Citation: CPT Pharmacometrics Syst. Pharmacol. (2017) 6, 357–364; doi:10.1002/psp4.12194

ORIGINAL ARTICLE

Translational Modeling of Drug-Induced Myelosuppression and Effect of Pretreatment Myelosuppression for AZD5153, a Selective BRD4 Inhibitor

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In this work, we evaluate the potential risk of thrombocytopenia in man for a BRD4 inhibitor, AZD5153, based on the platelet count decreases from a Han Wistar rat study. The effects in rat were modeled and used to make clinical predictions for human populations with healthy baseline blood counts. At doses >10 mg, a dose-dependent effect on circulating platelets is expected, with similar predicted changes for both q.d. and b.i.d. dose schedules. These results suggest that at predicted efficacious doses, AZD5153 is likely to have some reductions in the clinical platelet counts, but within the normal range at projected efficacious doses. The model was then extended to incorporate preexisting myelosuppression where bone marrow function is inhibited by acute myeloid leukemia. Under these conditions, duration of platelet count recovery has the potential to be prolonged due to drug-induced myelosuppression.
CPT Pharmacometrics Syst. Pharmacol. (2017) **6**, 357–364; doi:10.1002/psp4.12194; published online 5 April 2017.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THIS TOPIC?
☑ BRD4 inhibitors are indicated for acute myeloid leukemia and are known to cause thrombocytopenia in clinical studies. Myelosuppression has been shown to be predictable in the clinic from preclinical studies.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ This study aims to make a prospective clinical prediction from rat data for AZD5153 with different doses/schedules, and also considers the additional impact of myelosuppression caused by acute myeloid leukemia in clinical predictions.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
☑ This study exemplifies how risk of drug-induced myelosuppression is assessed prior to clinical studies and how models may be adapted for specific patient populations.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?
☑ This approach may be adopted for future drugs where myelosuppression is of concern.

Anticancer treatments frequently induce hematopoietic toxicity (myelosuppression) clinically due to their antiproliferative effects, both for cytotoxic agents[1] and targeted therapies.[2] Due to the frequency and dose-limiting nature of these adverse events, assessing compounds' potential to induce myelosuppression clinically is of importance during preclinical testing. Better understanding of the propensity for myelosuppression can aid safety margins and optimization of dose level/schedule for first-time-in-man studies, through mathematical modeling of preclinical findings and interpretation in the context of expected clinical activity.[3]

BRD4 is emerging as an important epigenetic target in oncology,[4] playing a role in stem cell survival and differentiation.[5,6] The BRD4 protein controls expression of large parts of the genome[7] and has the potential to promote cMyc activity.[8] Knockdown of BRD4 *in vivo* is associated with loss of stem cells in the gastrointestinal tract as well as loss of Lin-Sca1+cKit+ hematopoietic stem cells,[9] which are precursors of many circulating cells in the blood, including platelets and erythrocytes. Furthermore, the target has been identified as a potential therapeutic target for acute myeloid leukemia (AML),[10] and is currently pursued as a target for treatment of AML by several companies.

Recent reports for OTX015 (MK-8628), a BET-bromodomain BRD4 inhibitor currently in phase I for treatment of nonleukemia hematological malignancies, has reported thrombocytopenia (TCP) as a dose-limiting toxicity (DLT) in some populations.[11,12] Further, patients with hematological malignancies have preexisting disease-induced myelosuppression.[13] These data motivate developing a deeper understanding of the drug-induced myelosuppression of AZD5153, a brd-domain selective bivalent inhibitor[14] of BRD4 being developed for the treatment of AML.

AML is characterized by the presence of leukemic blasts (>20%) in the bone marrow (BM) and circulation, resulting in abnormally low complete blood counts (CBC) in AML patients. For example, platelet counts are reported to range between 7 and 358 (median 60–68) $\times 10^9$/L in AML diagnosed patients,[15] compared with a normal range of 140–400 $\times 10^9$/L. Platelet counts lower than the threshold for

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Received 21 October 2016; accepted 22 March 2017; published online on 5 April 2017. doi:10.1002/psp4.12194

common terminology criteria (CTC) for adverse events grades 3 and 4 (<50 and 25 x10^9/L, respectively) leads to increased risk of clinical complications. Treated patients can be classified (among others) as complete remission (CR) or complete remission with incomplete recovery, CRi, where platelets and neutrophils do not recover.[16]

Existing semimechanistic models of myelosuppression have been developed for leukopenia/neutropenia,[17] anemia,[18–20] and TCP,[21–24] with consistent system properties across several drugs,[17] and have been applied to drug-induced myelosuppression, but not disease-induced myelosuppression. The models tend to share consistent features: a self-renewing compartment (representing progenitor cell population), a series of transit compartments (representing cell population expansion and differentiation), and a circulating compartment (representing mature counts in blood), which regulates the self-renewing compartment. These models have demonstrated an ability to quantitatively and accurately predict drug effect in man based on WBC data from rats.[25] To accomplish this, the model utilizes measurements of preclinical circulating cell counts (rat) to quantify the drug effect, as well as species differences in protein binding (f_u) and compound potency *in vitro*.[25]

The investigation conducted here explores how these studies can be conducted and analyzed to support safety risk assessment prior to first-time-in-man. It exemplifies the application of modeling and simulation to explore human situations including when the disease (AML) has impact on safety endpoints (TCP) that may not be otherwise testable with preclinical toxicological experiments alone.

Aim

The aim was to understand and predict the risk of TCP with AZD5153 in the clinic, first in subjects with healthy pretreatment baseline, and second in patients with CR from preexisting myelosuppression using a mathematical myelosuppression model of circulating platelet counts. Data from a rat study were used to make human predictions for platelets at anticipated therapeutic doses and schedules. To investigate the behavior of the intended AML patient population, a range of recovery lengths were simulated.

METHODS

Animals

Female Han Wistar rats aged 9–10 weeks were obtained from Charles River (Wilmington, MA) and maintained under specific-pathogen-free conditions in an AAALAC-accredited facility. Irradiated food and autoclaved water were provided *ad libitum*. Animal protocols were approved by the AstraZeneca R&D Boston Institutional Animal Care and Use Committee. All animal work was conducted in accordance with ARRIVE guidelines.[26]

Rat study

For the pharmacodynamic (PD) study, rats were assigned randomly to groups based on their body weight so that each group had a similar average weight. Rats were treated orally with AZD5153 at 0.1 mg/kg (once daily (q.d.) for 10 days), 1.5 mg/kg (q.d. for 10 days), or 1 mg/kg (twice daily (b.i.d.) 3 days on treatment, 4 days off, 3 days on treatment) in 0.5% hydroxymethylcellulose, 0.1% Tween80. Blood samples were collected into microtainer EDTA tubes (Becton Dickinson, San Jose, CA) then analyzed on the Hemavet (Drew Scientific, Miami Lakes, FL) for complete blood counts, including platelets. Samples were collected from each rat 1 week prior to the start of dosing to establish pretreatment baseline, and on days 1, 4, and 7 during dosing to evaluate peak effect, and on days 11, 14, and 17 to assess recovery. To assess exposure of AZD5153 throughout the study, blood samples were collected into microtainer EDTA tubes, spun at 10,000 RPM for 5 min, then plasma was collected for liquid chromatography tandem mass spectroscopy. Samples for concentrations and pharmacokinetics (PK) were collected 2 h postdose on days 1, 4, and 7.

PK model

In the rat safety study, AZD5153 plasma concentrations were fitted to an appropriate compartmental PK model, based on the observations available. The human PK for AZD5153 were predicted from preclinical *in vitro* (rat, dog, and human intrinsic clearance) and low-dose rat and dog PK (other studies) *in vivo* (volume, absorption rate constant, bioavailability) data, and then integrated into a physiologically based PK six-compartment perfusion limited model in order to simulate concentration–time profiles in human,[27] accounting for differences in plasma protein binding across species.

As an approximation for the predicted PK, a two-compartment model with first-order absorption was used, assuming dose linearity over the studied dose range.

PD model

A model of myelosuppression first developed by Friberg[17] for leukocytes, was applied to platelet count effects in this analysis, the ODEs have been described elsewhere.[17] The ODE for the initial compartment includes an input rate that can be affected by drug concentration and circulating platelet levels according to:

$$\frac{dProl}{dt} = k_{tr} \cdot Prol \cdot (1 - slope_{AZD5153}.C).\left(\frac{Circ_0}{Circ}\right)^{\gamma} - k_{tr}.Prol \qquad (1)$$

where *Prol* represents the initial compartment, k_{tr} represents the rate constant, $slope_{AZD5153}$ represents the proportional drug effect, *C* represents concentration of AZD5153, $Circ_0$ represents initial baseline, *Circ* represents circulating (observed) cell counts, and γ represents feedback strength. An E_{max} model was also tested in replace of the proportional drug effect model.

Modeling platelet counts in the rat

All modeling and simulation was carried out in Phoenix NLME, v1.4 (Certara, St. Louis, MO) using first-order conditional estimation-extended least squares. The PK and PD models were fitted sequentially in the rat. Log-normally distributed between-subject variability (BSV) was considered for all PD parameters. The final model was selected based on objective function values, and the addition of a parameter was supported by a minimum difference of 3.84 between nested models. A visual predictive check was carried out (1,000 simulations) and used for normalized

prediction distribution errors (NPDE) using the npde package in R (v. 3.0.1).[28]

Translation of observed preclinical drug effect into human drug effect

The human predictions of platelet changes assuming healthy BM were based on the parameter estimates and associated uncertainty shown in **Table 2**. Human PK predictions, including uncertainty on clearance and volume, but only the mid-point clearance and volume, was used in human simulations of platelet changes. Therefore, these simulations should be considered representative of platelet counts at clinical doses, rather than accurately predict the planned phase 1 clinical trials. The rat and human plasma f_u were 0.05 and 0.25, respectively, so the human drug effect slope is assumed to be 5-fold higher than the slope estimated in rat driven by total concentrations. Cross-species *in vitro* experiments were not conducted with AZD5153, so potency was assumed to be the same in rat and human. System parameter values and their associated variabilities for platelet changes modeled using the myelosuppression model were obtained from literature reports[21–24,29] and averaged to give representative turnover rates and feedback for platelet changes clinically. IIV was included on $Circ_0$ and γ, but not MTT, as the majority of reports only included IIV on γ. One report of γ was more than 100-fold lower than the other reports, so this was excluded from the average for that parameter. Compared to the estimated system properties in rats, human $Circ_0$ is lower, γ is smaller, and mean transit time[17] (MTT, proportional to number of transit compartments and ktr) is longer ($\sim$134 h vs. $\sim$69 h). Compared to the original human leukocyte modeling,[17] circulating baseline levels of platelets are $>$30-fold higher than for leukocytes, MTT is similar, and feedback (typical value) is 40% higher.

Prediction of timecourse of platelet counts in humans with healthy BM

In the clinical simulations, AZD5153 was dosed q.d. continuously or b.i.d. with 3 days on drug treatment and 4 days off dosing schedule (hereafter described as 3 on 4 off) for 28 days. Human daily doses predicted to give equivalent unbound exposures (AUC) to the mouse at efficacious doses[14,30] for q.d. dosing was 56 mg, and for b.i.d. dosing (3 on 4 off) was 125 mg, so the range of clinical doses explored was 0.5–300 mg q.d. and 1.5–300 mg b.i.d. 3 on 4 off. In the simulations, 1,000 replicates were performed with observations during dosing and washout. Log-normally distributed IIV was included on $Circ_0$, γ and $slope_{AZD5153}$.

Prediction of timecourse of platelet counts during AML-induced BM dysfunction and recovery

Recovery duration for platelet counts is likely to be patient-specific and was therefore explored in the simulations from instantaneous recovery 1 h postdose, to longer recovery lengths of 10, 30, 100, 300, and 1,000 days. The capacity was expected to increase linearly over the recovery time from the start of treatment. The general equation to increase capacity (*fr*) over time is given by:

$$fr = \left(\frac{1-fr_b}{d}\right)t + fr_b \quad (2)$$

where fr_b is baseline fraction, d is recovery duration, and t is time. This additional term was introduced to the myelosuppression model on the initial compartment (*Prol*, see Eq. 1), according to:

$$\frac{dProl}{dt} = k_{tr} \cdot Prol \cdot fr \cdot (1 - slope_{AZD5153}.C).\left(\frac{Circ_0}{Circ}\right)^{\gamma} - k_{tr}.Prol \quad (3)$$

The initial baseline condition $Circ_0$ was reduced to account for the difference in steady state. The capacity (fr_b) was 0.65, producing a platelet count of $\sim 60 \times 10^9$/L at steady state, which is representative of median platelet counts in newly diagnosed AML patients.[15]

Simulations were carried out first by using recovery rate alone to drive platelet levels (i.e., $slope_{AZD5153} = 0$), and second observing the impact of 300 mg q.d. over a 14-week (98-day) dosing period (i.e., $slope_{AZD5153} = 0.45$).

To explore the impact of dose schedule on recovery, several schedules were dosed over a 300-day recovery period: q.d. continuous schedule, b.i.d. 3 on 4 off schedule, and q.d. dosing 7 on 7 off of 300 mg AZD5153. Finally the capacity *fr* was explored, ranging from 0.25–1.

RESULTS

Rat study and fit to myelosuppression model

The rat study showed negligible platelet changes at 0.1 mg/kg q.d., but clear effects at 1 mg/kg q.d. and 1.5 mg/kg b.i.d. (3 on 4 off). Only a single concentration was obtained on days 1, 4, and 7, which prohibited the construction of a PK model from these data alone. Similar doses were tested in a separate toxicology study so the absorption rate constant (k_a) and clearance were fixed to those estimates using a one-compartment PK model with first-order absorption, and volume was estimated from the observed data. This replicated the PK data well (not shown), while incorporating a degree of BSV. For the final reported PD model, BSV was included on $Circ_0$ and $slope_{AZD5153}$ (**Table 1**).

Figure 1 shows the individual observed platelet counts and model fitted results, with the parameter estimates in **Table 1**. Goodness-of-fit plots can be found in the **Supplementary Material**. For both q.d. and b.i.d. dosing, similar sampling schedules to that planned for the clinic were used. Samples were taken for blood counts prior to dosing, during the dosing period, and after cessation of dosing in order to capture the timecourse of drug effect and recovery. While the study did not have a vehicle group, platelet counts are not expected to change dramatically in untreated rats over the course of the study. The NPDE analysis indicated a departure from normality for the observations and, given the small size of the study (nine rats, 54 observations), some noise is not unexpected. As the majority of data are well described, this is unlikely to represent a significant misestimation in the slope.

Table 1 The rat PK and PD parameters estimated for platelet effects from the study where AZD5153 was dosed

Parameter (unit)	Population Typical Estimate (RSE)	%IIV (RSE)
AZD5153 PK		
k_a (h^{-1})	1.7 (-)	—
V (L/kg)	0.39 (16)	—
Cl (L/hr/kg)	0.15 (-)	—
PD model		
$Circ_0$ ($\times 10^9$/L)	562.4 (3)	5.4 (73)
MTT (h)	57.7 (4)	$5x10^{-4}$ (14)
k_{tr} (h^{-1})*	0.069	
γ	0.5 (10)	0.1 (63)
$slope_{AZD5153}$ (1/(μmol/L))	0.09 (14)	14 (85)

*Derived.

Prediction of platelet count timecourse in simulations with healthy BM

Simulations in patients with healthy BM were generated using predicted human PK (no IIV), the human slope predicted from rat platelet study, and human myelosuppression model system parameters. The human predictions at projected efficacious doses for q.d. and b.i.d. dosing (3 on 4 off) were simulated and the nadir over the entire dose range was investigated for q.d./b.i.d. dosing. Predicted human concentrations did not exceed those explored in the rat experimental study. At doses of 10 mg and below, there were only slight changes in the predicted platelet counts, increasing from 50 to 300 in a dose-dependent manner. At 300 mg the predicted median was reduced by ~80% at nadir for q.d. dosing and ~77% at nadir for b.i.d. dosing, although the median changes did not exceed CTC grade 3 threshold for TCP, with outliers predicted beyond this level. Comparison of the b.i.d. 3 on 4 off and q.d. dosing simulations demonstrated no improvement in the platelet count nadir with b.i.d. dosing (3 on 4 off) compared to q.d. dosing (**Figure 2**) when the weekly total dose was similar.

Based on these simulations representing patients with healthy platelet baseline counts, AZD5153 would be expected to have a dose-dependent effect on platelet counts clinically, and at predicted efficacious doses this is expected to be within the normal platelet count range.

Prediction of platelet counts in simulations AML–induced BM dysfunction and recovery

The model was further expanded to incorporate pretreatment disease-induced myelosuppression, and a range of recovery durations of platelet counts (**Figure 3a–c**). Upon recovery, when drug-induced myelosuppression was switched on, the platelet counts remain reduced relative to this $Circ_0$ (**Figure 3**) (~140 compared to 271.5 $x10^9$/L). Upon cessation of dosing, which effectively removes the drug-induced myelosuppression, platelet counts returned to the healthy baseline. This illustrates the opposing forces of recovery (secondary to efficacy on blast number) and drug-induced myelosuppression on platelet counts that can only be demonstrated when both these effects are taken into consideration. No other model modifications were considered, such as reduced strength of feedback which is taken into account by the reduced production rate in AML simulations, meaning the system cannot respond as efficiently to deviations from platelet baseline.

The 300-day recovery duration represents the typical remission rate of ~1 year,[31] and this was used to better understand the impact of varying dose schedule during recovery (**Figure 3d**). While negligible drug-induced myelosuppression is preferred, some drug-induced myelosuppression is unavoidable, although introducing a longer drug-free period (minimum 7 days) between periods of drug dosing can help to ameliorate this effect.

Impact of *fr* was explored (**Figure 3e**), which suggests that as *fr* decreases, absolute change at nadir caused by toxicity also decreases, but the percentage change is similar. Therefore, the myelosuppression caused by a drug such as AZD5153 in absolute terms could be lower in AML patients than the patients with healthy BM.

DISCUSSION

TCP model and translational approach

Prior to first-time-in-man, the only way to assess drug toxicity is to evaluate in preclinical species and extrapolate to human. Rodent models of BM toxicity have been demonstrated to predict outcome in clinical trials.[25,32] This study combined the drug effect in rat with system properties of platelets in human, to explore clinical platelet changes prior to

Table 2 Parameter values used in the human simulations for AZD5153

	AZD5153 estimate	IIV, CV%
PK model		
k_a (h^{-1})	0.25	—
V (L)	29.5	—
CL (L/h)	86.9	—
V2 (L)	34.3	—
Q (L/h)	52.5	—
PD model: healthy simulations		
$slope_{AZD5153}$ (1/(μmol/L))	0.45	100[b]
$Circ_0$ ($x10^9$/L)	271.5	36.9
MTT (h)	133.7	—
k_{tr} (h^{-1})[a]	0.030	—
γ	0.289	36.5
PD model: BM dysfunction simulations		
Recovery: $slope_{AZD5153}$ (1/(μmol/L))	0.0	—
Recovery + drug induced myelosuppression: $slope_{AZD5153}$ (1/(μmol/L))	0.45	—
$Circ_0$ ($x10^9$/L)	61.2	—
(Initial value)	271.5	—
(Baseline)		
MTT (h)	133.7	—
k_{tr} (h^{-1}) (derived)	0.030	—
γ	0.289	—
fr_b	0.65	—
d	1 h, 10, 30, 100, 300, 1,000 days	—

[a]Derived from MTT.
[b]Included as uncertainty.

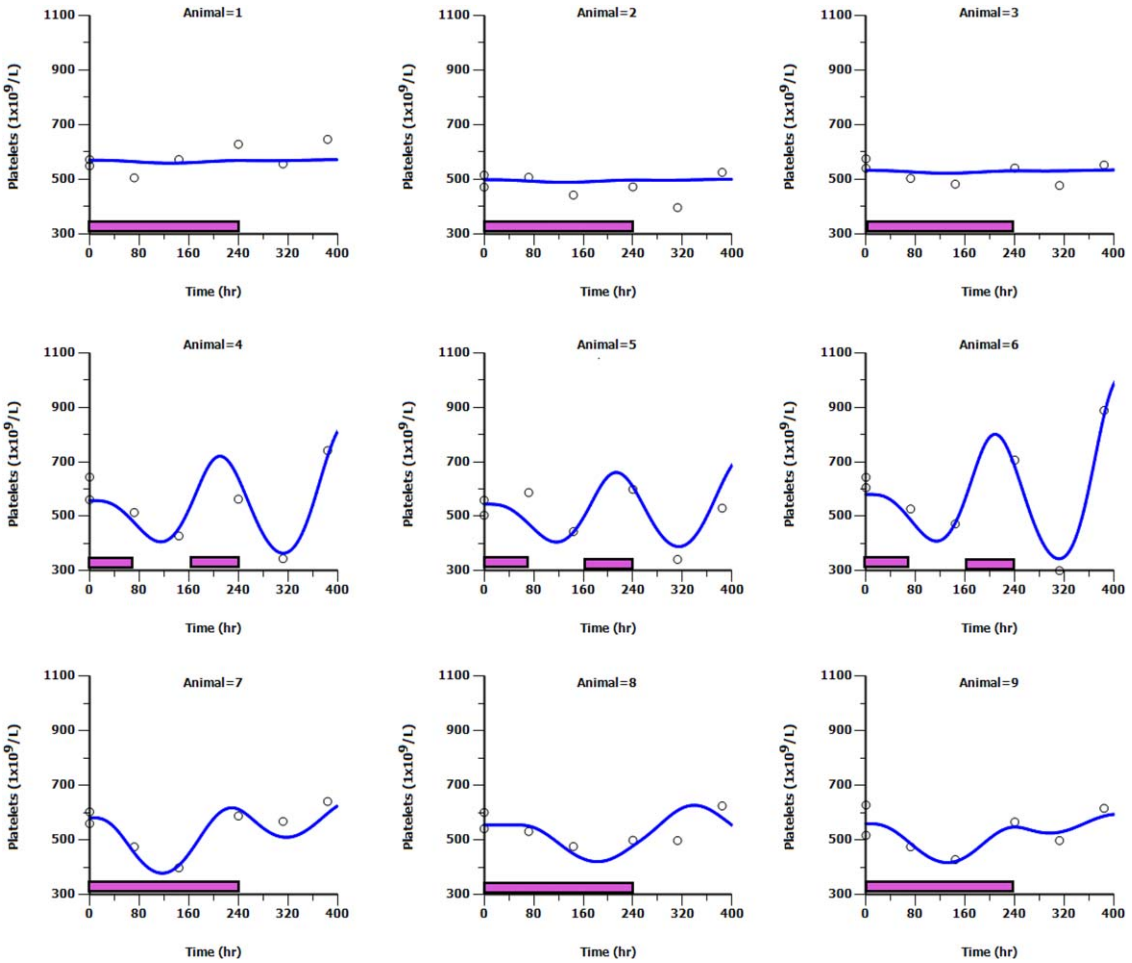


Figure 1 Individual fits to platelet data in the rat. Black circles indicate the observed platelet values, blue solid line is the individual fit, and the dosing periods are indicated by the pink shaded bars. Animals 1–3 received 0.1 mg/kg for 10 days, animals 4–6 received 1 mg/kg b.i.d. (3 days on, 4 days off, 3 days on), and animals 7–9 received 1.5 mg/kg q.d. for 10 days. Observations shown at time zero were taken in the days prior to dosing.

phase I studies, so a cross-species comparison cannot be made. The translational modeling approach has been studied elsewhere with leukocytes, but the translation of platelet changes has not been as thoroughly tested or explored. Rat safety is rarely reported in the literature, representing a gap in current knowledge. The intention of the clinical simulations was therefore not to predict with high confidence what proportion of patients would experience severe-grade TCP, but to exemplify the types of prospective predictions carried out before entering man that are used to inform decision making.

Model based predictions can influence clinical plans

Existing evidence that BRD4 inhibition is linked to effects on hematopoiesis and TCP gave cause to investigate TCP risk with AZD5153. The major concerns for AZD5153 were whether an efficacious dose level would induce TCP (narrow or no therapeutic index), comparison of schedules, and potential risk for AML patients, so this was addressed with simulations. With only preclinical safety data available at the first-time-in-patient stage, a quantitative, translational modeling prediction is an improved way to guide decision making on optimal dosing plan in phase I patients. For AZD5153, modeling provided guidance that the TCP risk for projected efficacious doses is low (given healthy BM), but would increase in a dose-dependent manner for b.i.d. and q.d. schedule options. A margin-based approach that simply records the dose level and exposure at which effects have occurred preclinically cannot provide this deeper understanding.

The predictions showed the proposed q.d./b.i.d. dose schedules were expected to give similar outcomes

Initially, two dose schedules were proposed: q.d. dosing continuously and b.i.d. dosing 3 on 4 off, with the hope that a break in dosing would improve tolerability while maintaining efficacy. The modeling analysis showed that q.d. and 3 on 4 off b.i.d. schedules were similar in terms of magnitude of effect at equivalent dose levels over a single cycle. The short, frequent drug-free periods in the 3 on 4 off b.i.d. schedules did not improve the nadir, and therefore the long-term tolerability risk of TCP. This suggests the total weekly dose is an important driver of platelet changes rather than the daily PK fluctuations being important,

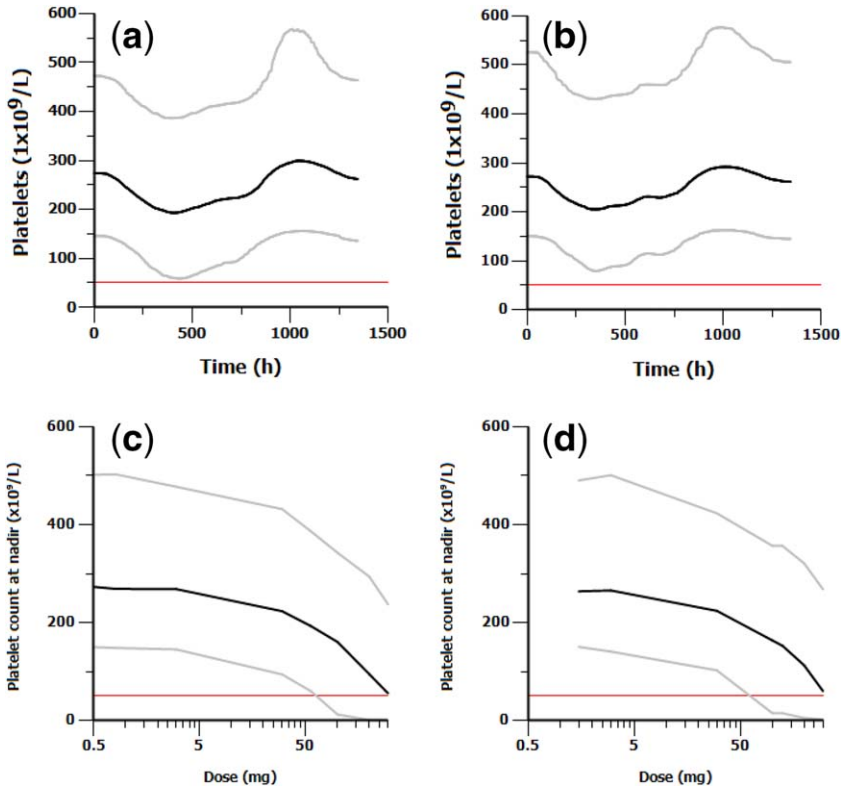


Figure 2 a: Simulation of human platelet counts starting from healthy baseline using a 28-day (672 h) dosing period and washout period of AZD5153 at predicted efficacious dose of q.d. dosing (56 mg). **b**: b.i.d. dosing (125 mg). **c,d**: The nadir values for platelet counts over the dose range studied for q.d. dosing and b.i.d. dosing 3 on 4 off, respectively. Three lines for each scenario indicate the median (black), 5th, and 95th percentiles (gray); red reference line is threshold for grade 3 TCP.

although 125 mg b.i.d. 3 on 4 off is a higher total weekly dose than 56 mg q.d., it would result in a lower platelet nadir. Exploring different schedule options provided model-based insight that could not be gained from the more traditional approach of margins.

Inclusion of a patient population provided additional insight and potential strategies to improve TI

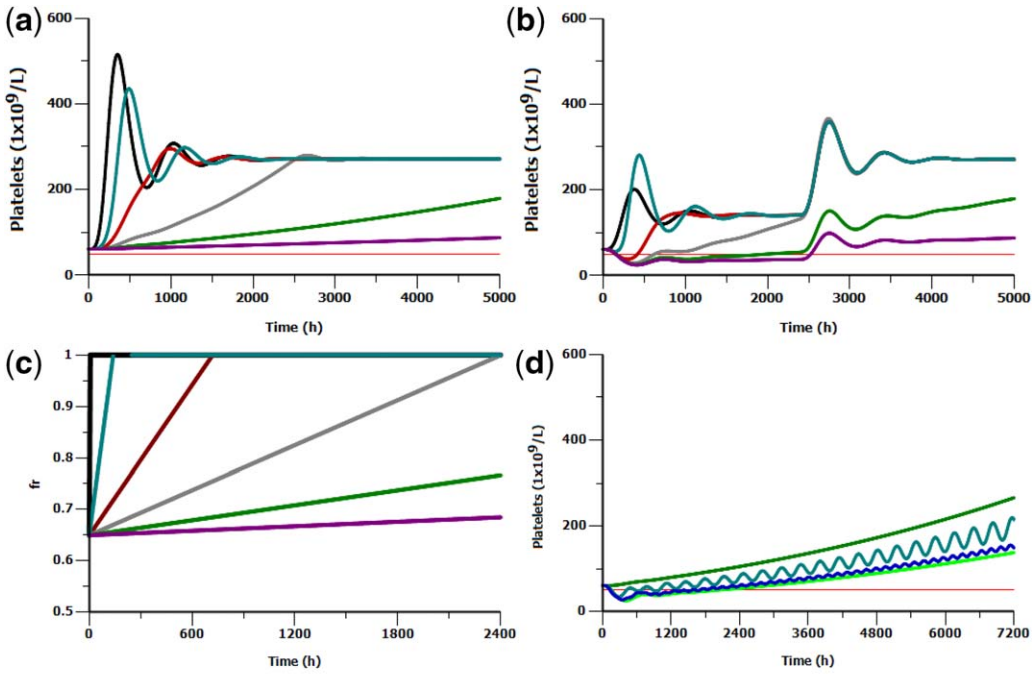
There were concerns about the intended AML patient population, where underlying BM dysfunction, efficacy, and toxicity may all affect the platelet counts. Generally, leukemia patients are not heme evaluable because of their BM infiltration (and thus low counts do not count as DLT). Therefore, they do not have specific inclusion criteria separate from standard of care.

AML patients would have patient-specific baseline platelet counts and BM function, and response to treatment would potentially allow for recovery in platelet counts. The simulations showed that recovery from disease-induced myelosuppression and drug-induced myelosuppression (exemplified using a dose exceeding efficacious exposure to illicit strong platelet changes) would be opposing processes. A high degree of drug-induced myelosuppression could increase the length of time a patient has severe (grade 3 or 4) TCP, even though absolute effects on platelet counts are expected to be smaller, given the reduced function of the BM. Potential mitigation strategies to overcome this are to reduce the dose level (as drug effect is proportional to drug concentrations) or, if required, a longer drug-free period (7 days or more) should be introduced (if efficacy can be maintained), which is in line with recommendations with neutrophils with similar MTT.[33] These results demonstrate the ability to tailor model-based predictions with reference to a patient population without additional preclinical studies. There may be other patient-specific aspects that have not been incorporated (CRi, platelet transfusions, relapse), however these scenarios are difficult to predict from preclinical studies.

Current limitations and future directions

The rat model is based on a limited dataset, and this may affect the overall model fit and subsequent extrapolation to human; for example, the RSE values were high for the estimation of interindividual variability. When introducing IIV terms, η-shrinkage was observed. However, the same model structure without any IIV (naïve pooled approach) gave similar parameter estimates. This gives confidence that despite shrinkage with the final rat model, the parameter estimates used (slope) in the human translation is representative of the effect.

In the human simulations shown here, no uncertainty in PK was included, which is likely to have clinical relevance. The simulations showed representative platelet changes at each dose level, and should not be interpreted as an accurate prediction of the proportion of patients that would



(e)

fr	Baseline (1x10⁹/L)	Nadir (1x10⁹/L)	Absolute change (1x10⁹/L)
0.25	2.2	1.2	1
0.5	24.7	10	14.7
0.75	100.4	41.3	59.1
1	271.5	107.2	164.3

Figure 3 Simulation of platelet count recovery: 1 h (black line), 10 day (turquoise), 30 day (maroon), 100 day (gray), 300 day (green), and 1,000 day recovery (purple). **a**: Only recovery ($slope_{AZD5153}$=0). **b**: Both recovery and drug-induced myelosuppression ($slope_{AZD5153}$ = 0.45) (cessation of dosing at 2,352 h, 14 week). **c**: *fr* time course based on different durations. **d**: Different dose schedules for 300-day recovery: recovery only ($slope_{AZD5153}$ = 0, green), 300 mg q.d. (light green), 300 mg b.i.d. (3 on 4 off) (blue), 300 mg q.d. (7 on 7 off) (turquoise), black line indicates CTC grade 3 threshold. **e**: Absolute and relative change in platelet counts of different *fr* values.

experience severe-grade TCP, but rather providing insight into the pharmacologically active exposures and relative comparison between dosing schedules. A major limitation to assessing the performance of the model is the lack of clinical data to validate the translational approach for this specific BRD4 inhibitor.

There are many limitations of the extrapolation to AML patients, since we have only considered a reduction in baseline and not a change in feedback or MTT, although a reduced baseline does reduce the impact of feedback, and in a report from patients including lymphoid malignancies the feedback parameter was quantified as ~100-fold lower. A recovery duration was introduced but it is likely that only a proportion of patients will respond and have the ability to recover to normal platelet counts. In the OTX015 phase 1 trial, severe TCP was more frequent in patients with low platelet counts at pretreatment baseline, indicating that the impact of hematological malignancies can have consequences for tolerability. In another study of TCP, patients with lymphoma required a reduced starting baseline, and further reduction of baseline over time to account for disease progression.[29] Both indicate that a reduced starting baseline is important for TCP risk, but that in addition to recovery considered here, platelet counts could remain low during treatment, or indeed worsen.

The semimechanistic model most likely represents a simplification of the hematopoiesis process. The rate constant k_{tr} gives an expected circulating half-life of platelets (~24 h), although it is widely reported that the lifespan of circulating platelets is around 10 days. Thrombopoietin may be represented by feedback.

Current preclinical efficacy models are solid-tumor AML cell lines, which are a different paradigm to the BM dysfunction expected in patients. In the future, assessment of drug efficacy and safety in an animal model with an AML phenotype would be of interest to better explore the interplay of efficacy and safety.

SUMMARY

This investigation sought to understand and predict risk of TCP caused by BRD4 inhibitor AZD5153 under healthy BM function and recovery from disease-induced myelosuppression in order

to guide planning of dose schedules for phase I studies. An existing myelosuppression model was used to make clinical predictions of platelet counts (based on rat data). Although platelet counts have previously been modeled in the clinic, this is the first time preclinical to clinical predictions of platelet counts have been reported. The model indicated that projected efficacious doses of AZD5153 were expected to have minimal impact on platelet counts when normal baseline platelets counts, the q.d./b.i.d. proposed schedules, were similar in terms of magnitude of effect, and there could be an interplay between recovery and toxicity in AML patients where significant drug-induced myelosuppression occurs. This work exemplifies the value of model-based predictions of safety endpoints prior to first-time-in-human trials in providing insight into the timecourse of platelet count changes, dose levels/schedules, and extrapolation from rat to the intended patient population.

Conflict of Interest/Disclosure. This study was wholly funded by AstraZeneca, of which all the authors are employees.

Author Contributions. T.A.C., M.H., J.Y., E.C., M.M., and J.T.M. wrote the article; T.A.C., M.H., E.C., and M.M. designed the research; M.H., M.M., and J.T.M. performed the research; T.A.C., J.Y., M.M., and J.T.M. analyzed the data.

1. Chatelut, E., Delord, J.-P. & Canal, P. Toxicity patterns of cytotoxic drugs. *Invest. New Drugs* **21**, 141–148 (2003).
2. Dy, G. & Adjei, A. Understanding, Recognizing, and managing toxicities of targeted anticancer therapies. *Ca Cancer J. Clin.* **63**, 249–279 (2013).
3. Venkatakrishnan, K. *et al.* Optimizing oncology therapeutics through quantitative translational and clinical pharmacology: challenges and opportunities. *Clin. Pharm. Ther.* **97**, 37–54 (2015).
4. Filippakopoulos, P. & Knapp, S. Targeting bromodomains: epigenetic readers of lysine acetylation. *Nat. Rev. Drug Discov.* **13**, 337–356 (2014).
5. Di Micco, R. *et al.* Control of embryonic stem cell identity by BRD4-dependent transcriptional elongation of super-enhancer-associated pluripotency genes. *Cell Rep.* **9**, 234–247 (2014).
6. Rodriguez, R.M. *et al.* Role of BRD4 in hematopoietic differentiation of embryonic stem cells. *Epigenetics* **9**, 566–578 (2014).
7. Muller, S., Filippakopoulos, P. & Knapp, S. Bromodomains as therapeutic targets. *Expert Rev. Mol. Med.* **13**, e29 (2011).
8. Mertz, J.A. *et al.* Targeting MYC dependence in cancer by inhibiting BET bromodomains. *Proc. Natl. Acad. Sci. USA* **108**, 16669–16674 (2011).
9. Bolden, J.E. *et al.* Inducible in vivo silencing of Brd4 identifies potential toxicities of sustained BET protein inhibition. *Cell Rep.* **8**, 1919–1929 (2014).
10. Zuber, J. *et al.* RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. *Nature* **478**, 524–528 (2011).
11. Stathis, A. *et al.* 5LBA Results of a first-in-man phase I trial assessing OTX015, an orally available BET-bromodomain (BRD) inhibitor, in advanced hematologic malignancies. *Eur. J. Cancer* **50**, 196 (2014).
12. Amorim, S. *et al.* Bromodomain inhibitor OTX015 in patients with lymphoma or multiple myeloma: a dose-escalation, open-label, pharmacokinetic, phase 1 study. *Lancet Haematol.* **3**, e196–e204 (2016).
13. Jodrell, D.I. *et al.* Relationships between carboplatin exposure and tumor response and toxicity in patients with ovarian cancer. *J. Clin. Oncol.* **10**, 520–528 (1992).
14. Bradbury, R.H. *et al.* Optimization of a Series of bivalent triazolopyridazine based bromodomain and extraterminal inhibitors: the discovery of (3R)-4-[2-[4-[1-(3-methoxy-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-4-piperidyl]phen oxy]ethyl]-1,3-dimethyl-piperazin-2-one (AZD5153). *J. Med. Chem.* 8, 7801–7817 (2016).
15. Huck, A. *et al.* Prior cytopenia predicts worse clinical outcome in acute myeloid leukemia. *Leuk. Res.* **39**, 1034–1040 (2015).
16. Döhner, H. *et al.* Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood* **115**, 453–474 (2010).
17. Friberg, L.E. Model of Chemotherapy-induced myelosuppression with parameter consistency across drugs. *J. Clin. Oncol.* **20**, 4713–4721 (2002).
18. Woo, S. & Jusko, W.J. Interspecies comparisons of pharmacokinetics and pharmacodynamics of recombinant human erythropoietin. *Drug Metab. Dispos.* **35**, 1672–1678 (2007).
19. Woo, S., Krzyzanski, W. & Jusko, W.J. Pharmacodynamic model for chemotherapy-induced anemia in rats. *Cancer Chemother. Pharmacol.* **62**, 123–133 (2008).
20. Mager, D.E., Woo, S. & Jusko, J.W. Scaling Pharmacodynamics from in vitro and preclinical animal studies to humans. *Drug Metab. Pharmacokinet.* **24**, 16–24 (2009).
21. Schmitt, A. *et al.* Factors for hematopoietic toxicity of carboplatin: refining the targeting of carboplatin systemic exposure. *J. Clin. Oncol.* **28**, 4568–4574 (2010).
22. Vong, C. *et al.* Semi-mechanistic PKPD model of thrombocytopenia characterizing the effect of a new histone deacetylase inhibitor (HDACi) in development, in co-administration with doxorubicin. <www.page-meeting.org/?abstract=2915> (2013).
23. Bender, B.C. *et al.* A population pharmacokinetic/pharmacodynamic model of thrombocytopenia characterizing the effect of trastuzumab emtansine (T-DM1) on platelet counts in patients with HER2-positive metastatic breast cancer. *Cancer Chemother. Pharmacol.* **70**, 591–601 (2012).
24. Xiong, H. *et al.* Exposure-response relationship of ABT-263-induced thrombocytopenia in cancer patients in phase 1 studies. *Mol. Cancer Ther.* B216 (2009).
25. Friberg, L.E., Sandstrom, M. & Karlsson, M.O. Scaling the time-course of myelosuppression from rats to patients with a semi-physiological model. *Invest. New Drugs* **28**, 744–753 (2010).
26. Kilkenny, C., Browne, W., Cuthill, I., Emerson, M. & Altman, D. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biol.* **8**, 1–5 (2010).
27. Poulin, P. *et al.* PHRMA CPCDC initiative on predictive models of human pharmacokinetics, part 5: prediction of plasma concentration-time profiles in human by using the physiologically-based pharmacokinetic modeling approach. *J. Pharm. Sci.* **100**, 4127–4157 (2011).
28. Comets, E., Brendel, K. & Mentre, F. Computing normalised prediction distribution errors to evaluate nonlinear mixed-effect models: the npde add-on package for R. *Comput Methods Programs Biomed.* **90**, 154–166 (2008).
29. Chalret du Rieu, Q. *et al.* Semi-mechanistic thrombocytopenia model of a new histone deacetylase inhibitor (HDACi) in development, with a drug-induced apoptosis of megakaryocytes. <www.page-meeting.org/?abstract=2503>.
30. Rhyasen, G.W. *et al.* AZD5153: A novel bivalent BET bromodomain inhibitor highly active against hematologic malignancies. *Mol. Cancer Ther.* **15**, 2563–2574 (2016).
31. Sievers, E. *et al.* Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. *J. Clin. Oncol.* 19, 3244–3254 (2001).
32. Pessina, A. *et al.* Pre-validation of a model for predicting acute neutropenia by colony forming unit granulocyte/macropahge (CFU-GM) assay. *Toxicol. In Vitro* **15**, 729–740 (2001).
33. Patel, M. *et al.* Dose schedule optimization and the pharmacokinetic driver of neutropenia. *PLoS One* **9**, e109892 (2014).

Supplementary information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website (http://psp-journal.com)