receptor signalling [4–6]. Daclizumab HYP was tested as a potential treatment for MS, based on the assumption that it would block the activation and expansion of activated T cells that are central to the pathogenesis of MS. In a phase 2 monotherapy study (SELECT) in subjects with replasing remitting MS (RRMS), subcutaneous (s.c.) daclizumab HYP significantly reduced the annualized relapse rate (ARR) compared with placebo [7]. A phase 3 study (DECIDE) of daclizumab HYP in subjects with RRMS demonstrated a consistently superior efficacy to IFN β-1a, along with a manageable safety profile [8].

Several pharmacodynamic (PD) parameters have been explored in daclizumab HYP MS studies based on their relationship to IL-2 signalling modulation. In clinical studies of MS subjects, the PD responses observed included sustained CD25 saturation on peripheral T cells, a decrease in regulatory T cells (Tregs) and an an increase in serum IL-2 and CD56^{bright} natural killer (NK) cells during daclizumab HYP treatment [9]. T cell and B cell counts decreased ≤10% from baseline during the first year of daclizumab HYP treatment, while total NK cell counts increased approximately 1.5-fold as a result of the change in CD56^{bright} NK cells [10]. Overall, the PD responses observed had a rapid onset of action after the first dose of daclizumab HYP, sustained during

daclizumab HYP treatment and reversible during a 6 month washout period [9]. This manuscript aims to characterize the population PK–PD relationships quantitatively for CD25 occupancy on target T cells, CD56^{bright} NK cell expansion and regulatory T cell reduction by daclizumab HYP in subjects with MS from four clinical trials.

Methods

Clinical studies

Two phase 2, one immunogenicity and one phase 3 studies were included in the population PK–PD analyses. 205MS201 (SELECT) was a phase 2, multicentre, randomized, double-blind, placeb -controlled, dose finding study in subjects with MS that tested 150 or 300 mg daclizumab HYP given s.c. every 4 weeks for 1 year. A total of 621 subjects were enrolled in a 52 week treatment phase. Subjects who completed the 52 week treatment phase without a major change in their medical status were eligible to enrol in the blinded extension study 205MS202 (SELECTION) to continue dosing with daclizumab HYP. In 205MS202 (SELECTION), a systematic evaluation of a treatment washout period was performed to determine the impact of treatment discontinuation. A total of 517 subjects were randomized to three treatment cohorts: subjects with continuing exposure to daclizumab HYP, subjects randomized to washout followed by re-initiation of daclizumab HYP and subjects with first exposure to daclizumab HYP. Subjects received either daclizumab HYP 150 or 300 mg s.c. every 4 weeks for a total of 13 doses or placebo s.c. every 4 weeks for a total of five doses (24 week washout) followed by daclizumab HYP 150 or 300 mg s.c. every 4 weeks for a total of eight doses. The trial design for SELECT and SELECTION is illustrated in Appendix S1 and was previously presented [7, 11].

205MS302 (OBSERVE) was a multicentre, single arm, open label study to assess the immunogenicity, PK, PD and tolerability of daclizumab HYP when administered s.c. using a pre-filled syringe in subjects with RRMS. A total of 113 subjects were enrolled, of whom 26 subjects were enrolled in the intensive PK/PD substudy. Eligible subjects received daclizumab HYP 150 mg s.c. injections every 4 weeks for a total of six injections in the main study. Following a screening period, subjects received daclizumab HYP over a 24 week treatment period and then entered a 20 week washout period

for monthly assessment of immunogenicity, PK, PD, safety and tolerability.

205MS301 (DECIDE) was a phase 3, multicentre, double-blind, randomized, parallel group, monotherapy, active control study to determine the efficacy and safety of daclizumab HYP *vs*. IFN β-1a (Avonex®) in subjects with RRMS. A total of 919 subjects were enrolled in the daclizumab HYP treatment group and the dosing regimen was 150 mg s.c. every 4 weeks for 96 to 144 weeks [8].

PD sampling schedules for the clinical studies are summarized in Table 1.

Bioanalysis

CD25 occupancy was measured by a validated fluorescence-activated cell sorter (FACS) assay, which detects CD25 expression and occupancy in the presence of anti-CD25 therapeutic antibody daclizumab HYP on the surface of peripheral blood T cells, by specifically gating on CD3+, CD4+ or CD8+ T cells. The CD25 assay utilized an anti-CD25 antibody that competes with daclizumab for binding to CD25 to assess receptor occupancy and a daclizumab non-competing anti-CD25 antibody to assess receptor expression and detect the CD25+ T cell population in the presence of daclizumab. Briefly, 100 μl whole blood aliquots were incubated with appropriate antibody cocktails containing antibodies to CD25 (competing or non-competing), CD3, CD4 and CD8, for 20 min in the dark at room temperature. After incubation, red blood cells (RBCs) were lyzed and the remaining leukocytes washed with 1x phosphate buffered saline with 2% normal calf serum (PBS-NCS). The samples were then resuspended, fixed (1% paraformaldehyde) and analyzed on a BD FACSCaliburTM flow cytometer. PD measurement is the percentage of CD4+ T cells staining positive for unoccupied CD25. Saturation of the CD25 occupancy is defined as ≤1% of the CD4+ T cells stained positive for unoccupied CD25 [12].

Validated FACS assays were used to quantify CD56bright NK cells and regulatory T cells (Tregs). The PD measurements are percentage of CD56bright NK cells among all lymphocytes (T, B and NK cells) and percentage of Tregs (CD4+CD127low/−/Foxp3+) among all CD4+ T cells.

Population PK–PD modelling

A sequential approach was implemented for the PK–PD modelling where the PK component was fixed based on the prior population PK analysis [13]. In summary, a two compartment model with first order absorption and elimination adequately described the PK of daclizumab HYP in subjects with RRMS. Clearance was 0.212 l day^{-1} and central volume of distribution was 3.92 l, scaled by [body weight(kg)/68] with exponents of 0.87 and 1.12, respectively. Peripheral volume of distribution was 2.42 l. Subcutaneous absorption half-life, lag time and bioavailability were 5 days, 1.61 h and 88% (100–300 mg), respectively. Time-varying neutralizing antibody positive status increased the daclizumab HYP CL by 19%. Inter-individual variability (IIV) for CL, V_2 and K_a were 27.2, 50.9 and 94.4%, respectively.

Both PK and PD observations were included in the PK–PD modelling to derive the PD parameters. NONMEM (version 7.2, ICON Development Solutions, Ellicott City, MD, USA), run within a UNIX-based cluster of computers, was used for the modelling. Perl Speaks NONMEM (PsN, Version 3.5.3) was used to conduct bootstrapping. Xpose4 (version 4.3.2, Pharmacometrics Research Group, Uppsala University, Sweden), a module written for the statistical program R, was used to assist in diagnostics plotting. First order conditional estimation with interaction (FOCEI) was used as the estimation method. Inter-individual variability (IIV) was modelled using log normal or normal distribution as appropriate. Residual variability was modelled with proportional and/or additive errors as appropriate.

Potential outliers were identified as observations with extreme CWRES in the base model. If these observations looked discordant, they were excluded from the dataset prior to the modelling. Seventy-one (0.9%), 15(0.2%) and 11(0.1%) PD records were excluded as outliers from the CD25, CD56bright NK and Treg datasets, respectively.

The statistical criterion for the model selection is based on comparison of the minimum objective function value (OFV), a value representative of minus twice the log likelihood value. Comparisons of nested models were based on the likelihood ratio test, which follows a chi-square distribution. A decrease in NONMEM OFV of 6.63 points was necessary to consider the improvement in model performance statistically significant at $P< 0.01$ and one degree of freedom.

Model selection was further aided by goodness-of-fit plots, including DV (observed PD measurement) *vs*. population prediction (PRED), DV *vs*. individual prediction (IPRED), conditional weighted residual (CWRES) *vs*. TIME and CWRES *vs*. PRED plots. Other diagnostics also were utilized to help

Table 1

PD sampling schedule

Study	PD sampling schedule
205MS302 (OBSERVE)	Intensive PK/PD subgroup: predose, 8 h, 24 h, 72 h, 120 h, day 7, 10, 14, 21 and post-dose at week 20
	Non-intensive PK/PD subgroup: Week 0, 2, 4, 8, 12, 16, 20, 24, (28, 32, 36, 44, 188)†
205MS201 (SELECT)	Week 0, 4, 8, 16, 24, 32, 40, 48, (52, 56, 64, 72)‡
205MS202 (SELECTION)	Week 0, 4, 8, 12, 16, 20, 24, 32, 40, 52, (72)§
205MS301 (DECIDE)	Week 0, 4, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 164

†Follow-up visits; ‡Subjects who do not rollover to 205MS202, follow-up period; §Subjects who do not rollover to 205MS203 (SELECTON extension), follow-up period.

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select the appropriate model, including parameter precision, IIV and CWRES distribution and IIV shrinkage.

Model evaluations

Robustness of the model was evaluated using a non-parametric bootstrap. Subjects were randomly sampled with replacement from the original dataset to form 2000 new datasets. The model parameters were then estimated using the bootstrap datasets. The median and 95% confidence interval (2.5^{th} to 97.5^{th} percentiles) for each parameter were calculated from the successfully converging runs and compared with point estimates from the original dataset.

Visual predictive check (VPC) was used to check the adequacy of the model. The final model PK parameters were utilized to simulate 2000 replicates of the observed data. Simulations were stratified by study, dose and group (intensive vs. non-intensive in OBSERVE; washout vs. non-washout in SELECT/SELECTION). Median, 10th and 90th percentiles of the observed and simulated were plotted along with the observations.

Results

Demographics and subject disposition

The number of subjects and records included in the PK–PD datasets are summarized in Table 2. Demographics and immunogenicity of subjects for the three studies (SELECT/SELECTION, OBSERVE and DECIDE) can be found in a population PK analysis published previously [13]. Twenty-five subjects in OBSERVE had intensive PK profiles and 174 subjects in SELECTION had a 24 week washout period. The treatment–withdrawal design of SELECT/SELECTION provided an opportunity to evaluate the effect of interruption of daclizumab HYP treatment on PD parameters during the daclizumab HYP washout period.

Modelling of CD25 occupancy

Because target T cells are in the blood stream instead of tissues, binding of daclizumab HYP in the circulation to CD25 is a direct rather than indirect effect. A sigmoidal maximum response (E_{max}) model was utilized to characterize unoccupied CD25 (denoted as CD25 in equation (1)) on peripheral T cells in subjects with RRMS upon treatment with daclizumab HYP.

C_p denotes daclizumab HYP serum concentration. Since CD25 occupancy can be completely saturated by daclizumab HYP, the E_{max} value was fixed at 1 in the model.

$$CD25 = E0 \cdot \left(1 - \frac{C_p^{\gamma}}{C_p^{\gamma} + IC_{50}^{\gamma}} \right) \qquad (1)$$

Since the OBSERVE intensive substudy contained the most informative sampling schedule about the saturation phase of CD25 occupancy, OBSERVE intensive data were singled out to characterize the saturation of CD25 occupancy by daclizumab HYP. However, the PD model estimates from the OBSERVE intensive data cannot describe well the desaturation phase for the SELECTION washout period (data not shown). Separate parameters were needed to characterize the rapid saturation of CD25 occupancy for the first dose and the apparently slower desaturation during the treatment discontinuation. This finding was consistent with a previous analysis of CD25 occupancy in healthy volunteers in phase 1 studies [14].

In the final model, the Hill coefficient and IC_{50} for the saturation phase were fixed as those from OBSERVE intensive data and for the SELECTION washout desaturation phase, separate Hill coefficients and IC_{50} were estimated. The parameter estimates are listed in Table 3. The mean (SD) for the baseline CD25 occupancy (percentage of CD4$^+$ T cells staining positive for unoccupied CD25) was 56 (11). IC_{50} and Hill coefficient for the saturation phase were 0.0135 mg l^{-1} (estimated for the OBSERVE intensive subgroup data) and 1 (fixed), respectively. A higher IC_{50} was estimated for CD25 desaturation during daclizumab HYP washout period (2.07 mg l^{-1}, with a Hill coefficient of 4.4 and IIV of 46.9%) than during the initial binding. A covariance step was not successful for the final model, therefore, the % relative standard error for the parameters are not presented. Bootstrap results for model 3 are listed in Table 3, which indicate that the model is stable. The diagnostic plots for this model are included in Appendix S2. The VPC plots are described in Appendix S3, which demonstrate consistency between simulated data and the observations. However, there were some outliers with faster CD25 desaturation resulting in some apparent bias in the diagnostic plots.

CD25 occupancy saturation following the first 150 mg s.c. dose for a typical subject is simulated in Figure 1A. Onset of CD25 occupancy is predicted to be rapid with complete saturation occurring after approximately 7 h, which is consistent

Table 2

Summary of the number of subjects and records in the PK–PD datasets

PD biomarker	Number	SELECT/SELECTION	OBSERVE	DECIDE	Total
CD25	Subject	580	113	766	1459
	PD record	5123	974	1525	7622
NK	Subject	561	107	737	1405
	PD record	5071	922	3637	9630
Treg	Subject	545	106	702	1353
	PD record	4835	891	3016	8742

Table 3

Summary of CD25 occupancy model

Parameter	Value*	Bootstrap median (2.5th, 97.5th)†
Baseline E_0‡, %	56	56 (55, 56)
IIV (additive)	11	11 (10, 11)
Saturation		
IC_{50} (mg l^{-1})	0.0135 (FIXED)§	---
Hill coefficient (γ)	1 (FIXED)§	---
Desaturation		
IC_{50} (mg l^{-1})	2.07	2.02 (1.50, 2.39)
IIV, %	47	47 (36, 79)
Hill coefficient (γ)	4.44	4.35 (3.19, 5.19)
Residual error (additive)	4.02	4.03 (3.13, 4.72)

†Summary from 1454 successful runs out of 2000 runs; ‡Percentage of CD4+ T cells staining positive for unoccupied CD25; §IC_{50} and γ fixed as that estimated using OBSERVE intensive subgroup PK data and 1, respectively; *ETA shrinkages for baseline E_0 and desaturation IC_{50} are 10.4% and 76.1%, respectively. EPS shrinkage for additive residual error is 9.1%
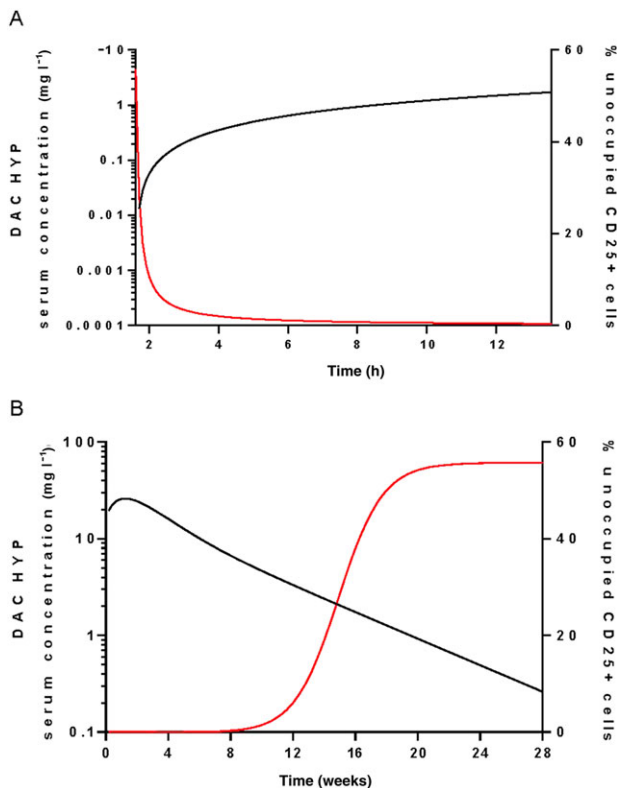

Figure 1

Simulated CD25 occupancy profile following A) the first daclizumab HYP s.c. 150 mg dose and B) the last daclizumab HYP s.c. 150 mg dose every 4 weeks at steady-state. — DAC HYP, — Unoccupied CD25

with the data from the OBSERVE substudy, which demonstrates 100% CD25 occupancy by daclizumab HYP for the majority of the subjects at the earliest time point 8 h post-dose. Saturation of CD25 occupancy is predicted to be maintained when daclizumab HYP serum concentration ≥ 5 mg l^{-1}.

Desaturation of CD25 occupancy after the last 150 mg s.c. dose at steady-state for a typical subject is plotted in Figure 1 B. After the last 150 mg s.c. dose at steady-state, unoccupied CD25 returned to baseline levels in approximately 24 weeks, with a daclizumab HYP serum concentration of approximately ≤ 1 mg l^{-1}.

Modelling of CD56^{bright} NK cell expansion

Expansion of CD56^{bright} NK cells by daclizumab HYP is hypothesized through the increased availability of T cell-derived IL-2 for NK cell signalling [15]. Thus an indirect response model was utilized with the drug effect modelled using a stimulatory S_{max} function on the zero order rate K_{in} of production of NK cells (Equation (2)). In the equation, K_{out} is the first order rate constant for elimination of CD56^{bright} NK cells, C_p denotes daclizumab HYP serum concentration and S_{max} is the maximum stimulatory factor attributed to daclizumab HYP.

$$\frac{d\text{NK}}{dt} = K_{in} \cdot \left(1 + \frac{S_{max} \cdot C_p}{EC_{50} + C_p}\right) - K_{out} \cdot A(\text{NK}) \qquad (2)$$

K_{out} was expressed as a function of K_{in} using baseline information in the modelling and therefore was not independently estimated. The model estimates are listed in Table 4. Diagnostic plots are presented in Appendix S4. The median and mean baselines for CD56^{bright} NK cell percentage are 0.6% and 0.75% among all the lymphocytes, respectively. A covariance step was not successful for the final model, therefore, the % relative standard error for the parameters are not presented. Median and 95% CI for the PD parameters, as well as IIV and residual variability from the bootstrap, are consistent with model estimates indicating that the model is stable. VPC plots also indicate the good performance of the model (Appendix S5).

Table 4

Summary of CD56^{bright} NK cell expansion model

Parameter	Value*	Bootstrap median (2.5th, 97.5th)†
K_{in}, %/h	4.12E-04	4.1E-04 (3.7E-04, 4.5E-04)
IIV (%)	97	97 (90, 104)
S_{max}	7.89	7.88 (7.12, 8.89)
IIV(%)	67	67 (64, 71)
EC$_{50}$ (mg l^{-1})	18.0	17.8 (14.2, 22.8)
Residual error (proportional), %	29.1	29.0 (28.2, 29.9)

†Summary from 1978 successful runs out of 2000 runs; *ETA shrinkages for K_{in} and S_{max} are 25% and 10.6%, respectively. EPS shrinkage for proportional residual error is 9.7%

The CD56^{bright} NK cell expansion time profile was simulated following daclizumab HYP 150 mg s.c. every 4 weeks for 48 weeks, then treatment discontinuation for 24 weeks and re-initiation of 150 mg s.c. every 4 weeks for 48 weeks (Figure 2). There is a significant expansion plateauing approximately at week 36, at which the CD56^{bright} NK cell maximum expansion ratio is 5.2. After dosing discontinuation at week 48, CD56^{bright} NK cell percentage gradually declines to approximately the baseline in 24 weeks. After re-initiation of treatment at week 72, CD56^{bright} NK cell expansion mirrored the first year of treatment.

Modelling of Treg reduction

A sigmoidal E$_{max}$ model was utilized to characterize the reduction of Treg (equation (3)).

$$Treg = E0 \cdot \left(1 - \frac{E_{max} \cdot C_p^{\gamma}}{C_p^{\gamma} + IC_{50}^{\gamma}} \right) \qquad (3)$$
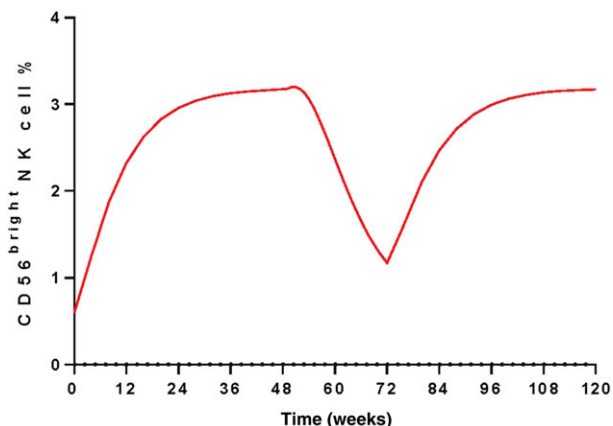
Figure 2

Simulated CD56^{bright} NK cell percent following daclizumab HYP 150 mg s.c. every 4 weeks for 48 weeks, then the treatment discontinuation for 24 weeks and re-initiation of daclizumab HYP 150 mg s.c. every 4 weeks at week 72 for another 48 weeks

Model parameters are listed in Table 5 and the diagnostic plots are presented in Appendix S6. An E$_{max}$ value of 0.610 indicates that the average maximum reduction by daclizumab HYP is approximately 60%.

To investigate whether there is a potential delay of effect of daclizumab HYP on Tregs relative to daclizumab HYP serum profile, a delay compartment was tested in the sigmoidal E$_{max}$ model. However, adding such a delay compartment did not improve the model fit.

A covariance step was not successful for the final model. Therefore, the % relative standard error for the parameters were not presented. Bootstrap results for the sigmoidal E$_{max}$ model are listed in Table 5. Median and 95% confidence interval for the PD parameters, as well as IIV and residual variability, were consistent with model estimates indicating that the model is stable. VPC stratified by study and subgroups also indicated the good performance of the model (Appendix S7).

Treg reduction following the first s.c. dose of daclizumab HYP 150 mg was simulated. Onset of Treg reduction upon daclizumab HYP treatment is slower than CD25 occupancy. The time to achieve 50% of the maximum effect after administering the 150 mg s.c. regimen for CD25 occupancy and Treg reduction are 1.7 h and 2.6 days, respectively. The onset of Treg reduction and CD25 occupancy is also reflected through IC$_{50}$ comparison. The IC$_{50}$ of the CD25 occupancy saturation process is 0.0135 mg l^{-1} while the IC$_{50}$ of Treg reduction is higher at 3.97 mg l^{-1}. Maximum Treg reduction of 60% occurs approximately 4 days post-150 mg s.c. dose (Figure 3A). After treatment discontinuation, Tregs are predicted to return to the baseline levels at approximately week 20 (Figure 3B).

Discussion

Daclizumab HYP (Zinbryta$^{™}$) was approved by the U.S. Food and Drug Administration (FDA) on May 27, 2016 for the treatment of RRMS and is under review with other health authorities. In a phase 3 (DECIDE) study, daclizumab HYP demonstrated superior efficacy to IFN β-1a across key clinical, radiographic and patient reported MS outcome measures, and has the potential to provide a new treatment option for subjects with RRMS as a once-monthly s.c. injection [8].

Table 5

Summary of Treg reduction model

Parameter	Value*	Bootstrap median (2.5th, 97.5th)†
Baseline E_0‡, %	12.1	12.2 (11.8, 12.6)
IIV (%)	42	42 (39, 44)
IC_{50} (mg l^{-1})	3.97	3.98 (3.34, 4.66)
IIV (%)	65	63 (46, 78)
Hill coefficient (γ)	2	1.92 (1.39, 2.91)
E_{max}	0.610	0.614 (0.584, 0.654)
IIV (%)	12	12 (9, 14)
Residual error (proportional), %	50.1	50.2 (48.8, 51.2)
Residual error (additive)	0.416	0.420 (0.154, 0.628)

†Summary from 1561 successful runs out of 2000 runs; ‡Percentage of Tregs (CD4 + CD127low/−/Foxp3+) among all CD4+ T cells; *ETA shrinkages for baseline E_0, IC_{50} and E_{max} are 15.6%, 60.5% and 57.7%, respectively. EPS shrinkages for proportional and additive residual errors are both 6.1%
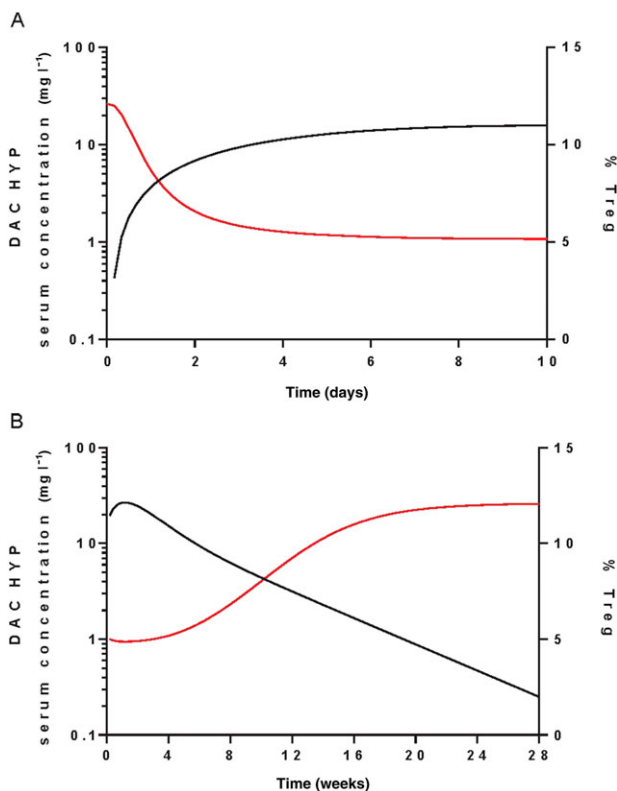

Figure 3

Simulated Treg percent following A) the first daclizumab HYP s.c. 150 mg dose and B) the last daclizumab HYP s.c. 150 mg dose every 4 weeks at steady-state. — DAC HYP, — Treg

Three PD measurements, i.e. CD25 occupancy on peripheral T cells, CD56^{bright} NK cells and Tregs, were evaluated for subjects with RRMS treated with daclizumab HYP from four clinical studies. Examination of observed data indicated that saturation of CD25 occupancy by daclizumab HYP was rapid with complete saturation achieved within 8 h of dosing (the first sampling time point in OBSERVE intensive subgroup). Significant reductions in Treg and increases in CD56^{bright} NK cells from baseline levels were detected in the first 2 weeks of treatment. The effects of daclizumab HYP were sustained. Treatment with daclizumab HYP maintained the CD25 occupancy saturation, reduced Treg and expanded CD56^{bright} NK cell populations during the treatment period. The effects of daclizumab HYP were reversible. CD25 occupancy, CD56^{bright} NK cell expansion and Treg reduction were reversible to the baselines during a 6 month washout period. After re-initiation of treatment, the PD effects mirrored the initial treatment phase (as demonstrated by the data in the SELECT/SELECTION studies).

The importance of target engagement with CD25 by daclizumab in treating MS was highlighted in a previous study which evaluated an investigational form of daclizumab (Daclizumab Penzberg, manufactured using a different process and different cell line) in subjects on stable interferon beta therapy for RRMS [16]. The effect of daclizumab Penzberg on new gadolinium contrast-enhancing lesions, the primary endpoint of that study, relative to placebo was robust and statistically significant in the high dose arm 2 mg kg^{-1} s.c. every 2 weeks, but was marginal and not statistically significant in the low dose arm 1 mg kg^{-1} s.c. every 4 weeks [16]. Population modelling predicted CD25 desaturation in patients whose serum daclizumab concentrations were in the lower fifth percentile for the low dose daclizumab group, but not the high dose daclizumab group.

Using a sigmoidal E_{max} model, two sets of IC_{50} and Hill coefficients were needed to characterize the saturation and desaturation phases of CD25 occupancy and a higher IC_{50} was estimated for CD25 desaturation during the daclizumab HYP washout period than during the initial binding. The majority of the subjects had CD25 rapidly occupied upon daclizumab HYP administration. The slope of the saturation part was very steep and the majority of unoccupied CD25 were close to zero at the first sampling point (8 h) in the OBSERVE intensive group. The desaturation during the drug discontinuation was apparently slower. This finding was consistent with

a previous analysis of CD25 occupancy by daclizumab HYP in healthy volunteers from phase 1 studies [14]. As discussed previously, it is possible that the rapid occupancy of CD25 is driven by CD25 binding in the lymphatic system, where daclizumab HYP concentrations are higher than those in the serum shortly after s.c. administration [14]. Saturation of CD25 occupancy was predicted to be maintained when daclizumab HYP serum concentration was more than 5 mg l^{-1} while the unoccupied CD25 returned to baseline levels when daclizumab HYP serum concentration decreased to approximately less than 1 mg l^{-1}. Unoccupied CD25 return to baseline levels around week 24 after dosing discontinuation. Overall, the kinetics of CD25 saturation and desaturation with the treatment of daclizumab HYP in this analysis including an extensive dataset of monotherapy treatment in MS patients were consistent with previous analyses of CD25 occupancy by daclizumab HYP in healthy volunteers [14].

CD56^{bright} NK cells represent an important immune regulatory population capable of selectively killing autologous, activated T cells [17]. The expansion of CD56^{bright} NK cells upon daclizumab HYP treatment is believed to be due to increased IL-2 bioavailability after CD25 blockade and the high concentration of intermediate affinity IL-2 receptors expressed on these cells [15]. In smaller published studies, higher numbers of the CD56^{bright} NK cells upon treatment were correlated with fewer new or newly enlarging MS lesions [16, 17]. In a recent analysis with SELECT/SELECTION data, CD56^{bright} NK cells appeared to mediate some of the treatment-related effects of daclizumab HYP, but their numbers do not account for the full effect of daclizumab HYP on MS-related outcomes [10]. CD56^{bright} NK cell expansion upon daclizumab HYP treatment was quantitatively characterized using an indirect response model and the drug effect was expressed as a stimulatory S_{max} function on K_{in}, the zero order rate constant for production of CD56^{bright} NK cells. The expansion of CD56^{bright} NK cells upon daclizumab HYP treatment was slow as indicated by the relatively high EC_{50} (18 mg l^{-1}). The time to reach 50% of the maximum CD56^{bright} NK cell expansion following initiation of the 150 mg s.c. every 4 weeks regimen was approximately week 8. Following daclizumab HYP 150 mg s.c. every 4 weeks, the expansion plateaus were reached approximately at week 36 and the ratio of expansion at plateau was 5.2.

In addition to being expressed on activated T cells, CD25 is expressed constitutively at high levels on Tregs, which mediate immune tolerance and depend on IL-2 for homeostasis [18]. Treg deficiency, instability, and dysfunction are implicated in the pathogenesis of numerous autoimmune diseases, including RRMS [19]. However, reduction of circulating Tregs was observed in subjects with RRMS treated with daclizumab HYP despite its clinical efficacy in subjects with RRMS [20–22]. These seemingly contradictory observations were evaluated for SELECT data and it was found that Treg measurements did not associate with daclizumab HYP treatment response or the development of adverse events of interest such as skin rashes [22]. Treg phenotype and lineage stability can be maintained despite CD25 blockade by daclizumab HYP [22]. In this analysis, Treg reduction was characterized by a sigmoidal E_{max} model. A model linking the effect directly without a delay to daclizumab HYP serum concentrations was adequate. The rapid decline in Treg counts (maximum decline in approximately 4 days following

150 mg s.c. dose) as well as their rapid return to the baseline at approximately week 24 when unoccupied target CD25 return to baseline levels suggest that the mechanism of the Treg effect by daclizumab HYP may be an indirect effect related to Treg trafficking, as opposed to direct effects on the development or survival of Tregs.

CD25 occupancy, CD56^{bright} NK cell expansion and Treg reduction represent important pharmacodynamic markers during daclizumab HYP treatment in subjects with MS. Therefore, the quantitative integration and understanding of the PK–PD data is a first essential step to allow future clinical utility of these PD markers. Further investigations of the daclizumab HYP effect on the balance of regulatory and effector immune cells and additional analyses linking the biomarker responses to clinical outcome measures are also warranted [15, 22].

In conclusion, the quantitative PK–PD relationships were characterized for CD25 occupancy on target T cells, CD56^{bright} NK cell expansion, and regulatory T cell reduction by daclizumab HYP in subjects with MS. CD25 occupancy data were characterized by a sigmoidal E_{max} model. Upon daclizumab HYP 150 mg s.c. treatment, CD25 saturation was rapid with complete saturation occurring approximately 7 h and maintained when daclizumab HYP serum concentration was ≥5 mg l^{-1}. After the last 150 mg s.c. dose at steady-state, unoccupied CD25 returned to baseline levels in approximately 24 weeks, with daclizumab HYP serum concentrations approximately ≤1 mg l^{-1}. CD56^{bright} NK cell expansion was characterized by an indirect response model. Simulation for a typical subject showed that treatment of daclizumab HYP 150 mg s.c. every 4 weeks resulted in a significant CD56^{bright} NK cell expansion, with the maximum expansion ratio relative to baseline of approximately 5 at week 36. After the last dose at steady-state, CD56^{bright} NK cells gradually declined to approximately the baseline level within 24 weeks. Daclizumab HYP induced decrease in Tregs was characterized by a sigmoidal E_{max} model. A maximum reduction of 60% occurred approximately 4 days post-150 mg s.c. dose. After the last dose at steady-state, Tregs were projected to return to the baseline levels in approximately 20 weeks.

Competing Interests

References

1 Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. N Engl J Med 2000; 343: 938–52.

2 Malek TR. The biology of interleukin-2. Annu Rev Immunol 2008; 26: 453–79.

3 Waldmann T, Tagaya Y, Bamford R. Interleukin-2, interleukin-15, and their receptors. Int Rev Immunol 1998; 16: 205–26.

4 Pfender N, Martin R. Daclizumab (anti-CD25) in multiple sclerosis. Exp Neurol 2014; 262 Pt A: 44–51.

5 Oh J, Calabresi PA. Emerging injectable therapies for multiple sclerosis. Lancet Neurol 2013; 12: 1115–26.

6 Vincenti F, Kirkman R, Light S, Bumgardner G, Pescovitz M, Halloran P, et al. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. Daclizumab Triple Therapy Study Group. N Engl J Med 1998; 338: 161–5.

7 Gold R, Giovannoni G, Selmaj K, Havrdova E, Montalban X, Radue EW, et al. Daclizumab high-yield process in relapsing–remitting multiple sclerosis (SELECT): a randomised, double-blind, placebo-controlled trial. Lancet 2013; 381: 2167–75.

8 Kappos L, Wiendl H, Selmaj K, Arnold DL, Havrdova E, Boyko A, et al. Daclizumab HYP versus Interferon Beta-1a in Relapsing Multiple Sclerosis. N Engl J Med 2015; 373: 1418–28.

9 Mehta D RK, Sheridan J, Elkins J, Amaravadi L. Rapid, sustained and reversible pharmacodynamics of DAC HYP in MS patients supports mechanism of action via modulation of the IL-2 pathway. In: ACTRIMS-ECTRIMS, 2014.

10 Elkins J, Sheridan J, Amaravadi L, Riester K, Selmaj K, Bielekova B, et al. CD56(bright) natural killer cells and response to daclizumab HYP in relapsing-remitting MS. Neurol Neuroimmunol Neuroinflamm 2015; 2: e65.

11 Giovannoni G, Gold R, Selmaj K, Havrdova E, Montalban X, Radue EW, et al. Daclizumab high-yield process in relapsing–remitting multiple sclerosis (SELECTION): a multicentre, randomised, double-blind extension trial. Lancet Neurol 2014; 13: 472–81.

12 Othman AA, Tran JQ, Tang MT, Dutta S. Population pharmacokinetics of daclizumab high-yield process in healthy volunteers: integrated analysis of intravenous and subcutaneous, single- and multiple-dose administration. Clin Pharmacokinet 2014; 53: 907–18.

13 Diao L, Hang Y, Othman AA, Nestorov I, Tran JQ. Population pharmacokinetics of daclizumab high-yield process in healthy volunteers and subjects with multiple sclerosis: analysis of phase I–III clinical trials. Clin Pharmacokinet 2016; 55: 943–55.

14 Minocha M, Tran JQ, Sheridan JP, Othman AA. Blockade of the high-affinity interleukin-2 receptors with daclizumab high-yield process: pharmacokinetic/pharmacodynamic analysis of single- and multiple-dose phase I trials. Clin Pharmacokinet 2015; 55: 121–30.

15 Martin JF, Perry JS, Jakhete NR, Wang X, Bielekova B. An IL-2 paradox: blocking CD25 on T cells induces IL-2-driven activation of CD56(bright) NK cells. J Immunol 2010; 185: 1311–20.

16 Wynn D, Kaufman M, Montalban X, Vollmer T, Simon J, Elkins J, et al. Daclizumab in active relapsing multiple sclerosis (CHOICE study): a phase 2, randomised, double-blind, placebo-controlled, add-on trial with interferon beta. Lancet Neurol 2010; 9: 381–90.

17 Bielekova B, Catalfamo M, Reichert-Scrivner S, Packer A, Cerna M, Waldmann TA, et al. Regulatory CD56(bright) natural killer cells mediate immunomodulatory effects of IL-2R alpha-targeted therapy (daclizumab) in multiple sclerosis. Proc Natl Acad Sci U S A 2006; 103: 5941–6.

18 Sakaguchi S. Naturally arising CD4(+) regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 2004; 22: 531–62.

19 Zozulya AL, Wiendl H. The role of regulatory T cells in multiple sclerosis. Nat Clin Pract Neurol 2008; 4: 384–98.

20 Oh U, Blevins G, Griffith C, Richert N, Maric D, Lee CR, et al. Regulatory T cells are reduced during anti-CD25 antibody treatment of multiple sclerosis. Arch Neurol 2009; 66: 471–9.

21 Milo R. The efficacy and safety of daclizumab and its potential role in the treatment of multiple sclerosis. Ther Adv Neurol Disord 2014; 7: 7–21.

22 Huss DJ, Mehta DS, Sharma A, You X, Riester KA, Sheridan JP, et al. In vivo maintenance of human regulatory T cells during CD25 blockade. J Immunol 2015; 194: 84–92.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

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Appendix S1 Phase 2 Clinical Studies 205M S201 (Select) and 205MS202 (Selection) Trial Design

Appendix S2 Diagnostic plots for the CD25 occupancy model. Black solid line is the unit line; red dashed line represents the linear regression line in (a) and (b) and the LOESS smoother in (c) and (d); DV is observed unoccupied CD25 percent; PRED is the population predicted unoccupied CD25 percent; IPRED is the individual predicted unoccupied CD25 percent; CWRES is conditional weighted residual; TIME is the time after dose and unit is h

Appendix S3 VPC for CD25 occupancy model. (Solid line: median; Dotted line: 10th and 90th percentiles; Black lines: observed; Red lines: simulated; Blue dots: observed.)

Appendix S4 Diagnostic plots for the CD56^{bright} NK cell expansion model. Black solid line is the unit line; red dashed line represents the linear regression line in (a) and (b) and the LOESS smoother in (c) and (d); DV is observed CD56^{bright} NK cell percent; PRED is the population predicted CD56^{bright} NK cell percent; IPRED is the individual predicted CD56^{bright} NK cell percent; CWRES is conditional weighted residual; TIME is the time after dose and unit is h

Appendix S5 VPC for CD56^{bright} NK cell expansion Model. (Solid line: median; Dotted line: 10th and 90th percentiles; Black lines: observed; Red lines: simulated; Blue dots: observed.) A. SELECT/?SELECTION (Upper left: 150 mg no washout; Upper right: 150 mg washout; Lower left: 300 mg no washout; Lower right: 300 mg washout). B. OBSERVE (Left: intensive; Right: Non-intensive) C. DECIDE

Appendix S6 Diagnostic plots for the Treg reduction model. Black solid line is the unit line; red dashed line represents the linear regression line in (a) and (b) and the LOESS smoother in (c) and (d); DV is observed Treg percent; PRED is the population predicted Treg percent; IPRED is the individual predicted Treg percent; CWRES is conditional weighted residual; TIME is the time after dose and unit is h

Appendix S7 VPC for Treg reduction model. (Solid line: median; Dotted line: 10th and 90th percentiles; Black lines: observed; Red lines: simulated; Blue dots: observed.) A. SELECT/?SELECTION (Upper left: 150 mg no washout; Upper right: 150 mg washout; Lower left: 300 mg no washout; Lower right: 300 mg washout). B. OBSERVE (Left: intensive; Right: Non-intensive) C. DECIDE