Impact of \textit{ABCB1} allelic variants on QTc interval prolongation

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STATEMENT OF TRANSLATIONAL RELEVANCE

Using romidepsin as an ABCB1 model substrate, this study shows that ABCB1 participates in a blood-tissue barrier in the cardiac endothelium. This blood-heart barrier limits the intracardiac concentration of romidepsin thereby reducing QT-prolongation induced by romidepsin. Analysis of clinical samples revealed that genetic variants in ABCB1 that were previously linked to increased expression of ABCB1 in the blood-cardiac barrier may be responsible for a less severe QT-prolongation phenotype in individuals receiving romidepsin. Therefore, a putative blood-heart barrier should be further explored with specific focus on ABCB1-substrate drugs that prolong the QT-interval, and ABCB1 alleles may serve as markers for QT prolongation induced by romidepsin.
ABSTRACT

Purpose: While the ABCB1 (P-glycoprotein) drug transporter is a constituent of several blood-tissue barriers (i.e. blood-brain and blood-nerve), its participation in a putative blood-heart barrier has been poorly explored. ABCB1 could decrease the intracardiac concentrations of drugs that cause QT-prolongation and cardiotoxicity.

Experimental design: ABCB1-related romidepsin transport kinetics were explored in LLC-PK1 cells transfected with different ABCB1 genetic variants. ABCB1 plasma and intracardiac concentrations were determined in Abcb1a/1b (-/-) mice and wild-type FVB controls. These same mice were used to evaluate romidepsin-induced QTc prolongation over time. Finally, a cohort of 83 individuals with available QTcB and ABCB1 genotyping data were used to compare allelic variation in ABCB1 versus QTc-prolongation phenotype.

Results: Here, we demonstrate that mice lacking the ABCB1-type P-glycoprotein have higher intracardiac concentrations of a model ABCB1 substrate, romidepsin, that correspond to changes in QT-prolongation from baseline (ΔQTc) over time. Consistent with this observation, we also demonstrate that patients carrying genetic variants that could raise ABCB1 expression in the cardiac endothelium have lower ΔQTc following a single dose of romidepsin.

Conclusions: To our knowledge, this is the first evidence that Abcb1-type P-glycoprotein can limit intracardiac exposure to a drug that mediates QT-prolongation and suggests that certain commonly inherited polymorphisms in ABCB1 may serve as markers for QT-prolongation following the administration of ABCB1-substrate drugs.
INTRODUCTION

Drug development is frequently discontinued, or stringent restrictions are imposed when treatment-associated QT prolongation is observed (1). The potential for drugs to lengthen the QT interval is now under major scrutiny by the FDA as QT interval prolongation is considered a surrogate for torsades de pointes (2). Pharmacogenetics studies have demonstrated that genotypic differences in metabolizing enzymes are associated with risk of QT interval changes and the development of arrhythmias through alteration in pharmacokinetics and pharmacodynamics (3-5), although to date involvement of drug transporters remains unexplored.

One such drug transporter, ABCB1 (P-glycoprotein, MDR1), has been shown to be protective in several tissues including heart (6-9), where it effluxes both endogenous and exogenous substrates away from these tissues back into the systemic circulation (10). ABCB1, expressed in the cardiac endothelium, may be a component of a proposed blood-heart barrier that protects cardiac tissues from damage induced from endogenous and exogenous substances. Meissner et al. demonstrated that ABCB1 is variably expressed in the cardiac endothelium based on two allelic variants (i.e. 2677G>T/A and 3435C>T) that have significant representation in many world populations (11); this study demonstrated increased expression associated with the variant alleles. Later, it was shown that the 1236C>T, 2677G>T/A, and 3435C>T SNPs were related to altered ABCB1 protein folding with reduced efflux for some substrates (12). We have previously reported that ECGs of patients treated with romidepsin often show measurable increases in QT interval when measured using the Bazett correction (13). Additionally, we have shown that the above ABCB1 polymorphisms were not related to the plasma...
pharmacokinetics of romidepsin (14); however, given that romidepsin concentrations still
may be increased locally within cardiac tissues, QTc prolongation could be altered by
differential efflux due to allelic differences in ABCB1 protein expression and function
(12, 15-18).

Using the HDAC inhibitor romidepsin (formerly FR901228 or FK228,
NSC630176) as a model substrate, this study aims to determine whether ABCB1 is a
constituent of a blood-heart barrier where the transporter limits intracardiac
concentrations of QT-prolonging substrate drugs (13, 19-21). First, we confirmed
previous reports that romidepsin is an ABCB1 substrate (22-24) and determined the
transport characteristics of wild-type and variant ABCB1 towards romidepsin. Secondly,
to test the hypothesis that ABCB1 limits the local intracardiac concentration of
romidepsin with ensuing effects on QT-prolongation, romidepsin was administered to
mice lacking the ABCB1-type P-glycoprotein. Both intracardiac and serum
concentrations of romidepsin were measured, and QT prolongation was assessed.
Finally, to test the hypothesis that allelic variation in ABCB1 is clinically associated with
QT prolongation, we evaluated the consequences of the ABCB1 1236C>T, 2677G>T/A
and 3435C>T variants on ΔQTc in 115 patients with cancer following administration of
romidepsin.
MATERIALS AND METHODS

Transport of romidepsin by ABCB1

Romidepsin was obtained as a gift from Gloucester Pharmaceuticals. LLC-PK1 cells were transfected with ABCB1-variants containing the exon 12 (1236T), the exon 21 (2677T) and the exon 26 wobble SNP (3435T). Membrane ABCB1 protein content was ascertained via flow cytometry using previously published methods (25). Transport assays were carried out in each transfectant as described (26), and romidepsin concentration was evaluated by mass spectrometry using previously published methods (27) and calculations (28). Transfection methods and transport assays are described in greater detail in the Supplementary Methods S1.

Study animals and drug administration

Pharmacokinetics and ECG studies were performed using female FVB.129P2-Abcb1atm1Bor-Abcb1btm1Bor N12 double knockout mice obtained from Taconic Farms (New York). All mice were administered a single bolus of romidepsin through the tail vein (3.6mg/kg dissolved in 100µL of 80:20 propylene glycol:ethanol) or vehicle only.

Pharmacokinetic Analysis in mice

Plasma and intracardiac romidepsin concentration was determined 5min, 15min, 30min, 1hr, 2hr, 4hr, 8hr, 16hr, and 24hr after drug injection (n = 3 per timepoint). Romidepsin concentrations in plasma and tissue samples were determined by previously published methods (27). More detailed information regarding sample collection can be found in the Supplementary Methods S1.
Transmitter implantation and ECG data collection in mice

The mice were divided into four groups: Group 1 consisted of Abcb1a/1b-/- mice receiving 3.6mg/Kg romidepsin; Group 2 consisted of Abcb1a/1b-/- mice receiving vehicle control; Group 3 consisted of wild-type FVB mice receiving 3.6mg/Kg romidepsin; and Group 4 consisted of wild-type FVB mice receiving vehicle control (n = 3 for each group). QTc correction methods in mice have been previously reported (29). Further information regarding ECG monitoring and data collection can be found in the Supplementary Methods S1 published online.

Statistical Considerations for mouse experiments

The plasma and intracardiac AUC were compared between wild-type and Abcb1a/1b knockout mice using Bailer’s Method for destructive sampling (30). For the analysis of ECG, the original data sets were filtered to remove extreme outliers. From each mouse's QT and RR interval lengths, means were calculated at 6-minute intervals (10 intervals per hour) using data obtained within 30 minutes of each interval. The deviations of all the observed values from these means were calculated, and the observations with deviations greater than the 95th percentile of the distribution or less than the 5th percentile were dropped from the analyses. For the QT intervals, these percentiles translated into deviations above or below the mean (~0.9 msec), and for the RR intervals, deviations of magnitude greater than about 6.5 msec above the mean or 5.7 msec below it were dropped. The cutoffs corresponded to approximately 1.5 standard deviations of their distributions. For the outcomes derived from the QTc values, comparisons between groups were assessed using the Wilcoxon rank sum test. Because
this test has low power when the groups are small, the results of the t test with Satterthwaite's approximate degrees of freedom are also provided.

Patient Population

We evaluated data from patients with T-cell lymphoma participating on a phase II clinical trial of romidepsin (n = 61). Eligibility criteria were reported previously (13). A separate cohort of patients was included in the analysis consisting of: a) patients participating on the same multi-institutional trial as the aforementioned cohort who were treated at institutions other than the NCI (n = 42);(13) and b) patients treated on the single-agent Phase I clinical trial of romidepsin previously conducted at the NCI (n = 12) (21). For the latter cohort, the common eligibility criteria were as described in the Phase II population, except that patients with malignancies other than T-cell lymphoma were also eligible. All patients also received premedication with the anti-emetic ondansetron or granisetron. Overall, samples were available from 115 patients (n = 12 from the Phase I trial, and n = 103 from Phase II trials; see Table 1). All patients were enrolled on protocols approved by the Institutional Review Board, and genotyping was approved as well.

Phenotypic evaluation of clinical data

Electrocardiograms (ECGs) were obtained immediately before romidepsin administration, and at 4 hours after the start of romidepsin administration (at the end of infusion). Only a subset of the original cohort had evaluable QTc data (n = 83). It should be noted that while ondansetron is known to prolong the QTc interval, this is
transient and no effect of ondansetron treatment on QTc interval would be expected at the end of the 4-hour romidepsin infusion when study ECGs were ascertained (31). More detailed information regarding determination of $\Delta$QTc$^{SB}$, $\Delta$QTc$^{MB}$, $\Delta$QTcF and $\Delta$QTcB is provided in Supplementary Results S2.

**Genotyping analysis**

Variants in the *ABCB1* gene were analyzed according to previous methodologies (14), and ABCB1 diplotypes were computed as described previously (17). Genotype-frequency analysis of Hardy-Weinberg equilibrium was conducted using Helix Tree Software v4.4.1 (Golden Helix Inc., Montana). The linkage between each pair of SNPs was determined in terms of the classical D’ statistic.

**Statistical considerations in the clinical data**

All data are reported as mean values with 95% confidence intervals, unless specified otherwise. Changes in QTc interval from baseline ($\Delta$QTc) as well as drug clearance based on genotype or haplotype were evaluated using the Fisher-Freeman-Halton test. Because of limited numbers of observations, subsequent analyses were based on grouping patients on the basis of the number of reference alleles in multiple loci, with these resulting two group statistical comparisons being evaluated using an exact Wilcoxon rank sum test. Repeated measures ANOVA was used to evaluate differences in $\Delta$QTcB and $\Delta$QTcF versus genotype over the course of repeated administrations of romidepsin. The Jonckheere-Terpstra trend test was employed in comparisons between trends in dose, number of variant alleles in genotype and haplotype categories. (32)
Differences between lab values and body composition versus genotype were evaluated using the Kruskal-Wallis test. Comparisons between the various ΔQTc data sets (i.e. ΔQTcB, ΔQTcB<sub>SB</sub>, ΔQTcB<sub>MB</sub>, and ΔQTcF) were evaluated using the Spearman rank correlation method. All P-values are two-tailed, and those obtained from nonparametric tests are from exact calculation, not asymptotic approximation. Given the exploratory nature of this study, P-values were not corrected for the large number of tests performed. Thus, P-values less than \( P = 0.01 \) were considered significant while all others \( 0.01 < P < 0.05 \) were considered to be notable trends.
RESULTS

Transport of romidepsin by ABCB1

We first confirmed that romidepsin is a substrate of ABCB1, and determined the transport characteristics of wild-type and variant ABCB1 towards romidepsin. Consistent with previous reports (22-24), we found that romidepsin (2 µM) is recognized by wild-type ABCB1 ‘CGC’ (Fig. 1A). We also noted that ABCB1 variant at the 1236, 2677, and 3435 loci (‘TTT’) has reduced transport capability (Fig. 1B), as indicated by an increase in romidepsin transport in the basolateral to apical direction in LLC-PK1 cell monolayers. The B-A/A-B ratio for wild-type ABCB1 ’CGC’ was 12.10 (95%CI = 10.87-13.32) while the ratio was significantly reduced at 5.846 (95%CI = 4.495-7.196, P < 0.001) for the TTT variant. Transport of romidepsin in the basolateral to apical direction by the ABCB1 ‘TTT’ variant was significantly impaired compared with wild-type ABCB1 (P = 0.028; Fig. 1C); the difference in transport was in spite of ‘TTT’ variant cells expressing more ABCB1 at the membrane than wild-type cells (Supplemental Figure 1A and 1B). Transport was not evaluated in mock transfected cells as there was an approximate 1000-fold increase in membrane-associated ABCB1 resulting from the transfection (Supplemental Figure 1C).

Plasma and intracardiac pharmacokinetics in mice

To test the hypothesis that ABCB1 expression impacts either plasma or intracardiac levels of romidepsin, drug was administered to mice lacking the ABCB1-type P-glycoprotein (Abcb1a/1b -/- mice) and romidepsin concentrations in the plasma and heart were monitored over 24 hours. Female mice were selected because females
may express more murine ABCB1 homologues and were thus expected to have a greater difference in Abcb1a/1b expression compared to female mice that lack the ABCB1-type P-glycoprotein (33). The mean plasma AUC_{0-8} for Abcb1a/1b knockout mice versus wild-type mice (291.76 ng\times h/mL vs. 255.64 ng\times h/mL, respectively) was not statistically different ($P = 0.11$; Bailor’s Method, Z-test; Figure 2A). However, when romidepsin exposure (AUC_{0-2}) was evaluated in heart tissue, a significant difference was observed ($P = 0.0026$; Bailor’s Method, Z-test). Mean heart AUC_{0-2} was 35% higher for Abcb1a/1b knockout mice than wild-type mice (0.23 ng\times h/mg vs. 0.17 ng\times h/mg respectively; Figure 2B). Similar, albeit non-significant, results were obtained using a ratio of the cardiac:plasma AUC_{0-2}; however, this primarily due to factoring in plasma AUC data that were not significantly different (Supplemental Results S1). These results suggested that there could be a difference in the ECG effects resulting from exposure to romidepsin.

Electrocardiogram phenotype of knockout vs. normal mice receiving romidepsin

To test the hypothesis that Abcb1 expression could alter electrocardiogram (ECG) measurements of the QT interval, Abcb1a/1b knockout mice were administered romidepsin and monitored over 72 hours via a surgically implantable ECG transmitter. For the analysis of the $\Delta$QTc values, the filtered values (e.g. $\Delta$QTc ± 1SD) were averaged in two-hour intervals from hours 0 to 72 for each mouse (see Figure 3), and the results are identified by the midpoints of the intervals. The mean time taken to achieve peak $\Delta$QTc (i.e. $\Delta$QTc\textsubscript{max}) was 16 hours in knockout mice and 31 hours in wild-type mice. This difference has a significance level of $P = 0.10$ by the exact Wilcoxon rank sum test, the smallest possible two-sided $P$ value in a comparison of N=3 vs. N=3. After a
variance stabilizing transformation, the difference reaches significance by the t-test \((P = 0.02)\). There is no apparent difference between the peak values (range 2.2-5.2 msec in KO mice vs. 1.8-2.7 msec in WT mice, \(P = 1.0\) by the Wilcoxon test). Therefore, while we did not observe a difference in \(\Delta QTc_{\text{max}}\), a decrease in the time taken to achieve \(\Delta QTc_{\text{max}}\) was noted in Abcb1a/1b knockouts receiving romidepsin.

**Relationship between dose and \(\Delta QTc\) in multiple \(\Delta QTc\) data sets obtained from patients**

All methods of calculating \(\Delta QTc\) data obtained in this study were highly correlated with only one exception ([Supplemental Results S1](#)). Therefore, we only utilized the \(\Delta QTcB^{SB}\) (change in QTc with Bazett correction relative to a single baseline), and the \(\Delta QTcB^{MB}\) (change in QTc with Bazett correction relative to multiple baselines) observations in subsequent analyses since more data were available and the different methods used to measure QTc were statistically similar. There was no association between dose and \(\Delta QTcB^{SB}\), although a weak trend was noted where mean \(\Delta QTcB^{SB}\) increased with increasing dose (mean \(\Delta QTcB^{SB}\) (msec) = -5.0, 11.9, 15.8, 22.2, and 29.0 for doses (mg/m\(^2\)) = 12.7, 14, 17.8, 18, 24.9 respectively with n = 1, 71, 6, 4, and 1; \(P = 0.11\); exact Jonckheere-Terpstra test for trend). Similarly, there was no association between dose and \(\Delta QTcB^{MB}\) \((P = 0.43)\); thus, comparisons between genotype and \(\Delta QTc^{MB}\) and \(\Delta QTc^{SB}\) were made by combining patients receiving different doses.
Genotype versus $\Delta Q_Tc$ relationships following infusion of romidepsin in romidepsin-naïve patients

As our *in vitro* and *in vivo* data indicated that variability in ABCB1 expression and function may alter the measurement of ECGs, we undertook to genotype patients treated with romidepsin, where ECG data were available, in order to test the hypothesis that $\Delta Q_Tc$ varied as a function of $ABCB1$ genotype. A summary of the patients and treatments evaluated, and genotype variation is included in Tables 1 and Table 2 respectively). No association between genotype and $\Delta Q_Tc$ versus romidepsin pharmacokinetics or laboratory test values was found in humans (Supplementary Results S1).

The initial analysis consisted of all patients for which both $\Delta Q_Tc_{BSB}$ and genotyping data were available (mean 8 msec; $n = 78$). The $ABCB1$ 2677G>T polymorphism was associated with inter-individual variation in $\Delta Q_Tc_{BSB}$ ($P = 0.017$; uncorrected) when individuals carrying 2677A alleles were excluded. Patients carrying two copies of variant alleles at the $ABCB1$ 2677G>T/A SNP were found to have the lowest $\Delta Q_Tc_{BSB}$ (5 msec; 95%CI = -3–13 msec; $n = 14$) as compared to heterozygous patients (13 msec; 95%CI = 7–18 msec; $n = 33$), and patients carrying 2677GG genotypes (18 msec; 95%CI = 12-23; $n = 25$; Figure 4A). While the $ABCB1$ 1236C>T SNP was marginally associated with $\Delta Q_Tc_{BSB}$ ($P = 0.042$), perhaps by virtue of its strong linkage to the 2677G>T/A SNP, the 3435C>T SNP was not related.

Functional analyses have demonstrated that these polymorphisms have at least an additive effect on altering protein folding and function (12, 16); thus we compared diplotype consisting of the $ABCB1$ 1236-2677-3435 variants (diplotypes 1-5), 1236-
2677 variants (diplotypes 6-10), and the 2677-3435 variants (diplotypes 11-15; Table S3) in order to evaluate the effects of multiple polymorphisms on phenotype as was done previously (17). Consistent with these observations (12, 16), considering the SNPs in the context of a haplotype increased the significance of the associations explored above with diplotypes 1-5, 6-10 and 11-15 being associated with $\Delta$QTcB$^{SB}$ ($P = 0.011$, $P = 0.020$ and $P = 0.010$ respectively). Diplotypes 1-5 were associated with $\Delta$QTcB$^{SB}$ with means equal to 22, 12, 12, 12, 3 milliseconds respectively (Figure 4B). Within diplotypes 6-10, individuals carrying diplotype 6 had the highest mean $\Delta$QTcB$^{SB}$ (20 msec; 95%CI = 13-27, n = 19), while diplotype 10 had the lowest mean (6 msec; 95%CI = -4-15; n = 12). Diplotypes 7-9 had roughly equal means (overall 12 msec; 95%CI = 7 -16; n = 44; Figure 4C). Diplotypes 11-15 were also associated $\Delta$QTcB$^{SB}$ with a similar trend in means ($\Delta$QTcB$^{SB}$ (msec) = 19, 13, 14, 10, 3 respectively; Figure 4D). No $ABCB1$ SNP or diplotype was associated with $\Delta$QTcB$^{SB}$ taken 24 hours following the start of the romidepsin infusion ($P > 0.05$).

Since the baseline QTc value may influence the variability in QTc, a subset of patients (n = 41) from the $\Delta$QTcB$^{SB}$ cohort had multiple baseline QTc measurements taken to increase the accuracy of the $\Delta$QTc values ($\Delta$QTcB$^{MB}$). Overall similar findings were observed, although the $\Delta$QTcB$^{MB}$ data tended to be better associated with $ABCB1$ genotypes in this subgroup. Both the $ABCB1$ 1236C>T and $ABCB1$ 2677G>T/A SNPs were associated with $\Delta$QTcB$^{MB}$ ($P = 0.026$, and $P = 0.0039$ respectively), while the $ABCB1$ 3435C>T allele was not associated. Individuals carrying the 1236CC alleles had the second highest $\Delta$QTcB$^{MB}$ (15 msec, 95%CI = 8-22, n = 14), those carrying 1236CT had similar values (19 msec, 95%CI = 14-24, n = 20), and those carrying 1236TT had...
lower ΔQTcBMB (5 msec, 95%CI = -8-18, n = 7; Figure 4E). The strongest association with ΔQTcBMB was determined in those carrying 2677GG (16msec, 95%CI = 11-21, n = 15) and 2677GT (19msec, 95%CI = 13-25, n = 18) genotypes that had similar ΔQTcBMB values, while those carrying the 2677TT genotype had the lowest ΔQTcBMB (0msec, 95%CI = -12-13, n = 6; Figure 4F). Diplotypes were not evaluated against ΔQTcBMB, given that this cohort was much smaller. Thus, it appears that more accurate measures of ΔQTcB increase the likelihood of an association with ABCB1 alleles. Taken together, the data suggest that individuals carrying wild-type alleles at ABCB1 have an increased likelihood of developing greater ΔQTc following romidepsin compared to individuals with variant genotypes.

No relationship was found between ABCB1 genotype and ΔQTcF or ΔQTcB over the course of repeated infusions (Supplemental Results S1). Thus, it seems that alterations in romidepsin-related QTc-prolongation due to allelic variation in ABCB1 may only be important following the first administration of romidepsin.
DISCUSSION

This study suggests that ABCB1 is involved in a previously unexplored blood-tissue barrier in the cardiac endothelium where it limits drug exposure in the heart. Interestingly, the observed associations with cardiac repolarization were found to be independent of an influence of ABCB1 on romidepsin plasma pharmacokinetic parameters in both mice and humans. Mice lacking the ABCB1-type P-glycoprotein had a greater intracardiac exposure to romidepsin. Although there was no difference in the QTc\text{max}, this difference in exposure may have altered cardiac repolarization since the time to maximum QTc was different between wild-type and knockout (31 vs. 16 hours). These data suggest that ABCB1 is a component of a blood-tissue barrier within the cardiac endothelium. Consistent with an impact of ABCB1 on cardiac exposure to romidepsin, we also show differences in the QT interval associated with common inherited variants in the \textit{ABCB1} gene. Unexpectedly, the variant alleles understood to be associated with reduced function of the ABCB1 transporter (12, 16) were in this study associated with a reduced impact of romidepsin on the QT interval. Taken together, these results are consistent with a hypothesis that patients carrying variant \textit{ABCB1} alleles have increased cardiac \textit{ABCB1} gene expression (11), which in turn limits the exposure of cardiac tissue to romidepsin.

The human ether-a-go-go related gene (\textit{hERG}) encodes the a-subunit of the rapid delayed rectifier current \textit{I_{Kr}} in the heart, which contributes prominently to terminal repolarization in human ventricular myocytes. Romidepsin has been shown to alter terminal cardiac repolarization by inhibiting the hERG protein, potentially by acetylation (34). Maximal QTc prolongation was delayed following romidepsin administration in
mice despite the rapid distribution and clearance of the drug demonstrating that romidepsin has a delayed, rather than acute, effect on ΔQTc in mice, similar to the effect observed in humans (13). This is inconsistent with direct hERG/\(I_{Kr}\) blockade (29, 35, 36); rather it is likely that the delay in QTc prolongation is related to an intracellular mechanism whereby romidepsin modulates certain pathways related to the hERG protein, such as by blocking hERG shuttling to the membrane by hsp90 as has been observed in several other drug treatments (i.e. fluoxetine, pentamidine, probucil, geldenamycin, radicicol, celastril; reviewed in (37)). Nonetheless, removal of Abcb1a/1b from the genome seems to be responsible for decreasing the time taken to ΔQTc\(_{\text{max}}\), most likely by increasing the overall exposure of the heart to romidepsin in mice. It is expected that this occurs because Abcb1a/1b is important for effluxing romidepsin from the heart, back into the systemic circulation thereby protecting the heart from QTc changes. In our hands, using whole heart homogenate, the exposure increase between wild-type and \(\text{Abcb1a/1b}\) -/- mice was relatively modest (~1.35-fold increase in knockouts); however, it is unclear to what degree cardiac exposure to romidepsin must increase in order to see an effect on QTc. Moreover, romidepsin exposure could have been even higher in cardiac myocytes of knockout mice than was apparent from whole heart homogenate as Abcb1 is expressed in cardiac endothelial cells and is thus expected to greatly limit drug penetration into highly vascularized cardiac muscle tissue.

Meissner et al. demonstrated that human individuals carrying the variant \(ABCB1\) 2677TT genotype have increased \(ABCB1\) mRNA levels in cardiac endothelial cells. Although the results of Meissner et al. contrast with the current understanding of \(ABCB1\) polymorphic variation (i.e. wild-type alleles are most often related to higher expression in
other tissues (38)), the current data obtained with romidepsin are consistent with Meissner et al. as individuals with higher copy numbers of variant \( ABCB1 \) 2677T alleles exhibited reduced lengthening of the QT-interval following romidepsin, compared to patients carrying increasing numbers of \( ABCB1 \) wild-type alleles. The association between \( \Delta QTc \) is strongest when all three of the studied alleles are considered together in a haplotype, consistent with functional studies of \( ABCB1 \) allelic variation (12, 16). Meissner et al. did not evaluate haplotypes in their study of mRNA expression. Since we determined that \( ABCB1 \) variant alleles confer a phenotype with lowered transport efficiency, the data suggest that gene expression in the cardiac endothelium may be a more important factor than altered protein folding and function induced by the non-synonymous 2677G>T/A (A893S/T) SNP, or the synonymous 1236C>T and 3435C>T transitions (12, 16).

The increase in time taken to achieve \( \Delta QTc_{\text{max}} \) in mice suggests that the human data may result from \( ABCB1 \) expression status modulating an early or later \( \Delta QTc_{\text{max}} \) and not necessarily a greater magnitude of \( \Delta QTc \) induced by romidepsin. This study found that \( ABCB1 \) alleles have an effect after 4 hours such that individuals carrying wild-type alleles, where ABCB1 expression is presumably low in the cardiac endothelium (11), also have a larger \( \Delta QTc \) at that time point. We did not find a relationship between \( ABCB1 \) SNPs and \( \Delta QTc \) at 24 hours or during subsequent administrations of the drug. However, since QTc was not monitored continuously in clinical trials, we were unable to assess \( \Delta QTc_{\text{max}} \) in patients, and future studies in humans must evaluate QTc prolongation with more resolution in order to determine whether or not ABCB1 expression is responsible...
for a greater magnitude, or a delayed $\Delta QTC_{\text{max}}$ induced by romidepsin in humans. Moreover, romidepsin may induce ABCB1 in humans (23) thereby facilitating its own elimination from the heart; this is possibly the reason that the difference in $\Delta QTc$ based on genotype is only apparent following the first administration, but not subsequent administrations of the drug. It also remains unclear whether other ABCB1 substrate drugs that prolong the QT interval (risperidone, clozapine, and potentially tamoxifen) are also limited from penetrating the heart tissue in a similar fashion, and whether genetic variation influences their intracardiac concentration. A single, small study in patients treated with doxorubicin appears to indicate that cardiomyopathy is more prevalent in patients with low ABCB1 mRNA and protein expression in cardiac endothelium (8). Thus, further research is required to ascertain the clinical importance of ABCB1 expression on substrate drugs that mediate QT-prolongation and cardiotoxicity.

This study contains several limitations and inconsistencies with the literature. First, the functional impact of allelic variation in $ABCB1$ gene expression presented herein add to a body of literature that has been controversial. Many investigations have demonstrated that in liver, intestine, and blood cells, the $ABCB1$ 2677G>T/A and 3435C>T wild-type alleles actually predict higher ABCB1 expression relative to variant alleles (39-47). However, functional studies have not consistently confirmed an impact of this higher gene expression on systemic drug exposure, with investigators reporting both increased and decreased drug clearance associated with these alleles. Nonetheless, our results are consistent with the observations set forth by Meissner et al. in human cardiac endothelial cells; cardiac expression of ABCB1 may be regulated in a tissue-dependent fashion (11). Second, due to data limitations, we were not able to ascertain QTcF in most
patients, and QTcF appears to be a better measure of romidepsin-induced QTc prolongation than QTcB in some patients (13). Third, this study didn’t evaluate the pharmacokinetics of reduced romidepsin, the active metabolite, and we are not able to conclude whether or not exposure to the active metabolite is greater. Thus our results should be considered exploratory and in need of further validation.

In conclusion, this study provides evidence that QTc interval changes following treatment with the ABCB1 model substrate romidepsin might be linked to the expression of the ABCB1 drug transporter in the cardiac endothelium. We believe that this is the first demonstration that ABCB1 expression alters QT-prolongation both in preclinical and clinical settings. This study is also the first to suggest that ABCB1 allelic variation may serve as a marker for prolonged QT-interval mediated by ABCB1 substrate drugs. These results warrant further evaluation for both romidepsin, and for other ABCB1 substrates that have the potential to cause cardiac side effects.

ACKNOWLEDGEMENTS:

We thank Dr. Rob Robey for his significant contribution of ABCB1 protein quantification via flow cytometry.
REFERENCES


Figure Legends

Figure 1
Transport of romidepsin by ABCB1. Data were obtained from an experiment in which transcellular transport of romidepsin was assessed in polarized monolayers of LLC-PK1 cells in the direction apical to basolateral (A to B) as well as the direction basolateral to apical (B to A). The latter reflects active transport by ABCB1. Results are shown for LLC-PK1 cells expressing wildtype ABCB1 (haplotype ‘CGC’) (panel A) or triple mutant ABCB1 (haplotype ‘TTT’) (panel B). Data in panel C show the apparent permeability coefficients ($P_{app}$), expressed in units of cm per sec. The star (*) indicates $P<0.05$ versus the B to A direction.

Figure 2
Romidepsin pharmacokinetics in mice. Mean romidespin concentration over time in: (A) mouse plasma where exposure was comparable between $Abcb1a/1b$ knockout mice and wild-type mice ($P = 0.11$); and (B) mouse cardiac tissues where approximately 35% greater romidepsin exposure was observed in $Abcb1a/1b$ knockout mice as compared to wild-type mice ($P = 0.0026$; Z-test).

Figure 3
Electrocardiogram measurements in romidepsin-treated wild-type and $Abcb1a/1b$ knockout mice presented as: Filtered $\Delta QTc \pm 1SD$ over 72 hours in knockout (solid line; $\Delta QTc = QTc$ (Group 1) – QTc (Group 2) for each timepoint) and wild-type mice (dashed line; $\Delta QTc = QTc$ (Group 3) – QTc (Group 4) for each timepoint)
**Figure 4**

Relationships between $ABCB1$ genotypes and the baseline corrected QTc interval following a 4-hour infusion of romidepsin. (A) $ABCB1$ 2677G>A/T genotype versus $\Delta$QTcB$_{SB}$ ($P = 0.017$); $ABCB1$ diplotypes (B) 1-5 ($P = 0.011$), (C) 6-10 ($P = 0.020$), and (D) 11-15 ($P = 0.010$) versus $\Delta$QTcB$_{SB}$; (E) $ABCB1$ 1236C>T genotype versus $\Delta$QTcB$_{MB}$ ($P = 0.026$); (F) $ABCB1$ 2677G>A/T genotype versus $\Delta$QTcB$_{MB}$ ($P = 0.0039$). * - $P$-value was initially significant ($P<0.05$), but did not withstand multiple comparisons testing. ** - $P<0.05$. *** - $P<0.01$. 
Table 1. Patient Demographics and Dosages

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase I (N = 12)</th>
<th>Phase II (NCI) (N = 103)</th>
</tr>
</thead>
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<tr>
<td>Age</td>
<td>58 (40–77)</td>
<td>57 (27–84)</td>
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<tr>
<td>Male:Female</td>
<td>6/6</td>
<td>71/39</td>
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<td>Race:</td>
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<tr>
<td>Caucasian</td>
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<td>17</td>
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<tr>
<td>Asian</td>
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<td>3</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Dose:</td>
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</tr>
<tr>
<td>12.7 mg/m²</td>
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<td>0</td>
</tr>
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<td>14.0 mg/m²</td>
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<td>98</td>
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<tr>
<td>17.8 mg/m²</td>
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<td>18.0 mg/m²</td>
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<td>5</td>
</tr>
<tr>
<td>24.9 mg/m²</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) All patients were diagnosed with cutaneous T-cell lymphoma except for 12 patients in Group 2 who were diagnosed with various refractory cancers;  
\(^b\) Data are presented as a median and range, and data were not available for 5 patients treated on the Phase II trial.
Table 2 - Genotype and allele frequencies of the studied variants

<table>
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<tr>
<th>Allelic variant</th>
<th>Effect</th>
<th>N</th>
<th>Wt</th>
<th>Het</th>
<th>Var</th>
<th>p</th>
<th>q</th>
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<td><strong>Caucasians (N = 90)</strong></td>
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<tr>
<td>ABCB1 1236C&gt;T</td>
<td>G411G</td>
<td>89</td>
<td>28 (31.4%)</td>
<td>41 (46.1%)</td>
<td>20 (22.5%)</td>
<td>0.54</td>
<td>0.46</td>
</tr>
<tr>
<td>ABCB1 2677G&gt;T</td>
<td>A893S</td>
<td>87</td>
<td>23 (26.4%)</td>
<td>43 (49.4%)</td>
<td>16 (18.4%)</td>
<td>0.52</td>
<td>0.45</td>
</tr>
<tr>
<td>ABCB1 2677G&gt;A</td>
<td>A893T</td>
<td>87</td>
<td>23 (26.4%)</td>
<td>2 GA (2.3%)</td>
<td>0</td>
<td>0.52</td>
<td>0.03</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ABCB1 3435C&gt;T</td>
<td>I1145I</td>
<td>90</td>
<td>20 (22.2%)</td>
<td>43 (47.8%)</td>
<td>27 (30.0%)</td>
<td>0.46</td>
<td>0.54</td>
</tr>
<tr>
<td><strong>African Americans (N = 19)</strong></td>
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<td></td>
</tr>
<tr>
<td>ABCB1 1236C&gt;T</td>
<td>G411G</td>
<td>19</td>
<td>8 (42.1%)</td>
<td>6 (31.6%)</td>
<td>5 (26.3%)</td>
<td>0.58</td>
<td>0.42</td>
</tr>
<tr>
<td>ABCB1 2677G&gt;T</td>
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<td>19</td>
<td>11 (57.9%)</td>
<td>5 (26.3%)</td>
<td>2 (10.5%)</td>
<td>0.74</td>
<td>0.24</td>
</tr>
<tr>
<td>ABCB1 2677G&gt;A</td>
<td>A893T</td>
<td>19</td>
<td>11 (57.9%)</td>
<td>1 (5.3%)</td>
<td>0</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>ABCB1 3435C&gt;T</td>
<td>I1145I</td>
<td>19</td>
<td>11 (57.9%)</td>
<td>2 (10.5%)</td>
<td>6 (31.6%)</td>
<td>0.63</td>
<td>0.37</td>
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<tr>
<td><strong>Other (N = 6)</strong></td>
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<tr>
<td>ABCB1 1236C&gt;T</td>
<td>G411G</td>
<td>6</td>
<td>0(%)</td>
<td>5 (83.3%)</td>
<td>1 (16.7%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>ABCB1 2677G&gt;T</td>
<td>A893S</td>
<td>6</td>
<td>1 (16.7%)</td>
<td>3 (50.0%)</td>
<td>1 (16.7%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>ABCB1 2677G&gt;A</td>
<td>A893T</td>
<td>6</td>
<td>1 (16.7%)</td>
<td>1 (16.7%)</td>
<td>0 (0.0%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>ABCB1 3435C&gt;T</td>
<td>I1145I</td>
<td>6</td>
<td>2 (33.3%)</td>
<td>2 (33.3%)</td>
<td>2 (33.3%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

\( ^a \) Number represent number of patients with percentage in parenthesis; the difference in the total number of patients is due to the fact that not all samples yielded sequencing data or showed PCR amplification; \( ^b \) Hardy-Weinberg notation for allele frequencies (p, frequency for wild type allele and q, frequency for variant allele); \( ^c \) Number represents position in nucleotide sequence; \( ^d \) Number represents amino acid codon; \( ^e \) genotype data were not available in all patients as not all samples yielded sufficient DNA or PCR amplified; \( ^f \) Wt, Homozygous wild-type allele patient; Het, Heterozygous patient; Var, Homozygous variant patient; G411G, G site of Codon 411 in ABCB1 gene; A893S, A site of Codon 893 in ABCB1 gene; A893T, T site of Codon 893 in ABCB1 gene; I1145I, I site of Codon 1145 in ABCB1 gene; 19 TA (2.3%)
Var, Homozygous variant patient;  The 2677G>T/A polymorphism is triallelic and two different SNPs are therefore presented; Three Hispanics and three Asians were included as “other” since accurate genotyping information could not be obtained with so few individuals from these populations.
Impact of ABCB1 allelic variants on QTc interval prolongation


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