A Phase I Study of Quisinostat (JNJ-26481585), an Oral Hydroxamate Histone Deacetylase Inhibitor with Evidence of Target Modulation and Antitumor Activity, in Patients with Advanced Solid Tumors

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Abstract

Purpose: To determine the maximum-tolerated dose (MTD), dose-limiting toxicities (DLT), and pharmacokinetic and pharmacodynamic profile of quisinostat, a novel hydroxamate, pan-histone deacetylase inhibitor (HDACi).

Experimental Design: In this first-in-human phase I study, quisinostat was administered orally, once daily in three weekly cycles to patients with advanced malignancies, using a two-stage accelerated titration design. Three intermittent schedules were subsequently explored: four days on/three days off; every Monday, Wednesday, Friday (MWF); and every Monday and Thursday (M-Th). Toxicity, pharmacokinetics, pharmacodynamics, and clinical efficacy were evaluated at each schedule.

Results: Ninety-two patients were treated in continuous daily (2–12 mg) and three intermittent dosing schedules (6–19 mg). Treatment-emergent adverse events included: fatigue, nausea, decreased appetite, lethargy, and vomiting. DLTs observed were predominantly cardiovascular, including nonsustained ventricular tachycardia, ST/T-wave abnormalities, and other tachyarrhythmias. Noncardiac DLTs were fatigue and abnormal liver function tests. The maximum plasma concentration (Cmax) and area under the plasma concentration–time curve (AUC) of quisinostat increased proportionally with dose. Pharmacodynamic evaluation showed increased acetylated histone 3 in hair follicles, skin and tumor biopsies, and in peripheral blood mononuclear cells as well as decreased Ki67 in skin and tumor biopsies. A partial response lasting five months was seen in one patient with melanoma. Stable disease was seen in eight patients (duration 4–10.5 months).

Conclusions: The adverse event profile of quisinostat was comparable with that of other HDACi. Intermittent schedules were better tolerated than continuous schedules. On the basis of tolerability, pharmacokinetic predictions, and pharmacodynamic effects, the recommended dose for phase II studies is 12 mg on the MWF schedule. Clin Cancer Res; 19(15): 4262–72. ©2013 AACR.

Introduction

Modification of histones by acetylation, in addition to other epigenetic processes, can play a key role in tumorigenesis (1). Histone deacetylase (HDAC) enzyme can affect chromatin condensation by deacetylating the lysine residues of histones that restrict access to transcriptional sites.
Quisinostat has been shown to have excellent tissue distribution and significant decrease in angiogenesis (8). Quisinostat has potent antitumor activity in murine models of multiple human cancers (7). In addition, quisinostat exhibits antitumor activity against a wide panel of cancer cell lines including lung, colon, breast, prostate, and ovarian cell lines at nanomolar concentrations. Pharmacodynamic efficacy was evidenced by increased acetylated histone 3 and decreased Ki67. A partial response lasting 5 months was seen in one patient with melanoma.

Translational Relevance
Epigenetic abnormalities are described in a wide variety of solid tumors and hematologic malignancies. Histone deacetylase inhibitors (HDACi) increase acetylation of histones and other proteins, leading to a modified histone-DNA complex and altered gene expression. This can lead to growth arrest, cellular differentiation, and apoptosis in preclinical models of cancer. Quisinostat is an orally available, potent, hydroxamate, pan-HDACi with broad activity in solid and hematologic tumor models. Here, we present the safety and efficacy data from a first-in-human, dose-escalation study in patients with advanced solid tumors treated on continuous and intermittent schedules. Quisinostat was well tolerated at the maximum-tolerated dose of 12 mg given 3 times weekly (Mon/Wed/Fri). The major dose-limiting toxicities were predominantly cardiovascular, including tachyarrhythmias and ST/T-wave abnormalities. Pharmacodynamic efficacy was evidenced by increased acetylated histone 3 and decreased Ki67. A partial response lasting 5 months was seen in one patient with melanoma.

Here, we report results of a first-in-human, phase I study in patients with advanced malignancies that determined dose-limiting toxicities (DLTs) and maximum-tolerated dose (MTD) of quisinostat and evaluated its safety and pharmacokinetic properties in patients with advanced stage or refractory solid malignancies and lymphoma. Also evaluated were the effect of food on pharmacokinetics of quisinostat, antitumor activity and pharmacodynamic effects in tumor and surrogate tissues.

Materials and Methods
Study population
Eligible patients were those 18 years of age or more with advanced solid tumors or lymphomas that were refractory to standard therapy, with Eastern Cooperative Oncology Group (ECOG) performance status score ≤2, life expectancy >3 months, and adequate gastrointestinal, hepatic, renal, bone marrow, and cardiac function. Patients at increased cardiac risk were excluded, including those with uncontrolled hypertension, unstable angina, myocardial infarction within the previous 12 months, left ventricular ejection fraction (LVEF) less than 50%, congestive heart failure of New York Heart Association (10) Class II–IV, any history of cardiomyopathy or ventricular arrhythmia, requirement for a cardiac pacemaker, or a family history of long QT syndrome. Patients were not allowed to take concomitant medications known to have a risk of causing QTc prolongation and torsades de pointes. Other exclusion criteria included: brain metastases, chemotherapy (in case of nitrosoureas or mitomycin C within 6 weeks) or radiotherapy or immunotherapy within 4 weeks before study drug administration, grade ≥2 neuropathy, or positive serology for hepatitis B, hepatitis C, or human immunodeficiency virus. Women who were pregnant, planning to become pregnant, or were nursing were excluded. Patients previously treated with HDACi were also excluded.

An Independent Ethics Committee at each study site approved the protocol. This study was conducted in accordance with the ethical principles originating in the Declaration of Helsinki and in accordance with International Conference on Harmonization (ICH) Good Clinical Practices guidelines, applicable regulatory requirements, and in compliance with the protocol. All patients provided written informed consent to participate in the study before undergoing any study-related procedures. A Data Review Committee was formed to ensure optimum study conduct.

Study design
This phase I dose-escalation study was conducted from September 2007 to September 2011 at 5 study centers in the United Kingdom. It included a 14-day screening phase, an open-label phase consisting of 21-day treatment cycles of quisinostat, and an end-of-study visit within 14 days after the last dose. The study was divided into 2 parts, a dose-escalation phase (part 1) and an expansion phase (part 2). The study drug was administered orally, once daily, starting with 2 mg/day dose with continuous treatment schedule. Cycle 1 was designated as the DLT period; DLT was defined as...
as either treatment interruption of more than 7 days for grade ≥2 toxicity during the 21-day cycle, grade 3 or grade 4 nonhematologic toxicity (excluding nausea or vomiting responsive to antiemetic treatment, alopecia, clinically tolerable diarrhea responding to antidiarrheal treatment, isolated grade 3 γ-glutamyl transeptidase elevations, grade 3 fatigue, grade 3 asthenia, and grade 3 troponin I rise in the absence of other evidence of myocardial damage indices), grade ≥3 nausea or vomiting despite adequate antiemetic treatment, persistent grade ≥2 nausea or vomiting despite antiemetic treatment for more than 7 days, grade ≥3 diarrhea despite adequate antidiarrheal treatment, or persistent grade ≥2 diarrhea despite antidiarrheal treatment for more than 7 days, grade 4 asthenia and grade 4 fatigue, or grade 4 hematologic toxicity (absolute neutrophil count of <500/mm³ for >7 days or with fever, platelet count <25,000/mm³). The MTD was defined as the highest dose at which less than 2 out of 6 patients experienced a DLT.

During part I of the study, a Korn’s two-stage accelerated titration design (11) was followed to minimize the number of patients exposed to subtherapeutic doses of quisinostat. Initially, 2 patients were included in each cohort, and 100% dose increments were applied. Following the first event of a drug-related grade ≥2 toxicity (except grade 2 fatigue, asthenia, anorexia and nausea, vomiting and diarrhea, if adequately treated within 7 days), subsequent treatment followed a conventional 3+3 dose-escalation design with up to 3 additional patients added if one patient exhibited a DLT. Further dose escalation was halted if at least 2 out of a maximum of 6 patients within a cohort exhibited a DLT. Dose-escalation decisions were based on the safety and pharmacokinetic data from cycle 1.

During part 2, additional patients were enrolled for safety, pharmacokinetics, antitumor activity, and pharmacodynamics assessments. In this phase, patients were treated at the MTD level and 19 patients were enrolled at 12 mg dosing on Monday, Wednesday, Friday (MWF) schedule.

Inpatient dose escalations were not allowed anytime during the study. Patients were discontinued from the study in the event of progressive disease or any unacceptable toxicity.

During cycle 1, pharmacokinetic, pharmacodynamic, and toxicity assessments were carried out on days -1, 1, 2, 8, 15, and 21. Interim safety evaluations were conducted on days 3, 8, 15, and 21 of cycle 1, on days 8, 15, and 21 of cycle 2, and on day 21 of subsequent cycles. During cycle 2, pharmacodynamic sampling was carried out on day 21. Modifications to the timing of pharmacokinetic, pharmacodynamic, and safety assessments were implemented for intermittent treatment schedules.

The study drug, quisinostat (Janssen Research and Development, LLC), was supplied as capsules for oral use, in strengths of 1, 5, and 20 mg. The study drug was administered within 5 minutes after breakfast and patients fasted at least for 2 hours after taking the study drug.

Continuous treatment schedule (starting with 2 mg/day dose) and intermittent treatment schedules, MWF, 4 days on/3 days off (starting with 6 mg/day dose), and Monday and Thursday (M-Th; starting with 8 mg/day dose) were evaluated in this study. The MTD was determined for all treatment schedules.

Safety evaluations

Safety assessments included monitoring for treatment-emergent adverse events (TEAE), DLTs, clinical laboratory tests, vital signs, physical examinations, and 12-lead electrocardiograms (ECG), and 24-hour Holter monitoring. Ejection fraction was assessed by either echocardiograms or multigated acquisition (MUGA) scans at baseline and at the end of cycle 2. Adverse events were evaluated in accordance with National Cancer Institute Common Terminology Criteria for Adverse Events, Version 3.0 (12), and were followed to a satisfactory resolution or a clinically stable endpoint.

Pharmacokinetic evaluations

Venous blood samples (3 mL) were collected for determination of plasma concentrations of quisinostat at following time points on day 1 and on the last day of study drug intake of the first cycle: predose (within 15 minutes before dosing), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 or 12, and 24 hours postdose across all schedules. In addition, predose sampling was done on days 8 and 15 in the continuous dosing regimen. In the intermittent dosing schedules, predose sampling was done on days 1 and 11 for both 4 days on/3 days off and M-Th schedules and on days 5 and 10 for MWF schedule. Plasma samples were analyzed for quisinostat using a selective and validated analytical method by Janssen Research & Development. The method is based on a protein precipitation extraction followed by liquid chromatography coupled to ion-spray tandem mass spectrometry in the positive ion mode. The method has a lower limit of quantitation of 0.1 ng/mL plasma.

Pharmacokinetic parameters. Noncompartmental analysis was conducted using WinNonlin (Pharsight Corporation, Version 5.2). Pharmacokinetic parameters calculated from plasma concentration–time data were maximum plasma concentration (Cmax), time to reach Cmax (tmax), and area under the plasma concentration–time curve (AUC) from 0 to the last measurable plasma concentration as calculated by the linear trapezoidal rule (AUC(0–last)); and apparent plasma terminal elimination half-life half-life t1/2 (whenever possible), where t1/2 = (ln2)/λz.

The plasma concentration–time curves were used to estimate Cmax tmax AUC from time 0 to 24 hours (AUC(0,24)) for days 1 and 21, and additionally the AUC from time 0 to infinity (AUC(0,∞)) for day 1.

Pharmacodynamic evaluations

Blood samples were collected for the assessment of acetylated histone 3 (AcH3) in peripheral blood mononuclear cells (PBMC). Sampling was conducted at predose, 2, 4, and 8 hours postdose on days 1 and 19 of cycle 1 on the MWF schedule and days 1 and 18 of cycle 1 for the 4 days on/3 days off.
off and M-Th schedules. PBMCs were collected only on the intermittent dosing schedules. Skin biopsies (punch biopsy), hair follicles, and tumor biopsies (in patients with anatomically accessible tumors, e.g., skin metastases) were collected during cycle 1 on day 1 (predose) and 3 to 6 hours postdose on days 18, 19, or 21 depending on the dosing schedule.

**Histone acetylation.** Blood samples collected in sodium citrate tubes (BD Vacutainer) were spun immediately at room temperature to separate plasma and PBMC. PBMCs were washed twice with phosphate buffered saline (also at room temperature), then snap frozen in dry ice, and stored at −80°C until further analysis.

AcH3 in PBMCs was measured by ELISA using Meso Scale Discovery (MSD) platform with Ruthenium-labeled goat anti-rabbit immunoglobulin G (detection antibody, MSD), Mouse pan antihistone monoclonal antibody (coating antibody, Millipore), and rabbit AcH3 polyclonal antibody (Millipore). The lower cutoff was defined as 0.0048 ng/ug protein based on the assay variability of the standard curve which was generated using an acetylated H3 peptide (Millipore).

AcH3 in skin and tumor biopsies from formalin-fixed paraffin-embedded blocks was detected in sections stained on a DAKO autostainer, which were deparaffinised and rehydrated for antigen retrieval by heat-induced antigen retrieval process in citrate buffer at pH 6 and 95°C for 30 minutes. Endogenous peroxidases were blocked with H2O2. Sections were incubated with primary antibody anti-histone 3 (Millipore) followed by peroxidase-labeled secondary antibody (Dako Envision system horseradish peroxidase-labeled polymer antimouse). Protein antibody complexes were visualized using 3,3-diaminobenzidine (DAB; Dako liquid DAB + substrate chromogen system). Hematoxylin was used for nuclear staining.

AcH3 in hair follicles were assessed by harvesting 3 to 4 hair follicles with intact bulb which was fixed in 10% formalin saline at 4°C. Antigen was retrieved at 95°C for 15 minutes with Target antigen retrieval solution (Dako, S1699) and blocked with 5% w/v nonfat dry milk in Tris buffered saline (50 mmol/L Tris, 150 mmol/L NaCl at pH 7.6). Follicles were incubated with Primary anti-Histone3 antibody (ab4729, Abcam) at 1:150 and secondary antibody, AlexaFluor 488 goat anti-rabbit (A11008, Invitrogen) at 1:1000; washed with carbocyanine monomer nucleic acid stain TO-PRO-3 at 1:10000 (T365 Invitrogen), and fluorescein isothiocyanate and Cy5 images were captured by confocal microscope (Leica Microsystems). The change in AcH3 was expressed as fold change (based on intensities of acetylated histone nuclear stain) between mean pretreatment and posttreatment staining.

**Ki67 analysis.** Ki67 was stained in skin and tumor biopsies as per immunohistochemical method for AcH3 estimation. Ki67 clone MIB-1 ready-to-use mouse monoclonal antibody (Dako) was used.

**Efficacy evaluations**

Baseline tumor assessments were conducted by CT or MRI within 4 weeks of starting study treatment, and efficacy was evaluated by repeat imaging and tumor markers (if appropriate) after every 2 treatment cycles until 8 cycles following which the imaging schedule was at investigators’ discretion. The tumor assessments were conducted using Response Evaluation Criteria in Solid Tumors version 1.0 (13) for solid tumors and the International Working Group Standardized Response Criteria (14) for lymphoma.

**Statistical analysis**

The pharmacokinetic and pharmacodynamic analysis sets included patients who had sufficient and interpretable

![Table 1. Demographic and baseline characteristics of patients](image-url)
data for treatment with quisinostat. The safety analysis set included all patients who received at least one dose of quisinostat. Descriptive statistics were used to summarize the pharmacokinetic, pharmacodynamic, safety, and efficacy results.

Results

Patient disposition and baseline characteristics
A total of 92 patients were enrolled in the study of which 52% were women. The median age of patients was 56 years (Table 1). Twenty patients received quisinostat on the original continuous treatment schedule. Subsequently, 3 intermittent treatment schedules were explored with 19 patients treated on 4 days on/3 days off treatment schedule, 38 patients on MWF treatment schedule and 15 patients on M-Th treatment schedule (Table 2).

Seventy-nine (86%) patients completed cycle 1. The median number of treatment cycles received by patients was 2 (range: 1–19); 7 patients received 6 or more cycles of treatment. The reasons for treatment discontinuations were disease progression (n = 73, 79.3%), adverse events (n = 12, 13%), withdrawal of consent (n = 2, 2.2%), death (n = 1, 1.1%), and other reasons (n = 4, 4.3%).

Safety

Dose escalation, DLTs, and determination of MTD. Ten (11%) patients experienced DLTs during the study (Table 2), which were predominantly cardiovascular. The MTD of quisinostat was found to be 8 mg with continuous treatment, 10 mg with 4 days on/3 days off treatment, 12 mg with MWF treatment, and 15 mg with M-Th treatment (Table 2). The MTDs were found to correlate inversely with dose intensity. Additional 19 patients were subsequently enrolled as an expansion of the MTD cohort at 12 mg MWF. Data obtained from the expansion cohort have confirmed 12 mg MWF as the recommended phase II dose.

DLT observed with the continuous treatment schedule included hyperbilirubinemia at 6 mg, an event that did not reoccur after dose reduction to 4 mg. Two patients experienced cardiac toxicity with asymptomatic nonsustained ventricular tachycardia (NSVT) in 1 patient at 8 mg and symptomatic NSVT (palpitations, dyspnea, and dizziness) in another patient at 12 mg. One patient at 12 mg experienced dose-limiting fatigue requiring repeated dose reduction. DLTs observed on the 4 days on/3 days off treatment at 12 mg included asymptomatic NSVT in one patient and significant electrocardiographic ST-T segment abnormalities in another. DLTs observed on the MWF treatment at 12 mg included QTc prolongation in one patient and hypertension in combination with asymptomatic troponin I increase in another patient that remained unexplained by further investigations. DLTs observed on the M-Th treatment at 19 mg included one event of supraventricular tachycardia/atrioventricular nodal reentry tachycardia in a patient with a medical history of supraventricular tachycardia.

Table 2. Dose-escalation scheme, DLTs observed, and determination of MTD for different schedules of quisinostat

<table>
<thead>
<tr>
<th>Treatment schedule/dose</th>
<th>2 mg</th>
<th>4 mg</th>
<th>6 mg</th>
<th>8 mg</th>
<th>10 mg</th>
<th>12 mg</th>
<th>15 mg</th>
<th>16 mg</th>
<th>19 mg</th>
<th>DLTs Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>QD schedule (n = 20)</td>
<td>n = 2</td>
<td>n = 2</td>
<td>n = 6</td>
<td>n = 8</td>
<td>–</td>
<td>n = 2, 1 DLT</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1 Hyperbilirubinemia, 2 Ventricular tachycardia, 3 Fatigue</td>
</tr>
<tr>
<td>4 on/3 off schedule (n = 19)</td>
<td>–</td>
<td>–</td>
<td>n = 3</td>
<td>n = 3</td>
<td>n = 6, 0 DLTs</td>
<td>n = 7, 2 DLTs</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4 Ventricular tachycardia, 5 T-wave inversion, ST depression, Chronic hepatic failure</td>
</tr>
<tr>
<td>MWF schedule (n = 38)</td>
<td>–</td>
<td>–</td>
<td>n = 3</td>
<td>n = 3</td>
<td>n = 6, 2 DLTs</td>
<td>–</td>
<td>n = 6, 0 DLTs</td>
<td>–</td>
<td>–</td>
<td>6 Ventricular tachycardia, 7 Prolonged QTc interval, 8 Hypertension, elevated troponin</td>
</tr>
<tr>
<td>M-Th schedule (n = 15)</td>
<td>–</td>
<td>–</td>
<td>n = 3</td>
<td>–</td>
<td>n = 3, 0 DLTs</td>
<td>–</td>
<td>n = 3, 2 DLTs</td>
<td>–</td>
<td>–</td>
<td>9 Cardiac T-wave abnormalities, 10 Atrioventricular nodal re-entry tachycardia</td>
</tr>
</tbody>
</table>

NOTE: Four different treatment schedules were evaluated. The DLTs were predominantly cardiovascular. At the recommended phase II dose of 12 mg MWF, 2 of 26 DLTs were seen.

Abbreviation: QD, every day.
A Continuous treatment.
B 4 days on/3 days off treatment.
C MWF treatment.
D M-Th treatment.
tachycardia and one patient showing significant changes in the ST segment/T-wave on ECGs (Table 2).

Treatment-emergent adverse events

Eighty-two (89%) patients experienced TEAEs that were considered at least possibly related to study drug; the most frequently reported (occurring in \( \geq 10\% \) of patients) drug-related TEAEs were fatigue, lethargy, nausea, decreased appetite, ventricular tachycardia, vomiting, and cardiac disorders (Table 3). The majority of these TEAEs were mild to moderate in severity. Fatigue or lethargy was the most common side effect reported and was seen in 57.6% of patients; however, only in one instance fatigue was the DLT and in general it was less common in the intermittent regimens (Table 3). Of note, at the recommended dose of 12 mg MWF, grade 3 fatigue/lethargy was seen in 3 of 26 (12%) patients. Nausea, decreased appetite, and vomiting were reported in 32%, 22%, and 17% of patients, respectively. There were no grade 3/4 events of nausea and one case of grade 3 vomiting was reported in the entire trial. These symptoms were well controlled by antiemetics such as domperidone and cyclizine.

Cardiac arrhythmia has been identified as an adverse effect of quisinostat. In 27% of patients, increased ventricular ectopic activity has been observed on serial Holter recordings (Supplementary Table S1). Most typically, this presented itself as a gradual increase in number of ventricular extrasystoles over time, sometimes associated with short runs of NSVT, and was asymptomatic in the majority of cases. In 2 patients on 16 mg MWF treatment, a substantial increase in ventricular extrasystoles up to >3,000/24 hours was associated with palpitations. The latter events were taken into account for setting 12 mg as the MTD on the MWF regimen. In one patient on 12 mg continuous regimens, increased ventricular ectopy was associated with palpitations, dizziness, and breathlessness requiring discontinuation of study drug. There was no evidence of QTc prolongation based on statistical analysis or based on a pharmacokinetic versus QTc correlation analysis. No effect on LVEF was observed in patients with serial measurements at baseline and on study drug. Two patients receiving 8 mg of quisinostat died during the study: one on continuous treatment schedule (pneumonia) and the other on MWF treatment schedule

<table>
<thead>
<tr>
<th>Table 3. TEAE considered at least possibly related to study drug, experienced by at least 3% of patients (highest grade per event per patient)</th>
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<tbody>
<tr>
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<tr>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
</tr>
<tr>
<td>Nausea</td>
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<tr>
<td>Decreased appetite</td>
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<tr>
<td>Ventricular tachycardia</td>
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<tr>
<td>Lethargy</td>
</tr>
<tr>
<td>Vomiting</td>
</tr>
<tr>
<td>Cardiac disorder(^e)</td>
</tr>
<tr>
<td>Palpitations</td>
</tr>
<tr>
<td>Diarrhea</td>
</tr>
<tr>
<td>Dysgeusia</td>
</tr>
<tr>
<td>Headache</td>
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<tr>
<td>Thrombocytopenia</td>
</tr>
<tr>
<td>Ventricular extrasystoles</td>
</tr>
<tr>
<td>Constipation</td>
</tr>
<tr>
<td>Edema peripheral</td>
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<tr>
<td>Dyspnoea</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Paraesthesia</td>
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<tr>
<td>Weight loss</td>
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</tbody>
</table>

Abbreviation: QD, every day.
\(^a\)Continuous treatment.
\(^b\)4 days on/3 days off treatment.
\(^c\)MWF treatment.
\(^d\)M-Th treatment.
\(^e\)Cardiac disorders reported: ST-T segment changes (n = 10), QTc prolongation G1 (n = 1), QTc prolongation G2 (n = 2), QTc prolongation G3 (n = 2), supraventricular tachycardia (n = 1), atrial fibrillation (n = 1), NSVT (n = 1).
Absorption of quisinostat was rapid with \( t_{\text{max}} \) ranging between 1 and 6 hours across all treatment schedules and doses. Generally, the \( C_{\text{max}} \) and AUC\(_{0-\text{last}} \) values increased roughly proportionally with dose on day 1 (Supplementary Table S2) as well as at the steady state (Table 4) although no formal testing was conducted as the dose range was limited. The concentration–time profile exhibited a biexponential decline; the median half-life with the 12 mg dose with MWF treatment schedule was 8.8 hours (range 2.4–11.7; Supplementary Table S2). Overall, pharmacokinetics of quisinostat was similar for continuous and intermittent treatment schedules apart from plasma drug exposure, which was numerically higher in MWF schedule. The pharmacokinetic profile at the recommended phase II dose achieved drug concentration that was known to be effective in animal models (Supplementary Fig. S1). In the 13 patients where pharmacokinetics was evaluated in fed and fasted state at 6 and 8 mg once daily dosing to ascertain food effect, no clinically meaningful effect was noted on drug absorption (AUC\(_{\text{fasted}} \)\/AUC\(_{\text{fed}} \) ratio: 1.03). Food intake, however, seemed to have a marginal effect on the rate of absorption (\( C_{\text{max}} \) fasted/fed ratio: 0.87).

**Pharmacodynamics**

Acetylation of histone 3 and Ki67. Treatment with quisinostat resulted in increased levels of AcH3 in PBMC, skin biopsies, and hair follicles at all evaluated doses in the intermittent treatment schedules. In PBMCs, the greatest change from baseline levels varied between 35% and 548% in individual samples, and mean increase per dose level ranged between 126% and 331% (Fig. 1A). There was evidence of an increase in levels of AcH3 measured in hair follicles following treatment with quisinostat (Fig. 1B) and there was a trend toward dose-dependent increase in histone 3 acetylation, starting at 6 mg dose level, \( (R^2 = 0.323; \text{Fig. 1C}) \). In skin biopsies, proliferation index (% Ki67-labeled nuclei) generally decreased after the treatment (data not shown). Three matched pre- and posttumor biopsies were conducted and it was possible to show changes in pharmacodynamic biomarkers in all the 3 paired samples (Fig. 1C).

**Efficacy**

There was one confirmed partial response observed in the study (Fig. 2A). A woman with metastatic melanoma who had previously been treated with dacarbazine had complete (progressive disease); both events were not considered to be study drug related.

No clinically relevant changes were observed in clinical laboratory values, vital signs, and physical examinations. There were no observations of grade 3/4 neutropenia or grade 3/4 thrombocytopenia.

### Table 4. Summary of pharmacokinetic parameters of quisinostat estimated at steady state

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( C_{\text{max}} ) (ng/mL)</th>
<th>AUC(_{0-\text{last}} ) (ng.h/mL)</th>
<th>( t_{\text{max}} ) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mg (( n = 5 ))</td>
<td>0.828 (0.387–1.71)</td>
<td>3.62 (2.09–9.21)</td>
<td>2.00 (0.50–3.23)</td>
</tr>
<tr>
<td>8 mg (( n = 5 ))</td>
<td>1.24 (0.227–2.19)</td>
<td>9.77 (0.110–20.7)</td>
<td>4.08 (0.50–4.12)</td>
</tr>
<tr>
<td>12 mg (( n = 2 ))</td>
<td>0.870–3.44</td>
<td>7.02–18.8</td>
<td>1.45–4.07</td>
</tr>
<tr>
<td>4 on/3 off</td>
<td>6 mg (( n = 2 ))</td>
<td>0.371–0.787</td>
<td>4.88–6.75</td>
</tr>
<tr>
<td>8 mg (( n = 2 ))</td>
<td>1.16–2.49</td>
<td>7.69–12.0</td>
<td>2.83–3.00</td>
</tr>
<tr>
<td>10 mg (( n = 4 ))</td>
<td>1.05 (0.443–1.41)</td>
<td>8.21 (7.29–11.0)</td>
<td>2.30 (0.98–4.10)</td>
</tr>
<tr>
<td>6 mg (( n = 3 ))</td>
<td>1.53 (0.647–1.79)</td>
<td>10.6 (1.96–10.6)</td>
<td>3.03 (0.98–3.05)</td>
</tr>
<tr>
<td>8 mg (( n = 2 ))</td>
<td>1.75–4.31</td>
<td>18.8–35.9</td>
<td>2.05–4.07</td>
</tr>
<tr>
<td>12 mg (( n = 16 ))</td>
<td>1.90 (0.693–6.57)</td>
<td>13.5 (7.23–27.0)</td>
<td>3.08 (1.56–6.00)</td>
</tr>
<tr>
<td>16 mg (( n = 6 ))</td>
<td>2.44 (1.41–3.35)</td>
<td>18.1 (10.8–56.1)</td>
<td>2.59 (1.43–7.97)</td>
</tr>
<tr>
<td>M-Th</td>
<td>8 mg (( n = 3 ))</td>
<td>0.653 (0.478–2.44)</td>
<td>3.96 (2.53–14.0)</td>
</tr>
<tr>
<td>12 mg (( n = 3 ))</td>
<td>2.78 (2.26–4.54)</td>
<td>16.3 (14.1–18.0)</td>
<td>3.08 (1.03–3.25)</td>
</tr>
<tr>
<td>15 mg (( n = 5 ))</td>
<td>1.82 (0.514–7.07)</td>
<td>14.2 (3.49–39.0)</td>
<td>3.08 (2.17–5.92)</td>
</tr>
<tr>
<td>19 mg (( n = 2 ))</td>
<td>1.66–1.86</td>
<td>12.2–13.0</td>
<td>2.05–2.37</td>
</tr>
</tbody>
</table>

Note: Four treatment schedules were studied. The data summarize the pharmacokinetic parameters at steady state.

Abbreviation: QD, every day.

\( ^a \)Continuous treatment.

\( ^b \)Days on/3 days off treatment.

\( ^c \)MWF treatment.

\( ^d \)M-Th treatment.
regression of subcutaneous metastases with 94% reduction in target lesions after one cycle of 12 mg every day, following which treatment was discontinued because of cardiac toxicity. The duration of response was sustained for 5 months. Tumor shrinkage was also shown in 2 additional patients with metastatic melanoma who were treated with 12 mg and 8 mg (both as continuous treatment) with 21% and 13% decrease in size of target lesions, respectively (Fig. 2B and C). Stable disease lasting 4 to 6 months was seen in 6 patients treated in continuous, 4 days on/3 days off, and MWF treatment schedules with the following tumor types: castrate-resistant prostate cancer, non–small cell lung cancer, nasopharyngeal carcinoma, melanoma, Bartholin’s gland adenocarcinoma, and cholangiocarcinoma.

**Recommendation of phase II dose**

The dose of 12 mg given 3 times a week (MWF) was chosen as the recommended phase II dose. The factors that were taken into consideration were toxicity, pharmacokinetic, and pharmacodynamic profiles. There were no events of ventricular tachycardia reported on 12 mg MWF schedule, and although there were no DLTs in 6 patients treated at 16 mg MWF, increased ventricular extrasystoles were present in 2 of 6 (33%) patients at that dose. An intermediate dose of 14 mg MWF was not explored as it was felt that there would be significant overlap in the drug concentrations between that dose and doses of 14 mg (MTF) and 15 mg (MT) where 1 of 6 (18%) patients expressed ventricular extrasystoles. In addition, pharmacokinetic parameters at the dose level of 12 mg suggested drug concentrations consistent with response in preclinical models. Pharmacodynamic studies suggested target modulation at doses of 8 mg or above. An expansion cohort at the 12 mg MWF dose confirmed the safety profile of quisinostat.

**Discussion**

Quisinostat is a pan-HDACi with potent activity against both class I and II HDACs at nanomolar concentrations (30-100 nmol/L), inducing acetylation of histones H3 and H4 (class I/II HDAC inhibition) and tubulin (HDAC6 inhibition). Quisinostat also induces the expression of p21 and E-cadherin (genes silenced by HDACi) and inhibits activity of HSP90 in tumor cells (7). Preclinical studies of quisinostat have shown prolonged and sustained
Acetylation of H3 in tumor tissue and activity in multiple xenograft models (7). This study shows that quisinostat can be safely administered orally in humans with a tolerable side effect profile and evidence of target modulation. The MTD of quisinostat was found to be 8 mg with continuous treatment. Higher doses were tolerated with intermittent treatment schedules; MTD values for intermittent treatments ranged between 10 to 15 mg. The recommended phase II dose of quisinostat is 12 mg MWF.

Overall, the safety profile of quisinostat in the present study was similar to other HDACi, which are in clinical development or approved for the treatment of patients with cancer. Previously reported HDACi toxicities include: fatigue, asthenia, nausea, vomiting, diarrhea, cardiac toxicities, and hematologic toxicity (predominantly thrombocytopenia; 4). Cardiac toxicities, in particular, conduction abnormalities and rhythm disturbances ranging from nonspecific T-wave and ST segment changes to ventricular tachycardia have been well documented with a number of other HDACi (4, 15–17). A phase II study of depsipeptide in patients with neuroendocrine tumors was terminated because of sudden death related to ventricular arrhythmia (18). In a previous study with romidepsin, 2 patients experienced drug-related asymptomatic ventricular tachycardia (19). The phase II study of romidepsin in patients with CTCL revealed ST segment changes and T-wave flattening in nearly two-thirds of patients and ventricular/supraventricular tachycardia in 34% of patients but without any deaths (20). Atrial fibrillation and prolonged QTc interval have also been associated with belinostat (16), and QT prolongation is a class effect of HDACi (21). In this study, extensive cardiac monitoring was incorporated and...
A Phase I Study of Quisinostat (JNJ-26481585), an HDAC Inhibitor

Preclinical studies of quisinostat have shown activity in variety of cell line models including lung, breast, ovarian, and prostate cancer (7) in addition to hematologic malignancies such as myeloma (22). However, the only previous indication where HDACi have been licensed is CTCL, and a study of quisinostat for the treatment of CTCL revealed significant anticancer activity (23).

Conclusion
Quisinostat was well tolerated and showed promising antitumor activity in patients with advanced solid tumors, in particular melanoma. The favorable pharmacokinetic and pharmacodynamic properties and acceptable safety profile of quisinostat observed in this study warrants further development of quisinostat as a monotherapy or in combination with other anticancer agents with synergistic activity and nonoverlapping toxicity. The 12 mg dose given 3 times weekly (MWF) is recommended for phase II development.

Disclosure of Potential Conflicts of Interest
R. Baird has received reimbursement from [JNJ] Pharmaceuticals for travel expenses to present trial results at ASCO. N. Fourneau is employed (other than primary affiliation; e.g., consulting) as an associate director in [JNJ]. P. Helleman is employed (other than primary affiliation; e.g., consulting) as a director clinical research in Johnson & Johnson. Y. Elsayed is employed (other than primary affiliation; e.g., consulting) as vice president, hematology malignancies, and has ownership interest (including patents) in Johnson and Johnson. J. Camm has a commercial research grant for reviewing cardiovascular events and is a consultant/advisory board member providing advice about cardiovascular adverse events. T.R.J. Evans has other commercial research support from Johnson & Johnson and is a consultant/advisory board member of Kanus Therapeutics. U. Banerji has a commercial research grant from Johnson & Johnson Pharmaceutical R&D and The Institute of Cancer Research. No potential conflicts of interest were disclosed by the other authors.

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cardiologist’s advice was sought wherever appropriate. There were no treatment-related deaths. Dose-limiting NSVT, which resolved after treatment discontinuation, was noted in 3 patients on 8 and 12 mg continuous treatment and on 10 mg 4 days on/3 days off treatment. The latter observations have led to the elimination of the continuous and 4 days on/3 days off schedules from further development. One patient out of 26 experienced grade 3 QTcF prolongation at 12 mg MWF. Safety and tolerability were improved with intermittent treatment compared with continuous treatment, which is similar to other HDACi. No cases of ventricular tachycardia were observed at the R2PD of 12 mg MWF. Unlike other HDACi, grade 3 or 4 hematologic toxicities were absent, and thrombocytopenia grade 1/2 was noted in only 5% of patients and none in the MWF schedule. Quisinostat displays linear pharmacokinetics for AUC and Cmax across all treatment schedules with single as well as multiple dosing. Overall, the pharmacokinetic profile of continuous and intermittent treatment administration was comparable, however, it should be noted that plasma exposure was higher in the MWF treatment schedule. Of note, the estimated median effective half-life of 8.8 hours in patients receiving the recommended dose, 12 mg on MWF treatment schedule is significantly longer compared with other approved HDACi; the plasma half-life of vorinostat is 91 to 127 minutes (17) and mean half-life of romidepsin is 0.42 hours (6). Thus, the pharmacokinetic profile of quisinostat is more likely to result in sustained drug exposure, a finding that is consistent with preclinical data. Quisinostat is rapidly absorbed upon oral administration and food intake does not seem to affect the pharmacokinetics, thus improving convenience for patients and treatment compliance.

Quisinostat displays evidence of interaction with its biologic targets by increasing the acetylation of histones both in tumor and surrogate tissues and also reduces the proliferation index of tumors. Acetylation of histones H3/H4 in surrogate and tumor tissue is widely accepted as a pharmacodynamic biomarker for HDAC inhibition (4). With quisinostat treatment, increased acetylation was noted in most surrogate tissue regardless of the dosing schedule. Similarly, the proliferation index, assayed by Ki67 staining was reduced in patients treated with quisinostat whose tumors and surrogate tissues were evaluable.

Treatment with quisinostat resulted in disease control (partial response or stable disease) in 9 (9.8%) patients who were heavily pretreated. Continuous daily dosing was associated with antitumor activity (Fig. 2) as evidenced in patients with melanoma but was associated with significant toxicity. Therefore, in addition to the continuous treatment schedule, alternative schedules of administration were evaluated in this phase I study.

On the basis of safety, efficacy, pharmacokinetic, and pharmacodynamic results, the recommended dose of quisinostat for phase II trials is 12 mg on the MWF treatment schedule.
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References

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