Research Article

A Mathematical Model of the Effect of Immunogenicity on Therapeutic Protein Pharmacokinetics

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Received 14 November 2012; accepted 22 July 2013; published online 30 August 2013

Abstract. A mathematical pharmacokinetic/anti-drug-antibody (PK/ADA) model was constructed for quantitatively assessing immunogenicity for therapeutic proteins. The model is inspired by traditional pharmacokinetic/pharmacodynamic (PK/PD) models, and is based on the observed impact of ADA on protein drug clearance. The hypothesis for this work is that altered drug PK contains information about the extent and timing of ADA generation. By fitting drug PK profiles while accounting for ADA-mediated drug clearance, the model provides an approach to characterize ADA generation during the study, including the maximum ADA response, sensitivity of ADA response to drug dose level, affinity maturation rate, time lag to observe an ADA response, and the elimination rate for ADA–drug complex. The model also provides a mean to estimate putative concentration–time profiles for ADA, ADA–drug complex, and ADA binding affinity–time profile. When simulating ADA responses to various drug dose levels, bell-shaped dose–response curves were generated. The model contains simultaneous quantitative modeling and provides estimation of the characteristics of therapeutic protein drug PK and ADA responses *in vivo*. With further experimental validation, the model may be applied to the simulation of ADA response to therapeutic protein drugs *in silico*, or be applied in subsequent PK/PD models.

KEYWORDS: anti-drug antibody; immunogenicity; mathematical model; pharmacokinetics; therapeutic proteins.

INTRODUCTION

Immunogenicity has become an important issue for therapeutic proteins during preclinical and clinical drug development phases. This topic has elicited much research interest and regulatory attention, resulting in a large number of recent scientific publications as well as FDA guidances (1–8). The root cause of immunogenicity is that the presence of antigenic components in therapeutic proteins (*e.g.*, foreign sequences or structures, aggregates, or impurities) can be recognized by the immune system, and elicit an immune response. This results in the production of anti-drug antibodies (ADA) against the administered protein drug, which may significantly influence the pharmacokinetics (PK), efficacy, and/or safety profiles of the therapeutic proteins. The PK profiles of the therapeutic proteins are often altered along with the appearance of ADA due to the modification of drug clearance (1). Although in rare cases ADA increase the drug exposure (9), they often accelerate drug

elimination and reduce drug exposure, even to a point where the pharmacological effect of the drug is obliterated, causing loss of efficacy (1). In addition, the loss of efficacy could be induced by the binding of ADA to the target binding site of the therapeutic proteins, which directly neutralize their pharmacological activities (1). ADA response can also lead to increased toxicity of a therapeutic protein through the formation of immune complexes which may result in anaphylaxis/hypersensitivity reactions (10). Last but not least, in the case of therapeutic proteins with endogenous counterparts, ADA may cross-react with the endogenous protein, leading to a deficiency syndrome which is a severe safety issue (11,12).

The central role of ADA in immunogenicity has attracted substantial research efforts aimed at characterizing the properties of ADA, such as the magnitude of ADA response, binding affinity, and neutralization capacity (13,14). Due to the heterogeneous nature of ADA and the lack of appropriate quantitative laboratory reference standards, the experimental characterization of ADA concentration has been limited to be quasi-quantitative (14). For example, instead of reporting ADA concentrations, titer values are routinely used for a qualitative estimation of the magnitude of ADA response (15). The binding affinity of ADA to the therapeutic protein is another important characteristic of the immune response, since it can undergo “affinity maturation” over time, a process that generates antibodies with higher binding affinity with time progression and repeated protein drug administration (16). Affinity maturation is an essential

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component of vaccination, as antibodies against the pathogen are expected to exhibit improved affinities with successive immunizations. Because of the polyclonal nature of the ADA, which results in heterogeneous populations of antibodies with varying affinities, a reliable approach for determining ADA affinity is not yet available.

Based on appropriate assumptions and reasonable simplifications of the immune system mechanisms, mathematical modeling could serve as a complementary approach to experiments for assessing the observed ADA response or even predicting the ADA response to therapeutic proteins. A few mathematical models have been developed for predicting the time course of various immune response components, mostly based on simulation. For example, Bell developed a mathematical model for predicting polyclonal antibody production based on the proliferation of relevant B cell species (17). Lee *et al.* simulated and predicted the adaptive immune response to influenza A virus infection, in which the virus triggers both an antibody response and cytotoxic T cell proliferation (18). The same philosophy underlying some of these models could be applied to the problem of predicting protein drug immunogenicity. That being said, these types of models may not generate therapeutic protein-specific prediction because of the lack of specific parameters to inform the model. Attempts to characterize immunogenicity using PK or statistical models were also reported. A recent approach was proposed by Xu *et al.* to consider immunogenicity status as a covariate in modeling the therapeutic protein PK (19). The authors analyzed the population PK of golimumab in patients with ankylosing spondylitis, and found anti-golimumab antibody status significantly influenced golimumab clearance. This model helps to account for the variability in PK between subjects when the ADA status is known *a priori*. Another statistical approach to characterize immunogenicity was proposed by Bonate *et al.* to model antibody titers using a zero-inflated Poisson random effects model (20). The model was able to identify patient-specific factors that might influence antibody titer. Although these models could account for the variability in immunogenicity, they could not be applied to assess/extract more ADA information such as putative ADA concentration. Despite growing efforts to develop quantitative methods, including modeling, to assess immunogenicity, a general approach to assess and ultimately predict therapeutic protein-specific ADA production and its impact on the drug's PK has not yet been described. In this article, we are proposing a PK/ADA mathematical modeling approach for quantitatively assessing ADA response. Recently, similar basic model structures were proposed by Chirmule *et al.* and Perez Ruixo *et al.* to evaluate the impact of immunogenicity on therapeutic protein pharmacokinetics (1,21). However, this paper applies a fully developed mathematical model to data fitting and simulation. This model can be informed from multiple and repeated dose PK studies in which the PK profiles are significantly altered by the presence of ADA. The PK/ADA model is inspired by traditional PK/PD models, and hypothesizes that ADA changes the PK profiles of therapeutic proteins by introducing a time-dependent ADA-mediated clearance route. This process can be regarded as a subtype of target-mediated drug disposition (TMDD) (22) called pharmacodynamics-mediated drug disposition (23), where as consequence of the drug effect (eliciting ADA response), the drug disposition is altered. By accounting for ADA-mediated drug clearance in the PK/ADA model, the model is able to take advantage of

relatively simple PK studies, and generate estimates of ADA response for specific therapeutic proteins, including concentration and binding affinity-time profiles of ADA. We speculate that once informed on existing studies, the model can also be potentially applied to immunogenicity prediction, such as simulating the expected ADA response following various dose regimens.

THEORY

Modeling Pharmacokinetics in the Presence of ADA: the Data

The PK data that are suitable for informing our proposed model need to fulfill the following criteria:

1. Pharmacokinetics recorded following repeated doses. Attention should be particularly paid to assess whether the drug concentration is free or total, and then incorporate that into the model accordingly. For example, if the assay measures total drug concentration, the PK data should be fitted to the total drug including ADA–drug complex in the model;
2. No preexisting anti-drug antibody (the ADA assay should be confirmed to be negative in pre-dosed animals);
3. Measurable changes in PK profile with repeated dosing (showing decreasing or increasing therapeutic protein concentration with repetitive dosing) that cannot be readily ascribed to expected target-mediated drug disposition;
4. Clear attribution of the PK changes to ADA-mediated drug disposition (ADA production is independently confirmed, *e.g.*, by a positive result in an ADA screening assay).

This type of PK data indicates the presence of ADA has altered the PK profile of the therapeutic protein, by affecting its elimination through the formation of ADA–drug complexes. Presumably, the magnitude of the change in therapeutic protein PK will depend on the ADA characteristics, such as concentration, binding affinity, rate of production and rate of clearance. Therefore, the changed PK profile of the therapeutic protein contains information about the ADA raised during the time course of the experiment. By building an empirical PK/ADA model, we intend to extract this information, and make assessments about the produced ADA which are suitable for experimental validation.

Modeling Pharmacokinetics in the Presence of ADA: the Model

The proposed mathematical model for therapeutic protein pharmacokinetics and ADA response (designated as “PK/ADA model”) is illustrated in Fig. 1.

The modeling of therapeutic protein PK is based on empirical PK modeling with the incorporation of ADA–drug interactions. When the therapeutic protein is repetitively dosed (*via* either subcutaneous or intravenous route), ADA is raised, binds to the free therapeutic protein reversibly with rate constants k_{on} and k_{off} , and forms an ADA–drug complex. The formation of the ADA–drug complex adds an elimination route for the free therapeutic protein, and therefore alters its PK.

Fig. 1. PK/ADA model for immunogenicity assessment. This PK/ADA model is based on the empirical modeling of protein drug–ADA interactions. With repeated dosing of the drug (e.g., via s.c. or i.v. route), ADA may be raised against the drug. The dashed arrow denotes the accumulated drug dose driving the generation of ADA [Eq. (1) in the text]. To account for the time delay to generate ADA, putative ADA doses are injected as boluses into a hypothetical compartment “ADA depot”. The ADA subsequently pass through a series of delay compartments with a time delay t_{lag} before entering the central compartment. The circulating ADA can bind to the drug reversibly with rate constants k_{on} and k_{off} , form complex, and alter the drug PK by adding additional elimination pathway. The affinity maturation of ADA is modeled by decreasing k_{off} in concert with the drug exposure time [Eq. (3)]. By accounting for the ADA-mediated drug clearance in the PK/ADA model, the ADA parameters can be estimated by fitting the drug PK profile

The modeling of ADA response is based on empirical functions. Putative ADA boluses are generated at the dosing time points of therapeutic protein, starting from the second dose (model assumptions 1 below). The dose of ADA is modeled as a saturable function depending on the therapeutic protein exposure (cumulative drug dose) [Eq. (1)]. The amount of ADA produced initially increases with cumulative drug dose (CD), until it reaches the plateau (A_{max}). To account for the time delay to develop an immune response, ADA boluses are injected into a hypothetical compartment (“ADA depot”), and subsequently enter the central compartment after going through a string of “delay compartments”, with a total time delay t_{lag} . The number of the delay compartments n can be flexible, but, as it is well known, increasing n results in better resolution and better approximation of a true (switch-like) delay. The value of n was chosen to be 5 in our model, which is supported by the report of Krzyzanski (24), that when the number of delay compartment is at least 5, the transit compartments model approximates lifespan based indirect response models. The transfer rate constant k_T between the ADA depot and all the delay compartments is automatically derived from t_{lag} [Eq. (2)]. The affinity maturation of ADA (i.e., the expected increase in affinity with time following successive drug doses) is modeled by decreasing k_{off} with the exposure time of the therapeutic protein [Eq. (3)]. When accounting for the ADA-mediated drug clearance in the PK/ADA model, the ADA parameters

can be estimated by fitting the drug's PK profile, as explained further. What follows here is a description of the model's parameters and their interpretation.

Amount of ADA depot bolus ($\ln_A$):

$$\ln_A = \frac{A_{max} \cdot CD}{(CD + K_m)} \quad (1)$$

The parameter A_{max} (in mole) is the maximum amount of ADA that can be produced by dosing the particular therapeutic protein being considered. CD (in mole per kilogram) is the cumulative drug dose. K_m (in mole per kilogram) is the accumulated drug dose at which the ADA production reaches 50% maximum.

Transfer rate constant (k_T):

$$k_T = \frac{n}{t_{lag}} \quad (2)$$

The rate k_T (in per hour) is derived by calculating the inverse of the mean time spent in each delay compartment. Since the mean time for ADA to remain in each compartment is t_{lag}/n , $k_T = 1/(t_{lag}/n) = n/t_{lag}$.

Affinity maturation:

$$k_{off} = k_0 \cdot e^{-a \cdot t} \quad (3)$$

Here, k_0 (in per hour) is the initial value for k_{off} , and is assumed to be constant among drugs (model assumption 7 below). The parameter a (in per hour) is the rate constant for k_{off} to change with time. Lastly, t (in hour) is the time.

The ADA–drug system can then be defined by the following differential equations:

$$\frac{dA_D}{dt} = \ln_A(t) - k_T \cdot A_D \quad (4)$$

$$\frac{dA_1}{dt} = k_T \cdot (A_D - A_1) \quad (5)$$

$$\frac{dA_i}{dt} = k_T \cdot (A_{i-1} - A_i) \quad i = 2, \dots, n \quad (6)$$

$$\frac{dA_p}{dt} = k_T \cdot A_n - k_{ADA} \cdot A_p - k_{on} \cdot \frac{D_p}{V_p} \cdot A_p + k_{off} \cdot C_p \quad (7)$$

$$\begin{aligned} \frac{dD_p}{dt} = & \ln(t) - (k_e + k_{pt}) \cdot D_p + k_{tp} \cdot D_T - k_{on} \cdot \frac{D_p}{V_p} \cdot A_p \\ & + k_{off} \cdot C_p \end{aligned} \quad (8.1)$$

$$\begin{aligned} \frac{dD_p}{dt} = & \ln(t) - \frac{V_{max} \cdot D_p}{K_{m,el} + D_p} - k_{pt} \cdot D_p + k_{tp} \cdot D_T - k_{on} \cdot \frac{D_p}{V_p} \cdot A_p \\ & + k_{off} \cdot C_p \end{aligned} \quad (8.2)$$

$$\frac{dC_p}{dt} = -k_{complex} \cdot C_p + k_{on} \cdot \frac{D_p}{V_p} \cdot A_p - k_{off} \cdot C_p \quad (9)$$

$$\frac{dD_T}{dt} = k_{pt} \cdot D_p - k_{tp} \cdot D_T \quad (10)$$

A_D is the amount of ADA in the ADA depot compartment, $A_1, A_2, \dots, A_n$ is the amount of ADA in the delay compartments, and A_p is the amount of free ADA in the plasma. D_p is the amount of free protein drug in the plasma, and C_p is the amount of drug-ADA complex in the plasma. D_T is the amount of free drug in the tissue. $In_A(t)$ is the bolus input for ADA, with the dose defined in Eq. (1). The ADA dosing time is essentially the drug dosing time, starting from the second dose (as discussed in the model assumption 2 and 3 below). $In(t)$ is the input dose for the drug. The parameter k_T is the transfer rate constant of ADA between delay compartments [Eq. (2)]. k_{ADA} is the elimination rate constant for ADA. In Eq. (8.1), k_e is the elimination rate constant for protein drug with linear elimination. The PK/ADA model can flexibly incorporate other clearance pathways, such as nonlinear clearance with Michaelis-Menten kinetics, or TMDD. In Eq. (8.2), Michaelis-Menten elimination is incorporated. $k_{complex}$ is the elimination rate constant for the ADA-drug complex. The parameters k_{pt} and k_{tp} are the distribution rate constant of drug between plasma and tissue; k_{on} and k_{off} are the association and dissociation rate constant for drug-ADA binding. Lastly, V_p is the volume of the central compartment. All the variables and the inputs in these equations are in the unit of mole/kg, normalized by body weight. As previously mentioned, n is the number of delay compartments. The parameters in the PK/ADA model are summarized in Table I. They can be categorized in three classes: (1) estimated from PK/ADA model, (2) fixed in PK/ADA model, and (3) derived from PK/ADA model.

Model Assumptions

The assumptions in the model are summarized below:

1. For the purpose of this modeling approach, it was assumed that induction of ADA results in a PK alteration. In situations when the ADA does not alter the drug PK, the current model may not be applicable. In cases where only part of the ADA has impact on drug PK, the model prediction may only account for these ADA fractions.
2. No ADA is elicited during the first dosing interval (from 3 to 7 days in the studies we presented here), mainly due to the time delay needed to raise an immune response. It has been reported that the lag period for the ADA to rise during primary immune response is very variable, as little as 7–10 days to as much as several weeks (25–27). Due to the model structure, the current model may not be directly applicable to a situation with preexisting antibody.
3. Starting from the second dose, ADA is produced, and injected as a bolus dose into a hypothetical depot compartment (“ADA depot”) at the time of drug dosing.
4. The amount of produced ADA is a saturable function of the accumulated therapeutic protein dose, which is an indicator for the accumulated drug exposure.
5. ADA enters the central compartment from the “ADA depot” through a string of delay compartments with an overall time delay t_{lag} .
6. PK parameters of free drug (*e.g.*, k_a , k_e) are estimated from the first dosing interval data only, and are kept

constant (at fixed values) during the entire PK study, independent of the ADA production.

7. ADA elimination rate (k_{ADA}) is assumed the same as endogenous IgG antibodies, and is species specific.
8. The k_{on} for ADA-drug binding is constant, while k_{off} changes with time. Previous studies suggested the k_{off} of antibodies is more variable while k_{on} is relatively constant during affinity maturation (28) and antibody affinity maturation exhibited time-dependency (29,30). The initial binding affinity of ADA (k_{on}/k_{off}) is assumed to be the same among different therapeutic proteins within the same species.
9. The volume of distribution for the ADA and the ADA-drug complex is assumed to be the same as that of the central compartment for the drug, V_p . It is assumed that there is no non-specific peripheral distribution for ADA and ADA-drug complex.

Modeling Process

Conduct PK Studies and Assess Available Data

Multiple and repeated dose PK studies are conducted, since repeated dosing is often needed to elicit strong ADA response and to significantly alter the PK of therapeutic proteins. In addition, it would be preferable to gather data at multiple dose levels to assess the presence of target-mediated disposition of the drug. Since therapeutic protein target or ADA can similarly modulate drug clearance, it is critical to account for any target-mediated clearance when modeling the altered drug PK profile before attributing the clearance to ADA.

Estimate Drug PK Parameters

The therapeutic protein PK parameters (*e.g.*, k_a , k_e) are estimated by fitting a suitable compartmental model using the PK data available during the first dosing interval. As mentioned, it is assumed that no ADA has been produced during the first dosing interval, due to the time delay to raise an immune response. Thus, during the first dosing interval, the drug PK is assumed to be unaffected by ADA, and the PK parameters are estimated and fixed for the entire PK study, assuming they are independent of ADA production. If target-mediated disposition of the therapeutic protein is observed, it is also assumed to be ADA-independent, and it is separately incorporated in the PK model.

Obtain ADA Parameters That Are Not Drug-Specific

Two ADA parameters are considered as not specific to the drug (system parameters), and they are acquired from the literature: (1) elimination rate of ADA (k_{ADA}), (2) on rate of the binding between ADA and drug (k_{on}). As described in the model assumptions, k_{ADA} is assumed to be the same as that of endogenous IgG antibodies, since the physicochemical properties of ADA is no different from the other endogenous antibodies. The half-life for polyclonal IgG in rhesus monkey is reported to be 8.3 days (31), corresponding to a k_{ADA} of 0.0035 h^{-1} . Since $K_a = k_{on}/k_{off}$, where K_a is the association constant for the binding between drug and ADA, a value of

Table I. Summary of Parameters in the PK/ADA Model

Category	Name	Definition	Unit	Parameter value (%cv)	
				Interferon-Fc fusion	mAb
Estimated from PK/ADA model	$A_{\max}$	Maximum amount of ADA bolus that can be produced	$\text{mol} \times \text{kg}^{-1}$	6.32×10^{-7} (4.1)	8.66×10^{-8} (12.7)
	K_m	Cumulative drug dose to achieve 50% $A_{\max}$ production	$\text{mol} \times \text{kg}^{-1}$	2.37×10^{-10} , fixed	5.83×10^{-11} (197)
	t_{lag}	Lag time for ADA to enter the central compartment	h	98.57 (8.51)	28.39 (4.71×10^{-3})
	a	Rate constant for k_{off} to decrease	h^{-1}	4.58×10^{-3} (78.8)	1.56×10^{-4} (15.3)
	k_{complex}	Elimination rate constant of the ADA-drug complex	h^{-1}	0.042 (143)	0.033 (23.8)
Fixed in PK/ADA model	f	Bioavailability (subcutaneous injection only)	N/A	0.82 (for 100 mg/kg)	N/A ^a
	k_a	Absorption rate constant (subcutaneous injection only)	h^{-1}	0.054	N/A ^a
	k_e	Elimination rate constant of the drug	h^{-1}	0.019	N/A ^b
	$V_{\max}$	Maximum elimination rate	$\text{mol} \times \text{h}^{-1} \times \text{kg}^{-1}$	N/A ^b	1.39×10^{-10}
	$K_{\text{m,el}}$	Drug amount in central compartment at which the elimination rate is 50% of $V_{\max}$	$\text{mol} \times \text{kg}^{-1}$	N/A ^b	7.15×10^{-10}
	k_{tp}	Distribution rate constant of the drug (2 compartment models only)	h^{-1}	0.094	0.020
	k_{pt}	Distribution rate constant of the drug (2 compartment models only)	h^{-1}	0.034	0.034
	V_p	Volume of the central compartment/plasma	$\text{L} \times \text{kg}^{-1}$	0.057	0.042
	k_{ADA}	Elimination rate constant of the ADA	h^{-1}	3.5×10^{-3}	3.5×10^{-3}
	k_{on}	On rate for ADA-drug binding	$\text{M}^{-1} \times \text{h}^{-1}$	3.60×10^9	3.60×10^9
	k_0	Initial value for k_{off} , which is the off rate for ADA-drug binding	h^{-1}	3.60×10^3	3.60×10^3
	t_{ADA}^c	Total lag time before ADA appear in the circulation	h	170.57	196.39
Derived from PK/ADA model	DI	Dosing interval	h	72.00	168.00
	$t_{1/2, \text{drug}}^d$	Elimination half-life of the drug	h	36.47	N/A ^b
	$t_{1/2, \text{ADA}}^e$	Elimination half-life of the ADA	h	199.20	199.20
	$t_{1/2, \text{complex}}^f$	Elimination half-life of the complex	h	16.50	21.00

^a mAb is intravenously injected^b The clearance of mAb is nonlinear and was modeled by Michaelis–Menten kinetics with parameters of $V_{\max}$ and $K_{\text{m,el}}$. Therefore, the elimination rate and half-life were not available since they are concentration dependent^c ADA lag time (t_{ADA}) represents the mean time ADA spend before appearing in the central compartment. It was calculated as $t_{\text{ADA}} = t_{\text{lag}} + \text{DI}$, where t_{lag} is the mean time for ADA to spend in the delay compartment and DI is the dosing interval, with an assumption that ADA response initiates from the second dose^d The elimination half-lives of the drugs were estimated by fitting the drug PK data during the first dosing interval^e The elimination half-life of the ADA was assumed to be the same as endogenous antibody (199.20 h in monkey)^f The elimination half-lives of the complexes were estimated from the PK/ADA model

$3.6 \times 10^9 \text{ M}^{-1} \text{ h}^{-1}$ for k_{on} (32) and $3.6 \times 10^3 \text{ h}^{-1}$ for k_{off} (28,32,33) results in a K_a of 10^6 M^{-1} . Here, we fixed the value of k_{on} , and let the value of k_{off} decrease with time to account for affinity maturation during immune response (28). The resulting starting K_a value of 10^6 M^{-1} was suggested by many studies measuring antibodies produced early after immunization (28,34–36).

fixed in order to let the fitting process converge. For fitting drug PK parameters and ADA parameters, currently, naïve-pool-based methods are applied by fitting drug concentrations to multiple subjects simultaneously. For data sets that have high inter-individual variability, population-based approach may be more suitable and this is further addressed in the “Discussion” section.

Fit the PK/ADA Model and Estimate the Drug-Specific ADA Parameters

The therapeutic protein-specific ADA parameters (e.g., $A_{\max}$, K_m , and t_{lag}) are estimated by fitting the full PK profiles arising from repeated dosing to the empirical PK/ADA model. Depending on the data set, some parameters may be

METHODS

Case Studies

The current case studies are adapted from multiple and repeated dose toxicokinetic studies which are originally designed to assess the safety and immunogenicity of the

studied therapeutic proteins in preclinical species. Pharmacokinetic studies were performed in cynomolgus macaques in compliance with US national legislation and subject to local ethical review. At all stages, consideration was given to experiment refinement, reduction in animal numbers and replacement with *in vitro* techniques. Blood from cynomolgus monkey was collected using manual restraint. All blood samples were collected following ethical guidelines. The data from these animal studies were later fitted with the PK/ADA model to estimate the ADA responses in animal models. As mentioned in the “Theory” section, the nature of the drug concentration (whether it is free or total) may influence the modeling process. For the current two case studies, the drug concentrations were measured *via* ELISA, and they were assumed to be free drug concentrations. In cases when all the ADA are neutralizing antibodies (which bind to the receptor/target binding site on the drug), the immune complex will not be captured by the ELISA assay, which uses receptor/target as capture/detection reagents. Therefore, the PK assay measures the true free drug concentration and the free drug assumption is solid. Otherwise, if some ADAs bind to other portions of the therapeutic protein, these immune complexes may be captured by the assay, and our model may underpredict the amount of the ADA that is actually present in the samples. The limitation is caused by the inability of the assay to distinguish free and total drug. Nevertheless, one should be aware of these limitations when applying the model.

Interferon-Fc Fusion

The PK study and the ADA assays for this case study come from a recent report (37). The fusion protein was dosed to cynomolgus macaques at 20, 50 and 100 mg/kg *via* the subcutaneous route twice a week for 25 days prior to a 3-week wash out. Two animals were included in each dose group. The drug's exposures in all three groups were gradually reduced during repetitive dosing. The ADA was confirmed to be negative in pre-dosed animals. In the 20 mg/kg group, reduced exposure was observed, consistent with the induction of an ADA response, as demonstrated *via* experimental measurements. Although the ADA response in the higher-dosing groups (50 and 100 mg/kg) cannot be confirmed experimentally due to the interference of high concentration of residual drug, we assume that the altered PK profile indicates the presence of ADA. The interferon-Fc fusion's PK parameters were obtained by fitting the PK data during the first dosing interval with a two-compartment PK model with linear elimination [Eq. (8.1)], and were then fixed in the PK/ADA model. The data from the multiple and repeated dose PK study were then fitted with the full PK/ADA model to estimate the ADA-specific parameters.

Monoclonal Antibody

Cynomolgus macaques were dosed intravenously with monoclonal antibody (mAb) at the dose levels of 1 and 10 mg/kg, once a week for 4 weeks. In this study, some of the animals (six of ten for 1 mg/kg and two of ten for 10 mg/kg group) exhibited altered PK profile after repetitive dosing of mAb. The animals which exhibited reduced mAb exposure with repetitive dosing were chosen for the PK/ADA model

and the ADA presence in these animals was confirmed with titer ADA assays. Different from the previous case study, mAb exhibited nonlinear elimination, observed in other previous PK studies. The PK parameters were obtained by fitting the PK data during the first dosing interval using a two-compartment PK model with saturable Michaelis-Menten elimination [Eq. (8.2)], and were then fixed in the PK/ADA model. The PK data from the multiple and repeated dose PK study were then fitted with the PK/ADA model to estimate the ADA parameters.

Simulation of Drug Dose-ADA Response Curves

To explore the relationship between therapeutic protein dose level and the resultant cumulative free ADA exposure, simulations were performed by changing drug dose levels in a wide range. As described above, the PK parameters of each compound were estimated from PK data during first dosing interval. The ADA parameters were obtained by fitting the PK/ADA model from multiple and repeated dose PK data. The PK and ADA parameters were then fixed in the PK/ADA model for simulation purposes, assuming these parameters do not vary with changing drug dose levels. The only parameter that changes between simulations is the drug dose level. At each drug dose level, simulation of ADA response was performed, and the cumulative free ADA concentration ($AUC_{0-1008\text{ h}}$) was calculated over the simulation time. This calculation yielded one data point (cumulative free ADA concentration *vs.* drug dose). By repeating the simulation at various drug dose levels, a curve describing the cumulative ADA responses at all drug dose levels was obtained. To make the calculated response curves comparable between different drugs, the same dosing regimen (once a week for 4 weeks) was used for each therapeutic protein in the simulation, and the ADA responses were calculated as the area under concentration curve (AUC) for an arbitrarily chosen fixed time period (time 0 to 6 weeks).

Sensitivity Analysis

We performed a sensitivity analysis for the PK/ADA model to elucidate the role of individual parameters.

Control coefficients of variable x to parameter p (CC_p^x) were calculated using the following equation (38,39):

$$CC_p^x = \frac{\partial x}{\partial p} \cdot \frac{p}{x}$$

The finite difference method was used to approximate the derivative in the above equation, where h is the step size:

$$CC_p^x \approx \frac{x(p+h) - x(p)}{h} \cdot \frac{p}{x}$$

The step size h was chosen as: $h = 1\% \cdot p$, because 1% increase in the value of p has been shown to be the most numerically stable quantity of variation for this type of sensitivity calculation (38). A positive CC_p^x suggests the parameter increase results in an increase in x value. A negative CC_p^x means the parameter increase causes a decrease in x value.

Sensitivity analysis was conducted for interferon-Fc fusion and mAb by calculating the control coefficients of the five model parameters for three state variables, including drug, ADA, and complex concentration. The analysis was performed at one representative dose level for each drug (20 mg/kg for interferon-Fc fusion and 1 mg/kg for mAb).

Modeling Platform

SAAM II (The Epsilon Group, Charlottesville, VA) was used as the model-building platform. The model parameters were estimated by using a nonlinear least squares methods (weighted least squares or extended least squares). Due to the complexity of the model, there might be a number of local minima in the objective function-parameter space, and thus our estimated results might be local minima. However, the global minimum can be possibly achieved by more vigorous algorithms: our main purpose in this study is to illustrate the potential application of the PK/ADA model. The coefficient of variation (%CV) was calculated for the estimated parameters. The goodness of fit was assessed by weighted residual patterns, correlation matrix and visual inspection.

RESULTS

In this report, we investigated a proposed PK/ADA model using two case studies and generated estimates for the properties of ADA raised against these therapeutic proteins in preclinical species. The estimated parameters for the ADA models are summarized in Table I. A derived parameter, t_{ADA} (ADA lag time, the mean time ADA spend before appearing in the central compartment), was calculated by adding the t_{lag} (mean time for the ADA to spend in the delay compartment) and the dosing interval, with an assumption of no ADA response before the second dose (no longer than 7 days in our studies). The estimated t_{ADA} are summarized in Table I. The estimated elimination half-lives for drug, ADA, and complex are also listed in Table I.

By fitting the two PK data sets, the estimated concentration-time profiles for the drug, ADA and complex, as well as the affinity maturation time profile, were generated for interferon-Fc fusion and mAb. The results are presented in Figs. 2, 3, 4, 5, 6, and 7.

Interestingly, a reverse relationship between drug dose level and calculated ADA concentration was observed for the current case studies. For interferon-Fc fusion (Fig. 3b), the level of ADA response is 20 > 50 > 100 mg/kg. Similarly, for mAb, the level of calculated ADA response was also higher in 1 mg/kg group than 10 mg/kg group for mAb (Fig. 6b).

These results motivated us to explore the expected relationship between drug dose level and cumulative free ADA exposure. To make results comparable between different drugs, the dosing regimen was kept the same, and the free ADA exposures were calculated as the AUC for a fixed time period (6 weeks). By performing simulations with a wide range of drug dose levels, it can be seen that the drug dose-free ADA exposure curves exhibit bell shapes (Fig. 8). This observation agrees with the notion that immune response has a bell-shaped relationship with the amount of antigen (40–42).

Lastly, analysis was conducted to calculate the sensitivity of drug, ADA and complex concentration to the five model parameters. The results are presented in Fig. 9. Overall, A_{max} , t_{lag} , and k_{complex} are the most sensitive parameters for the three variables. Affinity maturation rate (a) is less sensitive, while K_m (accumulated drug dose to achieve 50% maximum ADA production) is the most insensitive parameter.

DISCUSSION

Immunogenicity, particularly ADA production, has been an important, yet challenging, research topic for therapeutic protein development. Its importance lies in the unanticipated, potentially significant impact on the PK, PD, efficacy, as well as safety of protein therapeutics (1,10–12). Underestimation of immunogenicity may bring in higher risks during development. On the other hand, overestimation of immunogenicity may result in unnecessary studies and higher overall costs.

Various strategies for assessing or predicting immunogenicity have been suggested and applied (10,15,43,44). As for characterizing ADA, many limitations exist within the current technology frameworks, such as the inability to measure their absolute concentration or affinity. The lack of quantitative measurements causes difficulties in interpreting the results. For example, the magnitude of ADA responses, which are usually reported as quasi-quantitative titer values, can vary depending on the assay platform, and therefore, may not directly relate to the true response *in vivo*, and cannot be easily compared between different studies (45). Another challenge is to predict the immunogenicity of a therapeutic protein. Because of the complexity of the *in vivo* immune system, a generalized approach for predicting immunogenicity has not been established yet.

Mathematical modeling is sometimes considered as an alternative/complementary approach to experiments. Here, an empirical PK/ADA model which simultaneously models the drug PK and the ADA response was constructed for assessing the immunogenicity of therapeutic proteins. The mathematical modeling approach permits assessment or potentially simulation of ADA production in preclinical species based on repeated dosing PK studies. When the expected PK profiles of the drugs are modified by the binding of ADA, ADA information can be extracted using this modeling approach.

As mentioned in the “Introduction,” similar model structures were proposed recently by Chirmule *et al.* (1) and Perez Ruixo *et al.* (21) independently to evaluate the effect of immunogenicity on therapeutic protein pharmacokinetics. Similar to the above two models, the PK/ADA model is also based on the assumption that the drug disposition can be affected by ADA-mediated clearance. However, there are several differences that can be pointed out: (1) the PK/ADA model was developed to quantitatively assess ADA response by fitting the model to altered drug PK data which are affected by ADA-mediated clearance; (2) the PK/ADA model assumes the amount of ADA produced in the body is driven by the cumulative drug dose which accounts for the immunization history; while in Perez Ruixo's model, the synthesis rate of ADA is driven by the drug concentration. Choosing cumulative drug dose as the ADA driver is also supported by the finding in Bonate's report (20). With

Fig. 2. Fitted drug concentration–time profile for interferon–Fc fusion dosed at 20 mg/kg (**a** logarithmic scale; **b** linear scale), 50 mg/kg (**c** logarithmic scale, **d** linear scale), 100 mg/kg (**e** logarithmic scale, **f** linear scale). The notations “S1,” “S2,” *etc.* represent the data from subject 1, subject 2, *etc.* The drug concentrations are fitted to multiple subjects simultaneously through a naïve-pool approach

repetitive dosing, cumulative dose was identified to influence the probability of seroconversion, and dosing time after seroconversion (which correlates with cumulative dose) influenced the titer value (20). (3) ADA affinity maturation is specifically, albeit parsimoniously, modeled in the PK/ADA model, since both ADA concentration and affinity affect immune complex formation and subsequent drug clearance; (4) unlike the previous models, TMDD caused by drug target/receptor is not explicitly modeled in the PK/ADA model to focus on ADA-mediated clearance, and also to avoid confusion when there is no TMDD for certain therapeutic proteins (*e.g.*, enzymes). The current model structure is very flexible to include TMDD when it is applicable.

The PK/ADA model was fitted to the two PK data sets using maximum likelihood. As shown in Figs. 2 and 5, the model fitting was able to reasonably describe the PK data of the drug. The model fitting for mAb was able to converge to stable parameter estimates, while the model for interferon–Fc fusion converged with K_m being fixed at a relatively small value compared to the drug dose ($K_m/\text{dose}=1.1\times 10^{-3}$)

(Table I). The inability to accurately estimate K_m for the interferon data sets is probably because, in the current studies, the true K_m is very small compared to the accumulated drug dose in the experiment, so that the amount of produced ADA reaches plateau at the very beginning ($A\approx A_{\max}$ when $K_m\ll CD$, since $A=A_{\max}\times CD/(CD+K_m)$), where CD is the accumulated drug dose). Therefore, it is difficult to estimate the K_m value at which the ADA production reaches 50% maximum. This type of nonidentifiability may be a posteriori nonidentifiability that is caused by the experimental design, similarly to a lack of dose–response in traditional PK/PD studies. In order to get a more precise estimation of K_m , smaller drug doses can be used to elicit ADA production in future PK experiments.

Our model appears to be capable of capturing differences in the antigenic properties of protein drugs. The model generates estimates for five parameters (Table I), which represent different aspects of ADA response including: maximum ADA response ($A_{\max}$), sensitivity of ADA response to drug dose level (K_m), time lag to observe ADA

Fig. 3. Calculated concentration-time profiles for interferon-Fc fusion. **a** Drug concentration-time profile, **b** ADA concentration-time profile, **c** Complex concentration-time profile

response (t_{lag}), elimination rate for ADA-drug complex ($k_{complex}$), and affinity maturation rate (a).

Fig. 4. Calculated time profile of K_a for ADA produced against interferon-Fc fusion

The extent of the ADA responses are described in the model by the parameters A_{max} and K_m . The A_{max} values (maximum amount of ADA bolus against the drug) are about 7.3-fold different for the two presented drugs, and the K_m values (dose level to elicit 50% of maximum response) display about 4-fold difference. These results indicate high variation in immunogenic potentials between different drugs, and they also suggest that A_{max} and K_m are drug-specific characteristics.

The timing of the ADA response is depicted in the model by estimating t_{ADA} (ADA lag time, Table I). The parameter t_{ADA} was calculated by adding the dosing interval and the ADA lag time (t_{lag} , estimated from model fitting), based on the assumption that no ADA is produced during the first dosing interval. The estimated t_{ADA} for the two current drugs is fairly comparable, and is about 7–8 days. This is probably because the lag time for developing ADA response after immune stimulation is fairly constant in the same species, due to its universal underlying mechanisms. It has been widely observed that the primary immune response (more specifically, ADA production) lags behind the first antigen challenge for several days, since immune cells need to become activated and differentiate to produce antibodies. Our estimate of t_{ADA} appears to be consistent with many experimental observations (25–27).

Importantly, the extent and timing of the ADA responses can be directly visualized by the model-estimated concentration-time profiles of ADA (Figs. 3b and 6b). The overall concentration of ADA increased with repeated drug dosing, consistent with the observed faster drug elimination over time. The periodic drops in the ADA concentration are in accord with the rises in drug concentration due to the drug dosing. The delayed ADA response can also be directly observed on the plots when ADA concentration rises significantly after several days.

The model was capable of describing the complex concentration-time profiles (Figs. 3c and 6c), and also estimated the complex elimination rate ($k_{complex}$, Table I), which has direct impact on drug elimination. For the two therapeutic proteins studied here, both complexes exhibited shorter half-lives compared to free ADA, indicating a faster elimination for ADA-drug complexes compared to free ADA. When comparing the half-life of complex to that of free drug, the complex for interferon-Fc fusion exhibited shorter half-life compared to the free drug, suggesting that ADA binding facilitated drug elimination.

The present model was also able to quantitatively describe the affinity maturation process of ADA. The a values (rate constant for affinity maturation) are listed in Table I, and the affinity-time profiles are presented in Figs. 4 and 7. Time-dependent affinity maturation would result in tighter binding between drug and ADA, push the equilibrium towards complex formation, and increase drug clearance over time. A month after repeated dosing, the drug binding affinities of ADAs (K_a) increased by various magnitudes, about 101.15- and 1.17-fold for interferon-Fc and mAb, respectively. This result indicates that drugs may possess different capacities to stimulate the affinity maturation of ADA (28). Depending on the antigen and immunization protocols, the affinity of raised antibodies can increase several to 100-fold a month after the primary immunization

Fig. 5. Fitted drug concentration–time profile for mAb dosed at 1 mg/kg (**a** logarithmic scale, **b** linear scale), and 10 mg/kg (**c** logarithmic scale, **d** linear scale). The notations “S1,” “S2,” *etc.* represent the data from subject 1, subject 2, *etc.* The drug concentrations are fitted to multiple subjects simultaneously through a naïve-pool approach

(28,30,45). Our model estimations fall within a reasonable range of literature reports.

Different from current quasi-quantitative experimental methods, the model was able to estimate the absolute ADA concentration and affinity during the PK study. Due to the fact that ADA is polyclonal with a broad range of affinities, our estimated ADA concentration and affinity might reflect the “average” or “typical” ADA characteristics among the total ADA population. Limited by the arbitrary nature of titer values, it is difficult to directly correlate our estimation to measured titer. The model-generated estimations can be further validated by appropriate experiments. For instance, by mathematically modeling the titer assay, the estimated ADA concentration and affinity can be translated into a “theoretical titer”, which can then in principle be directly compared with actual titer values generated from specifically designed experiments. The estimated ADA binding affinity may be validated by measuring the binding affinities of the ADA repertoires taken from experimental animals at desired time points (46).

By simulating the ADA responses to a wide range of drug dose levels, we observed a bell-shaped relationship between drug dose and free ADA exposure (Fig. 8). The bell-shaped behavior exhibited by this model is caused by the binding equilibrium between the free ADA and the drug. When the drug level is low, the amount of produced ADA increases with drug dose [Eq. (1)]. Therefore more ADAs are introduced into the system at higher drug dose, and the free ADA exposure increases with drug dose. When drug dose level is high enough [we assume a saturable relationship between cumulative drug dose and ADA production; Eq. (1)], the amount of ADA production reaches the plateau

regardless of the increase of the drug dose. Excessive drug at higher drug dose then binds to the ADA and forms the complex, and therefore, drives down the amount of free ADA. This observation agrees with the notion that the relationship linking the immune response with the amount of antigen is bell shaped (40–42). When the amount of the antigen is either too little or too much, the observed immune response (usually assessed by the magnitude of antibody response) is small. (40–42). Although a large amount of antigen can elicit pathways (*e.g.*, activating T regulatory cells) to suppress the immune response (47), our model suggested another possible cause, drug interference, for the observed “immune tolerance” phenomenon at very high drug dose. The observed diminished antibody response at high dose levels could be caused by the drug binding to the ADA. When there are excess drug molecules to drive down the amount of free ADA, the “detectable” (which is usually free) antibody response decreases. It would be helpful to employ methods that can differentiate and quantify both free and total ADA. Extra caution should be taken to rule out the possibility of drug interference before concluding the therapeutic protein induces immune tolerance, which should literally indicate that the drug elicits suppressive mechanisms to inhibit the immune response to the drug (47).

Our simulations also demonstrate that the magnitude of ADA response, as well as the optimal drug dose level to induce peak ADA response, varies between drugs, which may reflect the immunogenicity of the drug in the studied animal models. To further validate the predictive power of the current dose–response relationship, it will be very interesting to experimentally determine the corresponding PK profiles responses over a wider range of drug dose levels,

Fig. 6. Calculated concentration-time profiles for mAb. **a** Drug concentration-time profile, **b** ADA concentration-time profile, **c** complex concentration-time profile

and evaluate whether our model simulations match the experimental observations. The validated model may be applied towards differentiating candidate protein drugs or selecting the appropriate drug dose level with minimal immune response.

We conducted sensitivity analysis for the model parameters. Looking specifically at sensitivity for drug concentration (Fig. 9a, d), the overall sensitive parameters are $A_{\max}$, t_{lag} , and k_{complex} , and a is less sensitive, with K_m the least

Fig. 7. Calculated time profile of K_a for ADA produced against mAb

Fig. 8. Simulated drug dose-ADA response curves based on current PK/ADA model for interferon-Fc fusion and mAb

sensitive parameter. Interestingly, the overall sensitivity of $A_{\max}$, k_{complex} , and a increase with time. The better sensitivity of $A_{\max}$ and k_{complex} is probably because the magnitude of ADA increases over time, and has a greater and greater impact on drug PK, allowing better estimation of the ADA magnitude ($A_{\max}$) as well as complex-mediated drug clearance (k_{complex}). Therefore, the PK measurement at later time points would have a bigger impact on model estimation. The increase in sensitivity to a (affinity maturation rate) is reasonable since a longer time frame allows affinity to increase. This finding suggests it might be helpful to conduct the PK study for longer duration for better estimation of ADA affinity. As discussed before, the insensitivity of K_m for these two case studies is a posteriori nonidentifiability caused by the experimental design. By conducting studies at suitable doses, K_m would be more sensitive.

Future plans include extensive model validation by comparing model predicted ADA concentration against experimental measurements. Currently, model validation through experiments is very difficult due to the lack of correlation between absolute ADA concentration and titer measurements (48). The development of a reliable ADA concentration measurement method would be helpful.

Another future plan would be to apply the PK/ADA model in a population framework by using mixed effects models which can analyze data from both ADA-positive and ADA-negative individuals. Population models that include ADA status as a covariate for drug PK have been proposed by Xu *et al.* in the population PK of golimumab (19). Bonate *et al.* developed a conditional model using random effects for population antibody titer data (20). The current PK/ADA model could be expanded to incorporate these features, and a full population model would be able to accommodate heterogeneous data from both ADA-positive and ADA-negative individuals.

With further experimental validation, the model may in principle be applied to assess the immunogenic potential of therapeutic proteins in conjunction with appropriate PK studies and ADA assays. The model may help simulate ADA response when different drug dose regimens are used, or be applied in subsequent PK/PD models that incorporate ADA response.

Fig. 9. Sensitivity analysis for interferon-Fc fusion (panels a, b, c) and mAb (panels d, e, f) at 20 and 1 mg/kg. As stated in “METHODS” section, the sensitivity metrics (CC_p^x) of the five model parameters were calculated for three state variables, including free drug, free ADA, and complex

LIMITATIONS OF THE CURRENT WORK

Although our PK/ADA model presents a novel approach to estimate inaccessible ADA parameters by fitting the ADA-impacted drug PK profiles, the model results should be interpreted or applied with caution, since the model possesses several limitations that need further improvement. One major limitation is the lack of independent experimental validation for the model prediction, due to the tremendous technical challenges this would require. To be directly comparable with the model prediction, an assay that measures absolute ADA concentration and affinity is necessary. However, as widely recognized by the scientific community, most of the current ADA assays are quasi-quantitative, due to the polyclonal nature of the ADA and the lack of reference standards (1,2). Currently, results of ADA measurement are expressed as quasi-quantitative values, such as titer, which are affected by ADA concentration, affinity,

polyclonality, as well as assay reagents, assay condition, cut-point setup, *etc.* On the other hand, the PK/ADA model estimates absolute ADA concentration and binding affinity, which are not directly comparable with currently available experimental results and thus cannot be directly validated. With a primary focus on the modeling aspect of immunogenicity, the current work merely aims to suggest the potential of applying the lessons from PK-PD and computer modeling to help understand immunogenicity. The present work should be recognized as hypothesis generating about the time course and dynamics of development of ADA, rather than a proven way to actually predict the ADA response. Hopefully, the current work may motivate others to address the issue of experimental validation, by developing new assays and experimental approaches which can be linked to the model predictions better than currently available paradigms, or alternatively by extending the model predictions so that they can be compared to the currently available experimental

paradigms. Upon the availability of appropriate assays, experimental validation can certainly be conducted and be presented as a follow-up report.

CONCLUSION

In summary, the proposed model may serve as a potential tool for assessing immunogenicity, particularly ADA response, based on repeated dose PK studies. This model aims to generate estimates for five parameters (Table I), which reflect different aspects of the immunogenic potential of the studied therapeutic protein: maximum ADA response ($A_{\max}$), sensitivity to drug dose level (K_m), affinity maturation rate (a), time lag required to observe ADA response (t_{lag}), and elimination rate for ADA–drug complex (k_{complex}). The model also provides an assessment of the concentration–time profiles for ADA, ADA–drug complex, and binding affinity of ADA (Figs. 2, 3, 4, 5, 6, and 7). By simulating ADA responses to various drug dose levels, bell-shaped curves were generated (Fig. 8). This observation appears to relate to the known phenomenon of bell-shaped immune response with various antigen levels.

Immunogenicity has shown increasing importance in drug development and immunogenicity assessment and prediction have become more and more prevalent practices. An empirical PK/ADA model is reported here, and was successfully applied to two case studies of protein drugs for evaluating their immunogenic potentials, particularly ADA production. Our model provides an approach to analyzing and accessing immunogenicity and reveals interesting characteristics, including a bell-shaped dose response curve relating ADA response with drug dose.

ACKNOWLEDGMENTS

We would like to thank the anonymous reviewers, Scott Fountain, Kenneth Luu, Mary Spilker, and Michael Zager for their valuable suggestions. We thank the PDM department colleagues and especially the immunogenicity strategy group (Li Xue, Bonita Rup, Pratap Singh, John Harrold, Denise O'Hara, Boris Gorovits, Jim McNally, Mengmeng Wang, Anup Zutshi, Ryan Nolan, Michel Awwad, and Anson Abraham) for their help and suggestions.

Conflict of Interest All authors are employed by Pfizer Inc. This work was partially presented as a poster presentation at the 2012 AAPS National Biotechnology Conference, May 21–22 in San Diego, CA, USA. P. V. receives royalties from the University of Washington Center for Commercialization, the licensor of the SAAM II software.

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