ARTICLE

Model-informed drug development for immuno-oncology agonistic anti-GITR antibody GWN323: Dose selection based on MABEL and biologically active dose

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Abstract

GWN323, an agonistic human anti-GITR (glucocorticoid-induced TNFR-related protein) IgG1 antibody, was studied clinically as an immuno-oncology therapeutic agent. A model-based minimum anticipated biological effect level (MABEL) approach integrating in vitro and in vivo data informed dose selection for the first-in-human (FIH) study. Data evaluated included pharmacokinetics (PK) of DTA-1. mIgG2a (mouse surrogate GITR antibody for GWN323), target-engagement pharmacodynamic (PD) marker soluble GITR (sGITR), tumor shrinkage in Colon26 syngeneic mice administered with DTA-1.mIgG2a, cytokine release of GWN323 in human peripheral blood mononuclear cells, and GITR binding affinity. A PK model was developed to describe DTA-1.mIgG2a PK, and its relationship with sGITR was also modeled. Human GWN323 PK was predicted by allometric scaling of mouse PK. Based on the totality of PK/PD modeling and in vitro and in vivo pharmacology and toxicology data, MABEL was estimated to be 3–10 mg once every 3 weeks (Q3W), which informed the starting dose selection of the FIH study. Based on tumor kinetic PK/PD modeling of tumor inhibition by DTA-1.mIgG2a in Colon26 mice and the predicted human PK of GWN323, the biologically active dose of GWN323 was predicted to be 350 mg Q3W, which informed the dose escalation of the FIH study. GWN323 PK from the FIH study was described by a population PK model; the relationship with ex vivo interleukin-2 release, a target-engagement marker, was also modeled. The clinical PK/PD modeling data supported the biological active dose projected from the translational PK/PD modeling in a "learn and confirm" paradigm of model-informed drug development of GWN323.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
Agonists of GITR have shown promising anti-tumor activities in preclinical animal models by stimulating the activation and proliferation of effector memory T cells and decreasing regulatory T cell tumor infiltration.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study features model-based approach and translatability of preclinical in vitro and in vivo data to inform clinical study dose selection and study design for a GITR agonist antibody.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This study utilized model-informed approach to determine minimum anticipated biological effect level and predict biologically active dose in clinic. The approach integrated preclinical results from in vitro and in vivo models of anti-tumor activities of GITR antibody to inform the design of a first-in-human (FIH) phase I study.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

A model-informed drug development approach integrating pharmacokinetic/ pharmacodynamic (PK/PD) modeling, in vitro and in vivo pharmacology, and preclinical toxicology data provided the scientific rationales and informed FIH dose selection for the immune agonistic GITR antibody GWN323, and was verified by clinical PK/PD modeling approach in a "learn and confirm" paradigm.

INTRODUCTION

Glucocorticoid-induced TNFR-related protein (GITR) is a cell surface receptor expressed on activated T cells, natural killer cells, macrophages and dendritic cells, and constitutively expressed on regulatory T (T_{reg}) cells as well; its expression is upregulated on many immune cells by a number of different stimuli, including T cell receptor stimulation with anti-CD3/CD28, concanavalin A, or phorbol 12-myristate 13-acetate.[1,2] Its ligand, GITR-L, is expressed on many activated cells of the immune system as well as non-immune cell types, such as endothelial cells.[3]

Despite recent advances in immunotherapy, especially with checkpoint inhibitors, there continues to be a high unmet medical need for patients with advanced malignancies who relapse on conventional therapies and/or experience disease progression following checkpoint inhibitor treatment. Triggering the GITR signaling pathway has been recognized as a therapeutic target for immunotherapy.[4] The manipulation of GITR/GITR-L axis resulted in favorable enhancement of immune response and modulated the ratio of T effector (T_{eff}): T_{reg} cells within the tumor microenvironment, correlating preclinically with a positive survival prognosis in animals.[1,5–7]

GWN323 is an agonist humaneered IgG1 monoclonal antibody targeted specifically against GITR with high binding affinity.[8] GITR agonists exhibited synergistic antitumor effect in mouse models when combined with programmed cell death protein 1 (PD-1) inhibitors, depleting T_{reg} and co-stimulating T_{eff} cells.[4,9–15] GWN323 demonstrated functional activity both in vitro in human T-cell assays and in vivo in preclinical models. A murine surrogate antibody

of GWN323 (DTA-1.mIgG2a) has shown compelling anti-tumor activity in syngeneic mouse tumor models.[13,16] The agonist properties of DTA-1.mIgG2a contributed to the antitumor activity by promoting the activation and expansion of cytolytic CD8+ T cells and NK cells.[12] Receptor shedding upon ligand stimulation is a hallmark of several TNF receptor superfamily members. Production of soluble GITR (sGITR) in vitro and in vivo can be achieved with the agonistic anti-GITR antibody.[1,17] GWN323 cross reacts with monkeys' but not rodents' GITR and thus monkey is the most appropriate toxicology species. Because the monkey does not have a tumor present, the pharmacokinetic/ pharmacodynamic (PK/PD) and pharmacological effect of GWN323 was studied via DTA-1.mIgG2a.

Traditional empirical approaches for estimating the starting dose in humans are based on human equivalent dose (HED) of the no observed adverse effect level (NOAEL) or the highest non-severely toxic dose (HNSTD) for oncology compounds in animals and applying a safety factor.[18,19] For biopharmaceuticals with immune agonistic properties, the regulatory agencies recommend to consider using a minimum anticipated biological effect level (MABEL) in the selection of the start dose, utilizing all relevant and available in vitro and in vivo information.[20,21]

To inform dose selection and escalation of the first-in-human (FIH) study of GWN323 in patients with cancer, a model-based approach integrating preclinical and translational PK/PD modeling, in vitro and in vivo pharmacology, and toxicology data were used. The MABEL and biologically active dose in humans were projected. When the clinical data from the FIH study[22] became available, clinical PK/PD modeling further confirmed

the prediction, implementing the "learn and confirm" paradigm in a model-informed drug development (MIDD) process.[23,24]

METHODS

Preclinical studies and PK/PD modeling

PK and efficacy study in Colon26 tumor mouse model

The concentration-time data of serum DTA-1.mIgG2a (anti-murine GITR antibody produced and purified in-house) and sGITR were obtained from the Colon26 syngeneic tumor mouse model following a single intravenous (i.v.) dose of 0.3, 1, 3, 10, or 15 mg/kg of DTA-1.mIgG2a. Blood samples were collected from six animals per group at 1 h, 24 h, 4 days, 7 days, and 14 days after dosing. Serum DTA-1.mIgG2a concentrations were quantified using a liquid chromatography-tandem accurate mass spectrometry (LC-MS/MS) assay with a lower limit of quantification (LLOQ) of 0.1 µg/ml. Detection of the mouse sGITR was carried out by enzyme-linked immunosorbent assay (ELISA) with an LLOQ of 1767 pg/ml. Blood samples collected at the same timepoint from different animals in the same dose group were pooled for the measurement of serum DTA-1.mIgG2a and sGITR concentrations due to the small sample volume. Anti-drug antibodies (ADAs) developed 7 days following dose administration, resulting in decreased serum DTA-1.mIgG2a. Therefore, only the DTA-1.mIgG2a and sGITR concentrations prior to ADA formation were used in the data analysis. Tumor sizes were measured three times a week once tumors were palpable and tumor volumes were calculated with the formula (length × width × width)/2.

Human peripheral blood mononuclear cell cytokine release assay

Human peripheral blood mononuclear cells (PBMCs) from eight donors were incubated for 24 h with GWN323 at concentrations ranging from 0.3 to 600 µg/ml for the assessment of GWN323 mediated cytokine release (IL-2, IL-4, IL-6, IL-10, TNFα, and IFNγ). To assess the impact of GITR upregulation associated with potential immune activation, cytokine release was assessed with or without αCD3/αCD28-coupled beads. Due to high cytokine levels induced by αCD3/αCD28-coupled beads, assessment of cytokine release relative to the isotype-matched isotype control was only reported for unstimulated cultures (i.e., without GITR induction induced by αCD3/αCD28-coupled beads).

PK analysis and model development for DTA-1.mIgG2a

The DTA-1.mIgG2a PK data were analyzed by noncompartmental analysis (NCA) to determine area under the concentration-time curve from time zero to infinity (AUC$_{inf}$). A one-compartment PK model with linear and nonlinear clearance (Figure S1) was developed to describe the PK of DTA-1.mIgG2a in Colon26 mouse.

Exposure–response analysis and estimation of MABEL for DTA-1.mIgG2a

The maximum serum sGITR concentration (C$_{max,sGITR}$) was determined by NCA. A maximum effect (E$_{max}$) model was developed to describe the exposure-response relationship for serum sGITR elevation by DTA-1.mIgG2a in Colon26 mouse using the following equation:

$$E\left(C_{max,sGITR}\right) = E_0 + \frac{E_{max} * AUC_{inf}}{EAUC_{50} + AUC_{inf}}$$

where AUC$_{inf}$ is the AUC$_{inf}$ of DTA-1.mIgG2a, E_0 is the baseline sGITR level in untreated and IgG2a control groups because no baseline data were available from the treatment group, E$_{max}$ is the maximal response (C$_{max}$ of sGITR), EAUC$_{50}$ is the AUC$_{inf}$ values of DTA-1.mIgG2a that result in 50% of the E$_{max}$ or maximal response. Mean data of the same dose group was used in the modeling. MABEL in the mouse was defined as the dose that causes 10% of maximal response of serum sGITR C$_{max}$: $\frac{E-E_0}{E_{max}-E_0} = 1/10$.

Tumor kinetic PK/PD modeling for DTA-1.mIgG2a

Based on the tumor volume and PK data, the tumor growth inhibition was described by an exponential model with a first-order growth and a second-order killing driven by DTA-1.mIgG2a concentration and first-order transit capturing the delay in tumor cell death.[25] The PK model developed was linked with the tumor kinetic model to fit the tumor volume versus time data (Figure S2).

Projection of human MABEL and biologically active dose for GWN323

Different approaches were evaluated to determine the starting dose of the FIH study of GWN323, including two MABEL approaches and the NOAEL/HNSTD approach

(Table 1). To ensure patients' safety, the MABEL approach was used instead of the NOAEL/HNSTD approach, which is based on HED to NOAEL determined in animal toxicology studies divided by a safety factor such as 10,[18] and for anticancer agents, 1/6 HNSTD if the nonrodent is the most appropriate species.[19] Monkey is the most appropriate toxicology species for GWN323 and the HNSTD was 100 mg/kg (unpublished data). To better represent the clinical setting of GWN323, the MABEL approach based on integration of preclinical PK/PD modeling, predicted human PK, and the in vitro cytokine release and GITR binding affinity data were used, rather than the approach only based on the in vitro receptor occupancy (GITR binding affinity) data.

PK/PD data of DTA-1.mIgG2a was modeled to estimate both the starting and efficacious dose in humans. The data used in the human dose projection are presented in Table S1. The dose prediction was based on the following assumptions: (1) exposure-serum sGITR relationship of DTA-1.mIgG2a in Colon26 mice represents that of GWN323 in patients with cancer; (2) GWN323 PK in patients with cancer can be scaled from the PK of DTA-1.mIgG2a in Colon26 mice following allometric scaling; (3) in vitro binding difference of DTA-1.mIgG2a to mice GITR versus GWN323 to human GITR, as represented by in vitro K_D, can account for the in vivo differences.

The following analyses were conducted to estimate the MABEL of GWN323 in humans: (1) estimating mouse MABEL based on mathematical modeling of PK/sGITR data from Colon26 syngeneic tumor model with the surrogate antibody DTA-1.mIgG2a; (2) estimating human MABEL based on mouse MABEL with or without correction for the difference in the in vitro GITR binding affinity between DTA-1.mIgG2a to mice GITR and GWN323 to human GITR; and (3) scaling PK parameters from the preclinical species to humans. In addition, cytokine data in human PBMC were incorporated to estimate human MABEL.

To estimate the biologically active dose in humans, the following analyses were conducted: (1) estimating the efficacious dose in tumor-bearing mice based on mathematical modeling of PK/efficacy data in Colon26 syngeneic tumor model with the surrogate antibody DTA-1.mIgG2a; (2) incorporating in vitro GITR binding data in mice and humans; and (3) scaling PK parameters from the preclinical species to humans.

Clinical study and PK/PD modeling in patients with cancer

Clinical study and PK/PD evaluations

The study design of phase I FIH study of GWN323 (Clinical Trials.gov identifier NCT02740270) and the PK assessment were described previously.[22] GWN323 (10–1500 mg) or

TABLE 1 Comparison of different approaches to project the starting dose for the FIH study for GWN323

Approach	Criteria	Data	Projected human equivalent dose (mg)[a]
MABEL approach based on receptor occupancy	• Predicted dose resulting in 10% receptor occupancy in humans[b]	• In vitro binding affinity data of DTA-1.mIgG2a • Predicted human PK based on DTA-1.mIgG2a PK data	0.3
Model-based MABEL approach integrating PK/PD modeling and in vitro data	• Predicted dose resulting in 10% sGITR elevation • Human C_{max} similar or lower than minimal concentration for cytokine release in human PBMC	• PK and sGITR data of DTA-1.mIgG2a and PK/PD modeling • Predicted human PK for GWN323 based on DTA-1.mIgG2a PK data • In vitro binding affinity of DTA-1.mIgG2a and GWN323 to GITR • Human PBMC cytokine release by GWN323	3–10
NOAEL/HNSTD approach	• 1/6 of the HNSTD (100 mg/kg) in monkeys	• In vivo toxicology data of GWN323 in monkeys	700[c]

Note: All the doses are i.v.

Abbreviations: C_{max}, maximum serum concentration; FIH, first-in-human; HNSTD, highest non-severely toxic dose; MABEL, minimum anticipated biological effect level; NOAEL, no observed adverse effect level; PBMC, peripheral blood mononuclear cell; PD, pharmacodynamic; PK, pharmacokinetic; sGITR, soluble GITR.

[a]Assume 70 kg body weight and a scaling factor of 0.8.

[b]Receptor occupancy (RO) calculated based on predicted human C_{max} and in vitro binding affinity of GWN323: $\%RO = C_{max}/(K_D + C_{max})$.

[c]Scaling factor 0.8.

GWN323 and PD-1 antibody spartalizumab (GWN323 10–750 mg + spartalizumab 100–300 mg) were administered i.v. at Q3W schedule during the dose-escalation phase.

Target engagement PD were assayed using the interleukin-2 (IL-2) ratio of staphylococcal enterotoxin B (SEB) stimulated PBMCs samples in increasing GWN323 concentration and untreated samples. GWN323-pretreated human PBMCs from healthy donors was used to study endotoxin-inducible IL-2 release and compared to the untreated PBMC. GWN323 pretreated PBMCs are expected to result in a greater release of IL-2, compared to baseline. The IL-2 stimulation ratio assay resembles the inhibitory nature of PD-1–binding interaction[26] and was run as follows: samples in duplicate were either untreated or treated with GWN323, starting at 50 μg/ml, decreasing in 1:3 dilution for a total of nine concentrations in the dose–response relationship for 1 h before a 1 ng/ml SEB was added and then incubated for 4 days at 37°C. The supernatants were collected, diluted 1:5, and ran in IL-2 ELISA assay using the human IL-2 MSD VPLEX kit. The IL-2 concentration was measured in both aliquots (LLOQ: 4 pg/ml). The stimulation ratio was calculated by dividing the IL-2 concentration in the GWN323-supplemented aliquot by that in the aliquot treated with SEB alone.

Population PK/PD modeling

Patients were considered evaluable for PK if they had at least one GWN323 administration and a corresponding evaluable concentration. The population PK model was a linear two-compartment model with first-order elimination, parameterized on clearance (CL), intercompartmental clearance, central and peripheral volumes (V_C and V_P) of distribution. For the PD model, a sigmoidal E_{max} model was used to evaluate the relationship between ex vivo GWN323 concentration spiked on PMBC and IL-2 stimulation ratio from baseline when no GWN323 was present.

A log-normal distribution was assumed for between-subject variability (BSV) of model parameters. The BSV was incorporated to all four parameters in the PK model, whereas BSV was associated with only E_{max} and EC_{50} parameters of the PD model. Residual variability for the PK model was described by a combined proportional and additive model, whereas an additive model was used to describe the residual variability of the PD model.

The covariates tested in the population PK model included height, weight, body mass index, age, race, gender, and spartalizumab combination. Stepwise covariate model algorithm with $p = 0.05$ for forward inclusion and $p = 0.01$ for backward elimination based on change in log-likelihood ratio test was utilized. No covariate was evaluated in the PD model.

RESULTS

Preclinical PK/PD modeling

PK modeling of DTA-1.mIgG2a in Colon26 mouse and prediction of human PK for GWN323

A one-compartment PK model with linear and nonlinear clearance adequately described the nonlinear PK of DTA-1.mIgG2a in the Colon26 mouse. The predicted and observed DTA-1.mIgG2a PK profiles are shown in Figure 1 and estimated PK parameters are shown in Table S2. GWN323 PK parameters in humans were estimated by allometric scaling of DTA-1.mIgG2a PK parameters in Colon26 mice. The scaling factor (exponent) was set to 0.85 for CL, 1 for V, 0.75 for maximal rate of elimination (V_{max}), and Michaeli-Menten rate constant (K_m) did not change with species. The predicted human PK parameters and PK profiles of GWN323 are shown in Table S3 and Figure S3, respectively.

Projection of human MABEL of GWN323

Exposure–response (sGITR) analysis for DTA-1.mIgG2a in Colon26 mouse

The serum sGITR concentration-time profiles in the Colon26 mouse are shown in Figure S4. Upon visual inspection, C_{max} of serum sGITR was correlated with DTA-1.mIgG2a exposure (AUC_{inf}). The E_{max} model adequately described the exposure-response relationship of DTA-1.mIgG2a exposure (AUC_{inf}) and sGITR response (C_{max}) in the Colon26 mouse (Figure 2).

Estimation of MABEL for GWN323 in humans

Based on model-estimated parameters, DTA-1.mIgG2a PK exposure (AUC_{inf}) at 10% of maximal response was determined to be 52 μg*h/ml. The in vitro binding affinity of DTA-1.mIgG2a to mice GITR ($K_D = 0.1$ nM ± 0.02) versus GWN323 to human GITR ($K_D = 4.3$ nM ± 0.1) is 43-fold different. The model parameters of GWN323 in humans are assumed to be the same as those of DTA-1.mIgG2a in mice, except for $EAUC_{50}$, which is either assumed to be the same as in mice (without correction by the GITR binding affinity difference of DTA-1.mIgG2a and GWN323) or 43 times that of the mouse by correcting for the GITR binding affinity difference of DTA-1.mIgG2a and GWN323. Based on the estimated human exposure at MABEL without or with the correction for in vitro binding affinity difference between DTA-1.mIgG2a to mice GITR
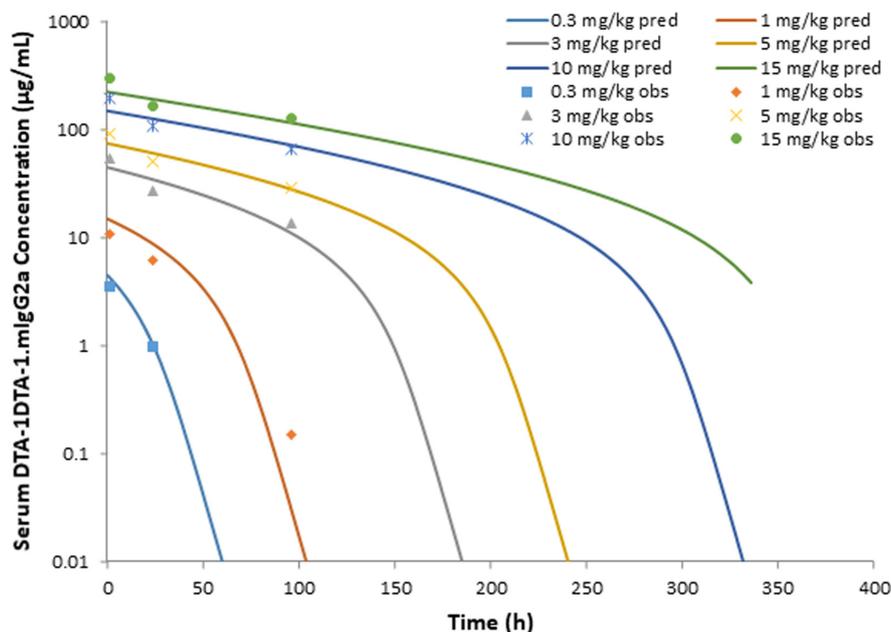
FIGURE 1 Predicted (pred) and observed (obs) concentration-time curves in Colon26 mouse following single intravenous administration of 0.3–15 mg/kg DTA-1.mIgG2a, showing target-mediated drug disposition profile. Symbols and curves represent observed and predicted data, respectively. DTA-1.mIgG2a concentrations of all samples at the same timepoint from the same dose group were averaged and the mean was used in the modeling.

FIGURE 2 Predicted and observed exposure (AUC_{inf} of DTA-1.mIgG2a)-response (C_{max} of sGITR) relationship in the Colon26 mouse following single i.v. administration of DTA-1.mIgG2a. An exposure of 52 µg*h/ml AUC_{inf} was associated with 10% MABEL. Symbols and curves represent observed and predicted data, respectively. AUC_{inf}, area under the concentration-time curve from time zero to infinity; C_{max}, maximum serum concentration; MABEL, minimum anticipated biological effect level.
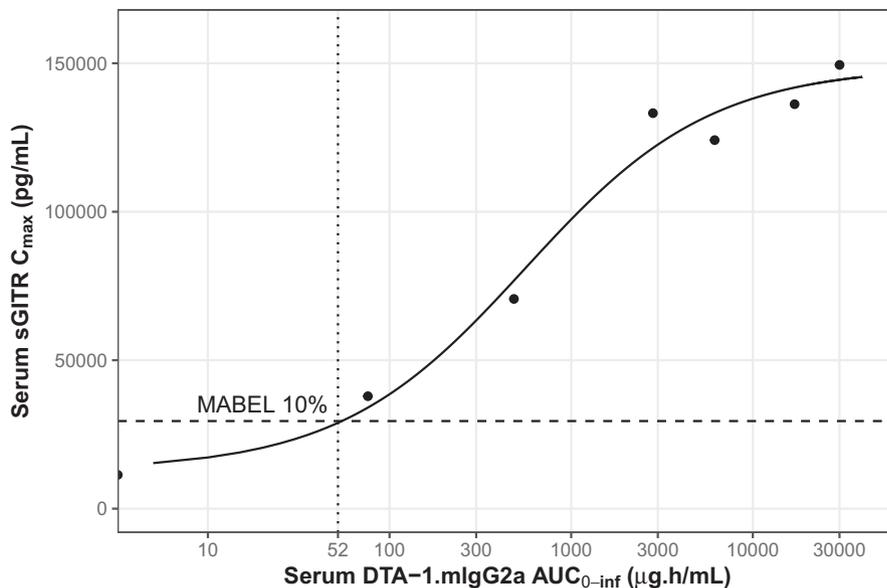
and GWN323 to human GITR, and the predicted human PK parameters by allometric scaling of DTA-1.mIgG2a PK parameters in the mouse, the human MABEL resulting in 10% sGITR response was determined to be 3.5 or 55 mg Q3W, respectively. Q3W was selected as the dosing schedule to align with the schedule of the combination partners to be studied. It would also not result in significant accumulation at steady-state.

In the human PBMC cytokine release assays, TNF-α and IL-6 are the most sensitive cytokines to GWN323

stimulation out of those tested (IL-2, IL-4, IFNγ, and IL-10 were not induced), with the minimal GWN323 concentration for their induction ranging from 20–2000 nM (3–300 µg/ml). At the doses of 3.5 and 55 mg, the model-projected mean steady-state C_{max} of GWN323 in humans following a Q3W dosing was 4.6 and 70 nM, respectively. Therefore, the predicted exposure of 55 mg is 3.5-fold higher than the lower end of the minimal concentration for cytokine induction (20 nM), whereas the predicted exposure of 3.5 mg is below it. At 10 mg, model-projected

mean steady-state C_{max} is 13.7 nM (2 µg/ml). Therefore, 3–10 mg was determined to be the MABEL representing the minimal biological response of GWN323.

Projection of human biologically active dose of GWN323

Tumor kinetic PK/PD modeling for DTA-1.mIgG2a in Colon26 mouse

Single i.v. dosing of DTA-1.mIgG2a demonstrated potent tumor suppression with complete response at 1 mg/kg and was used to estimate the tumor static concentration (TSC). The tumor volume-time profiles following the treatment of vehicle, 0.3 and 1 mg/kg of DTA-1.mIgG2a in Colon26 mice were modeled (Figure 3) to estimate tumor kinetic parameters of GWN323 (Table S4). TSC in Colon26 mice following single i.v. administration of DTA-1.mIgG2a was 0.742 µg/ml (k_{ng}/k_2).

Estimation of human biologically active dose for GWN323

The human tumor kinetic model parameters, k_{ng}, k_1, and w_0, were assumed to be the same as those of DTA-1.mIgG2a in mice except tumor death rate constant (k_2), which was adjusted by the in vitro binding affinity difference between DTA-1.mIgG2a to mice GITR and GWN323 to human GITR ($k_{2,human} = k_{2,mouse}/43$). The corresponding TSC of GWN323 in human (32 µg/ml) was calculated from mouse TSC of DTA-1.mIgG2a by correcting for GITR binding affinity difference between DTA-1.mIgG2a and GWN323. The projected human tumor kinetic model parameters are shown in Table S5.

Based on the projected human parameters, tumor stasis was predicted to be achieved at the end of 3 weeks following a single i.v. dose treatment of 350 mg GWN323, which represents the dose with a pharmacologically active response. The PK profile and tumor volume versus time curve following single i.v. administration of GWN323 were simulated, and the human biologically active dose is predicted to be 350 mg Q3W i.v (Figure 4).

Clinical PK/PD modeling for GWN323

The starting dose of 10 mg was selected in the FIH clinical trial of GWN323 based on the projected human MABEL, and was demonstrated to be safe and well-tolerated.[22]

GWN323 population PK

A two-compartmental model adequately described the PK of GWN323 in patients with solid tumors. Figure 5 shows the overlay of the individual observed and model-predicted
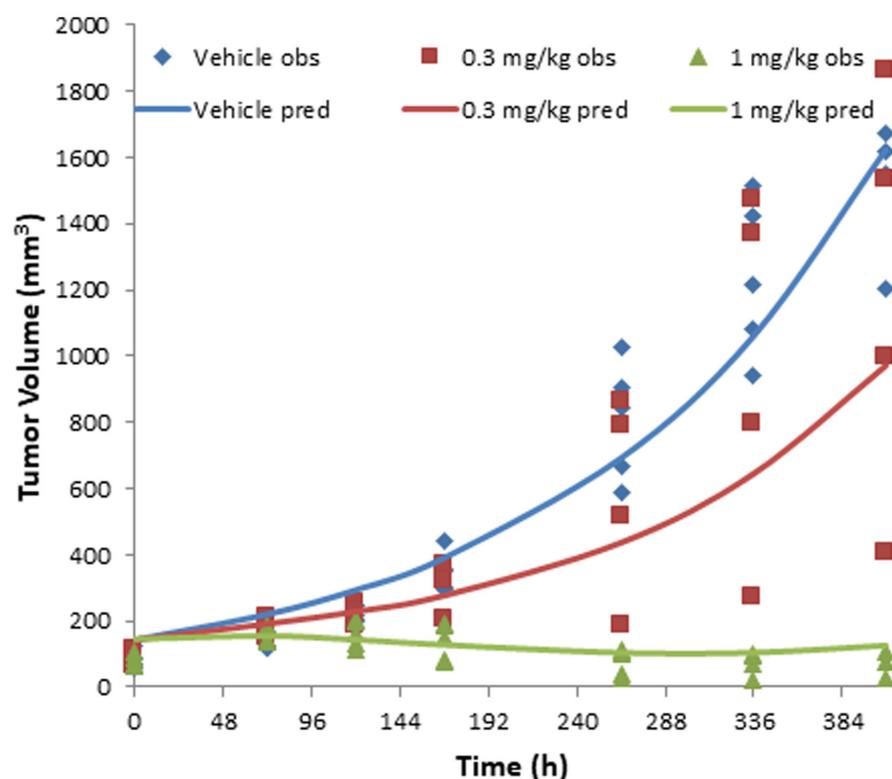
FIGURE 3 Predicted (pred) and observed (obs) tumor volume-time profiles following single intravenous administration of DTA-1.mIgG2a in Colon26 mice. Tumor stasis concentration (TSC) in mice was determined as 0.742 µg/ml.

FIGURE 4 Simulated PK profile and tumor volume-time curve following single intravenous administration of vehicle or 350 mg of GWN323. PK, pharmacokinetic; TSC, tumor stasis concentration.
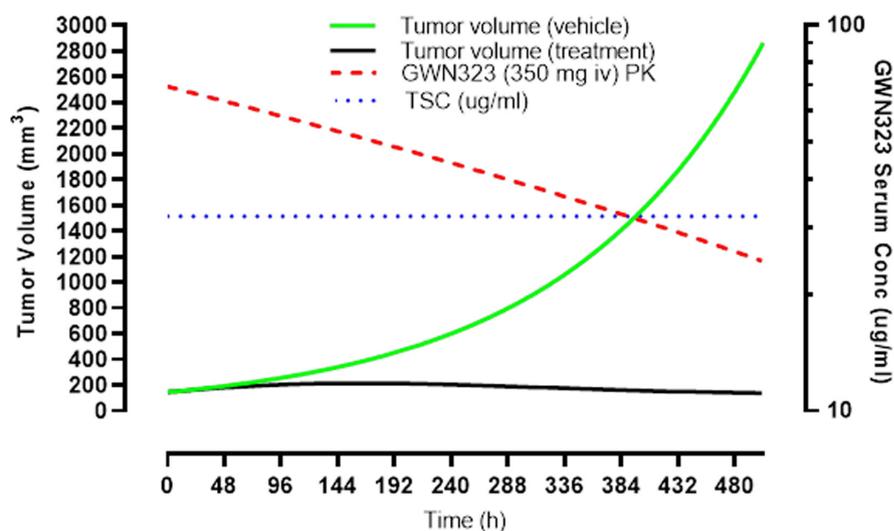
population mean concentrations over 3 weeks after the first and steady-state (Cycle 4) doses. The exposure of GWN323 increased linearly with the doses studied (10–1500 mg Q3W).

GWN323 clearance and its steady-state volume of distribution ($V_C + V_P$) were 0.54 L/day and 6.0 L (Table S6), which are characteristics of IgG antibodies. Body weight was a significant covariate and was implemented as a power model of CL and V_C, centered at 70 kg; the exponents were 0.8 and 0.7, respectively. The goodness-of-fit plots for the model showed good agreement between individual-predicted and observed drug concentrations; there was no bias in the conditional weighted residuals over time and across predicted concentration values (Figure S5).

For the 10 mg Q3W regimen, the model-predicted C_{max} after first and fourth cycles were 2.75 and 3.5 µg/ml, respectively. The dose that generated an average steady-state trough concentration above TSC of 32 µg/ml was between 350 mg and 750 mg Q3W. At these two doses, the proportion of the population whose steady-state trough concentrations reaches TSC were 41% and 74%, respectively, whereas greater than 90% of the population achieved steady-state trough concentration of at least TSC at 1500 mg Q3W (Figure 5).

GWN323 PD modeling – ex vivo IL-2 stimulation

The PD of GWN323 was evaluated using an ex vivo IL-2 stimulation. The relationship between GWN323 concentration and IL-2 stimulation in human PBMC is shown in Figure 6. The basal IL-2 stimulation ratio was 1.15 when no GWN323 is present. The GWN323 concentration that produced half the maximum IL-2 stimulation ratio (EC_{50}) was approximately 6 µg/ml (Table S7). The model

predicted a 70% maximum stimulation for the population occurs at the TSC of 32 µg/ml (Figure 6). The model-predicted population maximum stimulation ratio is at 4.2 (i.e., $E_{max} + E_0$). There was a large BSV in the model parameters, which is reflected in the range of stimulation ratios shown in Figure 6.

DISCUSSION

Among immuno-oncology therapeutics, novel GITR agonists have entered phase I clinical trials as a potential treatment for advanced solid tumors, including GWN323, MEDI1873, MK-4166, BMS-986156, AMG-228, and TRX-518.[22,27–30] Here, we described a MIDD-based paradigm of GWN323 which included estimation of MABEL and biologically active dose based on preclinical in vitro and in vivo data and translational PK/PD modeling with the confirmation by clinical PK/PD modeling.

Because GWN323 is an agonist GITR antibody and clinical data or dosing information of this class were not available at the time when the FIH study was planned, the purpose of the model-based MABEL approach was to inform the starting dose of GWN323 in the study. If the MABEL approach based on in vitro receptor occupancy had been used, the initial dose would have been much lower and might result in a lack of biological activity in patients; the FIH trial would have resulted in an unnecessarily long dose escalation process to reach a biologically active dose. On the other hand, if the NOAEL/HNSTD approach had been used, the human equivalent dose would have been much higher than the recommended dose based on the model-based MABEL approach and might not ensure patients' safety.

A model-based translational approach integrating PK/PD modeling as well as comprehensive assessment
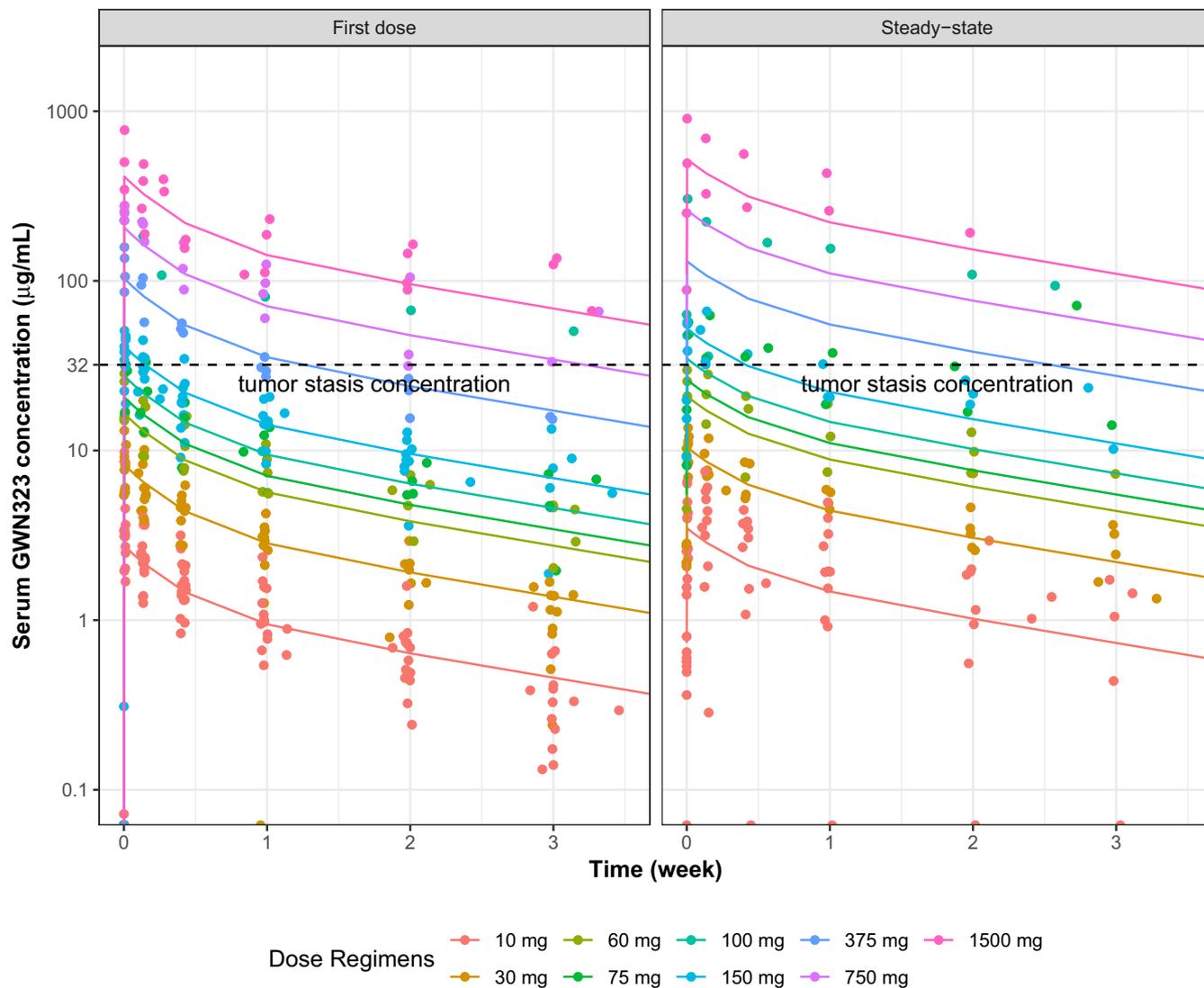
FIGURE 5 Population PK model-predicted average GWN323 concentration profiles and observed GWN323 concentrations over the dose range of 10–1500 mg Q3W (top graph); probability of steady-state GWN323 trough concentration achieving projected human tumor stasis concentration of 32 µg/ml across increasing GWN323 dose (bottom graph). Lines represent model-predicted population mean profile whereas symbols represent observed data. Doses are represented by color. PK, pharmacokinetic.

FIGURE 6 Model-predicted IL-2 stimulation ratio from baseline over increasing GWN323 concentration using an E_{max} model. A 70% population maximum IL-2 stimulation ratio (E_{max}) occurs at the projected human tumor stasis concentration of 32 µg/ml. Symbols represent observed data while lines represent individual model-predicted data from individual blood donors. Black line represents population mean data predicted from the population E_{max} model. E_{max}, maximum effect.
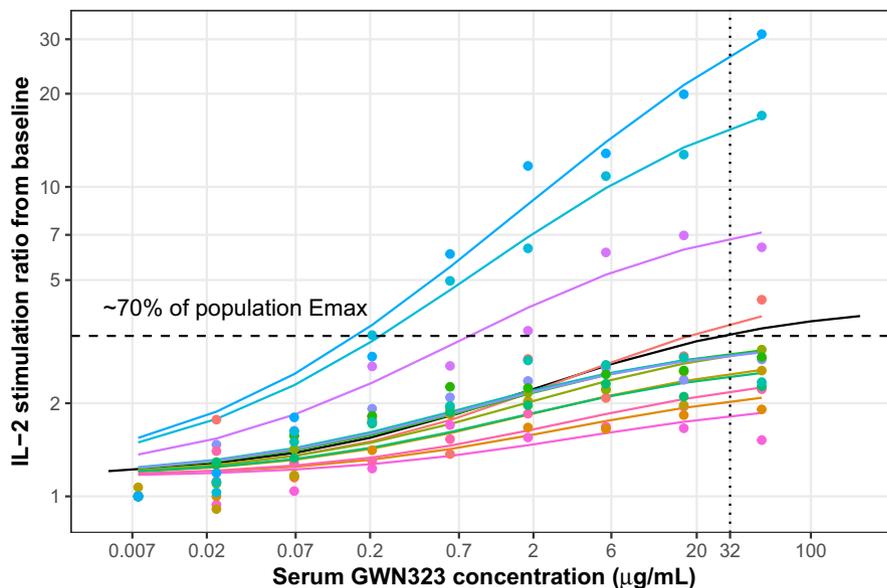
of in vitro and in vivo pharmacology and toxicology data is a more balanced strategy to determine human MABEL for GWN323. Because sGITR is a sensitive target-engagement biomarker in the murine model, MABEL was estimated based on the equivalent human exposure at 10% of maximal sGITR response in syngeneic Colon26 mice, and was determined to be 55 and 3.5 mg, with or without correction by GITR binding affinity difference between DTA-1.mIgG2a and GWN323, respectively. The projected steady-state exposure of GWN323 in humans at 55 mg is well covered by HNSTD (100 mg/kg), with the exposure margin of ~200- and 560-fold based on AUC and C_{max}, respectively (data on file). However, the human PBMC cytokine release data supports the dose around 10 mg, because the predicted steady-state C_{max} of 10 mg was similar to the minimal concentration of GWN323 for the release of the sensitive cytokines tested in PBMCs (TNF-α and IL-6), whereas that of 55 mg would exceed it. Based on the totality of the in vitro and in vivo pharmacology and toxicology data, the human MABEL of GWN323 was determined to be 3–10 mg. Therefore, 10 mg was selected as the starting dose to ensure the safety of patients in the FIH study, and was later demonstrated to be safe and well-tolerated.[22]

The projection of the human biologically active dose was accomplished using tumor PK/PD modeling to describe tumor inhibition kinetics in Colon26 mice. Efficacious dose was determined as the trough concentration that corresponded to the TSC in mice with adjustment for differences in binding affinities between mouse and human antibodies. The human TSC was estimated to be 32 µg/ml and the dose of 350 mg i.v. Q3W was projected to result in stable disease (tumor stasis). The projected dose was estimated to have sufficient exposure coverage by the HNSTD in monkeys. It provided the target level for

dose increments in the FIH study and thus informed dose escalation.

In the FIH study, single-agent GWN323 showed a dose-proportional increase in the PK exposure and moderate accumulation at Cycle 4.[22] The population PK model predicted-C_{max} values after the first dose and Cycle 4 were well within the C_{max} predicted from the animal model. Unlike in preclinical models where nonlinear PK were observed, target-mediated drug disposition was not apparent in human PK, which could suggest a high target engagement of GWN323 to GITR even at the lowest dose (10 mg Q3W) administered to patients. Simulations using human PK data suggested that PK exposure at the dose range between 350 mg and 750 mg Q3W would achieve a steady-state trough concentration corresponding to the population EC_{70} of the ex vivo IL-2 stimulation experiment. Soluble GITR was determined in the FIH study but the majority of the samples were undetectable.

A consistent recommendation of 350 Q3W as a biologically active dose was demonstrated both by preclinical projection based on model-estimated TSC and by clinical modeling based on PK simulation and ex vivo IL-2 data. One limitation of the clinical modeling was a lack of robust anticancer effect for GWN323 as monotherapy in the heavily pretreated patients in the FIH study.[22] Lack of or low response was also reported for other GITR antibodies.[27,28] Given the difference between human and mouse immune systems and heterogeneity of human tumors, mouse syngeneic tumor models may not quantitatively correlate with human tumor response, but serves as a surrogate system to assess the biological activities in humans. Of note, although single-agent of GWN323 showed limited efficacy, synergistic activity with PD-1 inhibitor was expected when GWN323 is combined with spartalizumab.[4,9–15] For GWN323, the TSC projected from modeling mouse tumor

inhibition is similar as the exposure threshold of a biologically active response observed in humans (IL-2 stimulation), showing the consistency of clinical findings and demonstrating the robustness of the translational modeling approach.

CONCLUSIONS

This study featured an MIDD approach using PK/PD modeling as well as a comprehensive assessment of in vitro and in vivo pharmacology and toxicology data to determine MABEL and biologically active dose to inform dose selection of the FIH study for a GITR agonist antibody in patients with cancer. Implementation of the "learn and confirm" paradigm in an MIDD process was carried out by evaluating clinical PK/PD from an early phase I trial results, and clinical PK/PD modeling further verified the preclinical projection.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.