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ORIGINAL ARTICLE

Semimechanistic pharmacokinetic/pharmacodynamic model for hepatoprotective effect of dexamethasone on transient transaminitis after trabectedin (ET-743) treatment

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Abstract
Purpose Reversible transient elevations in transaminases have been observed after trabectedin administration. A semimechanistic pharmacokinetic and pharmacodynamic (PKPD) model was developed to evaluate the time course of alanine aminotransferase (ALT) elevation, tolerance development, and the hepatoprotective effect of dexamethasone on trabectedin-induced transient transaminitis following different dosing schedules in cancer patients.
Patients and methods Trabectedin was administered to 711 patients as monotherapy (dose range: 0.024–1.8 mg/m^2) as 1-, 3-, or 24-h infusions every 21 days; 1- or 3-h infusions on days 1, 8, and 15 every 28 days; or 1-h infusions daily for five consecutive days every 21 days. Population PKPD modeling was performed with covariate evaluation [dexamethasone use (469/711 pt), ECOG performance status scores (89.7% pts ≤ 1), and body weight (36–122 kg)] on PD parameters, followed by model validation. Simulations assessed the influence of dosing regimen and selected patient factors on the time course of ALT and the effectiveness of the dose reduction strategy.
Results A precursor-dependent PKPD model described the temporal relationship between ALT elevation and trabectedin concentrations, where the transfer process of ALT from hepatocytes to plasma is stimulated by trabectedin plasma concentrations. Overall, 66% of patients had transaminitis. Mean predicted (%SEM) baseline ALT (ALT_o) and $t_{1/2}$ in plasma were 31.5 (5.1) IU/L and 1.5 days, respectively. The magnitude of the trabectedin stimulation of the ALT transfer rate from hepatocytes to plasma was 11.4% per 100 pg/mL of trabectedin plasma concentration. Dexamethasone decreased the rate of trabectedin-induced ALT release from hepatocyte by 63% ($P < 0.001$). Model evaluation showed that the model predicted incidence of grade 3/4 transaminase elevation was similar to the observed values. Simulations showed that severity of ALT elevation was dose- and schedule-dependent. The dose reduction strategy decreased the incidence of grade ≥3 toxicity by 13 and 39% following two and four cycles of therapy, respectively.
Conclusions A PKPD model quantifying the hepatoprotective effect of dexamethasone on transient and reversible transaminitis following trabectedin treatment has been developed. The model predicts that co-administration of dexamethasone and the suggested dose reduction strategy based on the serum concentration of liver enzymes will enhance the safe use of trabectedin in the clinic.

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Introduction

Trabectedin (ET-743, Yondelis™) is a novel tetrahydroisoquinoline compound isolated originally from the marine ascidian *Ecteinascidia turbinata* and is now produced synthetically [16]. It is the first of a new class of antitumor agents with a transcription-targeted mechanism of action [23]. Trabectedin is a unique DNA-interacting agent with covalent binding to the DNA minor groove [49]. This agent blocks cell cycle progression in G_2/M phase through a p-53 independent apoptotic process [9], inhibits the transcriptional activation of inducible genes [11, 30], and its antiproliferative activity is dependent upon transcription-coupled nucleotide-excision repair [39]. In addition, trabectedin has shown important preclinical activity against a number of human solid tumor cell lines and xenografts, including sarcomas, ovarian, breast, and prostate, with minimal or no cross-resistance to several conventional chemotherapeutic agents [19, 20, 25]. In clinical Phase I/II studies of trabectedin, promising responses were observed in patients with sarcoma, breast, and ovarian [12, 24, 25, 38, 40, 42, 43, 46, 48]. Trabectedin has been recently recommended for approval by the EMEA as treatment for patients with advanced soft tissue sarcoma after failure of anthracyclines and ifosfamide. Currently, a Phase III study is evaluating the efficacy of trabectedin in ovarian cancer and several Phase II clinical studies in breast, and prostate cancer are also ongoing.

In Phase I studies of trabectedin monotherapy, reversible increases in serum concentrations of transaminases, bilirubin, and alkaline phosphatase were observed [12, 23, 38, 40, 42, 43]. These changes were non-cumulative and transient at the studied doses and schedules. In general, increases in transaminases began 2–5 days after trabectedin administration, reached a maximum at Day 5 through Day 9, and resolved within 3–4 weeks. The severity of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) changes has also been related to exposure parameters such as maximum plasma concentration (C_{max}) or area under the concentration-time curve (AUC) of trabectedin [38, 41, 42].

The profiles of liver enzyme increases reported in Phase II studies of trabectedin have been consistent with those observed during the Phase I investigations [24, 46, 48]. The results of a multivariate analysis indicated that in part, severe or multi-organ toxicity related to trabectedin could be predicted based on specific elevations of intercycle transaminase or alkaline phosphatase peaks, or baseline bilirubin levels [14]. Since dose reduction strategies were implemented in subsequent clinical trials, the occurrence of liver toxicity has substantially decreased [3].

Interestingly, in a Phase I study including a trabectedin dose range of 0.050–1.8 mg/m^2, it was observed that the severity of the increases in transaminases after drug administration decreased with successive treatment cycles while plasma pharmacokinetics (PK) were unaltered [38, 41]. The mechanism underlying this observation is unknown. However, a similar type of tolerance phenomenon has been observed previously for other therapeutic agents [8], and has been characterized and quantified using pharmacokinetic/pharmacodynamic (PK/PD) modeling [13].

Nausea and vomiting remain common and debilitating side effects of therapy with many anticancer drugs. Dexamethasone is one of the recommended corticosteroids that has proven efficacious for acute and delayed emesis [22]. It is also known to significantly increase the activity of hepatic CYP3A isoenzymes [27, 35] and to induce the expression of membrane transporters in the rat, like Bsep, mdr2, oatp2, and mrp2, and it suppresses expression of rat liver microsomal carboxyl esterase [6, 21, 28, 45]. Dexamethasone has been shown to increase the clearance of trabectedin in humans [32, 33]. Interestingly, dexamethasone administered prior to trabectedin also diminishes hepatotoxicity in humans and rats [7, 15]. In addition, dexamethasone increases expression of glucuronyl transferases [26] and can prevent liver inflammatory damage [7].

Even though elevations in both ALT and AST are markers for hepatotoxicity, ALT is considered a more specific marker as it is primarily found in the liver, whereas AST is also found in other tissues including cardiac muscle, kidneys and lungs. The objective of this analysis was to develop a semimechanistic population PK/PD model to characterize the time course of ALT concentrations following intravenous administration of trabectedin in cancer patients, including the development of tolerance to this drug effect and the dexamethasone effect, and explore the influence of selected patient characteristics. In addition, model-based simulations were used to examine the impact of dosing regimen and covariate effects on the temporal relationship between ALT and trabectedin exposure. The effectiveness of the recommended dose reduction strategy to diminish ALT elevation was also investigated through model-based simulations. The knowledge gained through these PK/PD analyses has important application in the assessment of the safety outcomes for trabectedin in cancer patients.

Patients and methods

Study design and patient data

Data from fourteen clinical studies (711 patients in five Phase I and nine Phase II studies) were available for the PK/PD analysis of liver toxicity following trabectedin administration. The designs of these studies are outlined in Table 1. More detailed information about some of these studies has been published elsewhere [12, 24, 25, 38, 40–43]. All studies were conducted in accordance with the principles of the *Declaration of Helsinki* and were approved by the Human Investigational Review Board of each study center. Informed consent was obtained from each patient after being told the potential risks and benefits, as well as the investigational nature of the study.

Patients were eligible if they had histological or cytological confirmation of a malignant tumor not amenable to established forms of effective therapy. Other eligibility criteria included an ECOG performance status of zero to two, anticipated life expectancy of at least 3 months, and age >18 years. Anticancer radiation therapy and/or chemotherapy, if given, had to be discontinued for at least 4 weeks before entry into the study, or 6 weeks in the case of pretreatment with nitrosoureas or mitomycin C. Before entry into the study, patients had to have a negative pregnancy test (only for female patients with reproductive potential), and normal hepatic and renal function, defined as bilirubin ≤1.5 times the normal upper limit, AST and ALT ≤2.5 times the normal upper limit (≤5 times the normal upper limit in case of hepatic metastases), and serum creatinine ≤1.5 times the normal upper limit. All patients had to have acceptable bone marrow function, defined as white blood cells >3,500/μL, neutrophil count >1,500/μL, and platelets >100,000/μL. Patients with any of the following criteria were not selected: prior extensive radiation therapy (>25% of bone marrow reserve); prior bone marrow transplantation or high dose chemotherapy with stem cell rescue; concurrent radiation therapy, chemotherapy, hormonal therapy, or immunotherapy; participation in a clinical trial involving an investigational drug in the past 30 days or concurrent enrollment in another investigational trial; and any coexisting medical condition that was likely to interfere with study procedures and/or results.

In these studies, trabectedin was administered intravenously as a single agent at doses ranging from 0.024 to 1.8 mg/m^2, in six different dosing schedules: 1-, 3-, and 24-h infusions every 21 days (Q3W); 1- and 3-h infusions on Days 1, 8, and 15 every 28 days (QW3); and 1-h infusions daily for five consecutive days every 21 days

Table 1 Study designs

Study type[a] (Ref. #)	Dataset (*n*)[c]	Indication	Dose range (mg/m^2)	Dosing days	Cycle (days)	Infusion duration (h)
Phase I: Maximum tolerated dose						
1[40, 42]	Index (15)	Advanced solid tumor	0.8–1.1	Day 1	21	1
2 [40, 42]	Index (30)	Advanced solid tumor	1.0–1.6	Day 1	21	3
3 [38]	Index (52)	Advanced solid tumor	0.05–1.8	Day 1	21	24
4 [43]	Index (36)	Advanced solid tumor	0.024–0.38	Day 1 to 5	21	1
5[b]	Index (31)	Advanced solid tumor	0.46–0.92	Day 1, 8 and 15	28	1
Phase II: Safety and efficacy						
6[b]	Index (16)	Breast Cancer	1.3–1.65	Day 1	21	3
7[b]	Index (19)	Melanoma	1.3–1.65	Day 1	21	3
8 [25]	Index (37)	Soft Tissue Sarcoma	1.5	Day 1	21	24
9 [46, 48]	Index (42)	Breast cancer, melanoma, renal cancer and sarcoma	1.5	Day 1	21	24
10[b]	Index (38)	Soft Tissue Sarcoma	1.3–1.65	Day 1	21	3
11[b]	Index (17)	Colorectal Cancer	1.3–1.65	Day 1	21	3
12[b]	Index (23)	Breast Cancer	0.58	Day 1, 8 and 15	28	3
			1.30	Day 1	21	24
13[b]	Index (142)	Ovarian Cancer	0.58	Day 1, 8 and 15	28	3
14[b]	Test (213)	Soft Tissue Sarcoma	0.58	Day 1, 8 and 15	28	3
			1.50	Day 1	21	24

[a] Reference number

[b] Data on file, Johnson & Johnson Pharmaceutical Research and Development

[c] *n* number of patients in each study

(QD5) (Table 1). In general, liver transaminase measurements, such as ALT, were obtained on Days 1, 8, 15, and 22 for QW3 dosing regimens and on Days 1, 4, 5, 8, and 15 for Q3W and QD5 dosing regimens prior to drug administration. In the case of grade ≥3 liver toxicity, more frequent measurements of ALT were taken until the levels returned to normal. ALT measurements were determined using a spectrophotometric enzyme activity assay.

Data analysis

An index dataset and a test dataset were used to develop and evaluate the PK/PD model, respectively (Table 1). The index dataset consisted of data from 498 patients with various cancer types and 8919 ALT measurements, whereas the test dataset included data from an additional 213 cancer patients with soft tissue sarcoma and 4068 ALT measurements. After model evaluation, final parameter estimates were obtained by fitting the model to the combined dataset. A total of 711 patients and 12,987 ALT measurements were used to determine parameter estimates for the combined dataset. A summary of patient characteristics is provided in Table 2.

Software

The population PK/PD model of ALT following trabectedin administration was fit simultaneously to the data from all patients using nonlinear mixed-effects modeling with the first-order (FO) approximation method as implemented in the NONMEM® V level 1.1 software package (GloboMax, Hanover, MD, USA) [2]. The first-order conditional estimation (FOCE) minimization method was also investigated. Exploratory analyses, graphical displays, and other statistical analyses, including evaluation of NONMEM outputs, were performed using SAS® Version 8.2 and S-PLUS® 6.2 for Windows (Insightful, Seattle, WA, USA).

Pharmacokinetic-pharmacodynamic analysis

Structural model development

The development of the PK/PD model was performed using a sequential process [50]. Initially an open, four-compartment disposition model with linear elimination from the central compartment, linear and nonlinear distribution from central to deep and shallow compartments,

Table 2 Patient characteristics prior to trabectedin administration

Patient characteristics[a]	Index dataset (n = 498)	Test dataset (n = 213)	Combined dataset (n = 711)
Age (year)	56 (19–83)	53 (20–80)	55 (19–83)
Body weight (kg)	70 (36–122)	76.2 (41–148)	71 (36–148)
Sex (no %)			
Male	157 (31)	83 (39)	240 (34)
Female	341 (69)	130 (61)	471 (66)
ALT (IU/L)	42 (1–3820)	43 (3–1386)	43 (1–3820)
Liver metastasis (no %)	105 (21)	4 (2.0)	109 (15)
Soft tissue sarcoma (no %)	98 (20)	213 (100)	311 (44)
Performance status (no %)			
0	289 (43)	104 (49)	393 (44)
1	313 (46)	108 (51)	421 (48)
2	55 (8)	1 (<1)	56 (6)
3	15 (2)	–	15 (2)
4	2 (<1)	–	2 (<1)
Dexamethasone use[b]			
No	50 (10)	NA	50 (7)
Yes	256 (49)	213 (100)	469 (64)
Unknown	213 (41)	NA	213 (29)

ALT alanine aminotransferase, *NA* not applicable

[a] Continuous variables are expressed as median (range), whereas categorical variables are expressed as counts (%)

[b] Study 10 included a crossover design to evaluate the effect of concomitant administration of dexamethasone on trabectedin pharmacokinetic and pharmacodynamics, resulting in the number of patients analyzed before and after the crossover. As a consequence they are being counted twice for the purpose of this analysis

respectively, and a catenary compartment off the shallow compartment was used to describe the pharmacokinetics of trabectedin in plasma (Fig. 1) [32]. Individual PK parameters obtained from this model were fixed and the predicted individual trabectedin concentration-time profiles were used as input functions into the PD model.

Based on graphical exploration of the data, an adaptive precursor-dependent model of indirect pharmacodynamic (ALT) effect was evaluated (Fig. 1) [13]. This model consisted of two compartments: one represents the ALT in the hepatocyte [*ALT.H*] and the other represents the circulating plasma ALT [*ALT.P*]. The basic premise of this model is that the ALT elevation after trabectedin administration is produced by an indirect mechanism, and the development of tolerance and rebound is due to alteration in amounts of ALT in the hepatocytes (or the amount of hepatocytes). The model assumes a continuous production of ALT in the hepatocytes and the release of ALT from the hepatocytes into the blood is stimulated by trabectedin. The constant ALT production in the hepatocytes is characterized by a zero-order rate constant, k_p, which can be affected by a stimulatory feedback mechanism that modulates the rebound effect implicit in the system. The feedback mechanism is modeled as a power function of the ratio of

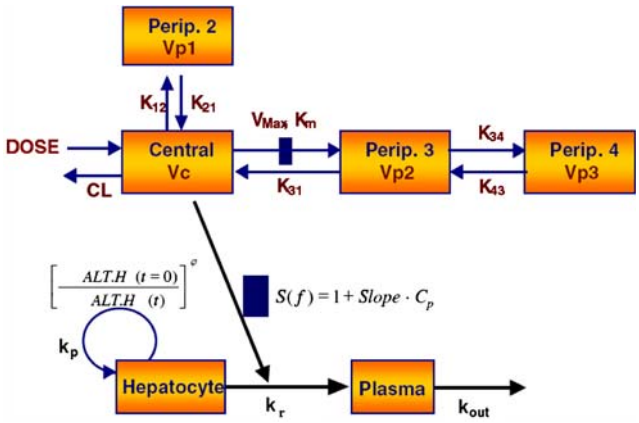

Fig. 1 Indirect response adaptive pool pharmacokinetic/pharmacodynamic model for ALT. K_{12}, K_{21}, and K_{31} are intercompartmental rate constants for the transfer of trabectedin to and from plasma and tissue compartments; K_{34} and K_{43} are intercompartmental rate constants for transfer of trabectedin to and from deep tissue compartments; V_{max} and K_m are the capacity constant and equilibrium rate constant of the transfer rate of trabectedin plasma concentrations to a peripheral (perip.) tissue site; CL is the clearance of trabectedin from the body; Vc, Vp1, Vp2, and Vp3 are central and peripheral volumes of distribution; k_p is the production rate constant of ALT in hepatocytes; k_r is the release rate constant of ALT from hepatocyte to plasma; k_{out} is the removal rate constant of ALT from plasma; *ALT.H* is the concentration of ALT in hepatocytes; C_p is the trabectedin plasma concentration; *SLOPE* is the relationship of the stimulatory effect of trabectedin on ALT elevation, and φ is the exponent for the feedback function

baseline ALT in the hepatocyte [$ALT.H_0$] to *ALT.H* at any given time, thus, [$\{ALT.H_0/ALT.H\}^{\varphi}$], where φ is the estimated parameter that determines the magnitude of the influence of the feedback system. This function facilitates a decrease in the production rate of ALT in hepatocytes at high concentrations of ALT in hepatocytes.

ALT produced in the hepatocytes is then released to the plasma according to a linear process characterized by the first-order rate, k_r. In addition, ALT is removed from the plasma following a linear process characterized by the first-order rate, k_{out}. The release of ALT from hepatocytes to plasma is stimulated by a linear function of trabectedin plasma concentrations [C_p], equivalent to $1 + \text{Slope} \cdot C_p$, where the "Slope" represents the fractional increase in k_r per unit of plasma concentration. Although the mechanism of the apparent tolerance is not known, the model assumes that the stimulatory effect of trabectedin on the release of ALT from hepatocytes to plasma depletes the amount of ALT in the hepatocyte (or the number of hepatocytes) and produces the development of the tolerance phenomena. This assumption is conceptual in essence and likely an over-simplistic representation of the actual mechanism. The differential equations used to describe the model are as follows:

$$\frac{dALT.H}{dt} = k_p \left[\frac{ALT.H_0}{ALT.H}\right]^{\varphi} - k_r(1 + \text{Slope} \cdot C_p) \cdot ALT.H \quad (1)$$

$$\frac{dALT.P}{dt} = k_r(1 + \text{Slope} \cdot C_p)ALT.H - k_{out} \cdot ALT.P \quad (2)$$

In this model, it was assumed that the only loss of *ALT.H* is into the *ALT.P* compartment. In absence of drug effect, the change of *ALT.P* and *ALT.H* over time is equal to 0 (steady-state condition) and, thus, the baseline *ALT.P* [$ALT.P_0$] is the ratio k_p/k_{out}, and the $ALT.H_0$ is equal to $ALT.P_0 \cdot k_{out}/k_r$.

The interindividual (IIV, between patient) and residual variabilities in the PK/PD model were assumed to follow lognormal distributions and, consequently, exponential error models were used.

Covariate analysis

Covariates explored in the analysis included demographic factors (age, body weight, BSA, sex) [18], ECOG performance status [31], liver metastases, concomitant administration of dexamethasone and tumor type. Continuous covariates were included in the model using power equations after centering on the median, while categorical covariates were incorporated using an additive function, following forward inclusion ($P < 0.005$) and backward

elimination ($P < 0.001$) [44]. The likelihood ratio test (LRT) was used for statistical comparison.

Model refinement

A majority of patients appeared to have some elevation in ALT following trabectedin administration, but not all patients had an observable increase. A mixture model [10] was implemented in NONMEM, which allowed the separate characterization of patients with or without ALT elevation following trabectedin administration according to maximum likelihood theory. The population with no ALT elevation had no drug effect on k_r, thus the administration of trabectedin had no influence on predicted ALT values.

Model evaluation and final model development

The model developed using the index dataset was externally evaluated based on its predictive performance on the test dataset, which included one Phase II study that was not used to develop the PK/PD model. Graphical analysis and prediction errors were used as complementary methods to evaluate the PK/PD model. Population and individual Bayesian predictions for the ALT measurements in the test dataset were obtained and the diagnostic plots were examined for bias and scatter. Model evaluation was done by comparing the mean and the standard deviation of the absolute prediction error in the log-domain obtained from the index and test datasets [36]. The estimates of the PK/PD model parameters and their standard errors obtained from fitting the PK/PD model to the index dataset were updated using the combined dataset.

Model-based simulations

In order to evaluate the relationship between trabectedin dosing regimen and ALT time course, the population PK/PD model was used to predict the typical ALT profile following 6 months of treatment with trabectedin 0.58 mg/m^2 3-h infusion weekly for 3 weeks out of a 4-week cycle, as well as 1.3 mg/m^2 3-h infusion and 1.5 mg/m^2 24-h infusion every 3 weeks.

Stochastic simulations incorporating interindividual and residual variability were performed to explore the influence of concomitant dexamethasone use. ALT profiles for a total of 1,000 hypothetical patients (500 males and 500 females) were simulated following administration of trabectedin 1.5 mg/m^2 24-h infusion every 3 weeks for 6 months in absence of any dose reduction and using the final estimates of the model parameters. For every patient, body weight and BSA were obtained by resampling from the patient covariate dataset. The resampling procedure was stratified by dexamethasone use and sex. Since the final PK/PD model for ALT included a mixture model for distinguishing between patients with and without ALT elevation, a logistic regression model was developed and used to predict if a randomly re-sampled patient was susceptible to having ALT elevation as a function of dose and infusion duration.

Stochastic simulations were also performed to assess the effectiveness of the dose reduction strategy (Fig. 2) implemented in a Phase II clinical trial (Study 14, Table 1). The time course of ALT values following the administration of trabectedin 1.5 mg/m^2 24-h infusion every 3 weeks for four cycles was simulated for 1,000 hypothetical patients with and without the dose reduction strategy. Consistent with the Phase II clinical trial in the test dataset, simulated patients remained on dexamethasone for all four cycles of therapy. Body weight, BSA, and sex were resampled from their distributions in the combined dataset. Body weight and BSA resampling was stratified by sex. The percentage of patients that was randomly assigned to the sub-population with ALT elevation was based on the logistic regression model previously developed. The simulated baseline ALT value for each patient was evaluated to determine baseline ALT toxicity grade. ALT values throughout these simulations were graded (0, 1, 2, 3, or 4) for liver toxicity according to NCI CTC [5]. Toxicity Grade 0 means that ALT values are within the normal limits, Grade 1 toxicity implies that ALT values are above the upper limit of normality (ULN) but within 2.5-fold the ULN, Grade 2 toxicity includes

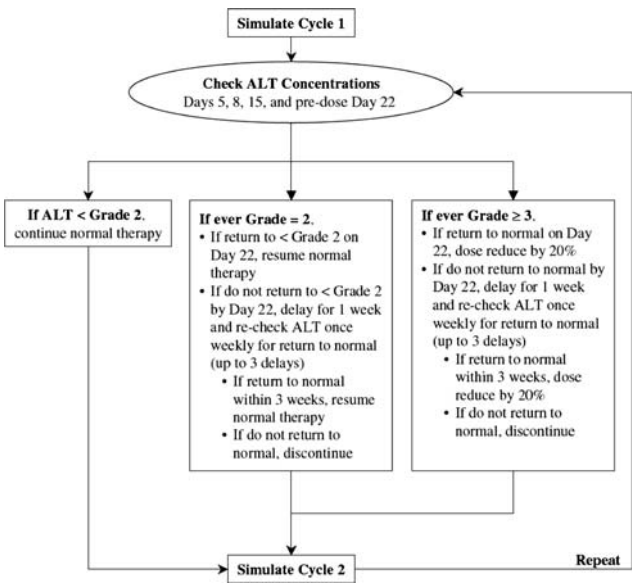

Fig. 2 Dose reduction simulation process

the ALT values between 2.5 and 5-fold the ULN, Grade 3 toxicity is established when the ALT values are between 5 and 20-fold the ULN and, finally, Grade 4 toxicity is determined when ALT values are higher than 20-fold the ULN. Simulated patients with baseline ALT values >2.5 times the normal upper limit of ALT were excluded from the analysis of simulated results in order to mimic eligibility criteria.

Results

Since a correlation exists between the elevation of ALT and AST after trabectedin administration [AST (IU/L) = 8.93 + 0.615·ALT (IU/L); $r^2 = 0.877$], this analysis utilized ALT as a representative measure of hepatocyte leakage to characterize the relationship between trabectedin exposure and the time course of transaminase elevation. ALT is considered a more specific marker as it is primarily found in the liver, whereas AST is also found in other tissues including cardiac muscle, kidneys and lungs.

Reference model

A two-compartment, adaptive precursor-dependent model for indirect pharmacodynamic effects was found to adequately describe the temporal relationship between ALT and trabectedin plasma concentrations (Fig. 1). This model incorporates the drug's stimulatory effect on the release rate of ALT from the hepatocytes to the plasma compartment as a linear relationship with trabectedin plasma concentration, and accounts for the development of tolerance to the drug's effect on ALT elevation in a vast majority of the population following several treatment cycles. An E_{max} stimulatory function was also tested to describe the drug effect, but without success.

Covariate analysis

Statistically significant relationships were observed between concomitant use of dexamethasone and "Slope" (ΔMVOF: 109.2; *df*: 2, $P < 0.001$), along with the effect of body weight on $ALT.P_0$ (ΔMVOF 61.8; *df*: 1, $P < 0.001$). Following the backward elimination analysis, the effect of body weight on $ALT.P_0$ (ΔMVOF: 1.3, *df*: 1, $P = 0.2542$) was finally excluded from the model. Diagnostic plots for the reference model and the model including covariates showed random uniform scatter around the identity line, indicating the absence of bias and confirming the adequacy of the model to describe the data.

Model evaluation

The PK/PD model developed on the index dataset was qualified by its application to data containing only soft tissue sarcoma patients from a Phase II study. Goodness-of-fit plots for the final model applied to this dataset revealed that the overall fit of the model was reasonable and is representative of patients with soft tissue sarcoma. The mean (standard deviation) of the absolute population prediction error (APE) for the index, 0.58 (0.51), and test, 0.54 (0.43) datasets were comparable. The mean (standard deviation) of the absolute individual prediction error (AIPE) for the index, 0.36 (0.36), and test, 0.32 (0.29), datasets were also comparable. These results suggest that the prediction errors are acceptable given the objective of this exercise.

Model refinement

One significant piece of the model refinement process included a mixture model to characterize patients with (66%) and without (34%) ALT elevation following administration of trabectedin (ΔMVOF: 1851). Further improvements to the model included the estimation of the covariance between the random effects on $ALT.P_0$ and "Slope" (ΔMVOF: 109) and the simplification of the dexamethasone effect on "Slope" by combining the categories of known and unknown dexamethasone use as the magnitude of the effect in both categories was similar.

Final model

The final parameter estimates for the combined dataset are shown in Table 3. Similar results were obtained using FO and FOCE method. The RSE for fixed and random effects parameters were ≤30% and <40%, respectively, indicating a good precision in the estimation of model parameters, except the between-patient variability in k_{out}. Diagnostic plots for the final model demonstrate that the overall fit of the model provides a relatively unbiased fit to the data (Fig. 3a). Individual model-predicted and observed ALT concentrations versus time illustrate that the model adequately characterizes at the individual level the observed data following several cycles of therapy for patients with and without transaminitis (Fig. 3b).

Overall, baseline ALT values are within the upper limit of normal for ALT. The effect of trabectedin on the release rate constant, k_r, is increased by approximately, 115-fold when 1,000 ng/mL of trabectedin is present in plasma, indicative of the pronounced impact that trabectedin has on the stimulation of ALT release from hepatocytes to plasma. In the presence of dexamethasone treatment, there is an

Table 3 Pharmacodynamic parameter estimates for the final PK/PD model

Parameter[a]	Typical value		Between-patient variability (%)	
	Final estimate	RSE (%)	Final estimate[b]	RSE (%)
k_{out} (1/h)	0.0193	11.2	67.68	42.6
Slope (mL/pg)	0.114	20.1	143.87[a]	18.9
Shift in slope due to known and unknown concomitant dexamethasone use (mL/pg)	–0.0744	28.9	NE	NE
Percent of patients in elevated ALT population (Population 1)	65.7	4.4	NE	NE
$ALT.H_0$ (IU/L)	5860	15.6	171.76	40.0
$ALT.P_0$ (IU/L)	31.5	5.1	52.54[a]	12.0
φ	0.865	25.7	NE	NE
RV (%)	52.73	12.8	NE	NE

RSE (%): relative standard error of the mean (%), *NE* not estimated

[a] k_{out}: elimination rate constant of ALT from plasma; Slope: the trabectedin effect on stimulation of ALT transfer rate from hepatocyte to plasma for patient population with elevated ALT; $ALT.H_0$: baseline ALT hepatocyte concentration; $ALT.P_0$: baseline ALT plasma concentration; φ: degree of feedback contribution; RV: residual variability

[b] The estimate of the covariance (%SEM) between the random effects of *Slope* and $ALT.P_0$ was –0.398 (16.2), equivalent to a correlation coefficient (r^2) of 0.277

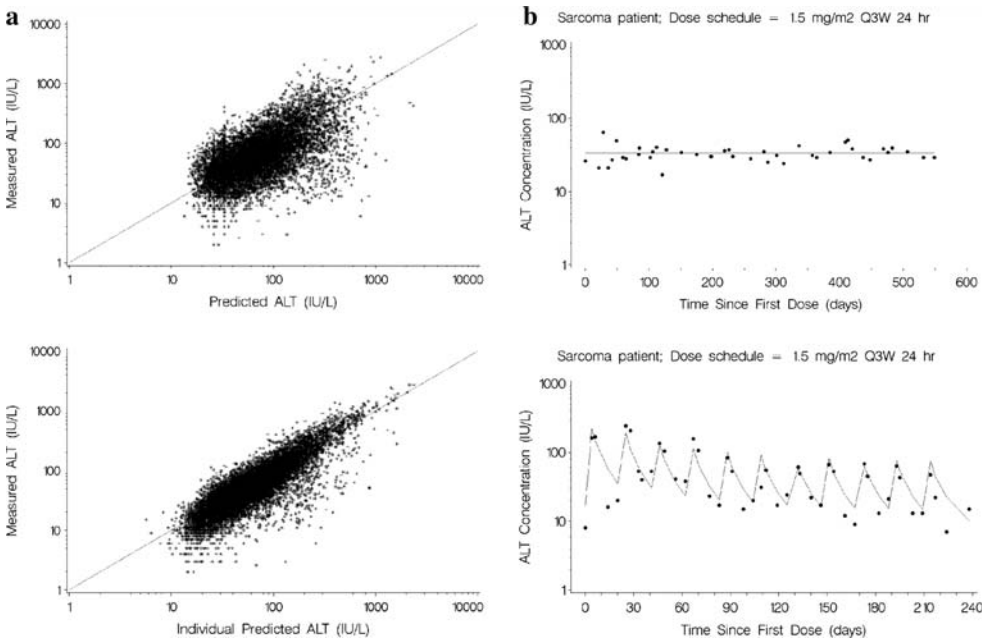

Fig. 3 **a** Observed and predicted ALT values following trabectedin administration. **b** Observed and predicted time course of ALT elevation following trabectedin administration in soft tissue sarcoma patients. *Top panel* patient with no ALT elevation, *Bottom panel*; patient with ALT elevation, *Filled circles* observed ALT concentrations, *Line* represents model-predicted ALT concentrations

approximate 63% reduction in the trabectedin effect, quantified through the "Slope" parameter. This finding is consistent with the modulation effect of dexamethasone on the elevation of ALT following trabectedin treatment, and the observed hepatoprotective effect of dexamethasone in these clinical studies. In addition, k_{out} was estimated to be 0.0193 1/h, which is reflective of an elimination plasma half-life of ALT of approximately 1.5 days, which is similar to published values of approximately 0.9 days [37]. The population mean values of $ALT.H_0$ and φ were estimated to be 5860 IU/L and 0.865, respectively. The φ parameter was significantly different than 1 (ΔMVOF: 7.945, *df*: 1, $P = 0.0048$). Of note, no statistically significant differences in model parameters across the different types of cancer patients evaluated were discerned.

Model-based simulations

Deterministic simulations clearly show the relationship between trabectedin exposure and the time course of ALT elevation (Fig. 4a). Maximum ALT values for the

population with ALT elevation were the highest during Cycle 1 and decreased with additional cycles of trabectedin. The model-predicted magnitude of ALT elevation was similar between 1.5 and 1.3 mg/m^2 regimens, with the initial peak concentration representative of at least a Grade 3 liver toxicity (Fig. 4a). On the other hand, the time above a given toxicity threshold (for instance, 150 IU/L) was longer for the 0.58 mg/m^2 QW3 regimen, which is reflective of a lower liver toxicity Grade of 1 or 2 that lasts longer. Thus, the degree and duration of elevated ALT are related to both the overall dose received and the frequency of trabectedin administration.

The final logistic regression model used to predict the prognostic factors influencing the ALT elevation revealed duration of infusion and dose as statistically significant predictors ($P < 0.0001$). The model-predicted probabilities of ALT elevation were 0.41, 0.89, and 0.85 for QW3 0.58 mg/m^2 with a 3-h infusion, Q3W 1.3 mg/m^2 with a 3-h infusion, and Q3W 1.5 mg/m^2 with a 24-h infusion, respectively. These probabilities of ALT elevation were then used to randomly assign the appropriate percentage of patients to ALT elevation in each regimen of the stochastic simulations.

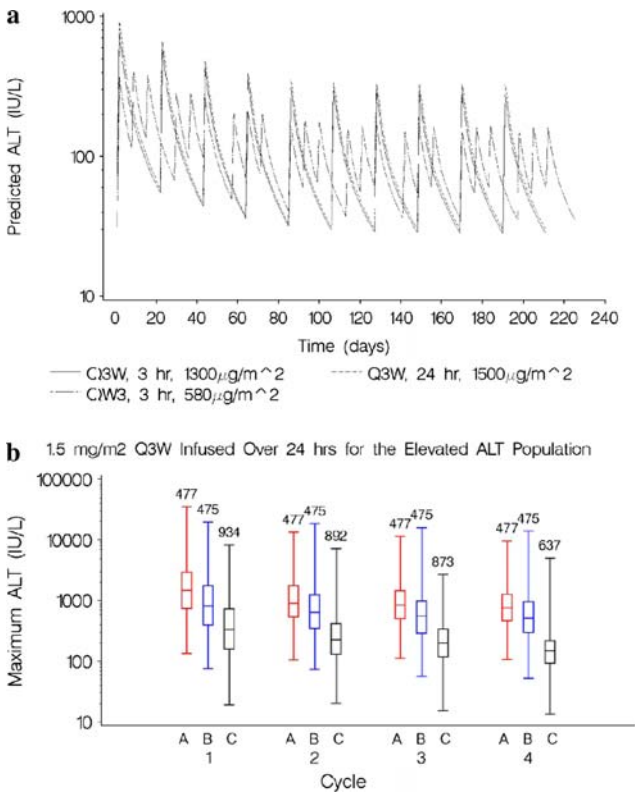

Fig. 4 **a** Effect of dose and inter-dose interval on the simulated time course of ALT elevation. **b** Effect of dexamethasone on simulated maximum ALT elevation. *A* No dexamethasone + no dose reduction *B* Dexamethasone + no dose reduction *C* Dexamethasone + dose reduction

Stochastic simulations investigating the effect of concomitant dexamethasone use revealed that maximum ALT elevation decreased by approximately 2- and 1.6-fold within the first and the fourth cycles when patients were administered dexamethasone in conjunction with a trabectedin dose of 1.5 mg/m^2 Q3W (Fig. 4b). The observed reduction in ALT elevation further illustrates the development of tolerance following trabectedin administration.

Overall, most patients had a dose reduction during Cycle 2, and fewer patients required dose reductions at Cycles 3 and 4, respectively (Table 4). Approximately 26, 18, and 11% of simulated patients required a dose delay in Cycles 2, 3, and 4, respectively. Dose delays of 1, 2, and 3 weeks occurred during Cycles 2, 3, and 4, with a median dose delay of 7 days. During Cycle 3, the largest percentage (25%) of simulated patients discontinued treatment with trabectedin due to ALT elevations. Furthermore, the validity of the results obtained from the simulations undertaken is supported by the fact that the severity of toxicity due to ALT elevation was comparable between the simulations and the observed toxicity in various Phase II clinical trials of trabectedin following the 1.5 mg/m^2 24-h Q3W dosing regimen (Table 5).

Table 4 Summary of dose reductions and dose delays, stratified by cycle, for the simulated trabectedin dosing regimen: 1.5 mg/m2 administered as 24 h intravenous infusion every 3 weeks

Cycle	Total patients in cycle	Dose reduction		Dose delay		
		n	%	*n*	%	Median (range) (days)
1	1000	0	0	0	0	NA
2	958	556	58	249	26	7 (7–21)
3	939	404	43	166	18	7 (7–21)
4	703	126	18	74	11	7 (7–21)

n number of hypothetical patients

Table 5 Comparison of simulated and observed Grade ≥3, Grade 3, and Grade 4 transaminitis following trabectedin 1.5 mg/m^2 administered as 24 h intravenous infusion every 3 weeks

ALT/SGPT	Simulated incidence, %[a]	Observed incidence, % (95%CI)[b]
Grade ≥3	53.2	50.2 (45.6–54.9)
Grade 3	38.5	41.9 (37.3–46.5)
Grade 4	14.7	8.3 (5.7–10.9)

ALT alanine aminotransferase, *SGPT* serum glutamic pyruvic transaminase, *CI* confidence interval

[a] Simulated incidence of liver toxicity grade on Day 5 post infusion during Cycle 1 of trabectedin treatment

[b] Incidence of transaminitis in Phase II studies conducted with trabectedin 1.5 mg/m^2 administered as 24 h intravenous infusion every 3 weeks ($N = 444$)

Discussion

Transient reversible transaminitis and subclinical cholangitis have been observed following administration of trabectedin [41]. This is indicative of potential intrinsic hepatocellular injury [3], resulting in release of transaminases from the hepatocyte [4]. However, it has been shown that pre/post-treatment liver biopsies following trabectedin administration showed no change in nonalcoholic steatohepatitis patients and one case of minimal post-treatment steatosis [47]. This acute, transient and reversible hepatotoxicity has been shown for other anticancer agents such as methotrexate, docetaxel, and gemcitabine [1, 17, 34]. To date, the temporal relationship between elevation in liver enzymes and trabectedin exposure has not been fully elucidated. The investigation of this relationship is critical as it could provide evidence that trabectedin transaminitis are predictable.

Results of the current PK/PD analysis demonstrate that a two-compartment adaptive precursor-dependent model of indirect pharmacodynamic effect on ALT concentration characterized the time course of transaminitis following different dosing schedules and infusion rates of trabectedin. This model demonstrates that the ALT elevation is reversible and transient. In addition, apparent tolerance to this adverse event develops following subsequent cycles of trabectedin chemotherapy (Fig. 3b). Examination of the simulations indicates that this tolerance is predicted to lead to lower ALT elevations upon the second administration and tolerance development is essentially complete following the fourth cycle of treatment.

The influence of pretreatment with dexamethasone use on the "Slope" parameter, reflective of stimulation of ALT release from the hepatocyte pool, was the most significant contributor to variability in the transaminitis. The dexamethasone pretreatment attenuated elevations in ALT by approximately a 63% reduction in the trabectedin stimulation of ALT release from the hepatocyte pool. The results presented above demonstrate unambiguously that trabectedin-induced transaminase elevation is amenable to pharmacological attenuation. Pretreatment of patients with dexamethasone afforded dramatic protection against the effect of trabectedin as reflected by plasma levels of liver enzymes ALT.

In general, the model-predicted incidence of Grade 4 transaminitis was 13–25% lower with dexamethasone use than without concomitant use. In patients who exhibited ALT elevation, co-administration of dexamethasone tended to have a 40–50% reduction in maximum ALT values as compared to patients without dexamethasone use, independent of dosing schedule (Fig. 4b). For the 1.5 mg/m^2 Q3W, 24-h infusion, stochastic simulations revealed that elevation to Grade 4 toxicity occurred in approximately 73% of simulated cancer patients who did not receive dexamethasone, compared to approximately 55% of simulated cancer patients who did receive dexamethasone comedication. The hepatoprotective effects of dexamethasone may be attributable, in part, to the modest decrease in trabectedin plasma concentrations observed with concomitant administration [32]. However, the current model has taken into account the effects of dexamethasone on trabectedin pharmacokinetics, and the magnitude of the pharmacodynamic effects described in the current model is only attributable to the hepatoprotective effects. Thus, dexamethasone may have additional effects on liver specific disposition of trabectedin or other pharmacodynamic influences, which modulate the degree of ALT elevation [15], and cause a reduction in hepatotoxicity. In fact, the pleiotropic pharmacological activities of dexamethasone, including the activation of many transcription factors and anti-inflammatory stimuli, could conceivably contribute to its hepatoprotective ability. It has been shown that pretreatment with the corticosteroid engendered a dramatic suppression of hepatic trabectedin concentrations in rats and, thus, effectively decreased hepatic exposure to the drug [31]. This suppression may have been the corollary of the propensity of dexamethasone to induce CYP3A enzymes and, thus, to increase the metabolic removal of trabectedin [27, 35]. It is also conceivable that the accelerated generation of nontoxic metabolites is responsible for the hepatic depletion of trabectedin observed in rats pretreated with dexamethasone [7]. At the same time, dexamethasone could exert its effect, at least in part, by increasing the bile flow and consequent elevated rate of trabectedin secretion. It is important to stress that, consistent with this hypothesis, dexamethasone pretreatment increases trabectedin systemic clearance by 19% in patients [32].

Comparisons of ALT elevation across different doses and dosing regimens of trabectedin were also made. Following trabectedin administration, the simulated magnitude of ALT elevation following the 1.5 mg/m^2 24 h infusion or 1.3 mg/m^2 3 h infusion Q3W regimens revealed an initial peak ALT concentration representative of at least Grade 3 liver toxicity on the day following dosing with trabectedin in each cycle, with a decreasing percentage of patients experiencing a longer duration of time between the day of dosing and the time that maximum ALT occurred. In contrast, the time above a given toxicity threshold (for instance 150 IU/L) was longer for the 0.58 mg/m^2 QW3 regimen, but only resulted in changes representative of a grade 1 or 2 toxicity, albeit lasting longer.

In comparing 0.58 mg/m^2 3 h infusion QW3, 1.3 mg/m^2 3 h infusion Q3W, and 1.5 mg/m^2 24 h infusion Q3W regimens, the total dose administered in a 3-week period is approximately 3, 2.25, and 2.6 mg, respectively, assuming

a BSA of 1.73 m^2. Interestingly, the total ALT elevation over this 3-week period, indicated by the ALT AUC (data not shown), is similar between the dosing regimens of 0.58 mg/m^2 QW3 and 1.5 mg/m^2 Q3W, with and without concomitant use of dexamethasone. Although the incidence of liver toxicity is higher and dose-dependent, as previously stated, the overall ALT elevation (i.e., AUC) across regimens is not substantially different. These results provide insight regarding the relationship between trabectedin exposure and the time course of transient ALT elevation, reduction in severity of transaminitis by dexamethasone, and decrease in degree of ALT elevation after several cycles of therapy. Although in previous analyses, the severity of ALT or AST changes have been related to exposure parameters such as C_{max} or AUC [38, 41, 42], our findings suggest that severity of ALT elevation is related more closely to trabectedin C_{max} than AUC, as prolonged Grade 2 toxicity had the same ALT AUC as observed with Grade 3 and 4 toxicities. Overall, these results support that the severity and frequency of ALT elevation is dose- and schedule-dependent, which makes ALT elevation manageable through monitoring of plasma levels of liver enzymes.

The main purpose of the model evaluation was to explore whether the model is able to predict the typical time course of ALT following the administration of trabectedin in the presence of dexamethasone in the target patient population, soft tissue sarcoma. Notably, the fact that no differences in pharmacokinetic and pharmacodynamic parameters have been seen across the different cancer types also confirms the validity of the test dataset for the model evaluation purpose. Nevertheless, the test dataset selected does not allow for the evaluation of the effect of dexamethasone or the typical time course of ALT in a different cancer population. However, for safety reasons raised during the clinical development, trabectedin treatment is recommended to always be administered in the presence of dexamethasone and, therefore, no additional data are available where trabectedin is administered in the absence of dexamethasone. The results presented show that the PKPD model developed with the index dataset is suitable to explain the typical profile of ALT and its variability after trabectedin administration in the presence of dexamethasone and, therefore, the model can be further used to explore different clinically relevant scenarios of interest and contribute in the decision making process. Although the model has not been validated to predict the individual time course of ALT and forecast the time and extent of ALT elevation for a particular patient, the model developed could be useful as an initial step to further develop and validate dose reduction strategies based on the maximum a posteriori Bayesian estimation of pharmacokinetic and pharmacodynamic parameters.

Simulations of the effect of the dose-reduction and dose-delay algorithm utilized in the clinical development of trabectedin on the predicted ALT profile for the 1.5 mg/m^2 24-h infusion Q3W dosing schedule were performed to evaluate the effectiveness at managing ALT elevation following drug administration. The incidence of elevated liver toxicity grade after incorporating dose reductions was compared (Tables 4, 5) between simulated and pooled laboratory data from Phase II studies for the 1.5 mg/m^2 Q3W regimen of trabectedin. A good agreement was revealed between simulated and observed grade ≥3 toxicity. Nevertheless, the simulated incidence of Grade 4 toxicity was found to be slightly higher than the observed, most probably because during the evaluation of the dose reduction strategy the assumption was made that only ALT elevation would lead to a dose reduction, while in reality the clinical management of other toxicities of lower incidence would lead to additional trabectedin dose reductions. As a consequence, the simulation results obtained are conservative with respect to the incidence of grade 4 ALT toxicities.

Furthermore, the incidence of grade ≥3 liver toxicity decreased by approximately 39% from Cycle 1 to Cycle 4 of treatment when the dose reduction strategy is considered (53.2% for Cycle 1 vs. 14.5% for Cycle 4). These findings demonstrate that the results of the model developed are consistent with results from the clinical trials, which allow the use of the model to make inference about the efficiency of the dose reduction strategy. This dose reduction strategy has been used recently in a randomized multicenter Phase II study, which characterized the efficacy and safety of two dosing regimen of trabectedin in patients with liposarcoma and leiomyosarcoma after failure of prior treatment with anthracylines and ifosfamide. Two hundred seventy patients were randomized to receive intravenous trabectedin, 1.5 mg/m^2 as 24 h infusion every 3 weeks (Q3W) or 0.58 mg/m^2 as 3 h infusion on days 1, 8 and 15 of a 28-day cycle (QW). Significantly better (hazard ratio: 0.734; $P = 0.0302$) median time to progression was observed for the Q3W dosing regimen, 3.7 months (95% CI: 2.1–5.4 months), as compared to the QW dosing regimen, 2.3 months (95% CI: 2.0–3.5 months). Although the incidence of neutropenia and transaminitis grade 3 and 4 was higher for Q3W dosing regimen relative to the QW dosing regimen, these toxicities were predictable, non-cumulative, reversible and did not have any clinical consequences. As a consequence, trabectedin can provide clinical benefit to patients with liposarcoma and leiomyosarcoma sarcoma following failure of all conventional treatment options [29].

In summary, a mechanism-based pharmacokinetic and pharmacodynamic model for the hepatoprotective effect of dexamethasone on the transient and reversible transaminitis after trabectedin treatment has been developed. The

validated model developed predicts that the administration of dexamethasone and the suggested dose reduction strategy based on the concentration of liver enzymes in serum will enhance the safe and efficacious use of trabectedin in the clinic. The model developed might be used in other populations to explore the dose reduction strategies for new dosing regimen in clinical development.

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