Electrocardiographic Studies of Romidepsin Demonstrate Its Safety and Identify a Potential Role for K\textsubscript{ATP} Channel

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Statement of Translational Relevance

Romidepsin is a histone deacetylase (HDAC) inhibitor FDA approved for the treatment of cutaneous and peripheral T cell lymphoma and continues in development. Cardiac safety has been intensively investigated for romidepsin and other HDAC inhibitors, generating confidence in the safety of the class. However, ST and T wave changes in the ECG of unclear etiology are observed in a majority of patients. This report documents a consistent increase in heart rate in all patients treated without evidence of a pro-arrhythmic effect. A high fraction of patients with T-cell lymphoma require potassium and magnesium supplementation. We present a mechanistic hypothesis for the ST segment and T-wave changes observed on serial ECGs, explaining why electrolyte replacement would be important. The report affirms the safety of romidepsin in the context of attention to potassium and magnesium supplementation; the data are important for both the academic and clinical communities, among them physicians prescribing romidepsin.
Abstract

Purpose: Romidepsin is a histone deacetylase inhibitor (HDI) approved for the treatment of both cutaneous and peripheral T cell lymphoma (CTCL and PTCL). During development, a thorough assessment of cardiac toxicity was performed.

Experimental Design: A phase II single-agent non-randomized study of romidepsin was performed in patients with CTCL and PTCL who had progressed after at least one prior systemic therapy.

Results: Results for the first 42 patients enrolled on the NCI 1312 Phase II study of romidepsin in CTCL or PTCL demonstrated no cardiac toxicity based on serial electrocardiograms, troponins and MUGA scans/echocardiograms. The cardiac assessments reported herein confirm the safety of romidepsin among 131 enrolled patients, while supporting a role for electrolyte replacement. Heart rate increased an average 11 bpm following romidepsin infusion; there was no evidence of increased arrhythmia. Criteria for potassium/magnesium replacement were met prior to 55% of 1365 romidepsin doses; an association with hypoalbuminemia was confirmed. We propose a mechanism for ST segment flattening and depression, the most common electrocardiogram abnormalities observed: HDI-induced alteration of the activity or expression of K_{ATP} channels. In addition, examination of the variants of the active transporter of romidepsin, ABCB1, demonstrated a trend towards smaller heart rate changes in the peri-infusion period among wild type compared to variant diplotypes.

Conclusions: We conclude that in the context of appropriate attention to electrolyte levels, the data support the cardiac safety of romidepsin.
Introduction

Histone deacetylase inhibitors (HDIs) are a novel class of epigenetic agents, among which two, were approved by the FDA for patients with progressive or recurrent cutaneous T-cell lymphoma (CTCL): vorinostat (Zolinza®, Merck) and romidepsin (Istodax® Celgene Inc). Romidepsin is also approved for the treatment of progressive or recurrent peripheral T-cell lymphoma (PTCL). The HDIs promote acetylation of lysine residues of histone proteins present as an octomer surrounded by DNA in the nucleosome chromatin complex. They have been shown to modulate gene expression, and to provoke cell cycle arrest, cell differentiation, and cell death.

Clinical trials with romidepsin, vorinostat and other HDIs including belinostat, panobinostat, entinostat, and moctinostat are ongoing, with the hope of extending the activity of HDIs into solid tumors. During the development of this class of agents, concerns were raised about cardiac toxicity due to observed ECG changes, although some preclinical data have suggested that in the setting of cardiac hypertrophy, HDAC inhibition may reduce atrial arrhythmia inducibility (1). Phase I and II clinical trials of HDIs have demonstrated non-diagnostic electrocardiogram changes including T-wave flattening and ST segment depression as well as QT interval prolongation and tachyarrhythmias (2-10). No clear mechanistic explanation has been identified. Several sudden deaths occurring early in the development of romidepsin led to amendment of the protocol to exclude patients with significant underlying cardiac disease at risk for sudden death and also to avoid concomitant medications that prolong the QT interval or inhibit CYP3A4. A strict electrolyte replacement regimen was also instituted during conduct of the protocol, coupled with continued ECG monitoring, and development continued without further incident.

Piekarz et al. previously reported cardiac studies for 42 of the first 43 patients enrolled on the NCI 1312 Phase II trial of romidepsin in T-cell lymphoma (2). Serial electrocardiograms, serial troponin I levels and MUGA or echocardiograms were reviewed, with no evidence of cardiotoxicity.
detected. T wave flattening (grade 1) and ST segment depression (grade 2) seen in more than half of the electrocardiograms obtained were of short duration and reversible (2).

We now provide new cardiac assessment studies for NCI 1312. We observed a consistent increase in heart rate following romidepsin treatment, an observation not previously reported. We report the remarkable frequency with which potassium and magnesium supplementation was required, and provide the cardiac adverse events for the complete 131 patients on the trial. The effect of variants of the active transporter of romidepsin, ABCB1, on heart rate following romidepsin is assessed. These data support the overall cardiac safety of romidepsin and other histone deacetylase inhibitors but they also support continued vigilance regarding the patient population to be treated, and potassium and magnesium supplementation.

**Patients and Methods**

Patients with relapsed or refractory cutaneous or peripheral T-cell lymphoma were enrolled in a phase II trial evaluating the safety and efficacy of romidepsin, [NCI protocol 1312]. The patients and methods and results for the cutaneous T cell lymphoma and the peripheral T cell lymphoma cohorts (11, 12) have been reported in detail. The trial was registered at ClinicalTrials.gov, NCT00007345, and approved by the NCI Institutional Review Board. All participants gave written informed consent. All toxicities were graded according the NCI Common Toxicity Criteria, version 2.0. At the NIH site, ECGS were obtained prior to commencement of therapy, prior to the infusion of each dose, within 1 hour after completion of the infusion, and on the day following treatment. An additional electrocardiogram was obtained on day 2 of the first cycle. Electrocardiograms were recorded using a HP Pagewriter XLi or a GE Marquette MAC 1200 and recorded at 25mm/s, with an amplitude of 10mm/mV and with 60Hz filtering. ECGs were analyzed using Pagewriter A.0.4.01 electrocardiogram analysis software (Philips Medical Systems, Andover, MA). In this program, the QT interval measurement is obtained by averaging the five longest QT intervals with a T or T’ wave
amplitude of >0.15 mV. T wave and ST segment abnormalities were assessed by either R.L.P or S.E.B. and graded using NCI Common Toxicity Criteria version 2.0.

**Cardiac Exclusion Criteria and Electrolyte Replacement**

Following protocol amendment to refine the cardiac eligibility criteria, patients with the following cardiac risk factors were excluded from the study: congenital long QT syndrome, QTc interval >480 milliseconds; myocardial infarction within 12 months of study entry; active coronary artery disease or screening ECG suggestive of cardiac ischemia (ST depression ≥2mm); NYHA class II-IV congestive heart failure or ejection fraction <45% by MUGA scan or <50% by echocardiogram and/or cardiac MRI; history of dilated, hypertrophic or restrictive cardiomyopathy; uncontrolled hypertension (SBP >160 mmHg, DBP>95 mmHg); cardiac arrhythmia requiring antiarrhythmic other than beta blocker or calcium channel blocker; Mobitz type II second degree heart block not controlled by a pacemaker.

After amendment, potassium and magnesium were monitored. For patients with a serum potassium level <3.5mMol/L, a total of 80mEq of potassium were administered given as 40mEq intravenously and 40mEq orally. For serum potassium >3.5mMol/L but <4.0mMol/l, 40mEq was administered either intravenously or orally. For patients with a serum magnesium level <0.85mMol/L, 1g MgSO₄ (8.12mEq) was administered intravenously for every 0.05mMol/L below 0.85mMol/L to a maximum of 4g MgSO₄ (32.48mEq).

**Statistical Methods**

The Wilcoxon rank sum test, Fisher’s exact test and Jonckheere-Terpstra trend test were used to test the relationship between low albumin and low potassium and magnesium levels. ANOVA was
used to test the differences in albumin and potassium and magnesium levels by histology and by cycle and day of treatment.

Diplotype arrangements of the ABCB1 SNPs were calculated using Haploview (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview) [See Supplementary Table S1 (13)]. Heart rates and romidepsin-induced heart rate changes versus genotype or beta-blocker therapy were evaluated using Kruskal-Wallis, ANOVA, Wilcoxon rank-sum test, and Jonckheere-Terpstra trend test. Since multiple tests were conducted, comprising three individual genotypes and three diplotype organizations (accounting for the coinheritance of ABCB1 alleles), tests that were $P<0.05$ were considered marginally significant. The $a$ priori level of nominal significance was set at $P<0.01$ rather than accounting for a strict Bonferroni adjustment since repeated measures of heart rates were correlated and ABCB1 alleles were coinherited (i.e., correlated). Data are reported as mean ± SD unless otherwise indicated.

**Results**

**Patient Characteristics**

Table 1A illustrates the patient characteristics for all 131 patients treated in the study and Table 1B outlines the characteristics for the 63 patients treated at the NIH Clinical Center only. The CTCL cohort was almost twice as large as the PTCL cohort. The majority of patients had an ECOG performance status of 1. Thirty-three percent of patients received 3 to 5 cycles, and 40% received 6 or more cycles of romidepsin. Fifty-one percent of all patients in the trial had received prior doxorubicin (52% of the NIH cohort). Excluding the differences in ECOG performance status, the NIH cohort is mostly representative of all patients in the trial. In all, the 131 patients received 3,358 doses of romidepsin in 1,198 cycles; the 63 patients enrolled at the NIH received 1,292 doses in 475 cycles.
**ECG Changes**

Electrocardiograms for the 63 patients treated at the NIH site were analyzed; in total, 3,633 electrocardiograms were reviewed. Table 2 summarizes the commonly observed T-wave (grade 1) or ST segment (grade 2) abnormalities noted on electrocardiograms following romidepsin treatment. On examining electrocardiograms from all doses given (Table 2A), 42% percent had grade 1 and 1% had grade 2 abnormalities immediately after completion of romidepsin infusion and on the day following infusion 66% had grade 1 and 4% had grade 2 abnormalities. Electrocardiogram abnormalities observed with the administration of the first dose of romidepsin are detailed in Table 2B. Last, considering the worst electrocardiogram grade obtained at any time during the study for each of the 63 patients, 73% had grade 1 and 25% had grade 2 abnormalities at some point during the treatment protocol (Table 2C). These results were comparable to the findings in the original analysis of 42 patients (2).

**Heart Rate Changes Following Romidepsin**

A new observation was that the heart rate modestly but consistently increased in patients receiving romidepsin. Supplementary Figures S1A -C demonstrate heart rate increases following Day 1, 8 and 15 dosing in cycles 1. To determine whether the heart rate increase varied by baseline heart rate, the change in heart rate was also analyzed in sets of 10, commencing with patients whose baseline heart rates were in the 40s. Notably, the magnitude of heart rate increase did not vary with baseline (Supplementary Figures S 2A -C). Median baseline heart rate was 85bpm ± 16bpm, increasing to 96bpm ± 15bpm on cycle 1 day 1. Cycle 2 day 1 values showed a similar increase (81bpm ± 15bpm to 93bpm ± 15bpm post-infusion). Supplementary figures S3-8 show similar patterns for cycle 1 and 2. Analysis was performed both including and excluding the patients who received beta-blockers.
Fourteen patients were treated with beta-blockers. Nine patients were noted to be on beta-blockers on cycle 1 day 1 - eight for hypertension and one for long QT and atrial flutter prior to romidepsin. Five patients were commenced on beta-blockers between cycle 1 day 2 and cycle 3 day 15 for tachycardia or arrhythmias observed during monitoring; these patients had pre-existing tachyarrhythmias on pre-enrollment Holter monitoring. Patients receiving beta-blockers prior to romidepsin start had lower heart rates (74bpm ± 15bpm vs. 85bpm ± 16bpm; P= 0.019). Beta-blockers did not prevent the heart rate increase following romidepsin. Values after infusion on cycle 1 day 1 were 86bpm ± 16bpm versus 96bpm ± 15bpm; P=0.053, respectively. Figure 1 shows the heart rate changes examined in quintiles for cycle 1 day 1 excluding patients on beta-blockers. Cycle 2 day 1 values were similar, with and without beta-blockers showing similar increases in magnitude (pre-infusion, 64bpm ± 10bpm versus 81bpm ± 15bpm; P = 0.0008, respectively, and post-infusion, 75bpm ± 12bpm versus 93bpm ± 15bpm; P=0.0011, respectively). We therefore accounted for beta-blocker therapy in subsequent analyses of heart rate.

**Holter Monitor and Telemetry Results in NIH Cohort**

Baseline 24hr holter data were available in 56 of the 63 patients. Cardiac rhythm monitoring with telemetry totaling 24hr during and after romidepsin infusion was available for 59 patients; 19 of these patients also had concurrent holter monitoring. Table 3 summarizes the holter and telemetry data for baseline and post cycle 1 day 1. Frequent atrial premature complexes (APCs) were defined as >200 APCs on 24hr holter based on two studies showing increased incidence of arrhythmia and stroke with >200 APCs/24 hours (14, 15). Frequent premature ventricular complexes (PVCs) were defined as >1000/24 hours (16). On baseline holter monitor recorded prior to commencing romidepsin infusion, 23 patients had grade 1 supraclavicular tachycardia (SVT), 5 patients had grade 1 ventricular tachycardia (VT) and 3 patients had accelerated idioventricular rhythm (AIVR). In addition, one
patient had grade 2 atrial flutter and was cardioverted prior to commencing on protocol. All events reported on telemetry and holter monitoring during and for 24 hours after the first romidepsin infusion were grade 1, apart from one patient considered to have grade 3 arrhythmia - frequent runs of SVT, VT and AIVR post romidepsin infusion in a patient who also had frequent APCs, frequent PVCs and AIVR on baseline holter prior to romidepsin. This patient had electrophysiologic testing within 24 hours of receiving his third dose of romidepsin, which showed no evidence of increased susceptibility to arrhythmia. One patient with grade 1 trigeminy was found to have concomitant hypomagnesemia (magnesium 0.59 mMol/L) and hypokalemia (potassium 3.4 mMol/L).

**Cardiac Adverse Events in the Entire Population**

**Supplementary Tables S2** outlines the cardiovascular adverse events reported for all 131 patients on the trial. The majority of events were grade 1. Two deaths were observed prior to institution of electrolyte monitoring; both were unexpected and considered episodes of sudden cardiac death, occurring in one patient with multivalvular cardiac disease and in a second patient with severe atherosclerotic heart disease. Fourteen episodes of SVT occurring in 13 patients were reported on or post-romidepsin infusion; 6 of these were atrial fibrillation two of which were grade 1, two grade 2 and two grade 3. Among the patients with atrial fibrillation, grade 1 hypomagnesemia was seen in one patient and magnesium below the replacement level was seen in another. Potassium levels below the replacement level were seen in three patients. One episode of grade 3 atrial flutter was reported in a patient whose potassium and magnesium were both above the replacement levels. Troponin levels were routinely measured in the NIH patients within 48 hours before romidepsin administration on day 1, and before and 1 day after each romidepsin dose (i.e. on days 1, 2, 8, 9, 15, and 16) of each treatment cycle; 2,353 were collected over all cycles. Sixteen events of elevated troponin I were observed in 11 patients. Eleven of these were normal on repeat assay or subsequent measurement within 24 hours and were thought to be lab errors, except for 1 patient with a lymphomatous myocardial wall mass (17) who had repeatedly elevated troponin levels.
Potassium and Magnesium Monitoring

Given that electrolyte monitoring and supplementation was required in patients enrolled on the clinical trial, we quantified the incidence of hypomagnesemia and hypokalemia requiring intervention as defined per protocol. Serum electrolyte measurements for 1,365 doses administered to 128 out of 131 patients (between cycles one and six assessed) were analyzed. Only 10 patients (7.6%) on the trial never required electrolyte replacement as defined in the protocol during their course of therapy. Figure 2 shows the number of doses requiring magnesium and potassium replacement. Fifty-five percent (746/1365) of doses of romidepsin administered between cycles one and six were associated with pre-treatment levels meeting criteria for protocol-mandated supplementation. Magnesium repletion was required more often than potassium repletion [529 doses (39%) versus 428 doses (31%)].

Because both our data and previous reports suggested that hypokalemia and hypomagnesemia were common in cutaneous T-cell lymphoma (18), and because we observed frequent hypoalbuminemia, we tested the association of these findings. Using the NIH lower limit of normal for albumin, 3.7 mg/dL, we asked whether potassium or magnesium below or equal to the lower limit of normal (potassium ≤3.5mmol/L or magnesium ≤0.75mmol/L) or below the protocol-defined replacement levels (potassium <4.0mmol/L or magnesium <0.85mmol/L) was associated with low albumin. This was analyzed initially by the Wilcoxon rank sum test. Doses from cycles 1–3, and days 1, 8, and 15 were analyzed. Results, as shown in Supplementary Table S3, showed that 28 of 98 analyses revealed a P<0.05. Similar results were obtained with Fisher’s exact test and the Jonckheere-Terpstra trend test (Table S3), indicating that higher albumin levels were associated with higher potassium or magnesium levels. However, many of the p-values were rather large, indicating a weak association. To further examine the association, a set of repeated measures of analysis of variance (ANOVA) was performed on the albumin data by histology (CTCL or PTCL or both), with data restricted to the first 3 cycles. Starting with the initial model, a backward selection process was performed on the model in a hierarchical manner. The results are summarized in

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Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

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Supplementary Table S4. Again, association of albumin with potassium and magnesium was found. Variation with cycle and day was also noted in some of the models. Together, the results of these 4 statistical analyses show that hypoalbuminemia, aggravated by the disease process in T-cell lymphoma, although not the only factor, is associated with low potassium and magnesium.

ABCB1 Genotyping

Genotype data for the ABCB1 (P-glycoprotein) transporter was available for 61 patients (13) [Supplementary Table S4]. We previously noted that several ABCB1 polymorphisms and diplotypes conferred a reduced impact on the QT interval on cycle 1 day 1 following romidepsin administration (13). As observed in Figure 3, the data (excluding patients who were on beta-blockers) point to a similar trend with romidepsin-induced heart rate changes (C1D1 Post-Baseline), although most trends are not statistically significant ($P_{\text{trend}} \leq 0.18$, Figure 3). Nonetheless, those carrying genotypes and diplotypes with increasing numbers of variant alleles have smaller heart rate changes than their counterparts carrying more wild-type alleles. Similarity between heart rate changes versus the various genotypes was expected given that all three $ABCB1$ loci are in significant linkage-disequilibrium. No apparent relationship was observed when the same SNPs and diplotypes were compared with heart rate data obtained on cycle 2 day 1 of romidepsin treatment ($P>>0.05$).
Discussion

Romidepsin is a histone deacetylase inhibitor approved by the FDA for treatment of CTCL and PTCL. While the drug functions as an inhibitor of deacetylase activity, the downstream events that emerge are pleiotropic. The HDIs as a class cause cell cycle arrest, gene induction, reactive oxygen species production, and apoptosis. The relative importance of these various activities likely depends on the context of pre-existing genetic aberrations. Clinically, the HDIs have similar efficacy and toxicity profiles. Active in T-cell lymphoma, with fatigue, nausea, and vomiting the most common AEs; along with thrombocytopenia and neutropenia; and ECG effects including minor QT prolongation and ST and T wave changes. The mechanisms underlying these cardiac effects have not been worked out although there was speculation that acetylation of the hERG channel might cause QT prolongation (19, 20). In recently reported data, intensive investigation of romidepsin has suggested minimal impact on the QT interval, no evidence of myocardial damage, and no cumulative effects (21, 22). We have theorized that the more interesting cardiac effect may be the ST and T wave change observed in as many as 75% of patients receiving romidepsin. This report summarizes our studies of the cardiac effects of romidepsin on heart rate and ST and T wave changes, including cardiac rhythm monitoring, which did not suggest an increase in the incidence of arrhythmias following romidepsin. However, the monitoring did suggest that the patient population with T-cell lymphoma is one with frequent ectopy at baseline. Finally, our data call attention to the preponderance of patients whose potassium and magnesium levels met protocol criteria for electrolyte replacement.

Notably, atrial fibrillation has been observed in the development of almost every HDI. It was observed as a dose limiting toxicity in the romidepsin Phase I, occurring at 24.9mg/m² on the day 1 and 5 schedule (a dose well above the approved 14mg/m² dose) (8). Atrial fibrillation or flutter was observed during the Phase I trials of panobinostat (LBH589) (23), LAQ824, belinostat, plitidepsin, and CHR-3996; often occurring in more than one patient for any given agent, and occasionally below doses that were defined as the maximum tolerated dose (23-28). This is in contrast to the QT interval-associated arrhythmia torsades de pointes, which was observed with only one agent, LAQ824, a cinnamic hydroxamate no longer in clinical development (29). Recent evaluation of romidepsin data
suggests a minimal effect on the QT interval using the Friderica (QTcF) correction, considered a
better assessor of the QT interval than the Bazett (QTcB) correction at faster heart rates (22, 30).

Romidepsin is the most potent HDAC inhibitor currently in development largely targeting
Class I HDACs. Extensive cardiac safety data have been gathered, in part because it was the first
potent and effective HDAC inhibitor in development, and in part because of the early observation of
ECG changes. During early development, four unexpected deaths were observed in other trials in
addition to two in the NCI 1312 study that were suggestive of sudden death. Upon review it was
recognized that each of the deaths occurred in patients with risk factors for cardiac events, including
severe valvular pathology, severe atherosclerotic heart disease, sarcoidosis, and uncontrolled
hypertension in a patient with neuroendocrine tumor (24, 30-32). Notably, sudden death has also been
reported in patients receiving other HDIs (29, 33-35). After these observations, the protocol was
amended to exclude patients with significant cardiac disease and to require potassium and magnesium
supplementation to maintain levels in a high normal range, since electrolyte deficiencies increase
arrhythmia risk (36-39). Hypomagnesemia is a known risk factor for cardiac arrhythmia and sudden
cardiac death (SCD) (37, 38). Data from the Nurses’ Health Study reported in 2011 showed an inverse
linear relationship between plasma magnesium level and SCD in which each 0.25mg/dL increment in
plasma magnesium was associated with a 41% lower risk (38). Hypokalemia is also a well-
documented risk factor for cardiac arrhythmia and SCD (39, 40). Consistent with a previous report of
hypomagnesemia in patients with advanced stage CTCL (18), we observed that half of doses required
supplementation with either magnesium or potassium.

The most reproducible electrocardiographic effect of the HDIs appears to be ST segment
flattening and depression and not QT prolongation. ST shift is an unusual drug effect, and in
cardiology practice this ECG finding is most frequently associated with ischemia, particularly
occurring in the subendocardial region of the left ventricle. Certain drugs can induce cardiac ischemia
and ST depression by inducing vasospasm of the coronary arteries, with cocaine and amphetamines
being the most notorious culprits. These drugs may also result in myocardial necrosis. In addition,
some anticancer drugs, such as 5FU, are known to cause coronary vasospasm (41). Importantly, objective evidence of ischemia was sought and excluded in the case of romidepsin (2). Subjects with ST depression did not manifest mechanical dysfunction or enzyme release by troponin assay, a particularly sensitive measure. Instead, the evidence is clear that ST depression occurs in the absence of significant ischemia.

The mechanism underlying ST segment shifts induced in ischemia is somewhat controversial, but likely involves activation of the ATP sensitive potassium conductance (K\textsubscript{ATP}) in the ischemic region due to energy deprivation (42). Often considered to be cardioprotective, opening of K\textsubscript{ATP} channels can also promote arrhythmias. In response to a decrease in the ATP:MgADP ratio opening of just 1% of the K\textsubscript{ATP} channels shortens the action potential by 50% (43, 44). When channel opening occurs in one region of the heart but not another, a voltage gradient can develop between the de-energized subendocardium with shortened action potentials and adjacent tissue with normal action potentials, resulting in arrhythmogenic dispersion in repolarization. In addition, the shortened action potential duration and refractory period can promote phase 2 reentry (45).

The dual capability of K\textsubscript{ATP} channels to promote reentry (e.g. atrial fibrillation) and ST segment shifts may suggest that HDIs affect the activity or expression of K\textsubscript{ATP} channels. In cardiac myocytes, K\textsubscript{ATP} channels are composed of two different subunits - a pore forming Kir6.2 and a regulatory member of the ATP-binding cassette family, either SUR1 or SUR2A (46). HDIs are known to affect the expression of ATP binding cassette (ABC) proteins, and in recently reported data, Flagg and co-workers found that HDIs downregulate SUR1 but upregulate SUR2 in the transformed HL-1 atrial cell line (47). Further, we recently reported romidepsin-induced increased trafficking to the cell surface of an ABC protein ABCG2 due to changes in both expression and protein processing (48). In mice, K\textsubscript{ATP} channel structure is chamber specific (atrial SUR1+Kir6.2 vs. ventricular SUR2A+Kir6.2) (49, 50); however both combinations appear to exist in both chambers in the human heart (51). Increased activity of the K\textsubscript{ATP} channel in the atrium has been associated with an increased incidence of atrial fibrillation, an arrhythmia that is rarely seen with QT prolonging drugs (51). Although
functional data are needed to determine whether the effects of HDI on SUR2 expression results in increased channel activity, these data support a hypothetical explanation for the reported observations, i.e. differential downregulation of SUR protein in the ventricle (resulting in ST depression) and upregulation in the atria (resulting in atrial fibrillation).

Subjects carrying variant alleles in the gene encoding an active transporter of romidepsin, ABCB1 (P-glycoprotein, MDR-1), are thought to express higher levels of ABCB1 in the cardiac endothelium (52). Consistent with these data, we recently showed that ABCB1 variant carriers have a modestly lower risk of developing QT-prolongation following romidepsin than similar subjects carrying wild-type alleles (13). We previously reported that ABCB1 expression directly limits intracardiac exposure in a murine model, and that mice lacking Abcb1-type P-glycoprotein are consequently more susceptible to romidepsin-induced ECG changes. In our current data, there were no clinically significant differences in heart rate changes between genotypes, but a trend to lower heart rate change in the variants did appear to be consistent with the QT observations.

Taken together, these studies again support the cardiac safety of romidepsin in the treatment of CTCL and PTCL, and support its continued development in solid tumors. The studies also highlight the frequency with which low potassium and magnesium levels are observed in the TCL patient population. Mechanistically, it is not a simple matter to tie together the ST- T wave changes, the heart rate increase, and the observation of atrial fibrillation occurring as a dose limiting toxicity. We have here hypothesized that some effects are linked to the variable penetration of romidepsin (and equally the other HDIs) through the myocardium from the perfusing vessels. A gradient could result that altered repolarization and thereby affected the ST and T wave, as well as the potential for ectopy. An increase in heart rate due to increased catecholamines, a decrease in electrolytes due to disease, and an alteration in repolarization due to increased activity of SUR in one part of the myocardium relative to another could create a perfect storm that in a patient with a hypertrophic myocardium or a poorly perfused myocardium could induce arrhythmia. This is a hypothesis that argues for, as
recommended in the FDA-approved package insert, careful monitoring of potassium and magnesium, and close observation of patients with known underlying cardiac disease.
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References


Blood. 2009;114(Abstract #3709).
Table Legends

Table 1A shows the baseline characteristics and cycle and dose data for all 131 patients treated on NCI 1312. Table 1B shows similar data for the NIH cohort.

Table 2 demonstrates the T-wave and ST-segment changes at baseline and following romidepsin administration for the 63 patients in the NIH cohort.

Table 3 Holter Monitor and Telemetry Results in NIH Cohort
### Tables

#### Table 1A

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<tr>
<td></td>
<td>3 (1-82)</td>
</tr>
<tr>
<td><strong>Doses</strong></td>
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</tr>
<tr>
<td>12.0</td>
<td>12.0 (1-244)</td>
</tr>
<tr>
<td></td>
<td>12.5 (2-158)</td>
</tr>
<tr>
<td></td>
<td>9 (1-244)</td>
</tr>
<tr>
<td><strong>Total Number of Doses</strong></td>
<td>All Patients</td>
</tr>
<tr>
<td></td>
<td>CTCL</td>
</tr>
<tr>
<td></td>
<td>PTCL</td>
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</table>

Abbreviations: CTCL, cutaneous T cell lymphoma; PTCL, peripheral T cell lymphoma; ECOG, Eastern Cooperative Oncology Group
Table 1B

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. Patients</th>
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<tbody>
<tr>
<td></td>
<td>All Patients</td>
<td>CTCL</td>
<td>PTCL</td>
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<tr>
<td><strong>Gender</strong></td>
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<tr>
<td>Male</td>
<td>40</td>
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<td>11</td>
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<tr>
<td>Female</td>
<td>23</td>
<td>13</td>
<td>10</td>
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<td><strong>Diagnosis</strong></td>
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<td>CTCL</td>
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<td><strong>Prior doxorubicin</strong></td>
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<td>Yes</td>
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<td>12</td>
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<td>No</td>
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<td>30</td>
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<td><strong>ECOG</strong></td>
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<td>4</td>
<td>5</td>
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<td>1</td>
<td>46</td>
<td>32</td>
<td>14</td>
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<tr>
<td>2</td>
<td>8</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td><strong>Cycles administered</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>19</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>3-5</td>
<td>19</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>≥6</td>
<td>25</td>
<td>18</td>
<td>7</td>
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<tr>
<td><strong>Characteristic</strong></td>
<td>Median (range)</td>
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<tr>
<td>Age</td>
<td>53.3 (27.6 – 80.3)</td>
<td>55.5 (28.2 – 80.3)</td>
<td>57.6 (27.6 – 79.4)</td>
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<td>Cycles</td>
<td>4 (1 – 79)</td>
<td>4 (1-79)</td>
<td>2 (1-29)</td>
</tr>
<tr>
<td>Doses</td>
<td>12 (1 – 158)</td>
<td>12 (2-158)</td>
<td>6 (1-81)</td>
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<tr>
<td><strong>Total Number of Doses</strong></td>
<td>1403</td>
<td>1005</td>
<td>398</td>
</tr>
</tbody>
</table>

Abbreviations: CTCL, cutaneous T cell lymphoma; PTCL, peripheral T cell lymphoma; ECOG, Eastern Cooperative Oncology Group
<table>
<thead>
<tr>
<th></th>
<th>No. Electrocardiograms</th>
<th>Grade 0 [n, (%)]</th>
<th>Grade 1 [n, (%)]</th>
<th>Grade 2 [n, (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. T-wave and ST-segment abnormalities by time after administration of romidepsin</strong> (n=63, NIH cohort)</td>
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<td></td>
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<tr>
<td>Pretreatment</td>
<td>1101</td>
<td>898 (82)</td>
<td>192 (17)</td>
<td>11 (1)</td>
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<tr>
<td>Immediate posttreatment</td>
<td>1061</td>
<td>604 (57)</td>
<td>446 (42)</td>
<td>11 (1)</td>
</tr>
<tr>
<td>Day after treatment</td>
<td>1024</td>
<td>309 (30)</td>
<td>672 (66)</td>
<td>43 (4)</td>
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<tr>
<td>Unscheduled</td>
<td>447</td>
<td>246 (55)</td>
<td>181 (40)</td>
<td>20 (5)</td>
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<tr>
<td><strong>B. T-wave and ST segment abnormalities associated with the first dose of the first cycle (n = 63)</strong></td>
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<tr>
<td>Pretreatment</td>
<td>63</td>
<td>59 (94)</td>
<td>3 (5)</td>
<td>1 (&lt;2)</td>
</tr>
<tr>
<td>Immediate posttreatment</td>
<td>61</td>
<td>48 (79)</td>
<td>12 (20)</td>
<td>1 (&lt;2)</td>
</tr>
<tr>
<td>Day 2</td>
<td>62</td>
<td>27 (44)</td>
<td>33 (53)</td>
<td>2 (3)</td>
</tr>
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<td>Day 3</td>
<td>49</td>
<td>26 (53)</td>
<td>22 (45)</td>
<td>1 (2)</td>
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<td><strong>C. T-wave and ST segment abnormalities observed at any timepoint</strong></td>
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<tr>
<td>Cycle 1 only</td>
<td>63</td>
<td>8 (13)</td>
<td>49 (78)</td>
<td>6 (&lt;10)</td>
</tr>
<tr>
<td>All cycles</td>
<td>63</td>
<td>1 (&lt;2)</td>
<td>46 (73)</td>
<td>16 (25)</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Frequent APCs &gt;200/24 hours [n, (%)]</td>
<td>Frequent PVCs &gt;1000/24 hours [n, (%)]</td>
<td>Supraventricular tachycardia (SVT) [n, (%)]</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------</td>
<td>-------------------------------------</td>
<td>--------------------------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Baseline Holter Pre romidepsin</td>
<td>56</td>
<td>10 (18)</td>
<td>5 (9)</td>
<td>23 (41)</td>
</tr>
<tr>
<td>Telemetry during &amp; for 24 hours after romidepsin infusion*</td>
<td>59</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>6 (10)</td>
</tr>
<tr>
<td>Cycle 1 Day 1 Holter during &amp; for 24 hours post romidepsin infusion‡</td>
<td>19</td>
<td>5 (25)</td>
<td>0 (0)</td>
<td>6 (30)</td>
</tr>
</tbody>
</table>

†One event was due to line misplacement
*Some patients had both SVT and VT
~Percentages calculated using denominator of 57
‡This subset of patients had both a holter and telemetry on cycle 1 day 1
Figure Legends

Figure 1. Analysis of heart rate changes by quintile for cycle 1 day 1 of romidepsin, excluding patients on beta-blockers. Heart rate increases were noted at the end of the 4 hour infusion of romidepsin. The majority returned to baseline heart rate level 24 hours post romidepsin infusion.

Figure 2. Number of doses of romidepsin during which replacement of magnesium or potassium or both electrolytes was required per protocol. Only 619 doses (45%) did not require potassium or magnesium replacement.

Figure 3. Trends were observed in romidepsin-induced heart rate (HR) changes for patients carrying (A) ABCB1 1236C>T (15.5, 12.3, and 6.4 msec respectively; P=0.033), (B) ABCB1 2677G>T/A (14.4, 11.3, 5.6, 19.7, and 10.0 msec respectively; P=0.050 when GA and AA alleles were not accounted for), (C) ABCB1 3435C>T (15.2, 12.5, and 8.4 msec respectively; P=0.062), or combinations of these genotypes, including: (D) Diplotypes 1-5 (15.0, 14.8, 11.2, 9.8, and 6.2 msec respectively; P=0.047), (E) Diplotypes 6-10 (15.8, 13.5, 11.6, 8.5, and 6.5 msec respectively; P=0.062), and (F) Diplotypes 11-15 (14.6, 15.1, 11.2, 10.3, and 6.2 msec respectively; P=0.071). All P-values are derived from the Jonckheere-Terpstra test for trend, and patients who received beta-blockers were not included in this analysis.
Figure 1.

Heart Rate Changes For Cycle 1 Day 1 of Romidepsin Infusion
Excluding Patients on Beta-Blockers

- Quintile 1
- Quintile 2
- Quintile 3
- Quintile 4
- Quintile 5
Figure 2.
Figure 3
Electrocardiographic Studies of Romidepsin Demonstrate Its Safety and Identify a Potential Role for the KATP channel


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