The Utility of Modeling and Simulation Approaches to Evaluate Immunogenicity Effect on the Therapeutic Protein Pharmacokinetics

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Abstract. While therapeutic proteins (TP), particularly recombinant human proteins and fully human monoclonal antibodies, are designed to have a low immunogenic potential in humans, a clinical immune response does sometimes occur and cannot be predicted from preclinical studies. Changes in TP pharmacokinetics may be perceived as an early indication of antibody formation and serve as a surrogate for later changes in efficacy and safety in individual subjects. Given the substantial increase in number of biological products, including biosimilars, there is an urgent need to quantitatively predict and quantify the immune response and any consequential changes in TP pharmacokinetics. The purpose of this communication is to review the utility of population-based modeling and simulation approaches developed to date for investigating the development of an immune response and assessing its impact on TP pharmacokinetics. Two examples of empirical modeling approaches for pharmacokinetic assessment are presented. The first example presents methods to analyze pharmacokinetic data in the presence of anti-drug antibody (ADA) and confirm the effect of immunogenicity on TP pharmacokinetics in early phases of drug development. The second example provides a framework to analyze pharmacokinetic data in the absence or with very low incidence of ADA and confirm with enough power the lack of an immunogenicity effect on TP pharmacokinetics in late phases of drug development. Finally, a theoretical mechanism-based modeling framework is presented to mathematically relate the complex interaction among TP, their targets, and ADA.

KEY WORDS: immunogenicity; modeling and simulation; pharmacokinetics; therapeutic proteins.

INTRODUCTION

Therapeutic proteins (TP) including monoclonal antibodies, proteins, and peptides are macromolecules that can potentially trigger a vigorous cellular and humoral immune response, manifested as an anaphylactic reaction, cell-mediated reaction, or production of anti-drug antibodies (ADA). If an immune response is induced, ADA may bind to native endogenous proteins or to the TP, which could alter its pharmacokinetics and, thereby, lead to the loss of therapeutic effect. Although TP are often designed to reduce their immunogenic potential in humans, they may still elicit an immune response in some patients.

The immunogenicity of a TP is influenced by a variety of factors, including factors related to the product (e.g., variation from human sequence and glycosylation), process (e.g., storage conditions, aggregates, contaminants or impurities during processing, dose and duration of treatment, route of administration, and formulation), and patient characteristics (e.g., genetic background and immune status of the patient due to disease) that have been extensively reviewed recently (1–4). Underlying immunological abnormalities such as the presence of pre-existing pro-inflammatory cytokines in rheumatoid arthritis patients could increase the incidence and intensity of an immune response to a TP. Concomitant treatment affecting the immune system may also change the frequency of immune response. For instance, the broad and nonspecific immunosuppressive or cytotoxic effects of chemotherapy have the potential to impair the immune system, which could reduce the incidence of ADA in patients receiving biologic drugs in combination with chemotherapy (5,6). Some anticancer agents have gastrointestinal toxicities and, consequently, have the potential to affect local and systemic immunity.

Following the Gell and Coombs classification of hypersensitivity reactions (7), the type II and type III immunogenic effects of TP can be quantitatively investigated in population pharmacokinetic and pharmacodynamic analyses with clinical data; this is particularly applicable in susceptible populations due to particular genetic characteristics or disease states. When ADA binds to TP, the circulating immune complexes formed may result in decreased or increased systemic exposure of TP; therefore, changes in TP pharmacokinetics may be perceived as an early indication of antibody formation

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from an immune response. The mechanism of the ADA effect on TP pharmacokinetics can (a) lead to enhanced reticulo-endothelial system uptake, resulting in reduced bioavailability and/or enhanced clearance; (b) limit tissue penetration, resulting in reduced volumes of distribution; or (c) serve as storage depot, resulting in more sustained TP concentrations (8). While immune responses to a TP can occur in nonclinical animal species, it is often not possible to predict immunogenicity in healthy subjects and patients from animal observations (9). Hence, clinical assessment of immunogenicity in the target population and evaluation of the resulting changes in pharmacokinetics (PK) as a surrogate for pharmacodynamic variables, safety, and efficacy in the individual subject is absolutely necessary (10,11). Moreover, there is an urgent need for further research in this area given the substantial increase in the number of biological products, including biosimilars, under clinical development. Nonetheless, the recordable incidence of TP-induced immune response is infrequent and hard to quantify and predict during clinical development. The advance in population methodology provides a viable way to address the need as it allows sparse sampling of PK data collected from a study, pooling of PK data across multiple clinical studies of different nature and development phases, and accounting for a multitude of interacting factors (12,13). Hence, the purpose of this communication is to review the utility of population-based modeling and simulation approaches developed to assess the impact of immunogenicity on TP pharmacokinetics during early and late stages of clinical development.

QUANTIFYING THE IMMUNE RESPONSE DEVELOPMENT

The quantification of the immunogenicity effect in an individual subject is normally defined with a tiered approach (14). In the first tier, immunoassays are used to identify whether or not a subject has seroconverted. In the second tier, assays further characterize the nature of these antibodies in seroconverted subjects (binding *vs*. neutralizing antibodies). While binding antibodies either enhance or diminish clearance, neutralizing antibodies prevent the biological effects of the antigens. The amount of anti-drug antibodies developed is assessed by measuring antibody titers, which are often expressed as the largest serum dilution that still gives a positive result. Therefore, antibody titers are a "quasi-quantitative" surrogate for the magnitude of the immune response and reflect both antibody concentration and affinity. Of note, antibody titers are influenced by the assay conditions, assay sensitivity, and the amount of TP present at the time of the assessment.

Identifying the factors involved in TP immunogenicity that are predictive of the level of antibody titer in a subject is critical in drug development. The challenge of quantitatively assessing the relationship between predictive factors and the level of antibody titers requires statistical modeling, which is not immediately intuitive because the antibody titer is not a continuous variable, but becomes more informative than a typical ordinal data type. Consequently, statistical models based on continuous random variables are not appropriate, and although ordinal models may be applicable, a loss of information and (potentially) power should be expected. The problem is further complicated because not all subjects exposed to TP would develop antibodies; hence, the population consists of seroconverter and non-seroconverter subpopulations. Recently, a statistical approach for analyzing antibody titer data conditional on seroconversion has been developed (15) and consists of (1) transforming the antibody titer data based on a geometric series, using a common ratio of 2 and a scale factor of 50, and then (2) analyzing the exponent using a zero-inflated or hurdle model (16), assuming a Poisson or negative binomial distribution with random effects to account for subject heterogeneity. Subject-specific factors can be incorporated into the modeling framework in order to quantify the probability of developing ADA and the magnitude of the antibody titers.

The method was applied to analyze antibody titer data collected from 87 seroconverted Fabry patients receiving Fabrazyme. Titers from five clinical trials collected over 276 weeks of therapy with anti-Fabrazyme IgG ranged from 100 to 409,600 after exclusion of seronegative patients. A zero-inflated Poisson model was used to explain the probability of seroconversion, which was dependent on the cumulative biweekly dose. There was an 80% chance of seroconversion when the cumulative biweekly dose reached 210 mg (90% CI 194–226 mg). Once seroconverted, antibody titers decreased in an exponential manner from an initial magnitude to a new lower steady-state value, which was estimated to be 870 (90% CI 630–1,109). The half-life associated with the exponential decrease in the antibody titers after seroconversion was 44 weeks (90% CI 17–70 weeks). The time to seroconversion did not appear to be correlated with titer at the time of seroconversion. This empirical method is suitable for modeling immunogenicity development and quantifying antibody titer in seroconverters. Together with mechanistic models, mathematical approaches intended to quantify the immunogenicity development should be considered in understanding the time course of the immunogenicity effect on TP pharmacokinetics.

QUANTIFYING THE IMMUNOGENICITY EFFECT ON TP PHARMACOKINETICS

Immunogenicity usually impacts clinical response to therapy in a negative manner by affecting bioavailability, pharmacokinetics, and pharmacodynamics (17). Mathematical modeling and simulation may hold the promise of elucidating the interaction between TP, their targets, and ADA. Various factors often make such an effort difficult. The effort is further impeded in studies where insufficient numbers of samples can be collected to characterize the TP pharmacokinetics. This is the case when only sparse samples of TP concentrations are collected due to issues of patient burden, study logistics, or other reasons. In such cases, there is not enough information to elicit a proper understanding of the interaction between the pharmacokinetics of TP and the ADA profiles. Furthermore, the majority of studies evaluating the influence of ADA on TP pharmacokinetics are based on solid-phase enzyme-linked immunosorbent assays (ELISA) in which the presence of circulating TP renders the test insensitive in detecting ADA (18).

Simplified empirical approaches using mixed-effects modeling, as a tool for studying pharmacokinetic variability,

can be useful to explore the immunogenicity effect on TP pharmacokinetics. Two examples of adapting population pharmacokinetic modeling and simulation analysis for this purpose are presented below. While the first example presents a methodological approach to analyze pharmacokinetic data in the presence of ADA, confirm the hypothesized immunogenicity effect on TP pharmacokinetics, and quantify the magnitude of its effect, the second example provides a framework for analyzing sparse pharmacokinetic data presumably in the absence of an immunogenicity effect on TP pharmacokinetics and conducting a sensitivity analysis for confirming the lack of effect with sufficient power. Both analyses were conducted with sufficient statistical consideration on data with typically large variability.

Example 1: Population-Based Modeling and Simulation Approach for Analyzing and Confirming the Immunogenicity Effect on the TP Pharmacokinetics in Early Clinical Development

AMG 317 (Amgen, Inc.) is a fully human IgG_2 monoclonal antibody that binds with high affinity to human IL-4Rα ($K_D = 0.18$ nM) and is in early clinical development for the treatment of asthma. Three phase 1 trials and one phase 2 trial were conducted in healthy subjects and patients with mild to moderate and severe asthma to evaluate the safety, pharmacokinetics, and pharmacodynamics of single or multiple weekly doses given as intravenous (i.v.) or subcutaneous (s.c.) injections. The i.v. doses ranged from 10 to 1,000 mg, and the s.c. doses ranged from 100 to 300 mg. The pharmacokinetics of AMG 317 is nonlinear, and the nonlinearity is a consequence of the saturable elimination pathway due to the target-mediated disposition of AMG 317, as is the case of many targeted TP [19,20]. A population pharmacokinetic modeling analysis of AMG 317 was conducted using 2,183 concentrations from 294 subjects for the purpose of identifying sources of variability. Although fully human, AMG 317 was found to be immunogenic in a subset of subjects. Thus, an additional objective was to confirm and quantify the immunogenicity effect on AMG 317 pharmacokinetics, which could be confounded with the concentration-dependent nonlinearity of the compound [21].

Serum samples were assayed for AMG 317 concentrations using an ELISA assay. The lower limit of quantification ranged from 9.7 to 10 ng/ml. Anti-AMG 317 binding antibodies (i.e., free ADA) were detected with a validated electrochemiluminescence immunoassay. The sensitivity of the assay was approximately 34.3 ng/ml of anti-AMG 317 in neat human serum. At concentrations of 29 and 108 µg/ml of AMG 317, the assay was able to detect 94 and 500 ng/ml of antibody, respectively. Based on the ADA assay, a variable (ABS) was defined and assigned a value of 0 or 1, if ADA status was negative or positive, respectively. ABS was set to 0 for samples collected within the first 2 weeks after treatment started because of the low probability that ADA would be present soon after exposure to the TP. If any ADA was present, it was assumed to be at low levels and, therefore, less likely to influence AMG 317 pharmacokinetics. If ADA status was negative at a particular time point, but the previous and successive measurements were positive, ABS was coded as 1.

A two-compartment quasi-steady-state (Qss) pharmacokinetic model [22], as a simplification to approximate the general target-mediated drug disposition model [23], with linear and nonlinear clearances was adopted and reasonably described the entire range of concentrations. Preliminary investigations with a Michaelis–Menten model as even a simpler approximation showed systematic bias in estimation of concentrations below 300 ng/ml. The bias was, however, eliminated with the Qss approximation. In the Qss model, the inter-individual random effects were assumed to be on clearance (CL), central volume of distribution (V_1), inter-compartmental flow (Q), absorption rate (k_a), and total receptor concentration (R_{max}), and were described by an exponential model. For these parameters, the effects of covariates including demographics and immunogenicity were explored. The residual variability was described by combined additive and exponential error model.

The population pharmacokinetic analysis was conducted in the nonlinear mixed-effects modeling software NONMEM (version VI, level 2.0 and version VII, ICON Development Solutions). The first-order conditional estimation method with interaction was used. Model development was guided by the standard diagnostic plots and plausibility of the parameter estimates with minimum objective function value as a secondary measure for model assessment. External model validation was conducted with the phase 2 study as the test data set. The precision of model parameters was investigated by stratified bootstrap. Specifically, 500 replicate data sets were generated through random sampling with replacement using individuals as sampling units. Stratification during the random sampling process was implemented to ensure that the bootstrap datasets adequately represented the original data with respect to continuous covariate distributions and categorical covariate percentages. The 90% CI was constructed by observing the 5th and 95th percentiles of the parameter distributions resulting from the bootstrap runs. The above modeling approach was found to be adequate in characterizing the population pharmacokinetics of AMG 317. The final model revealed the effects of body weight on CL and V_1, age on k_a, and formulation and route of administration on V_1. Details of pharmacokinetic parameter estimates were reported elsewhere [21].

In this review, the immunogenicity effect on AMG 317 clearance was further assessed based on the population pharmacokinetic model with three approaches of increased complexity. The first approach was to implement inter-occasion variability on the pharmacokinetic parameters of the model. This allowed the flexibility of generating estimates of the *post hoc* model parameters that were driven overtime according to the pharmacokinetic data observed at various specified occasions. If indeed these time-dependent parameters were influenced by ADA presence, they could be correlated with immunogenicity status as a time-dependent covariate and studied graphically. Thus, the effect of immunogenicity on AMG 317 pharmacokinetics could be ascertained with reasonable confidence. In this particular example, inter-occasion variability was implemented in the population pharmacokinetic analysis on CL and R_{max}, the two parameters considered to be responsible for AMG 317 clearance and potentially impacted by immunogenicity. This approach, although intuitive, should be considered exploratory since

the time-dependent individual parameters were generated based on the empirical Bayes method and could shrink toward the population parameter estimates if data from individual subjects were not informative. In addition, the definition of the occasion over the time was nevertheless arbitrary and conditioned on the study design.

An alternative approach was to include the time-dependent ADA status variable (presence or absence of ADA) directly as a model covariate and test it for its effect on the two clearance parameters. Significance of the effects was judged by the standard statistical criteria in model fitting. This was therefore the second approach, which was considered less exploratory and more definitive. Finally, a population pharmacokinetic model was established using early data that were not affected by ADA in order to predict later drug concentrations and compared with the observed concentrations. A significant difference, for example, would confirm the immunogenicity effect on AMG 317 pharmacokinetics, if other potential effects could be ruled out. The magnitude of that difference would quantify its effect. This approach avoided using the ADA status variable entirely, which may not always be accurate due to, for example, ADA assay limitations.

For the first approach, *post hoc* pharmacokinetic elimination parameters at different occasions were generated.

Figure 1 shows box plots of CL and R_{max} at different occasions comparing ADA-positive with ADA-negative patients. A trend of increased elimination in ADA-positive patients was observed relative to ADA-negative patients. Larger sample sizes at occasions were generally associated with more confidence of the median difference. Next, the ADA effect on CL and R_{max} was considered. With CL as an example, a form of $1+\theta\cdot ABS$ was coded with θ representing the increase in CL in the presence of ADA. The result showed that, typically, CL and R_{max} at ADA-positive occasions was 16% and 6% greater ($\theta=0.16$ and 0.06) than at ADA-negative occasions, respectively (Fig. 2). The difference in CL (but not in R_{max}) was significant. Based on the model with ADA status as a covariate on CL and R_{max}, the *post hoc* individual clearance parameters between ADA-positive and negative patients were also significantly greater for those patients with ADA always positive after the initial 2 weeks than for those with ADA always negative. The random effects had shrinkage in the range of 40–65%, implying that the *post hoc* individual parameters were moderately informative. For the final approach, the model-predicted concentrations were compared with the observed (Fig. 3). The upper-left panel in the plot shows a typical profile of the model based on week 1 data for the 300-mg
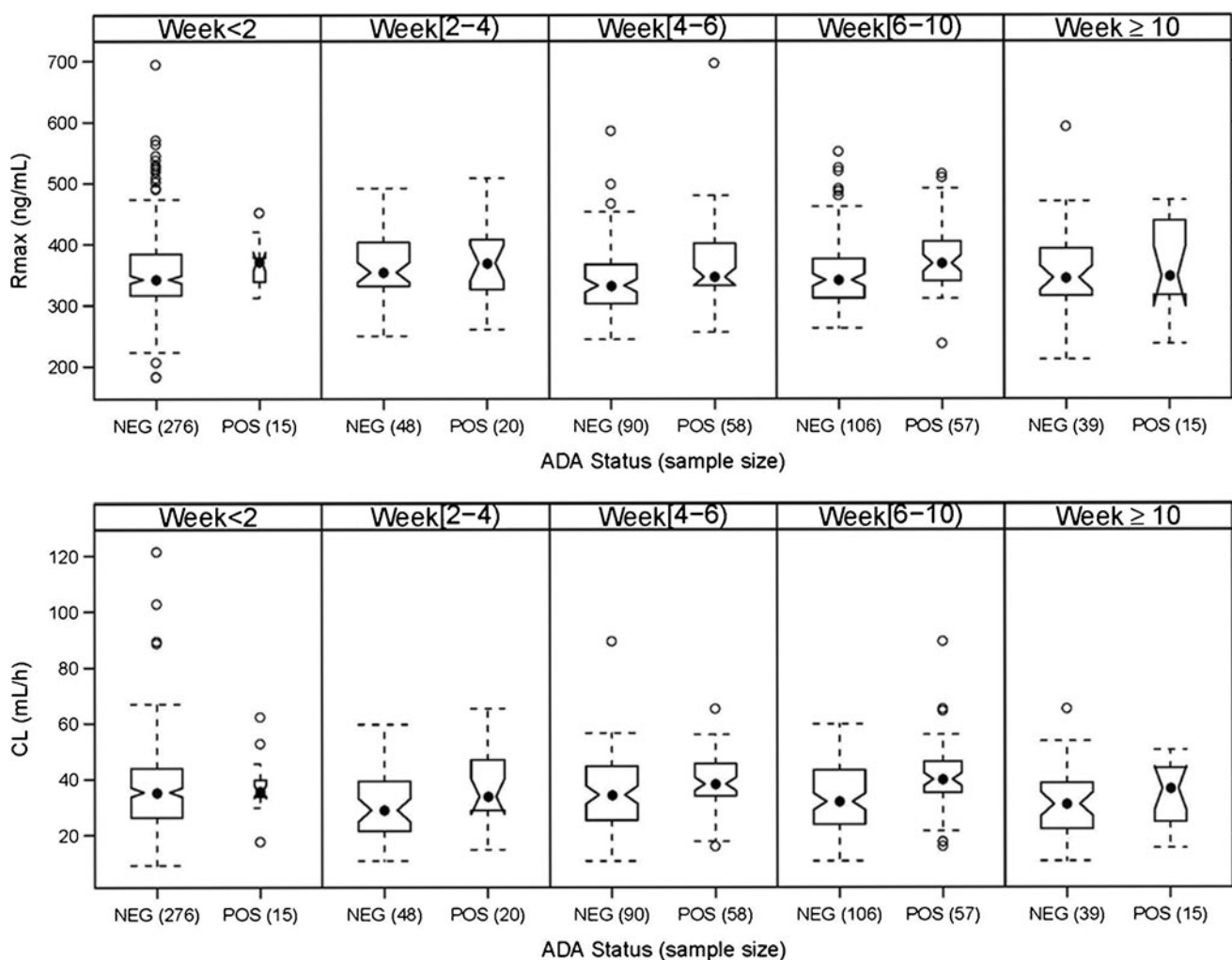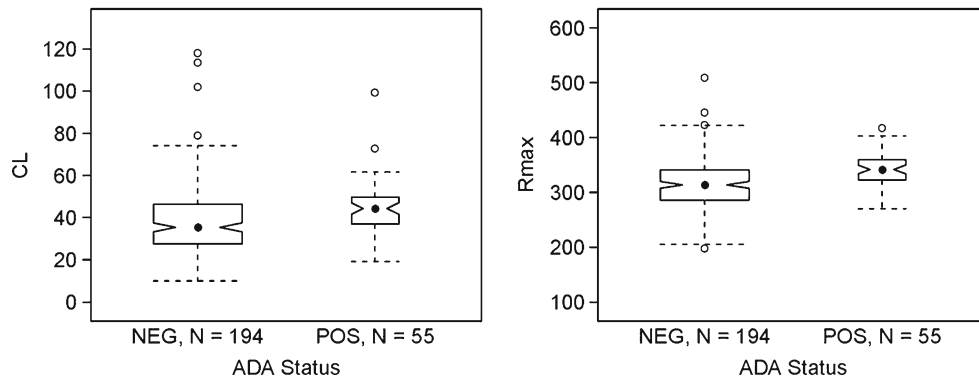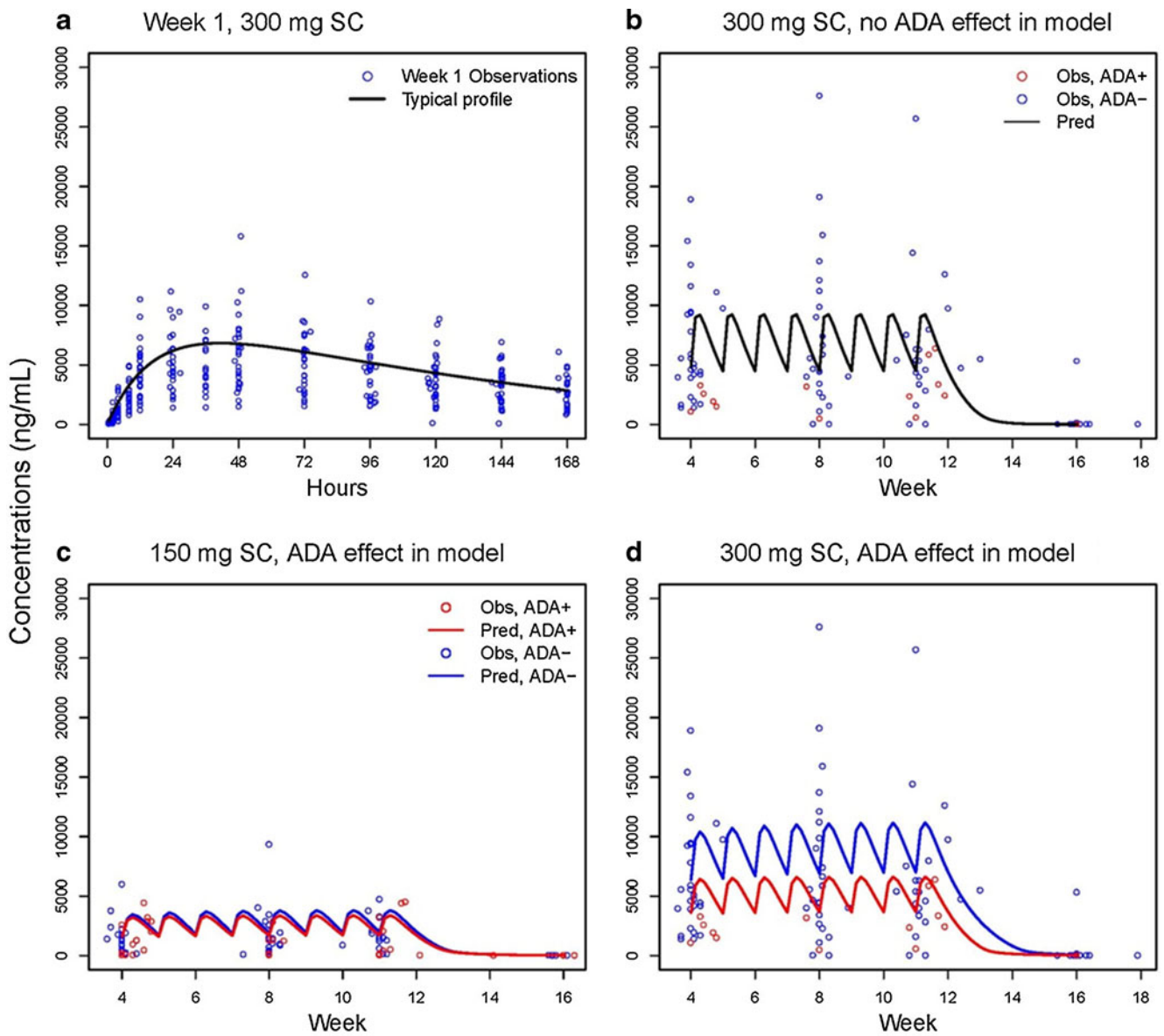

Fig. 1. *Box plots* of CL and R_{max} at different occasions (i.e., study weeks) comparing ADA-positive with ADA-negative subjects

Fig. 2. *Box plots* of CL and R_{max} in subjects with no change in ADA status over time or at steady state (*x*-axis label represents ADA status from week 4 and later)

Fig. 3. Time course of the observed concentrations and predicted curves from a model without accounting for ADA effects on CL and R_{max} **a** during week 1 when ADA were not yet detected and **b** during week 4 and later for the 300-mg-dose group. Time course of the observed concentrations and predicted curves from a model accounted for ADA effects on CL and R_{max} during week 4 and later for **c** 150 mg and **d** 300 mg

dose. Prediction beyond week 3 shows that ADA-positive observations tended to be below the typical population profile, which again confirmed the ADA impact on pharmacokinetics (upper-right panel). When using the model with ADA status as a covariate to predict later drug concentrations, the difference between ADA-positive and ADA-negative patients was not the same across dose levels; the 300-mg-dose group appeared to be more impacted by the immune response than the 150-mg-dose group (as shown in the lower panels). However, the result should not be viewed as definitive since the ADA response is known to be dependent on different aspects of the treatment: the limitation of the bioanalytical assays, the inclusion of multiple studies with different doses, administration schedules and routes. These variables can all influence the level and type (neutralizing *vs.* binding) of the ADA response, which in turn affects the TP pharmacokinetics, and should ideally be considered during the analysis.

By applying the population pharmacokinetic analysis on early-phase clinical trial data, we have assessed and confirmed the impact of immunogenicity on AMG 317 pharmacokinetics after i.v. and s.c. administration in healthy subjects and asthma patients. The approach using ADA status as a covariate is exploratory in nature since the interaction of ADA with AMG 317 was assumed to modify its pharmacokinetics in a simple fashion. Nevertheless, this pragmatic approach has proven to be useful in identifying the immunogenicity effect on the linear pharmacokinetics of other TP (10, 24–28).

Example 2: Population-Based Modeling and Simulation Approach for Analyzing and Confirming the Lack of Immunogenic Effect on the TP Pharmacokinetics in Late Clinical Development

In some instances, the immune response following the administration of a TP is relatively low. In such situations, getting the appropriate sample size to have an adequate power in assessing the lack of an ADA effect on the TP pharmacokinetics may be difficult. The problem can be further complicated if only sparse samples are available for assessing the pharmacokinetics, if the TP exhibits nonlinear pharmacokinetics due to target-mediated disposition and/or if a titrated dosing regimen is used in clinical trials. In these cases, pooling data from multiple studies may be helpful (29). However, the quality and quantity of the information obtained from multiple studies might be still limited, and therefore, the sample size may be too small to formally conduct any statistical comparison. In this circumstance, the lack of evidence of an ADA effect on TP pharmacokinetics is not evidence of its absence because the comparison is simply underpowered. Consequently, it is important to conduct a sensitivity analysis to identify the minimum magnitude of the immunogenicity effect on TP pharmacokinetics that can be detected with the available sample size.

An example of the methodology employed for such assessment has been recently published and applied to panitumumab (30). Panitumumab, a fully human IgG$_2$ against the epidermal growth factor receptor, is indicated for the treatment of metastatic colorectal cancer. Only 1.8% and 0.2% of patients treated with panitumumab in combination

with oxaliplatin- or irinotecan-based chemotherapy, respectively, developed binding and neutralizing anti-panitumumab antibodies. Since neutralizing antibodies have not been reported in cancer patients treated with panitumumab in monotherapy (31,32), it is unlikely that the low incidence of immunogenicity reported for panitumumab in combination with oxaliplatin- or irinotecan-based chemotherapy was due to the immunosuppressive effects of these chemotherapy regimens.

A population pharmacokinetic modeling and simulation approach was used to evaluate the impact of immunogenicity on serum panitumumab pharmacokinetics by comparing the observed panitumumab concentrations of ADA-positive patients with the predicted pharmacokinetic profiles based on population pharmacokinetic parameters from ADA-negative patients. An open two-compartment model with the Michaelis–Menten approximation of the target-mediated disposition, previously developed to describe the panitumumab pharmacokinetics in ADA-negative patients (33), was used to simulate 1,000 pharmacokinetic profiles for each ADA-positive patient according to the actual individual dosing history. The observed concentrations from each ADA-positive patient were superimposed with the model-predicted distribution. The panitumumab pharmacokinetics in ADA-positive patients would be considered similar to the panitumumab pharmacokinetics in ADA-negative patients if (1) the proportions of the observed concentrations from ADA-positive patients falling above, within, and below the 90% prediction interval were not statistically different (chi-square test; $\alpha < 0.05$) from hypothesized proportions (i.e., 5% above, 90% within, and 5% below the prediction interval) and (2) the quartiles of the panitumumab concentrations from ADA-positive patients were compared with the ADA-negative model-based distribution of the panitumumab concentration quartiles. A sensitivity analysis was conducted to evaluate the minimum difference in panitumumab concentration between ADA-positive and ADA-negative patients which could result in a statistically significant effect of ADA on panitumumab pharmacokinetics, given the available sample size.

Among the 38 observed panitumumab concentrations from the ADA-positive patients, 5 (13%) were above, 2 (5%) were below, and 31 (82%) were within the 90% prediction interval derived from pharmacokinetic profiles of ADA-negative patients. These proportions were not statistically different ($P = 0.0685$) from the hypothesized proportions (Table I) and were similar to those for the ADA-negative patients ($P = 0.8807$). Similarly, the observed concentration quartiles for ADA-positive patients fall within the 95% confidence interval of the empirical model-based prediction of the concentration quartiles for ADA-negative patients (Fig. 4). Taken together, the results of the two approaches suggest that no marked difference in the observed panitumumab concentrations was observed between the ADA-positive and ADA-negative patients. Results of the sensitivity analysis showed that a statistically significant effect of immunogenicity would have been observed if the serum concentrations of ADA-positive patients were at least 55% lower than the currently observed values given the available sample size. Therefore, this analysis ruled out the possibility that immunogenicity would cause a greater than 55% decrease in

Table I. Distribution of the Observed Concentrations of Panitumumab Relative to the 90% Predictive Interval

Sample distribution in relation to 90% prediction interval	Antibody-positive patients				Antibody-negative patients				
	N	%	95% CI	P value[a]	N	%	95% CI	P value[a]	P value[b]
Below	2	5.3	0.6–17.8	0.07	3	4.4	0.9–12.4	0.13	0.88
Above	5	13.2	4.4–28.1		7	10.3	4.2–20.1		
Within	31	81.6	65.7–92.3		58	85.3	74.6–92.7		

[a] Chi-square test with hypothesized proportions (5% below, 5% above, 90% within)
[b] Chi-square test of antibody-positive proportion *vs.* antibody-negative proportion

panitumumab concentrations. Since the relationship between pharmacokinetics and efficacy has not been established, it is unclear what level of a decrease in panitumumab concentration would result in a clinically significant change in efficacy. By assuming a less than 20% difference would be biologically unimportant, additional simulations were conducted to understand the sample size required to detect that level of difference. The results showed that 200 and 650 samples in ADA-positive patients would be needed to detect a 38% and 20% difference, respectively, in panitumumab concentration from ADA-negative patients (Fig. 5). To obtain this larger number of samples, it would require either sparse samples from a larger ADA-positive population or intensive samples from a smaller ADA-positive population, both of which would be challenging, considering the low rate of immunogenicity for panitumumab.

In order to better quantify the effect of ADA on the pharmacokinetics of TP, it is necessary to fully utilize prior information available from patients that were exposed to foreign proteins but never developed antibodies. Assuming a validated population pharmacokinetic model is available to describe the time course of drug concentration in non-seroconverted patients, an alternative approach to analyze the pharmacokinetic data in example 2 is to use the population pharmacokinetic parameters derived from non-seroconverted patients as prior information for a Bayesian estimation of the pharmacokinetic parameters in seroconverted patients, using only the data from the seroconverted patients (PRIOR subroutine in NONMEM)

(34). In order to assess whether or not the non-seroconverted and seroconverted patients can be considered within the same parameter distributions, it is possible to dichotomize the data by assuming two populations exist that do not share the same pharmacokinetic parameter distributions. After adjusting by relevant patient covariates, the results of a random dichotomization implemented by the MIXTURE subroutine in NONMEM can be compared with the results of an arbitrary dichotomization that stratifies by the seroconversion status (i.e., non-seroconverted less than 800 titers or seroconverted greater than 800 titers). Such an approach has been successfully used in other settings (35).

In addition, a parametric bootstrap approach can also be used to compare the pharmacokinetic parameter distribution between non-seroconverted and seroconverted patients. Briefly, the population pharmacokinetic model derived from non-seroconverted patient data can be used to simulate the seroconverted patient data, which are then analyzed using the PRIOR subroutine in NONMEM and the pharmacokinetic model parameters derived from non-seroconverted patient data as prior information. Repeating this process 1,000 times, using a different set of pharmacokinetic model parameters obtained from the replicates of a non-parametric bootstrap analysis of the non-seroconverted patient data, allows obtaining the expected distribution of the pharmacokinetic parameters in seroconverted patients, conditioning on the pharmacokinetic sampling times available for this subpopulation, and assuming no effect of the seroconversion on the drug pharmacokinetics (i.e., the null
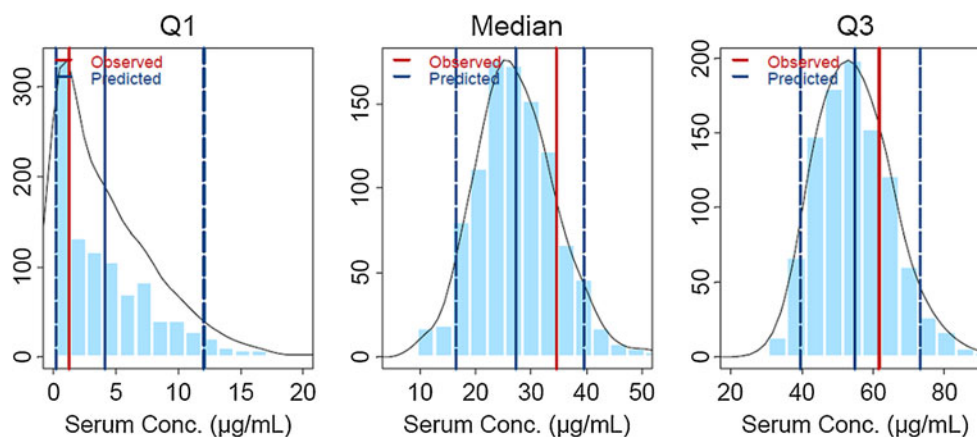
Fig. 4. Comparison of the observed serum panitumumab concentration quartiles for ADA-positive patients (*vertical red line*) and the empirical model-based distribution of the concentration quartiles for ADA-negative patients (*vertical light blue bars*). The *vertical blue lines* represent the median (*solid line*), and the 95% confidence interval (*dashed line*) of the model-based concentration quartiles for ADA-negative patients
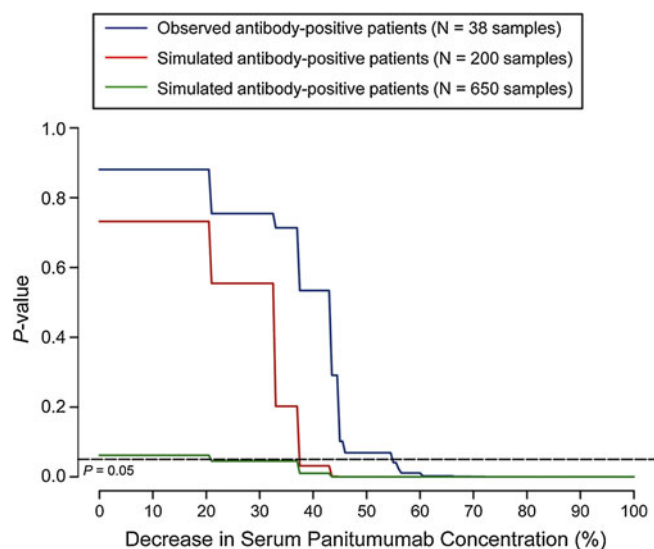
Fig. 5. Sensitivity analysis for estimating the minimum difference in panitumumab concentration between antibody-positive and antibody-negative samples that could be statistically significant (*P*<0.05) with respect to the model prediction

Fig. 6. Schematic of the immunogenicity-mediated disposition of a therapeutic protein

hypothesis). The distribution of model parameters under the null hypothesis can be compared with the Bayesian estimates of the pharmacokinetic parameters in seroconverted patients using only the data from the seroconverted patients. The utility of the proposed approach to detect differences in the distribution of pharmacokinetic parameters across two populations has been demonstrated recently (36) and can be also applied to detect the immunogenicity effect on drug pharmacokinetics.

MECHANISM-BASED MODEL FRAMEWORK TO UNDERSTAND IMMUNOGENICITY-MEDIATED DRUG DISPOSITION

Thus far, a modeling and simulation approach has only been used in empirical ways to investigate the immunogenicity effect on TP pharmacokinetics. Moving forward, mechanistic modeling techniques will be instrumental to improve the understanding of the complex interaction among the TP, their targets, and the ADA, provided a sufficient amount of information is available during drug development. A first step in this direction can be the development of a theoretical mechanism-based modeling framework that qualitatively explains the different experimental findings and concepts outlined in this review.

A schema of such theoretical mechanism-based model describing the immunogenicity-mediated disposition of TP is presented in Fig. 6. In the absence of immunogenicity effects, an arbitrary i.v. dose of a certain TP is administered into the central compartment and, for simplicity, assumed to follow a standard one-compartment target-mediated drug disposition model, with a volume of distribution, V. The TP can either be eliminated through a nonspecific, linear pathway, characterized by the first-order rate constant, k_{el}, or bound to its receptor (or target), R, forming the drug–receptor complex, CR, according to a second-order constant, k_{on}. Then, the drug–receptor complex can be dissociated according to a first-order process (k_{off}) or eliminated (i.e., internalized for
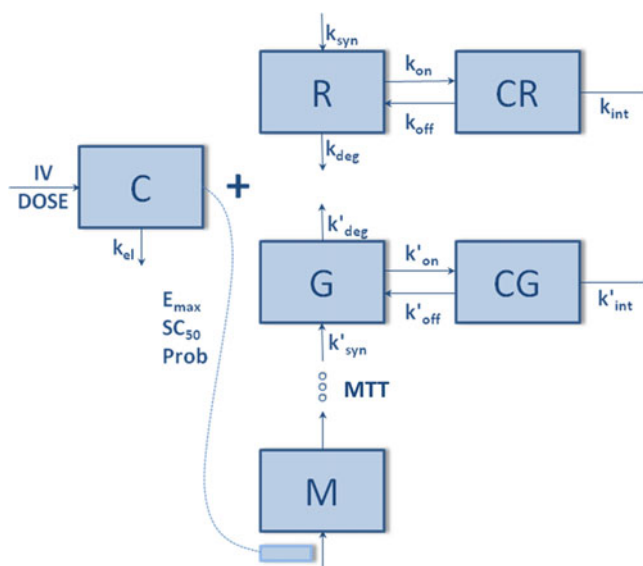
membrane-bound targets) also following a first-order process (k_{int}). The time course of the free and total drug concentrations as well as the free and the total receptor (or ligand) is displayed in Fig. 7 for a monthly dosing regimen, assuming V= 0.08 L, k_{el}=0.002 h^{-1}, R_0=25 mg/L, k_{syn}=1.25 mg/h, k_{deg}=0.05 h^{-1}, k_{on}=0.3 L/mg/h, k_{off}=0.015 h^{-1}, and k_{int}=0.01 h^{-1}.

A proportion of subjects (Prob) may develop an immune response as a consequence of TP administration. ADA isotype responses from IgM to various IgG subtypes have been reported. These responses have been shown to be directed to epitopes which target both the active site (ligand/receptor binding epitopes, which result in neutralizing activity) and non-neutralizing sites of TP (binding activity). Consequently, in a subset of patients, the TP stimulates B cells to produce first IgMs (M). The maximum immunogenic effect of the TP (E_{max}) determines the magnitude of the immune response, while the drug concentration that provides 50% of the maximum immune response (EC_{50}) represents
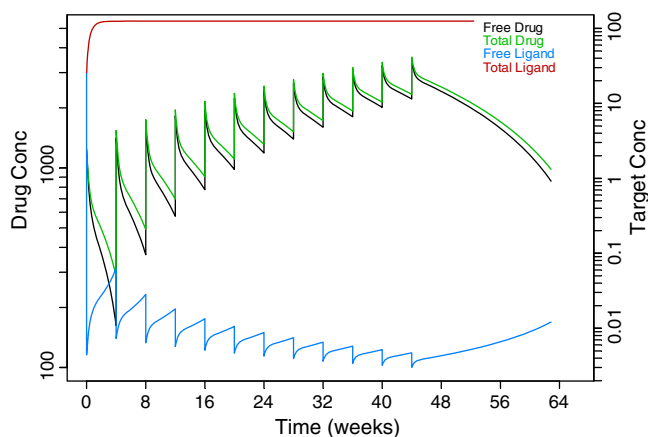
Fig. 7. Model simulation of the time course of the free and total drug concentrations as well as the free and the total receptor (or ligand)

the sensitivity of a subject to develop an immune response. After a certain amount of time (MTT), the initial IgM response disappears, and the specific immunogenic effects are driven by IgG (*G*). According to the model displayed in Fig. 6, if the IgG is a binding ADA that does not interfere with the ability of the TP to bind to its target, then the k_{el} may or may not be affected. If k_{el} is affected, it could be a function of the ADA concentration that results either in a reduction (sustained effect) or increase of TP clearance. An increase of TP clearance might be due to the formation of immune complexes in the blood, which trigger an endogenous elimination process mediated through the reticuloendothelial system, predominantly phagocytic cells in the liver and spleen such as monocytes and macrophages as well as endothelial cells. Furthermore, if a binding ADA interferes with the ability of the TP to bind to its target because of steric hindrance, then the apparent k_{on} might decrease, while k_d (k_{off}/k_{on}) may increase, and the drug affinity to the target may decrease, regardless of the effect of the binding ADA on k_{el}.

On the other hand, if the IgG is a neutralizing ADA, then the ADA competes with the receptor for binding the TP, resulting in immunogenicity-mediated TP disposition, a specific case of a pharmacodynamic-mediated drug disposition (37). The immune complexes circulating in the bloodstream are internalized (k'_{Int}) and undergo subsequent lysosomal degradation. Since this process results in the degradation of the TP and the ADA, ADA-induced clearance constitutes an additional elimination pathway for the TP. In this situation, k_{el} may or may not be affected. If k_{el} is not affected, an example of the time course of the free and total drug concentrations as well as the free and the total receptor (ligand) is displayed in Fig. 8 for a monthly dosing regimen, assuming the same parameters are used for Fig. 7 and Prob=1, MTT=56 days, k'_{syn}=0.012 mg/h, k'_{deg}=0.05 h^{-1}, k'_{on}=0.05 L/mg/h, k'_{off}=0.15 h^{-1}, k'_{int}=0.01 h^{-1}, E_{max}=1, and EC$_{50}$=200.

In order to characterize the time course of drug effects, it is critical to understand not only the pharmacokinetic profile of free and bound forms of the drug and the ligand, but also the time course of free ADA, immune complex, and total

ADA, and its relationship with drug and ligand concentrations (Fig. 9). Figure 9 also illustrates the reasons why the effect of high concentrations of circulating TP should be considered in developing and validating bioanalytical methods for measuring free circulating ADA. Assuming arbitrarily that a lower limit of quantification for measuring free circulating ADA is 1, Fig. 9 shows that the free circulating ADA would not be measurable until approximately more than 1 month after the last dose. Thus, assessment of the immunogenicity status of a subject is typically performed after the drug treatment has stopped. It is, however, possible that a subject with high concentrations of TP could score negative for the presence of neutralizing antibodies in the bioassay (Fig. 9), whereas ADA could influence the concentrations of TP as evidenced by comparing Figs. 7 and 8. In the absence of quantifiable free ADA concentrations, the free and/or total concentrations of TP can be used as a biomarker of a mature neutralizing antibody response, which allows monitoring the impact of the ADA in terms of pharmacodynamics, efficacy, and safety. In this setting, the appropriate timing of sample collection should be established, and sampling design should consider including samples at the peak and trough, at late time points after dosing, and after the circulating drug has been cleared. For instance, both infliximab and adalimumab (anti-TNF-α antibody therapeutics) have been shown to clear more rapidly when neutralizing ADA was formed and resulted in loss of efficacy (38,39). However, 2 to 4 weeks post-administration were needed to have detectable ADA and to confirm the immunogenicity impact. Therapeutic drug monitoring has been suggested in order to individualize the dosing regimens that deliver higher and/or more frequent doses to avoid the loss of efficacy due to immunogenicity.

The limitation of free ADA assays described above justifies the efforts in developing assays for measuring immune complex (Fig. 9). With this type of assay, the time window for immunogenicity assessment will no longer be that sensitive, will provide better understanding of ADA responses, and will allow more flexibility to incorporate rich or optimal sampling strategy to understand the interaction
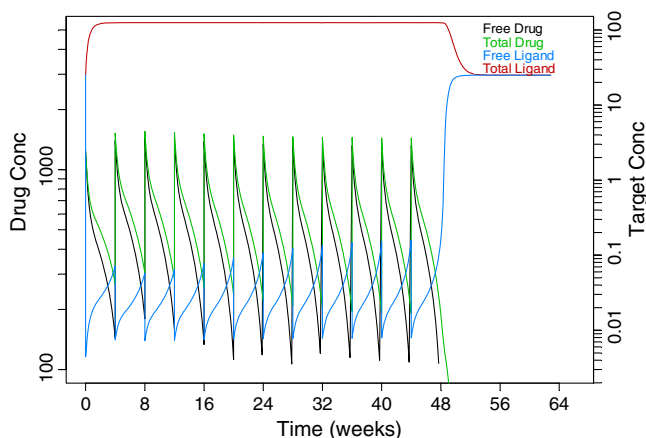
Fig. 8. Model simulation of the time course of the free and total drug concentrations as well as the free and the total receptor (or ligand) when k_{el} of free drug concentration is not affected by IgG neutralizing ADA
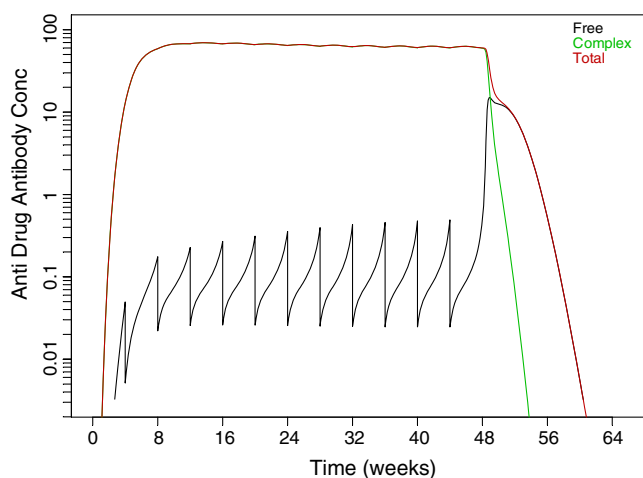
Fig. 9. Model simulation of the time course of free ADA (*black line*), the immunocomplex (*green line*), and the total ADA (*red line*)

between the pharmacokinetics of TP and the ADA profiles. Highly sensitive liquid-phase mobility-shift assays and liquid-phase radioimmunoassays that measure ADA in the presence of circulating TP are also emerging and should provide more accurate evaluation of the rate and intensity of the immunogenicity effect early in the course of TP treatment (13).

CONCLUSION

Many known and unknown factors exist that may influence immunogenicity of a TP. The sensitivity and specificity of current analytical methods are not always adequate to detect rapidly the occurrence and the magnitude of immune response when seroconversion happens. Furthermore, clinical studies are essentially observational in nature with respect to immune response. The factors that led to immune response of individual patients are themselves heterogeneous, and the incidence of observed immune response is generally low. Therefore, studies conducted are often ineffective for detecting, quantifying, and predicting changes in TP efficacy and safety that are particularly due to immunogenicity.

As reviewed, the recent advance in statistical approach for analyzing antibody titer data conditional on seroconversion offers a way to quantify the probability of developing ADA and the magnitude of the antibody titers following TP administration. By ruling out other influential factors, the changes in TP pharmacokinetics observed in clinical trials may better be perceived as an early indication of ADA formation and served as a surrogate for later changes in efficacy and safety in individual subjects. In this regard, simplified empirical approaches, using nonlinear mixed-effects modeling as a tool for studying pharmacokinetic variability, are shown to be useful to explore or even confirm the impact of immunogenicity on TP pharmacokinetics with adequate statistical rigor. Methods used thus far are empirical and data-driven which offer limited causality understanding. Moving forward, we believe a theoretical mechanism-based modeling framework such as the one presented in this communication could be instrumental to address the limitation. Such modeling framework explicitly links the complex interaction among the TP, its target, and ADA and considers both the target- and immune-response-mediated disposition of the TP. The approach should provide insight to the understanding of the causal relationship in TP immunogenicity and, by suggesting needed experimental areas, help to elucidate further PK mechanism of the TP.

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