ORIGINAL ARTICLE

Simultaneous Modeling of Biomarker and Toxicity Response Predicted Optimal Regimen of Guadecitabine (SGI-110) in Myeloid Malignancies

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Guadecitabine (SGI-110) is a novel next-generation hypomethylating agent (HMA) administered as s.c. injection with extended decitabine exposure. Dose/exposure-response analyses of longitudinal measures of long interspersed nucleotide element-1 (LINE-1) methylation and absolute neutrophil counts (ANC) pooled from 79 and 369 patients in 2 phase I/II trials, respectively, were performed to assist, through modeling and simulation, the selection of dosing regimens for phase III. Simulation of ANC predicted a decrease after a 5-day regimen of 60 mg/m^2 with partial recovery before the next cycle, whereas the nadir of 90 mg/m^2 on the same schedule was below 100/μl. ANC following a 60 mg/m^2 10-day regimen was predicted to be suppressed below 100/μl as long as treatment continued without recovery. The developed models provided useful tools to assist simultaneous evaluation of the relative dynamics of the two effects (DNA demethylation and the effect on ANC).

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ We postulate that two independent mechanisms (i.e., methylation and cytotoxicity), contribute to the action of hypomethylating agent guadecitabine on neoplastic cells, and, hence, therapeutic benefit.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ This analysis of phase I/II data characterized the dynamics of LINE-1 (a global metric of DNA methylation) and ANC (cytotoxic effect) following administration of guadecitabine, providing a useful tool to evaluate the two effects following different dosing regimens.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
☑ An indirect response model best described the LINE-1 time course, whereas a K-PD model was used to link the dosing rate to decreases in ANC. We show that a 5-day regimen of 60 mg/m^2 every 28 days is optimal, in the sense that the maximum effect of demethylation is reached when the cytotoxic effect is minimal and vice versa.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?
☑ A simultaneous quantitative characterization of relative dynamics of DNA-demethylation and effect on ANC could be used to optimize the dose regimen of guadecitabine.

Effective treatment for acute myeloid leukemia (AML) in patients, particularly the elderly who are not considered candidates for intensive remission induction chemotherapy, remains a persistent unmet medical need.[1] Gene hypermethylation is widespread in patients with myeloid malignancies. Hypomethylating agents (HMAs), azacitidine and decitabine have been approved in the United States for the treatment of myelodysplastic syndromes (MDS), a clonal myeloid disorder that may eventually evolve into AML. Decitabine was also approved to treat elderly patients with AML who are not candidates for intensive induction chemotherapy in the European Union.[1] These HMAs are known to induce gene expression following DNA-methyl transferase 1 sequestration. However, it has been controversial whether DNA methylation biomarkers (e.g., long interspersed nuclear element 1 (LINE-1)) could be used as predictors of clinical response.[2,3]

Guadecitabine is a dinucleotide of decitabine and deoxyguanosine, designed to protect the active moiety, decitabine, from inactivation by cytidine deaminase.[4] *In vitro* evidence suggests that guadecitabine has a longer half-life than decitabine in the presence of cytidine deaminase.[5] Prolonged exposure time is predicted to increase efficacy because activity of decitabine is dependent on its incorporation into DNA during DNA synthesis (i.e., S-phase of the cell cycle).[4,6] Prolonged exposure affects more cancer cells as they enter into S-phase and are susceptible to decitabine activity. These effects of decitabine are associated with a decrease of both global DNA and gene-specific methylation. In a phase I/II dose escalation randomized study in patients with intermediate or high-risk MDS or AML, daily exposure of the active metabolite decitabine increased slightly more than dose-proportionally following administration of guadecitabine from 3–125 mg/m^2.[7]

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Myelosuppression is identified as the primary side effect of guadecitabine, highlighting the need to account for its cytotoxicity profile into optimum drug dosing in myeloid malignancy, in which pancytopenia is a hallmark of disease. We also hypothesized that the cytotoxic effect, in addition to DNA hypomethylation, may result in the death of cancer blast cells in the bone marrow, and potentially contribute to therapeutic benefit of guadecitabine.

The value of integrating the dose/exposure-response relationship of relevant biomarkers together with safety to guide selection of an optimally tolerated and bioactive phase III clinical dose in oncology drug development has been shown.[8] To predict the relationship among guadecitabine dose/schedule, biomarker response, and clinical response in combination with dosing rate-safety relationship in patients with AML/MDS, we developed a pharmacokinetic/pharmacodynamic (PK/PD) model of longitudinal data of LINE-1 methylation, and a kinetic-PD (K-PD) model of absolute neutrophil counts (ANCs) that account for dosing rate and toxicity response. The biomarker model described the inhibitory effect of decitabine, derived from guadecitabine, on DNA methylation and this was linked to clinical response using logistic regression. The relationship between ANC and clinical response was also investigated. Using this framework, the clinical response rate for the 10-day regimen not used in phase I but included in phase II was predicted. The K-PD model was used to assess the effect of dose and schedule on ANC and together the two models were used to optimize the relative dynamics of the two drug effects.

METHODS
Study design and patient population
The study involved two stages: (1) the phase I dose-escalation stage was designed to determine the safety, tolerability, and pharmacokinetics of guadecitabine at dose levels of 3, 9, 18, 36, 60, 90, and 125 mg/m^2 administered s.c. in 93 patients with MDS and AML[7]. Three 28-day schedules were tested including 5-day (dosing days 1–5), weekly $\times$ 3 (dosing days 1, 8, and 15), and twice-weekly (dosing days 1, 4, 8, 11, 15, and 18); and (2) the phase II dose-expansion stage evaluated the safety and efficacy of 60 and 90 mg/m^2 5-day regimens in patients. After safety and preliminary efficacy were established with the 5-day regimen, a single arm 10-day regimen of 60 mg/m^2 was also investigated in phase II. Subjects from the phase I/II trials who had both plasma concentrations of guadecitabine/decitabine and LINE-1 methylation were used for PK/PD and subsequent exposure-response analyses. Neutrophil counts pooled from the phase I/II trials were used to develop the K-PD model of ANC time course, because no plasma concentrations were measured in phase II.

Plasma concentrations of guadecitabine and decitabine were determined by a validated high-performance liquid-chromatography tandem mass spectrometry method, as described previously.[7] Whole-blood samples for DNA demethylation were collected prior to treatment (baseline) and weekly during the treatment cycles. Global DNA methylation was measured by LINE-1 methylation assay, as previously reported.[9] Clinical response of patients with AML
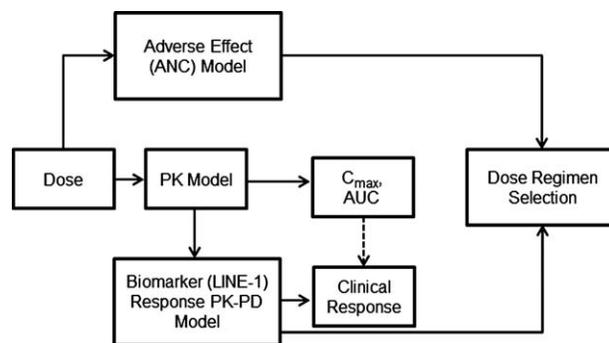
Figure 1 A schematic of integrated model analysis of biomarker time course, exposure-response, as well as safety endpoint of guadecitabine. ANC, absolute neutrophil count; AUC, area under the curve; C$_{max}$, peak plasma concentration; LINE-1, long interspersed nucleotide element-1; PK, pharmacokinetic; PK-PD, pharmacokinetic-pharmacodynamic.

and MDS was assessed based on revised recommendation of the 2003 International Working Group[10] and the 2006 International Working Group criteria,[11] respectively.

Model development
A schematic of integrated model analysis of biomarker time course, exposure-response, as well as safety endpoint are shown in **Figure 1**. The nonlinear mixed-effects modeling software (NONMEM version 7.2; ICON Development Solutions) was used for model development. The RStudio software[12] was used for preprocessing and postprocessing of data. Model selection and evaluation during model building included comparison of the objective function value (OFV) and inspection of a range of model diagnostics. For application of the likelihood ratio test in the case of comparing nested models, a significance level of $P < 0.01$ was applied, corresponding to a decrease in OFV of at least 6.63 when one extra parameter was added. The predictive performance of the final model was evaluated using prediction-corrected visual predictive checks (VPCs).[13] Prediction intervals with 95% confidence intervals were derived from 1,000 simulated datasets and compared with the observed data.

Pharmacokinetic model. A combination of one-compartment pharmacokinetic (PK) model with first-order absorption for guadecitabine and two-compartment PK model for decitabine, assuming that 100% of guadecitabine was metabolized to decitabine, was previously developed ($n = 98$). Time-varying absorption was described by a model event time model, in which a typical absorption rate was changed at an estimated time after dose administration. Individual empirical Bayes estimates (EBEs) of the parameters for those patients with LINE-1 measurements ($n = 79$) and the concentration-time profiles of guadecitabine and decitabine were derived from this previously developed model, which were used as an input function for the LINE-1 pharmacodynamic (PD) model via a sequential PK/PD approach.

Biomarker model. The time course of LINE-1 methylation was related to guadecitabine/decitabine plasma concentrations

using indirect response models with maximum effect (E_{max}) drug effect relationships. The demethylation effect of decitabine was assumed to be mediated through either stimulation of a first-order degradation rate of methylated DNA, k_{out}, or the inhibition of a zero-order production rate of methylation of DNA, k_{in}.[14]

In these models, baseline LINE-1 methylation is defined as the ratio of the zero order production rate and first order degradation rate of the response (k_{in}/k_{out}). The E_{max} is the maximal effect of decitabine to stimulate or inhibit, respectively, and half-maximal effective concentration (EC_{50}) is the decitabine plasma concentration that provided half maximal drug effect.

Kinetic-PD model of ANC. Because no PK data were available for the phase II trial, a "kinetics of drug action" model was used to quantify the effect of the drug on neutrophil counts.[15] The K-PD model used a virtual dose-driving rate that is defined as the product of the first order equilibration rate from the virtual compartment (into which the drug is administered). Subsequently, the amount in this compartment multiplied by the virtual rate is used as a forcing function to drive the effect on ANC. Structural models were developed in order of increasing complexity. The K-PD compartment was first coupled with an indirect response model and thereafter a lag time and/or a semiphysiological model of myelosuppression with or without a feedback loop was also tested.[16] Initial values of parameters were estimated from a subpopulation with baseline neutrophil counts $\geq 1 \times 10^9$/L. In order to reduce the number of parameters to estimate and improve model stability, degradation rate of neutrophil, representing the removal of existing cells from the systemic circulation as they die, was fixed at a value derived from the literature.[17] Modeling was performed in the log domain and a proportional error model was used to describe the residual variability associated with the neutrophil counts. Approximately 80% of the patients had baseline neutrophil measured. The missing baseline neutrophil values were imputed with the median for covariate assessment. Covariate effect of baseline neutrophil and disease type were examined by likelihood ratio test.

Simulations. In order to simultaneously evaluate the effect on biomarker response and neutrophil counts following 3 cycles of 60 and 90 mg/m^2 5-day regimen or 10-day regimen of 60 mg/m^2, the time-course of LINE-1 demethylation was simulated using EBEs from the LINE-1 model and ANC in a typical patient using the population prediction parameters.

Exposure-response analysis of efficacy
Clinical response (yes/no) was modeled using logistic regression analysis. The relationship of response to decitabine exposure (area under the curve (AUC), peak plasma concentration (C_{max})) and biomarker effects (maximal LINE-1 demethylation, AUC of demethylation, or ANC at nadir during the first treatment cycle) was evaluated. Because of the relatively small proportion of responders in the phase I trial, the efficacy data from patients with AML and MDS were pooled to enrich the exposure-response analysis.

RESULTS
Pharmacokinetic model
A previous population PK model of guadecitabine and decitabine was developed from 98 patients (Astex internal pharmacometrics report). The goodness-of-fit plots showed concentrations randomly distributed around the identity line, indicating that the individual PK parameters derived from the model described the concentration-time profiles of guadecitabine and decitabine well. The estimates of main model parameters were guadecitabine apparent clearance of 371 L/hr (95% confidence interval (CI): 330–412 L/hr), guadecitabine apparent volume of distribution of 550 L (95% CI: 455–646 L), decitabine apparent clearance of 405 L/hr (95% CI: 364–446 L/hr), decitabine apparent central volume of 52.8 L (95% CI: 38–67.5 L), decitabine apparent intercompartmental clearance of 368 L/hr (95% CI: 259–477 L/hr), and decitabine apparent peripheral volume of distribution of 187 L (95% CI: 168–206 L). Absorption was described by a first-order absorption process, which was variable with time. During the first 1.15 hours, absorption rate constant was estimated at 0.663 hour-1, whereas after 1.15 hours post-dose it increased to 2.00 hour-1. Allometric scaling with the fixed power coefficient of 0.75 and 1 for clearance and volume parameters, respectively, adequately described dependence of these parameters on body weight. Model parameters were independent of sex and disease. Intersubject variability on clearance, volume, and absorption rate constant of guadecitabine as well as clearance of decitabine ranged from 17.1–63.4%. Interoccasion variability on relative bioavailability and absorption rate ranged from 28.2–75.4%. The residual variability for both guadecitabine and decitabine concentrations was described as a combination of the proportional and additive errors. The parameter estimates of the final PK model are summarized in **Supplementary Table S1**. This model described the subset of the data with both PK and LINE-1 measurements ($n = 79$) adequately well (**Supplementary Figure S1**).

Biomarker model
The PK and LINE-1 measurements from 79 patients in the phase I trial were used to develop the biomarker model. An indirect response model with stimulation of degradation rate of LINE-1 methylation best described the data. The parameter estimates of the final biomarker model are summarized in **Table 1**. The typical value of decitabine EC_{50} was estimated to be 75.2 ng/mL. Both fixed and random effects were estimated with acceptable precision (**Table 1**), except for EC_{50} and E_{max}, in which the relative standard errors were higher. Despite this, the goodness-of-fit plots showed that observations were randomly distributed around the identity line, indicating the absence of systematic bias in parameter estimation and the overall adequacy of using EBEs to predict the LINE-1 methylation time course (**Supplementary Figure S2**). The prediction-corrected VPC (**Figure 2**) indicated adequate predictive ability of the model to describe LINE-1 demethylation dynamics following treatment with guadecitabine.

Table 1 Parameter estimates for the biomarker PD model

Variables (unit)	Typical value	RSE	95% CI
K_{in}	6.91	12.2%	5.31–14.6
K_{out}, h^{-1}	0.0979	12.2%	0.0746–0.206
EC_{50}, ng/mL	75.2	78.1%	17.2–2086
E_{max}	17.8	58.4%	6.08–365
IIV of K_{in}, %	57.7	38.4%	35.0–117
IIV of K_{out}, %	57.2	37.6%	33.0–116
IIV of EC_{50}, %	104	41.9%	65.3–143
Residual error, %	2.02	7.99%	1.68–2.35

CI, confidence interval; EC_{50}, half-maximal effective concentration; IIV, inter-individual variability; K_{in}, zero-order production rate of methylation of DNA; K_{out}, first-order degradation rate of methylated DNA; RSE, relative standard error.

Table 2 Parameter estimates for the K-PD model of ANC

Variables (unit)	Typical value	RSE	95% CI
KDE, mg/day	0.064	2.33%	0.00796–0.246
Lag time, day	7.47	2.12%	5.43–38.3
KS	0.133	6.98%	0.050–0.387
KD, day^{-1}	0.185 FIX	NA	NA
EKD50, mg/day	14.7	5.18%	6.21–45.2
Γ	2.49 FIX	NA	NA
IIV of KDE, %	100	14.5%	65.7–596
IIV of KS, %	112	9.36%	115–898
IIV of EKD50, %	97.2	15.5%	77.8–1030
Residual error, 10^9/L	2.63	0.392%	2.47–3.13

ANC, absolute neutrophil count; CI, confidence interval; EKD50, dose rate resulting in 50% inhibition of KS; IIV, interindividual variability; KD, ANC degradation rate constant; KDE, first-order equilibration rate constant; K-PD, kinetic-pharmacodynamic; KS, ANC synthesis rate; NA, not applicable; RSE, relative standard error.

Kinetic-PD model of ANC

The ANC from 369 patients in two phase I/II trials were used to develop neutrophil model. A K-PD model incorporating an inhibitory sigmoid E_{max} relationship on neutrophil synthesis rate with E_{max} fixed at 1 and a lag time was used to link the dosing rate to the time course of ANC. The sigmoidicity parameter (γ) was estimated and fixed at 2.49 according to likelihood profiling (data not shown). The goodness-of-fit plots showed the overall adequacy of the final model (**Supplementary Figure S3**) and it described the time course of ANC well up to 10 weeks post-treatment, but there was a tendency of underprediction thereafter (**Supplementary Figure S4**). More complex model structures did not result in an improved fit. The lag time was estimated to be ~7 days, indicating a delay in the onset of drug effect on neutrophils in the systemic circulation (**Table 2**). Guadecitabine is, therefore, most likely impacting on neutrophil precursor cells in the bone marrow. Baseline neutrophil counts and disease type were not

Figure 2 Prediction-corrected visual predictive check of pharmacokinetic-pharmacodynamic model of biomarker long interspersed nucleotide element-1 (LINE-1) shown as original raw data. CI, confidence interval.

associated with a statistically significant drop in OFV (likelihood ratio test) during covariate modeling, therefore, they did not impact the drug sensitivity in the present analysis.

Simulations. A more profound demethylation effect was observed following the 5-day regimen compared to the twice-weekly and once-weekly regimens. Based on these simulations, the time to nadir was estimated to be 5 days with recovery taking ~1 cycle. Although this model does not mathematically constrain the nadir to be the same at all doses, this was the case in the dose range investigated, indicating that these doses are most likely in the lower part of the dose response curve. The predicted average plasma concentration of decitabine receiving the 5-day regimen of 60 mg/m^2 dose was 3.75 ng/mL and predicted peak concentration of decitabine was 23.5 ng/mL. The simulated ANC following 60 mg/m^2 on days 1–5 of a cycle decreased from 700/uL to values between 200 and 500/μL under treatment with partial recovery of the ANC before the next cycle (**Figure 3a**). The nadir of 90 mg/m^2 on the same schedule was below 100/μL (**Figure 3b**). Neutrophil counts following the 60 mg/m^2 10-day regimen were suppressed below 100/μL, as long as treatment continue without recovery (**Figure 3c**).

Exposure-response analysis of efficacy

Among 79 patients, all previously treated for their disease with the majority having had prior hypomethylating therapy and who had both PK and biomarker measurements, 11 patients were determined to be clinical responders and consisted of 6 patients with AML and 5 patients with MDS. Simulated LINE-1 demethylation nadir of the first cycle showed a significant relationship to the probability of clinical responses ($P < 0.05$), whereas decitabine AUC during the first cycle ($P = 0.86$) and ANC nadir ($P = 0.81$) in the same patients did not (**Figure 4**). There were three nonresponsive patients who had extremely high nadir ANC counts $>10 \times 10^9$/L (see **Figure 4**). When these three patients were removed as part of a sensitivity evaluation, the relationship for ANC nadir changed but remained statistically not significant at the 5% level ($P = 0.154$). Using this framework, the clinical response rate for the 10-day regimen not
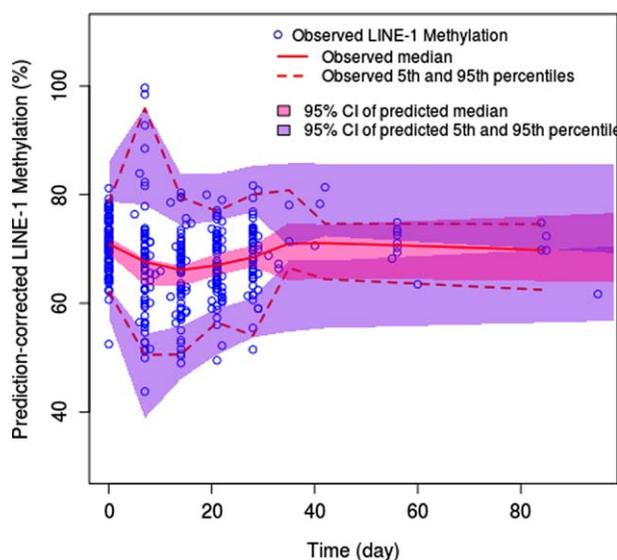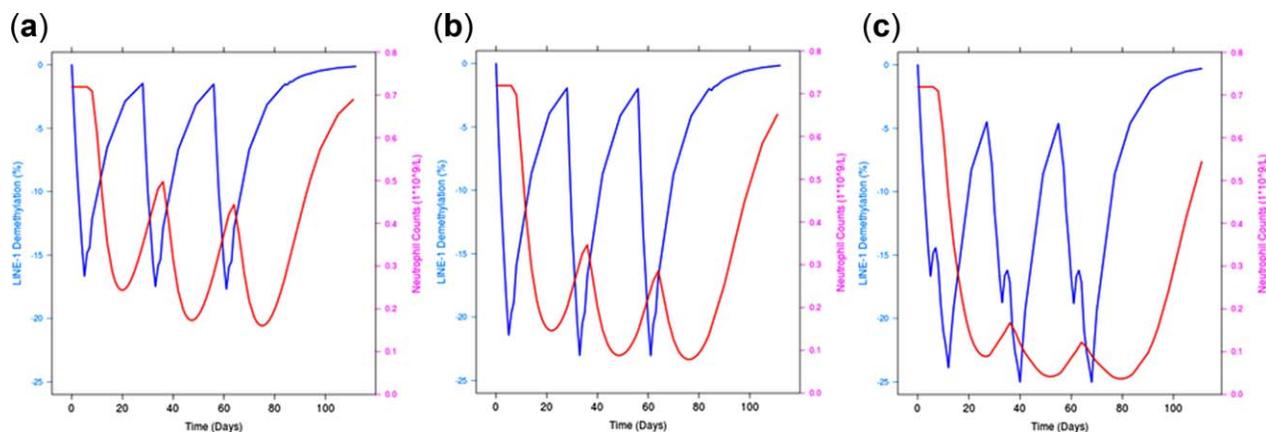
Figure 3 Time course of long interspersed nucleotide element-1 (LINE-1) demethylation shown as change from baseline (%) and absolute neutrophil count following 3 cycles of 5-day regimens of 60 mg/m^2 (**a**), and 90 mg/m^2 (**b**), 10-day regimens of 60 mg/m^2 (**c**) in a typical patient.

used in phase I but included in phase II was predicted to be about 25%, which was within 95% CI of observed data (data not shown).

DISCUSSION

An integrative approach of leveraging both efficacy and safety data at early clinical development to assist dose selection for a late-stage clinical trial has been explored in this study. This is the first study quantitatively characterizing the time courses of both hypomethylating and cytotoxic effect (manifested by a decrease in ANC) of guadecitabine. We further show that simulated LINE-1 demethylation nadir of the first cycle significantly correlated with the probability of clinical responses, whereas decitabine exposure and ANC nadir in the same patients did not. In addition, we also show that a 5-day regimen every 28 days is optimal compared with the predicted time courses of LINE-1 demethylation and ANC following a 10-day regimen, in the

sense that, the maximum effect of demethylation is reached when the cytotoxic effect is minimal and vice versa.

Both DNA methylation and apoptosis induction have been suggested to be involved in the mechanism of action of HMAs.[3] Therefore, we hypothesized that two independent mechanisms (i.e., hypomethylation and cytotoxicity), contribute to the action of guadecitabine on neoplastic cells, and, hence, therapeutic benefit. In the present study, the simulated LINE-1 demethylation nadir of the first cycle showed a significant relationship to the probability of clinical responses, whereas decitabine exposure and ANC nadir in the same patients did not, which implies that the therapeutic benefit is mostly attributed to hypomethylation and likely independent from cytotoxicity. Although the primary advantage of guadecitabine over decitabine is prolonged exposure of decitabine due to its resistance to cytidine deaminase, the absence of relationship between decitabine exposure and response is somewhat expected, because PD biomarkers rather than PK exposure is more likely to predict response and/or survival.[8] The lack of association between
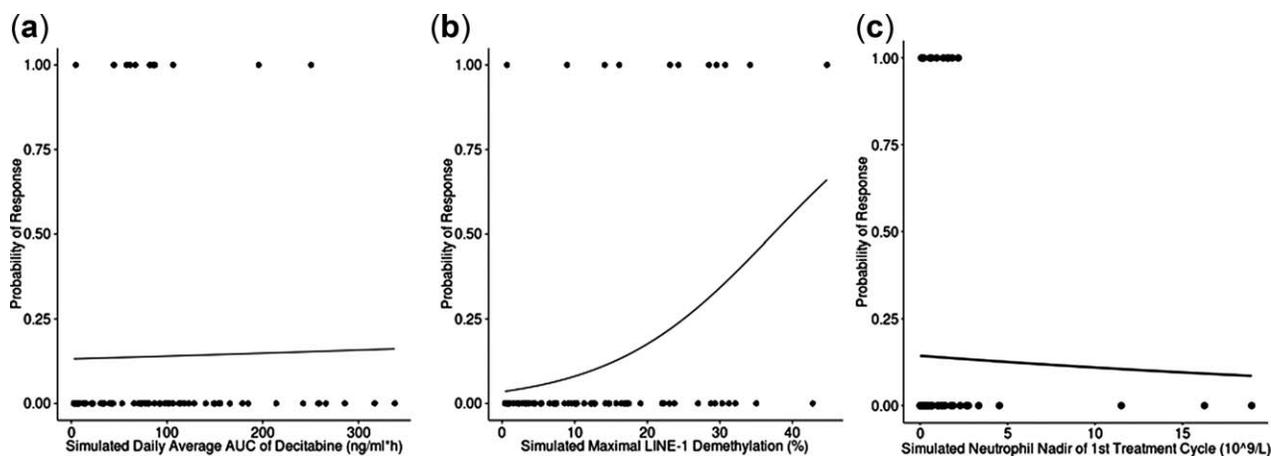
Figure 4 Logistic regressions of simulated decitabine area under the curve (AUC) (**a**), long interspersed nucleotide element-1 (LINE-1) demethylation (**b**), absolute neutrophil count nadir (**c**) and clinical response.

ANC nadir and response could have different explanations. One explanation may be that ANC in early cycles is more indicative of safety rather than efficacy. Another explanation could be that hypomethylation is a more dominant mechanism of activity, whereas cytotoxicity is a complementary pathway. Despite this poor correlation, the utility of modeling myelosuppression in dose adaptation has been summarized in literature and was used to facilitate dose selection in the present study.[8] Simulations showed that hypomethylation of LINE-1 exhibits rapid dynamics relative to drug administration, whereas the cytotoxic effect of ANC has classical and slow dynamics (**Figure 3**). The rapid reversibility of methylation (compared to cytotoxicity) explains the necessity for a maintenance treatment as long as response persists. We show that a 5-day regimen every 28 days is optimal due to its asynchrony that allows for recovery of neutrophils within the treatment cycle, whereas the drug effect is maintained through demethylation. In contrast, the 10-day regimen completely suppressed ANC throughout the treatment cycles and such asynchrony is not apparent from simulation, which indicates that such regimen may not be favorable for fragile patients whose capacity to fight infection is already low. Nevertheless, the 10-day regimen was predicted to have a more potent hypomethylation effect and, thus, likely higher response rate, 25% vs. 17%, compared to 5-day regimens of 60 mg/m². This is consistent with literature that a 10-day regimen of decitabine seems to have higher activity in myeloid malignancies.[18,19] But for MDS/AML, higher response rate is not always translated into better overall survival,[20] which further highlights the need to take both efficacy and safety into account while evaluating different regimens at early development.

Quantitative understanding of dose/exposure-PD relationship is crucial for dose/schedule optimization. In the present study, we applied a modeling and simulation approach to analyze LINE-1 methylation and determine a full time course of methylation changes. It predicted that the time to nadir was 5 days following a 5-day regimen with recovery taking ~1 cycle across the escalating doses. The predicted average plasma concentration of decitabine receiving the 5-day regimen of 60 mg/m² dose was 3.75 ng/mL and predicted peak concentration of decitabine was 23.5 ng/mL, respectively, which compared to an EC_{50} for LINE-1 effect of 75.2 ng/mL. This suggests that, in the dose range used in this study, we are likely in the lower part of the LINE-1 dose-response curve. Intersubject variability in sensitivity to this effect was very high, as evidenced by a coefficient of variation of 104% for EC_{50}. Despite a more potent methylation effect projected at a higher dose, a 5-day regimen of 60 mg/m² was still selected for the current ongoing phase III trial in adults with untreated AML not considered for intensive remission induction (NCT02348489), because it was predicted to have less cytotoxicity than 90 mg/m² using a K-PD model of ANC. The 10-day regimen was predicted to have even greater cytotoxicity. In patients with relapsed refractory AML, adverse events increased significantly over these three groups in a dose-dependent manner.[21] In addition, PK/PD modeling also facilitated the simulations of AUC and LINE-1 demethylation nadir, which were further correlated with response. This finding was consistent with a

previous study that showed reduced methylation over time was correlated with better clinical response,[2] although contradictory evidence is also available in literature.[3] This controversy could be due to the fact that sampling schedules for PD measurements are rarely optimized in different clinical studies and when the schedules are sparse and different they do not yield to empirical methods but require model-based methods for elucidation. In a phase II dataset, the LINE-1 demethylation effect was also found to be correlated with overall survival in about 100 patients with MDS and AML (unpublished data), which further supports LINE-1 as a potential PD endpoint for dose individualization to optimize response.

Neutropenia is the major dose-limiting toxicity, but difficult to evaluate due to the pancytopenia associated with the disease. Although the final model without feedback mechanism underpredicted ANC after 10 weeks, it described ANC reasonably well up to 10 weeks. We assumed that the sustained blood neutrophil number between 200/μL and 500/μL during first three treatment cycles would be more favorable than greater depletion to levels <100/μL. In this regard, the ANC profile following the 5-day regimen of 60 mg/m² is preferred to the 5-day regimen of 90 mg/m² and 10-day regimen of 60 mg/m². Recovery is also more complete within the dosing cycle for the 5-day regimen of 60 mg/m². The present model fulfilled our purpose to simulate the ANC time course following three cycles of treatment. A myelosuppression model coupled with a feedback mechanism accounting for a rebound of ANC was first introduced by Friberg *et al.*[16] However, more complex model structures did not improve the fit in the current study. During covariate modeling, different categorizations of disease were tested, starting from introduction of four disease categories (i.e., treatment naive AML; r/r AML; treatment naive MDS; and r/r MDS). The disease type was not found to have any impact on drug sensitivity parameter.

In conclusion, the PK/dose-response models of both biomarker (LINE-1) and toxicity (ANC) endpoints were developed for guadecitabine. Simulated LINE-1 matrices have further been related to clinical response. The developed models provided useful tools to assist simultaneous evaluation of the relative dynamics of the two effects (DNA demethylation and effect on ANC).

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