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Effects of *UGT1A1* Genotype on the Pharmacokinetics, Pharmacodynamics, and Toxicities of Belinostat Administered by 48-Hour Continuous Infusion in Patients With Cancer

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Abstract

The histone deacetylase inhibitor belinostat is eliminated through glucuronidation by UGT1A1. Polymorphisms that reduce UGT1A1 function could result in increased belinostat exposure and toxicities. We wanted to determine which single-nucleotide polymorphisms alter belinostat exposure and toxicity. In a phase I trial (belinostat over 48 hours in combination with cisplatin and etoposide), belinostat (400, 500, 600, or 800 mg/m²/24h, 48-hour continuous infusion) was administered to patients with cancer in combination with cisplatin and etoposide (n = 25). Patients were genotyped for *UGT1A1* variants associated with reduced function : *UGT1A1**6, *UGT1A1**28, and *UGT1A1**60. End points were associations between *UGT1A1* genotype and belinostat pharmacokinetics (PK), toxicities, and global protein lysine acetylation (AcK). Belinostat AUC

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Declaration of Conflicting Interests

No potential conflicts of interest were disclosed.

Presentation Elsewhere

This study was presented in part at the 2015 Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics, March 3–7, 2015, New Orleans, Louisiana; at the AACR Annual Meeting 2015, April 18–22, 2015, Philadelphia, Pennsylvania; and as abstract (online only) for the 2015 ASCO Annual Meeting, May 29–June 2, 2015, Chicago, Illinois.

was increased ($P = .003$), and $t_{1/2}$ increased ($P = .0009$) in *UGT1A1**28 and *UGT1A1**60 carriers who received more than 400 mg/m²/24 h. The incidence of grades 3–4 thrombocytopenia ($P = .0081$) was associated with *UGT1A1* polymorphisms. The US Food and Drug Administration-approved package insert recommends dose adjustment of belinostat for *UGT1A1**28. However, our data suggest dose adjustment is also necessary for *UGT1A1**60. *UGT1A1* polymorphisms were associated with increased systemic belinostat exposure, increased AUC, and increased incidence of toxicities, particularly at doses > 400 mg/m²/24h.

Keywords

belinostat; *UGT1A1*; pharmacogenomics; pharmacokinetics; pharmacodynamics

In 2014, the US Food and Drug Administration approved the histone deacetylase (HDAC) inhibitor belinostat (BELEODAQ, Spectrum Pharmaceuticals, Inc., Henderson, Nevada) for treatment of relapsed or refractory peripheral T-cell lymphoma.¹ Belinostat-induced HDAC inhibition is reversible,² and its elimination half-life ($t_{1/2}$) is only 1.1 hours. We therefore studied a continuous intravenous infusion (CIVI) of belinostat over 48 hours to prolong exposure in a phase 1 trial in patients with advanced cancer in combination with cisplatin and etoposide (BPE).

We began investigation of belinostat metabolism after a patient in the BPE trial had persistent QTc prolongation. This individual was found to have the highest steady-state plasma concentration (C_{ss}) of all study participants. We then identified several polymorphisms for this individual in the uridine diphosphate glucuronosyltransferase 1 family, polypeptide A1 (*UGT1A1*). Based on this observation, along with emerging preclinical data on belinostat metabolism through *UGT1A1*,³ we undertook a comprehensive evaluation of *UGT1A1* genotyping in patients enrolled in the BPE trial.

Several *UGT1A1* polymorphisms decrease gene expression, leading to reduced bilirubin conjugation and hyperbilirubinemia, which, for example, occurs in patients with Gilbert syndrome. The polymorphism generally associated with Gilbert syndrome is *UGT1A1**28 (rs8175347), which contains an extra TA repeat (A[TA]₇TAA) in the *UGT1A1* promoter region⁴ and is frequently expressed among whites (26%–31%).⁵ Furthermore, *UGT1A1**28 in patients treated with irinotecan is associated with increased SN-38 plasma concentrations leading to irinotecan-induced neutropenia,⁶ and therefore a dose reduction of irinotecan is recommended in patients homozygous for *UGT1A1**28.⁷ *UGT1A1**60 (3279T>G, rs4124874) is in linkage disequilibrium with *UGT1A1**28⁸ and is another frequent genetic variant in whites (allele frequency; 53%). This variant is associated with reduced glucuronidation and increased bilirubin levels in Japanese patients treated with irinotecan⁹ and with hematological toxicities in white patients.¹⁰ Similar to *UGT1A1**28 and *UGT1A1**60, *UGT1A1**6 (211G>A, rs4148323), with an allele frequency of 10%–23% in Asians,¹¹ is also associated with impaired UGT1A1 activity, reduced glucuronidation of SN-38, and irinotecan-induced neutropenia.¹²

Given its recent approval, there is limited information regarding the clinically important metabolic pathways of belinostat. The belinostat package insert recommends a dose

reduction of belinostat to 750 mg/m² in patients homozygous for *UGT1A1**28 to minimize dose-limiting toxicities.¹ However, this recommendation is only supported by 1 preclinical study, reporting that UGT1A1-glucuronidation is the main metabolic pathway of belinostat, and human liver microsomes (HLMs) heterozygous and homozygous for *UGT1A1**28 metabolized belinostat more slowly than wild-type HLMs.³ No clinical study has evaluated the effect of *UGT1A1* polymorphisms on the pharmacokinetics (PK) and pharmacodynamics (PD) of belinostat.

Based on this background, we conducted a pharmacogenomic (PG) analysis in which effects of *UGT1A1* polymorphisms on systemic belinostat exposure, protein lysine acetylation and toxicities were evaluated in patients with cancer receiving BPE.

Methods

Patients and Treatment

All patients provided written informed consent before study enrollment, and genotyping was approved by the Institutional Review Board of the National Cancer Institute (NCI) in Bethesda, Maryland.

PK, PD, and PG analyses were performed in patients participating in the BPE trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00926640) Identifier: NCT00926640) at the NCI. Patients with advanced cancer (n = 25) received belinostat (day 1: 400, 500, 600, or 800 mg/m²/24h as 48-hour continuous intravenous infusion) in combination with cisplatin (60 mg/m² intravenously on day 2) and etoposide (80 mg/m² intravenously daily on days 2–4). Patients > 18 years old were included with histologic or cytologic confirmation of cancer for which there is no known standard therapy capable of extending life expectancy. Other main inclusion criteria were Eastern Cooperative Oncology Group (ECOG) performance status 0–2, life expectancy ≥ 3 months, absolute neutrophil count ≥ 1500/mm³, platelets ≥ 100 000/mm³, total bilirubin ≤ 1.2 mg/dL, aspartate aminotransferase (AST) and alanine transaminase (ALT) ≤ 2.0 x upper limit of normal (ULN), creatinine ≤ 1.5 x ULN, or creatinine clearance > 50 mL/min/1.73 m². Exclusion criteria included treatment with another HDAC inhibitor for at least 2 weeks prior to enrollment, acute or chronic hepatitis, cardiovascular disease (NYHA class III or IV), QTc interval prolongation (> 450 milliseconds) at baseline, or use of concomitant medications associated with QTc interval prolongation.

PK Analysis of Belinostat

PK analysis of belinostat was performed during the first treatment cycle. BPE blood samples were collected before treatment and 30 minutes, 1 hour, and 2 hours after the start of belinostat infusion; on day 2 immediately prior to the start and at the end of cisplatin infusion and 15 minutes, 30 minutes, 1, 2, 4, and 6 hours post-end of cisplatin infusion; on day 3 immediately prior to the start of etoposide infusion and 5, 15, and 30 minutes and 1, 2, 4, 6, 8, and 12 hours post-end of belinostat infusion.

At the Clinical Pharmacology Program Blood Processing Core, blood samples were centrifuged at 1200g for 5 minutes at 4°C. Subsequently, the plasma layer was transferred into 2 cryovials and stored at –80°C until analysis. Belinostat plasma concentrations were

measured using a validated ultraperformance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) assay with a lower limit of quantification of 5 ng/mL. Belinostat was extracted from 100uL of plasma using 1mL of methyl tert-butyl ether, dried down and resuspended before injection into the UPLC-MS/MS system.

A noncompartmental analysis was performed using Phoenix WinNonlin 6.3 (Certara Pharsight, Cary, North Carolina), and the following PK parameters were obtained: area under the plasma concentration-time curve (AUC), maximum plasma concentration ($C_{\max}$), total body clearance (CL), and $t_{1/2}$.

PD Effects in Peripheral Blood Mononuclear Cells

The extent of global protein lysine acetylation (AcK) in peripheral blood mononuclear cells (PBMCs) was used as a surrogate marker for the HDAC inhibiting effects of belinostat. For this purpose, PBMCs were collected prior to belinostat infusion and 12, 36, and 60 hours after the start of infusion. Analysis of AcK was performed by multiparametric flow cytometry using a MACSQuant flow cytometer (Miltenyi Biotec, Bergisch Gladbach, Germany) as described previously,¹³ after which fold change of AcK postdose relative to predose was calculated.

Toxicities

Incidence of the following belinostat-related toxicities during the first treatment cycle was reported: thrombocytopenia, lymphocytopenia, neutropenia, nausea, vomiting, fatigue, and QTc as a surrogate marker for QTc-prolonging effects of belinostat (QTc = mean QTc interval during cycle 1 - QTc interval at baseline).

UGT1A1 Genotyping

*UGT1A1**6, *UGT1A1**28, and *UGT1A1**60 genetic variants were determined with fragment analysis or direct sequencing. Details about the used genotyping methods are presented in the Supplemental Methods.

Statistical Considerations

An exact Cochran-Armitage trend test was used for comparisons between dichotomous parameters (eg, grades 0–2/3–4 hematological toxicities, sex) and ordered categorized genotypes (ie, wild type vs heterozygous vs homozygous). Comparisons between continuous parameters (eg, PK parameters, AcK fold change, QTc change, baseline laboratory values) and ordered categorized genotypes were determined using a Jonckheere-Terpstra test for trend. Comparisons between 2 dichotomous parameters were performed with Fisher's exact test. Hardy-Weinberg equilibrium (HWE) of *UGT1A1**28 and *UGT1A1**60 genotypes was tested by the χ^2 test ($\alpha = .05$ was the threshold for statistical significance). The sample size for our study was determined by the number of patients who completed the BPE trial. No separate sample size determination was carried out for this report, because this is based on patients from the BPE study, which was powered to meet other clinical objectives. All *P* values are 2-tailed and reported without any formal adjustment for multiple comparisons. However, in view of the number of exploratory tests performed, only tests with $P < .01$ were interpreted as statistically significant, whereas those for which $.01 < P < .05$ were considered

to represent strong trends toward statistical significance. Statistical analyses were carried out with GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, California), SAS version 9.3 (SAS Institute, Cary, North Carolina), and IBM SPSS Statistics 22 (IBM Corp., Armonk, New York).

Results

Demographics and *UGT1A1* Genotyping

Twenty-five patients were enrolled on the BPE trial. Baseline characteristics and *UGT1A1* genotyping results are shown in Table I. In our patient population only 1 patient carried the *UGT1A1**6 variant; thus, this polymorphism was excluded from the PG analysis. *UGT1A1**28 and *UGT1A1**60 genotypes were in HWE ($P > .05$).

The majority of baseline laboratory values were comparable for genetic variants of *UGT1A1**28, *60 and combinations of these variants. Only albumin levels significantly trended downward in *UGT1A1**28 carriers compared with *UGT1A1**28 wild types ($P = .0022$; Supplemental Table S1). Other liver function tests did not significantly differ across the evaluated *UGT1A1* genotypes ($P > .01$). Overall, patients' demographics were comparable for the investigated *UGT1A1* genotypes, although men tended to be wild type and women tended to be variant in the combined *28/*60 analysis ($P = .0079$; otherwise $P > .01$; Supplemental Table S1). Because the stratified genotypes were generally comparable in regard to baseline liver function and demographics, these parameters were unlikely to be confounding factors in this PG analysis.

Effect of *UGT1A1* Genetic Variants on Belinostat PK

Table 2 (showing dose-normalized values) and Supplemental Table S2 list averaged and genotype-stratified PK parameters of belinostat during cycle 1. *UGT1A1**28 genetic variants were not associated with belinostat AUC, CL, $t_{1/2}$ or C_{max} ($P > .01$). However, patients heterozygous and homozygous for *UGT1A1**60 tended to have significantly increased $t_{1/2}$ compared with *UGT1A1**60 wild types ($P = .0077$; Supplemental Table S2). No significant associations between belinostat PK and genotype were found in the combined variant analysis of *UGT1A1**28 and *UGT1A1**60 ($P > .01$; Figure 1 A-D).

Because effects of the *UGT1A1* genotype on the risk of irinotecan-induced neutropenia are greater at high irinotecan doses, 14–15 we hypothesized that the *UGT1A1* effects on belinostat PK and toxicities could also be dose dependent. Therefore, associations between *UGT1A1* genotype and belinostat PK were reanalyzed after exclusion of patients at the lowest belinostat dose level of 400mg/m²/24h ($n = 10$). Now, dose-normalized AUC ($P = .003$; Figure 2A) and $t_{1/2}$ ($P = .0009$; Figure 2D) were significantly increased in carriers of *UGT1A1**28 and/or *UGT1A1**60, whereas a decreasing trend of CL was found in these patients ($P = .023$; Figure 2C). Also dose-normalized C_{max} tended to be increased in these patients ($P = .061$; Figure 2B). Evaluation of the separate effects of *UGT1A1**28 and *UGT1A1**60 on belinostat PK showed that dose-normalized AUC ($P = .0037$; Table 2) and $t_{1/2}$ ($P = .0017$; Supplemental Table S2) were significantly increased in carriers of *UGT1A1**60 variants. In addition, a strong decreasing trend was observed for belinostat CL in these

patients ($P = 0.02$), and a nonsignificant increase in absolute AUC ($P = .025$; Supplemental Table S2) and $C_{\max}$ was found (dosenormalized: $P = .068$, Table 2; absolute: $P = .034$, Supplemental Table S2). These findings suggest that at daily doses $> 400 \text{ mg/m}^2$ *UGT1A1**60 has a greater effect on belinostat PK than on *UGT1A1**28.

Effects of *UGT1A1* Genotype on AcK in PBMCs

As a surrogate marker for the acetylating effects of belinostat, AcK fold change in PBMCs 12, 36, and 60 hours after the start of infusion relative to predose was calculated. As expected, belinostat administration induced global protein acetylation in PBMCs, and AcK fold change was highest 36 hours after the start of infusion (Figure 3A). Figures 3B-D show AcK fold change at this point in PBMCs stratified per genotype (*UGT1A1**28, *UGT1A1**60, and both variants combined). AcK fold change in PBMCs tended to be increased in carriers of *UGT1A1**28 alone ($P = .075$; Figure 3B) and in combination with *UGT1A1**60 ($P = .094$; Figure 3D), the latter suggesting that higher belinostat plasma concentrations lead to greater HDAC inhibition in these patients. AcK fold changes were not significantly associated with genotype after exclusion of the $400 \text{ mg/m}^2/24 \text{ h}$ dose level (data not shown).

UGT1A1 Genotype Versus Belinostat-Related Toxicities

Table 3 shows associations between *UGT1A1* genotype and the incidence of belinostat-related toxicities. Patients carrying 1 or 2 *28 alleles tended to have a progressively increased risk for grades 3–4 neutropenia ($P = .045$) and thrombocytopenia ($P = .038$), and QTc values were marginally nonsignificantly increased compared with *UGT1A1**28 wild types ($P = .075$). The *UGT1A1**28 genotype did not significantly affect the incidence of grades 3–4 lymphocytopenia, nausea, vomiting, and fatigue ($P > .01$). *UGT1A1**60 was significantly associated with an increased incidence of grades 3–4 thrombocytopenia ($P = .0072$). No other significant associations with toxicities were found for this genotype ($P > .01$). Combined analysis of *UGT1A1**28 and *UGT1A1**60 showed a significant association between genotype and grades 3–4 thrombocytopenia ($P = .0081$) and a trend toward an increased risk for grades 3–4 neutropenia ($P = .063$). Supplemental Table S3 shows the incidence of the 2 most significant toxicities, thrombocytopenia and neutropenia, by genotype (*UGT1A1**28, *UGT1A1**60, and both mutations combined) and by belinostat dose (400, 500, 600, or $800 \text{ mg/m}^2/24 \text{ h}$).

Exclusion of patients receiving $400 \text{ mg/m}^2/24 \text{ h}$ resulted in a significant association between the *UGT1A1**28 genotype and severe thrombocytopenia ($P = .081$). In addition, the incidence of grades 3–4 neutropenia ($P = .064$) and thrombocytopenia ($P = .035$) were increased in patients carrying combinations of *UGT1A1**28 and *UGT1A1**60 variants but did not reach statistical significance. The incidence of other toxicities was not significantly affected by genotype after exclusion of the lowest dose level (Table 3).

Discussion

This is the first report on the clinical effects of *UGT1A1* polymorphisms on the PK, PD, and toxicities of belinostat. Taken together, the data suggest that, contrary to the package insert,¹ both *UGT1A1**28 and *UGT1A1**60 should be ascertained prior to altering belinostat dose

and that only patients receiving high doses of belinostat should be genotyped at these loci. Moreover, univariate analysis indicates that *UGT1A1** 60 is a much stronger marker for interpatient belinostat pharmacokinetic differences than is *UGT1A1** 28. Therefore, future clinical studies should account for both of these genotypes to reduce interpatient variability, and the package insert should be reconsidered because it is based on preclinical data alone.

Specifically, a model accounting for status of both *UGT1A1** 28 and *UGT1A1** 60 was typically better associated with the pharmacokinetics (eg, 2-fold greater AUC in patients homozygous for both *UGT1A1** 28 and *UGT1A1** 60), histone acetylation (eg, >2-fold increase of average AcK fold change in *UGT1A1** 28- and *UGT1A1** 60-homozygous patients) and severe hematological toxicities (eg, 100% of patients homozygous for *UGT1A1** 28 and *UGT1A1** 60 suffered from grades 3–4 neutropenia and thrombocytopenia compared with 0%–25% of wild type patients) than were individual genotypes. We suggest that this was likely caused by both singlenucleotide polymorphisms independently contributing to a reduction in belinostat metabolism that results in greater plasma concentrations and drug exposure. Genotype-phenotype relationships were also strongest in patients receiving 500, 600, or 800 mg/m²/24 h. This finding has been observed by others studying irinotecan^{14,15} and suggests that the glucuronidation rate is particularly important in patients receiving higher doses.

Analysis of the separate effects of *UGT1A1** 28 and *UGT1A1** 60 on belinostat PK indicated that at doses greater than 400 mg/m²/24 h, *UGT1A1** 60 appeared to have a greater impact on belinostat PK than did *UGT1A1** 28. *UGT1A1** 60 variants were also associated with greater risk of severe thrombocytopenia, whereas carriers of *UGT1A1** 28 variants had greater risk of QTc prolongation and also experienced greater HDAC inhibition. Because both alleles are coinherited, it was not possible to test their effects in isolation in the present data set; therefore, these findings justify ascertaining both alleles simultaneously.

We therefore developed a population PK/PD model accounting for both the *UGT1A1** 28 and *UGT1A1** 60 genotypes to simulate optimal belinostat dose to achieve equivalent exposures.¹⁶ Based on this model, it was determined that patients who were wild type for both *UGT1A1** 28 and *UGT1A1** 60 or carriers of 1 copy of *UGT1A1** 28 should receive a higher belinostat dose (600 mg/m²/24 h) than patients homozygous for *UGT1A1** 28 or those with at least 1 copy of *UGT1A1** 60 (400 mg/m²/24 h) to provide equivalent exposures of belinostat. These dose adjustments hopefully reduce the risk of belinostat-related toxicities in patients with impaired clearance of belinostat. The applicability of these dose recommendations will be prospectively evaluated in a genotype-directed expansion of the BPE trial. We expect that the results of this prospective trial will help to adjust the current dosing recommendation in the drug label of belinostat.

The significant *UGT1A1* effects on PK and the lack of a significant genotype-PD association at doses > 400 mg/m²/24 h suggest that the PBMC data were not a strong biomarker, presumably because of small numbers of patients.¹⁶ However, toxicity associations are likely caused by differences in drug inactivation; therefore, the present data suggest that genotype directly affects PK but indirectly affects PD.

Etoposide has an overlapping toxicity profile with belinostat (eg, nausea/vomiting, leukocytopenia, thrombocytopenia) and is also a UGT1A1 substrate. Therefore, the role of etoposide in contributing to the increased incidence of thrombocytopenia in patients carrying *UGT1A1**28 and *UGT1A1**60 cannot be completely ruled out. However, in contrast to belinostat, etoposide is metabolized by many more enzymes than UGT1A1, such as CYP3A4 and CYP3A5.¹⁷ In fact, etoposide is predominantly (44%) excreted unchanged, and less than 10% of the dose is glucuronidated.¹⁸ Therefore, we believe that the increased incidence of thrombocytopenia in *UGT1A1* variant carriers was most likely attributable to the use of belinostat.

The dosing schedule of belinostat in the BPE trial (400, 500, 600, or 800 mg/m²/24 h, 48-hour CIVI) differs from standard belinostat administration, which is 1000 mg/m² given as a 30-minute intravenous infusion on days 1–5 of a 21-day cycle. Therefore, the standard dose is higher, and it is given over a shorter period. The present study detected the *UGT1A1*-*belinostat* gene–drug interaction despite this lower dose and the potential confounding effects of etoposide. It is therefore reasonable to expect that the gene–drug interaction is *more likely* in patients treated with the standard dose, although there are no current data available in humans. This should add further concern that the present drug label, which is not based on clinical observations, includes *UGT1A1**28 and not *UGT1A1**60 genotyping data.

In conclusion, this PG analysis showed that *UGT1A1**28 and *UGT1A1**60 genotype status affected the risk of hematological toxicities of belinostat in the BPE trial. In addition, the impact of *UGT1A1**60 on belinostat PK was even greater than that of *UGT1A1**28 in this analysis, especially at doses > 400 mg/m²/24 h. Therefore, in addition to assessing *UGT1A1**28 status, *UGT1A1**60 status should also be assessed when making *UGT1A1* genotype-based dose adjustments of belinostat to minimize the risk of toxicities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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All dose levels

Figure 1.

Effects of combined variants of uridine diphosphate glucuronosyltransferase I family, polypeptide A I (*UGT1A1*)*28 and *60 on belinostat pharmacokinetics for all dose levels in the trial of belinostat (400, 500, 600, or 800 mg/m²/24 h, 48-hour continuous infusion) in combination with cisplatin and etoposide. (A) *UGT1A1* vs dose-normalized area under the plasma concentration-time curve (AUC, n = 23). (B) *UGT1A1* vs dose normalized maximum plasma concentration (C_{max} , n = 23). (C) *UGT1A1* versus total body clearance (CL, n = 19). (D) *UGT1A1* versus elimination half-life ($t_{1/2}$, n = 19). *P* values were obtained using the

Jonckheere-Terpstra test for trend ($\alpha = .01$ required for significance). WT, wild type; HT, heterozygous; VAR, homozygous.

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Figure 2.

Effect of combined variants of uridine diphosphate glucuronosyltransferase I family polypeptide A I (*UGT1A1*)* 28 and 60* on belinostat pharmacokinetics after exclusion of the 400mg/m²/24h does level in the trial of belinostat (400,500,600, or 800 mg/m²/24h, 48 hour continuous infusion in combination with cisplatin and etoposide. (A) *UGT1A1* vs does-normalized area under the plasma concentration-time curve (AUC) excluding 400mg/m²/24h (n=15). (B) *UGT1A1* versus does-normalized maximum plasma concentration (C_{max}) excluding 400 mg/m²/24h (n=15). (C) *UGT1A1* versus total body clearance (CL) excluding 400mg/m²/24h(n=13). (D) *UGT1A1* versus elimination half-life ($t_{1/2}$) excluding 400mg/m²/24h (n=13). P values were obtained using the jonckheere-Terpstra test for trend ($\alpha=.01$ required for significance). WT, wild type; HT, heterozygous; VAR, homozygous.

Figure 3.

Effects of uridine diphosphate glucuronosyltransferase I family, polypeptide A I (*UGT1A1*) genotype status on the fold change (relative to predose) of global protein lysine acetylation (AcK) in peripheral blood mononuclear cells (PBMCs) in patients receiving belinostat (400, 500, 600, or 800 mg/m²/24 h, 48-hour continuous infusion) in combination with cisplatin and etoposide. (A) Average ($\pm$ standard deviation) AcK fold change in PBMCs 12, 36, and 60 hours post-start of belinostat infusion. (B) Effect of *UGT1A1**28 on AcK fold change in PBMCs (n = 25). (C) Effect of *UGT1A1**60 on AcK fold change in PBMCs (n = 23). (D) Combined effect of *UGT1A1**28 and *UGT1A1**60 on AcK fold change in PBMCs (n = 23). P values were obtained using the Jonckheere-Terpstra test for trend ($\alpha = 0.05$ required for significance). WT, wild type; HT, heterozygous; VAR, homozygous.

Table I.

Patient Characteristics and Uridine Diphosphate Glucuronosyltransferase I Family, Polypeptide A I (UGTIAI) Genotyping Data From the BPE Trial

Characteristics	Number of patients or Sex median range
Number of patients or	25
Sex	
Male	15
Female	10
Age	
Body surface area (BSA, m ²)	54.5 (39.8–78.3)
ECOG performance status	1.90 (1.20–2.49)
0	1
1	23
2	1
Race	
White	21
African American	2
Hispanic	1
Asian	1
Primary tumor site	
Non-small cell lung cancer	3
Adrenocortical cancer	2
Cervix	2
Small cell lung cancer	6
Pheochromocytoma	2
Prostate	2
Endometrium	1
Pancreas	1
Mesothelioma	2
Cancer-prone syndromes	2
Brain	1
Not specified	2
Belinostat dose level (mg/m ² /24h)	
400	10
500	7
600	6
800	2
Baseline laboratory values	
Albumin(g/dL)	3.7 (2.1–4.2)
Allakine phosphatase(U/L)	99 (59–239)
Total bilirubin (mg/dL)	0.3 (0.1–0.6)
AST (U/L)	19 (10–49)

Characteristics	Number of patients or Sex median range
ALT(mg/dL)	28 (8–65)
Creatinine (mg/dL)	0.81 (0.48–1.13)
BSA-adjusted creatinine (mL/min) ^a	100.6(51.7–222.2)
UGT1A1 genotyping results	
<i>UGT1A1*6^b</i>	
<i>UGT1A1*1/*1</i>	22
<i>UGT1A1*6/*6</i>	0
<i>UGT1A1*6/*6</i>	1
<i>UGT1A1*28</i>	
<i>UGT1A1*1/*1</i>	11
<i>UGT1A1*1/*28</i>	11
<i>UGT1A1*28/*28</i>	3
<i>UGT1A1*60^c</i>	
<i>UGT1A1*1/*1</i>	5
<i>UGT1A1*1/*60</i>	11
<i>UGT1A1*60/*60</i>	7

ALT, alanine transaminase; AST, aspartate aminotransferase; ECOG, Eastern Cooperative Oncology Group.

^aBSA-adjusted creatinine clearance was calculated using the Cockcroft-Gault formula.

^bUnable to determine *UGT1A1*60* genotype in 2 patients.

^cUnable to determine *UGT1A1*60* genotype in 2 patients.

Table 2.
Pharmacokinetic Parameters of Belinostat in the BPE Trial (n = 25) During Cycle I

PK parameter	Mean (± SD)				
Dose-normalized AUC (ng · h/mL)/mg	11.26 (± 4.67)				
Dose-normalized C _{max} (ng/mL)/mg	0.37 (± 0.17)				
<i>UGT1A1</i> *28	<i>UGT1A1</i> *28 Excluding 400 mg/m ² /24h dose level (n = 15)				
PK parameter (median with range)	*I/*I (n = 11)	*I/*28 (n = 11)	*28/*28 (n = 3)	*I/*28 (n = 5)	*28I*28 (n = 2)
Dose-normalized AUC (ng · h/mL)/mg	10.0 (6.2 – 17.6)	10.2 (7.2 – 22.5)	12.7 (4.7 – 19.0)	11.7 (7.6 – 21.7)	15.8 (12.7 – 19.0)
Dose-normalized C _{max} (ng/mL)/mg	0.29 (0.22 – 0.53)	0.34 (0.21 – 0.88)	0.39 (0.17 – 0.74)	0.35 (0.23 – 0.53)	0.57 (0.39 – 0.74)
<i>UGT1A1</i> *60 ^b	Excluding 400 mg/m ² /24h dose level (n = 15)				
PK parameter (median with range)	*I/*I (n = 5)	*I/*60 (n = 11)	*60/*60 (n = 7)	*I/*60 (n = 7)	*60I*60 (n = 4)
Dose-normalized AUC (ng · h/mL)/mg	8.4 (6.2 – 0.0037)	11.7 (7.6 – 22.5)	11.2 (4.7 – 21.7)	11.7 (7.6 – 17.6)	15.8 (11.2 – 21.7)
Dose-normalized C _{max} (ng/mL)/mg	0.26 (0.23 – 0.068)	0.35 (0.21 – 0.88)	0.34 (0.17 – 0.74)	0.25 (0.23 – 0.42)	0.48 (0.29 – 0.74)
<i>UGT1A1</i> *28 <i>UGT1A1</i> *60 combined (all dose levels, n = 23 ^b)					
PK parameter (median with range)	*28 WT + *60 WT (n = 4)	*28 WT/HT + *60 WT/HT (n = 12)	*28 WT/HT + *60 VAR (n = 4)	*28 VAR + *60 VAR (n = 3)	
Dose-normalized AUC (ng · h/mL)/mg	7.9 (6.2 – 10.0)	11.4 (7.6 – 22.5)	9.7 (7.8 – 21.7)	12.7 (4.7 – 19.0)	0.23
Dose-normalized C _{max} (ng/mL)/mg	0.25 (0.23 – 0.42)	0.35 (0.21 – 0.88)	0.32 (0.22 – 0.56)	0.39 (0.17 – 0.74)	0.56
<i>UGT1A1</i> *28 and <i>UGT1A1</i> *60 combined (excluding 400 mg/m ² /24 h dose level, n = 15)					
PK parameter (median with range)	*28 WT + *60 WT (n = 4)	*28 *WT/HT + *60 *WT/HT (n = 7)	*28 WT/HT + *60 VAR (n = 2)	*28 VAR + *60 VAR (n = 2)	
Dose-normalized AUC (ng · h/mL)/mg	7.9 (6.2 – 10.0)	11.7 (7.6 – 17.6)	16.5 (11.2 – 21.7)	15.8 (12.7 – 19.0)	0.0030
Dose-normalized C _{max} (ng/mL)/mg	0.25 (0.23 – 0.42)	0.35 (0.21 – 0.53)	0.43 (0.29 – 0.56)	0.57 (0.39 – 0.74)	0.061

WT, wild type; HT, heterozygous; VAR, homozygous; PK, pharmacokinetics.

^a P-values obtained using the Jonckheere-Terpstra test for trend (α = .01 required for statistical significance).

^b Unable to determine *UGT1A1**60 genotype in 2 patients.

Table 3.

Relationship Between Uridine Diphosphate Glucuronosyltransferase I Family, Polypeptide A I (*UGT1A1*) Genotype and Toxicities During Cycle I in the BPE Trial

UGT1A1*28				UGT1A1*60 ^a			
Genotype	Grade		P ^b	Grade		P ^b	
	0 – 2 (n)	3 – 4 (n)		0 – 2 (n)	3 – 4 (n)		
Lymphocytopenia							
*1/*1	4	7	0.75	*1/*1	1	4	
*1/*28	2	9		*1/*60	4	7	
*28/*28	1	2		*60/*60	1	6	
Neutropenia							
*1/*1	8	3	0.045	*1/*1	4	1	
*1/*28	6	5		*1/*60	7	4	
*28/*28	0	3		*60/*60	2	5	
Thrombocytopenia							
*1/*1	8	3	0.038	*11*1	5	0	
*1/*28	5	6		*1/*60	5	6	
*28/*28	0	3		*60/*60	1	6	
	Grade		P ^b	Grade		P ^b	
	0 – 1 (n)	2 – 3 (n)		0 – 1 (n)	2 – 3 (n)		
Nausea							
*1/*1	9	2	0.73	*1/*1	4	1	
*1/*28	9	2		*1/*60	9	2	
*28/*28	2	1		*60/*60	5	2	
Fatigue							
*1/*1	8	3	0.76	*1/*1	3	2	
*1/*28	7	4		*1/*60	8	3	
*28/*28	2	1		*60/*60	4	3	

UGT1A1*28					UGT1A1*60 ^a				
Genotype	Grade		P ^b		Grade		P ^b		
	0 – 2 (n)	3 – 4 (n)			0 – 2 (n)	3 – 4 (n)			
	Grade		P ^b		Grade		P ^b		
	0 (n)	1 – 3 (n)			0 (n)	1 – 3 (n)			
Vomiting									
*1/*1	9	2	0.44		*1/*1	5	0	1.00	
*1/*28	10	1			*1/*60	9	2		
*28/*28	3	0			*60/*60	7	0		
QTc (average QTc in cycle 1 - baseline QTc.)									
	Median (ms, range)		P ^c			Median (ms, range)		P ^c	
*1/*1	12 (–16 – 54)		0.075		*1/*1	15 (12 – 54)		0.62	
*1/*28	15 (–7 – 45)				*1/*60	11 (–7 – 45)			
*28/*28	18 (17 – 49)				*60/*60	17 (–15 – 49)			
Excluding 400 mg/m ² /24 h dose level, n=15									
UGT1A1*28					UGT1A1*60				
Genotype	Grade	P ^b		Grade		P ^b			
	0 – 2 (n)	3 – 4 (n)			0 – 2 (n)	3 – 4 (n)			
Lymphocytopenia									
*1/*1	2	6	1.00		*1/*1	0	4	0.68	
*1/*28	0	5			*1/*60	2	5		
*28/*28	1	1			*60/*60	1	3		
Neutropenia									
*1/*1	7	1	0.051		*1/*1	3	1	0.25	
*1/*28	4	1			*1/*60	7	0		
*28/*28	0	2			*60/*60	1	3		
Thrombocytopenia									
*1/*1	7	1	0.0076		*1/*1	4	0	0.081	
*1/*28	1	4			*1/*60	3	4		

UGT1A1*28				UGT1A1*60 ^a				
Genotype	Grade		P ^b		Grade		P ^b	
	0 – 2 (n)	3 – 4 (n)			0 – 2 (n)	3 – 4 (n)		
*28,*28	0	2		*60,*60	1	3		
	Grade		P ^b		Grade		P ^b	
	0 – 1 (n)	2 – 3 (n)			0 – 1 (n)	2 – 3 (n)		
Nausea								
*I/*I	6	2	0.70	*I/*I	3	1	1.00	
*I/*28	4	1			*I/*60	6		1
*28,*28	2	0			*60/*60	3		1
Fatigue								
*I/*I	5	3	1.00	*I/*I	3	1	0.30	
*I/*28	3	2			*I/*60	5		2
*28,*28	1	1			*60/*60	1		3
	0 (n)	1– 3 (n)	P ^b		0 (n)	1– 3 (n)	P ^b	
Vomiting								
*I/*I	6	2	0.37	*I/*I	4	0	1.00	
*I/*28	5	0			*I/*60	5		2
*28,*28	2	0			*60/*60	4		0
QTc (average QTc in cycle 1- baseline QTc)								
	Median (ms, range)		P ^c		Median (ms, range)		P ^c	
*I/*I	13 (–15 – 54)		0.34	*I/*I	19 (12 – 54)		0.66	
*I/*28	17 (–7 – 45)				12 (–7 – 45)			
*28,*28	34 (18 – 49)				18 (–15 – 49)			
UGT1A1*28 and UGT1A1*60 combined, n = 23 ^a				Excluding 400 mg/m ² /24 h, n = 15				
Genotype	Grade		P ^b		Grade		P ^b	
	0–2 (n)	3– 4 (n)			0–2 (n)	3– 4 (n)		
Lymphocytopenia								

UGT1A1*28				UGT1A1*60 ^a			
Genotype	Grade		P ^b	Grade		P ^b	
	0 – 2 (n)	3 – 4 (n)		0 – 2 (n)	3 – 4 (n)		
*28 WT + *60 WT	0	4	1.00	0	4	0.34	
*28 WT/HT + *60 WT/HT	5	7		2	5		
*28 WT/HT + *60 VAR	0	4		0	2		
*28 VAR + *60 VAR	1	2		1	1		
Neutropenia							
*28 WT + *60 WT	3	1	0.063	3	1	0.064	
*28 WT/HT + *60 WT/HT	8	4		7	0		
*28 WT/HT + *60 VAR	2	2		1	1		
*28 VAR + *60 VAR	0	3		0	2		
Thrombocytopenia							
*28 WT + *60 WT	4	0	0.0081	4	0	0.035	
*28 WT/HT + *60 WT/HT	6	6		3	4		
*28 WT/HT + *60 VAR	1	3		1	1		
*28 VAR + *60 VAR	0	3		0	2		
	Grade		P ^b	Grade		P ^b	
	0 – 1 (n)	2 – 3 (n)		0 – 1 (n)	2 – 3 (n)		
Nausea							
*28 WT + *60 WT	3	1	0.79	3	1	1.00	
*28 WT/HT + *60 WT/HT	10	2		6	1		
*28 WT/HT + *60 VAR	3	1		1	1		
*28 VAR + *60 VAR	2	1		2	0		
Fatigue							
*28 WT + *60 WT	3	1	0.82	3	1	0.30	
*28 WT/HT + *60 WT/HT	8	4		5	2		
*28 WT/HT + *60 VAR	2	2		0	2		

UGT1A1*28				UGT1A1*60 ^a					
Genotype	Grade		P ^b		Grade		P ^b		
	0 – 2 (n)	3 – 4 (n)			0 – 2 (n)	3 – 4 (n)			
*28 VAR + *60 VAR			2	1	1	1			
			0 (n)	1 – 3 (n)	0 (n)	1 – 3 (n)	P ^b		
Vomiting									
*28 WT + *60 WT			4	0	0.76		4	0	1.00
*28 WT/HT + *60 WT/HT			10	2			5	2	
*28 WT/HT + *60 VAR			4	0			2	0	
*28 VAR + *60 VAR			3	0			2	0	
QTc (average QTc in cycle 1 - baseline QTc)									
	Median(ms, range)			P ^c		Median (ms, range)			P ^c
*28 WT + *60 WT	19 (12 – 54)			0.93		19 (12 – 54)			0.79
*28 WT/HT + *60 WT/HT	11.5 (–7 – 45)					12 (–7 – 45)			
*28 WT/HT + *60 VAR	11 (–15 – 17)					1 (–15 – 17)			
*28 VAR + *60 VAR	18 (17 – 49)					34 (18 – 49)			

WT, wild type; HT, heterozygous; VAR, homozygous.

^a n = 23; unable to determine UGT1A1* 60 genotype in 2 patients.

^b P-values were obtained using the Cochran-Armitage trend test (α =.01 required for significance).

^c P-values were obtained using Jonckheere-Terpstra test for trend (α =.01 required for significance).