Review

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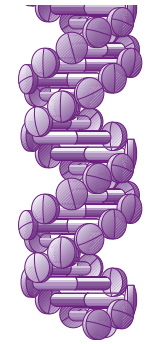
Pharmacogenomics



Pharmacogenomics and histone deacetylase inhibitors

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The histone deacetylase inhibitor valproic acid (VPA) has been used for many decades in neurology and psychiatry. The more recent introduction of the histone deacetylase inhibitors (HDIs) belinostat, romidepsin and vorinostat for treatment of hematological malignancies indicates the increasing popularity of these agents. Belinostat, romidepsin and vorinostat are metabolized or transported by polymorphic enzymes or drug transporters. Thus, genotype-directed dosing could improve pharmacotherapy by reducing the risk of toxicities or preventing suboptimal treatment. This review provides an overview of clinical studies on the effects of polymorphisms on the pharmacokinetics, efficacy or toxicities of HDIs including belinostat, romidepsin, vorinostat, panobinostat, VPA and a number of novel compounds currently being tested in Phase I and II trials. Although pharmacogenomic studies for HDIs are scarce, available data indicate that therapy with belinostat (*UGT1A1*), romidepsin (*ABCB1*), vorinostat (*UGT2B17*) or VPA (*UGT1A6*) could be optimized by upfront genotyping.

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Since the discovery of its anticonvulsant properties in 1962, valproic acid (VPA) has been widely used in the field of neurology and psychiatry. More recently, VPA has also been shown to inhibit HDAC and to exert cytotoxicity against tumor cells [1]. In the past decade, the US FDA approved the HDAC inhibitors (HDIs) romidepsin (2004), vorinostat (2006), belinostat (2014) and panobinostat (2015) for the treatment of T-cell lymphoma, which illustrate the increasing popularity of these class of agents in oncology. Since the pharmacokinetics (PKs) and pharmacodynamics of a significant number of HDIs are affected by polymorphic enzymes or drug transporters, certain genetic variants could impact therapeutic efficacy and the risk of toxicities of these agents. In this review, we aim to address the relevance of pharmacogenomics (PGs) for treatment with HDIs, including VPA, romidepsin, vorinostat, belinostat, panobinostat and a number of novel compounds currently being tested in Phase I and II trials.

Valproic acid

The metabolism of VPA is mainly characterized by glucuronidation via uridine diphosphate glucuronosyltransferase (UGT) isoforms (relative contribution 50%) [2] and beta-oxidation in mitochondria (relative contribution 40%) [3,4]. Among the UGT isoforms, UGT2B7 contributes the most to the intrinsic clearance of VPA followed by UGT1A6 and UGT1A9 [2,5]. A minor (~10%) metabolic pathway is oxidation through the CYP enzymes [4], in particular CYP2A6, CYP2B6 and CYP2C9 [6].

Pharmacogenomic studies on VPA have particularly focused on UGT polymorphisms

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(Table 1). When focusing on UGT1A6, three common SNPs *UGT1A6*3* (19T>G; rs6759892), *UGT1A6*5* (541A>G; rs2070959) and *UGT1A6*9* (552A>C; rs1105879) are associated with increased enzyme activity, increased VPA glucuronidation and requirement of higher VPA dosages than patients who are wild-type (WT) for UGT1A6 [7]. It has been shown that in recombinant *UGT1A6* variants, glucuronidation of VPA was twofold higher for the **2* haplotype comprising these three nonsynonymous polymorphisms compared with the *UGT1A6*1* reference haplotype [8]. Guo *et al.* also reported higher VPA doses and lower adjusted plasma VPA concentrations in 98 epileptic children carrying *UGT1A6*3*, **5* or **9* polymorphisms in both alleles, compared with WT patients or patients with polymorphisms in a single allele [9]. Furthermore, in 162 epileptic patients, carriers of *UGT1A6*3*, **5* and **9* polymorphisms tended to require a higher dosage of VPA and lower concentration-to-dose ratios than patients who were WT for *UGT1A6* [10]. These associations were also observed in haplotypes composed of *UGT1A6* (**3*, **5*, **9*) and *UGT1A9* (*I399T>C*, *I887T>G*) SNPs [10]. Sun *et al.* also reported lower VPA serum concentrations in patients heterozygous or homozygous for *552A>C* [11]. In contrast with these findings, other reports did not show clinically relevant effects of *UGT1A6* polymorphisms on VPA PK [12,13].

The reported effects of *UGT2B7* polymorphisms, for example, *UGT2B7*2* (802C>T; rs7439366), *UGT2B7*3* (211G>T; rs12233719) and *UGT2B7*4* (1192G>A; rs145725367) on VPA PK are more conflicting [7]. The majority of the pharmacogenomics analyses did not find significant associations between *UGT2B7*2* genotype and VPA glucuronidation [9,10,12,14]. However, one study reported significantly lower VPA trough plasma concentrations in patients with epilepsy carrying the TT and CT genotype at *UGT2B7*2* (rs7439366) than patients with the CC genotype, suggesting that a dose increase of VPA in carriers of a T allele may be necessary to avoid subtherapeutic treatment of these patients [15]. In contrast, significantly higher VPA concentrations were found in epileptic children carrying *UGT2B7* -161C>T (rs7668258) [16,17] or *UGT2B7*2* (rs7439366) polymorphisms [16] compared with children with WT genotypes. Furthermore, the *UGT2B7* -268A>G polymorphism (rs7662029) affected VPA PK in epileptic patients, since carriers of the AA genotype had higher VPA serum concentrations than patients carrying the GG genotype [18]. The *UGT2B7*3* polymorphism (rs12233719) had no significant effect on VPA PK in this study [18].

The presence of drug–drug interactions (DDIs) was not expected to be confounding factors, since in the majority of the clinical studies VPA was administered as monotherapy. In addition, in trials with combination regimens, drugs were coadministered that were known not to affect VPA PK, such as clobazam, zonisamide, levetiracetam, gabapentine [17], lamotrigine [16] and lorazepam [14]. Only coadministration of carbamazepine affected the PG outcome as shown by Chu *et al.* [12]. In patients cotreated with carbamazepine, the *UGT1A3*5* polymorphism was not associated with any effect on VPA exposure, while in the monotherapy group lower plasma concentrations of VPA were measured in carriers of this genetic variant.

Reports on the impact of other UGT polymorphisms on VPA metabolism are either lacking (e.g., *UGT1A4*, *UGT1A9* [7]) or scarce (e.g., *UGT1A3* [12]). Overall, only *UGT1A6* polymorphisms seem to be clinically relevant for VPA metabolism and dosing. The clinical relevance of genotyping other UGT enzymes remains unclear due to contradicting results (*UGT2B7*) or limited available data (*UGT1A3*, *UGT1A4*, *UGT1A9*).

Romidepsin

As a class, HDIs cause an increase in the time between the start of the Q wave and the end of the T wave after correction for heart rate (QTc prolongation) and abnormalities in the S and T waves [25]. While the precise mechanism of QT prolongation and ST- and T-wave changes have not been elucidated, acetylation of the hERG channel may cause QT prolongation [26,27]. Additionally, it appears that HDIs – including romidepsin – affect K_{ATP} subunit expression in ventricular myocytes through epigenetic modifications [25].

Romidepsin is a good substrate of ABCB1 and is also subject to *ABCB1* polymorphism-induced differences in efflux transport [20]: variant carriers at 1236C>T (rs1128503), 2677G>T/A (rs2032582) and 3435C>T (rs1045642) had a greater than twofold reduction in B->A/A->B ratio. Polymorphism-induced *ABCB1* gene expression differences also vary by tissues. For instance, hepatic *ABCB1* gene expression is lower in variant carriers, whereas cardiac endothelial *ABCB1* gene expression was higher [28,29]. Romidepsin-induced QT prolongation is likely a function of both hepatic elimination through hepatocellular *ABCB1* (leading to exposure differences) and cardiac-tissue elimination through cardiac endothelial *ABCB1* (leading to differences in intracardiac exposure). Therefore, *ABCB1* polymorphisms have highly pleiotropic phenotypic consequences.

We showed that *ABCB1* variant genotype (2677G>T/A; rs2032582) and diplotype carriers (only variant alleles at all three aforementioned *ABCB1* SNPs) typically have lower romidepsin clearance, although

Table 1. Clinical effects of polymorphisms on the pharmacokinetics & pharmacodynamics of histone deacetylase inhibitors.

Polymorphism	Number of subjects	Ethnicity	Monotherapy or combination therapy	Key findings	Ref.
Valproic acid					
*UGT1A6*3*: 19T>G (rs6759892) *UGT1A6*5*: 541A>G (rs2070959) *UGT1A6*9*: 552A>C (rs1105879) *UGT2B7*2*: 802C>T (rs7439366)	98 children with epilepsy	Asian (Chinese)	Monotherapy	*UGT1A6* double HT associated with higher VPA doses vs WT or single HT *UGT1A6* double HT associated with lower VPA plasma concentrations vs single HT *UGT2B7*2*: no significant effect on VPA PK	[9]
	162 patients with epilepsy	Asian (Chinese)	Monotherapy	Variant *UGT1A6* carriers tended to require higher VPA dosages and had lower exposure to VPA (concentration-to-dose ratios) *UGT2B7*: no significant effect on VPA PK	[10]
*UGT1A6*9*: 552A>C (rs1105879)	67 patients with epilepsy	Unknown	Monotherapy	Lower VPA serum concentrations in heterozygous or homozygous patients vs WT patients	[11]
*UGT1A6*5*: 541A>G (rs2070959) *UGT1A6*9*: 552A>C (rs1105879) *UGT2B7*2*: 802C>T (rs7439366) *UGT1A3*5*: 17A>G/31T>C/81G>A/477A>G (rs28898617/rs3821242/rs6706232/rs7574296)	242 patients with epilepsy	Asian (Chinese)	Monotherapy (n = 136) Combination therapy: – Carbamazepine (n = 38) – Topiramate (n = 19) – Phenytoin (n = 3) – Piracetam (n = 6) – Vitamins and cardiovascular agents (n = 23)	No significant effect of *UGT1A6* and *UGT2B7* SNPs on VPA PK Patients with the *UGT1A3*5* variant had lower trough plasma concentration of VPA than WT carriers No effect of UGT SNPs on VPA PK in patients cotreated with carbamazepine	[12]
*UGT1A6*5*: 541A>G (rs2070959)	147 children with epilepsy	Asian (Chinese)	Monotherapy	No significant effect on VPA PK	[13]
*UGT2B7*2*: 802C>T (rs7439366)	14 healthy subjects with homozygous for *UGT2B15*2*	Asian (Korean)	Combination therapy with lorazepam	Insignificant trend of increased AUC of VPA with increasing numbers of *UGT2B7*2* alleles	[14]
*UGT2B7*2*: 802C>T (rs7439366) *UGT2B7*3*: 211G>T (rs12233719)	102 patients with epilepsy	Asian (Chinese)	Monotherapy	Significantly lower VPA trough plasma concentrations in patients carrying the *TT* and *CT* genotype at *UGT2B7*2* vs patients with the *CC* genotype *UGT2B7*3:* no significant effect on VPA PK	[15]
UGT2B7: 161C>T (rs7668258) *UGT2B7*2*: 802C>T (rs7439366)	166 patients with epilepsy	Asian (Chinese)	Combination therapy with lamotrigine	Significantly higher VPA concentrations were found in carriers of *UGT2B7* (rs7668258) and -802C>T (rs7439366) vs WT	[16]

CLL: Chronic lymphocytic leukemia; HT: Heterozygosity; PFS: Progression-free survival; PK: Pharmacokinetic; $t_{1/2}$: Elimination half-life; VPA: Valproic acid; WT: Wild-type.

Table 1. Clinical effects of polymorphisms on the pharmacokinetics & pharmacodynamics of histone deacetylase inhibitors (cont.).

Polymorphism	Number of subjects	Ethnicity	Monotherapy or combination therapy	Key findings	Ref.
UGT2B7: 161C>T (rs7668258)	78 children with epilepsy	Asian (Japanese)	Monotherapy (n = 37) Combination therapy: – Clobazam (n = 17) – Zonisamide (n = 11) – Levetiracetam (n = 5) – Triple therapy (n = 8)	Patients with the CC genotype had lower adjusted plasma VPA concentrations than those with CT or TT genotype	[17]
UGT2B7: 268A>G (rs7662029) *UGT2B7*3*: 211G>T (rs12233719)	248 patients with epilepsy	Asian (Chinese)	Monotherapy	Carriers of the AA genotype had higher VPA serum concentrations than patients carrying the GG genotype *UGT2B7*3*: no significant effect on VPA PK	[18]
Romidepsin					
ABCB1: 2677G>T/A (rs2032582) 1236C>T (rs1128503) 3435C>T (rs1045642)	98 patients with T-cell lymphoma	Caucasian, African–American, Hispanic, Asian	Monotherapy	Nonsignificant trend toward reduced romidepsin clearance in carriers of *ABCB1* 2677G>T/A variant alleles	[19]
	83 patients with T-cell lymphoma and other cancers	Caucasian, African–American, Hispanic, Asian	Monotherapy	*ABCB1* variants associated with less severe QTc prolongation than WTs	[20]
Vorinostat					
*UGT2B17*2* (rs7439366)	26 patients with breast adenocarcinoma	Asian (Chinese, Malay, Indian)	Monotherapy	*UGT2B17*2* homozygotes had significantly lower mean AUC ratio of vorinostat-*O*-glucuronide/vorinostat, and trended toward having higher vorinostat AUC, more serious adverse events, higher clinical benefit rate and longer median PFS than patients with at least one WT allele	[21]
UGT1A1 *UGT2B17*2*	Seven patients with advanced cancers	Unknown	Combination therapy with vinorelbine	Both *UGT1A1* and *UGT2B17*2* polymorphisms had no significant effects on vorinostat PK No interaction between vorinostat and vinorelbine	[22]

CLL: Chronic lymphocytic leukemia; HT: Heterozygosity; PFS: Progression-free survival; PK: Pharmacokinetic; $t_{1/2}$: Elimination half-life; VPA: Valproic acid; WT: Wild-type.

Table 1. Clinical effects of polymorphisms on the pharmacokinetics & pharmacodynamics of histone deacetylase inhibitors (cont.).

Polymorphism	Number of subjects	Ethnicity	Monotherapy or combination therapy	Key findings	Ref.
Belinostat					
*UGT1A1*28*: A[TA]$_7$TAA (rs8175347) *UGT1A1*60*: 3279T>G (rs4124874)	25 patients with solid tumors	Caucasian, African–American, Hispanic, Asian	Combination therapy with cisplatin and etoposide.	Increased AUC, $t_{1/2}$ at doses >400 mg/m^2 per 24 h Increased incidence grade 3 or 4 thrombocytopenia	[23]
Panobinostat					
*CYP3A5*3* (rs776746)	14 patients with advanced or metastatic solid tumors	Caucasian	Monotherapy (day 1) Combination with ketoconazole (day 8).	No differences in panobinostat PK between patients heterozygous and homozygous for *CYP3A5*3*	[24]

CLL: Chronic lymphocytic leukemia; HT: Heterozygosity; PFS: Progression-free survival; PK: Pharmacokinetic; $t_{1/2}$: Elimination half-life; VPA: Valproic acid; WT: Wild-type.

this observation only approached statistical significance [19]. Thus, hepatobiliary transport of romidepsin in humans may be slightly reduced in variant allele carriers, which is consistent with previous observations that polymorphisms impart both a low expression and a low function phenotype on *ABCB1* in liver [20,28]. However, mice lacking *ABCB1*-type P-glycoprotein (Pgp) had similar clearance as their WT counterparts, which suggest that compensatory pathways are present that compensate for the lack of Pgp-mediated efflux [20]. Therefore, *ABCB1* polymorphisms do not appear to modulate romidepsin clearance to the extent that they would affect drug dosing.

Conversely, mice lacking *ABCB1*-type Pgp had a 35% increase in intracardiac romidepsin exposure and an earlier ΔQTc_{MAX} [20]. These data suggest that local cardiac exposure of romidepsin is strongly regulated by Abcb1 expression in the cardiac endothelium. We next showed that patients carrying *ABCB1* variants, who counterintuitively express more intracardiac ABCB1, were protected from QT prolongation [20] and had smaller changes in heart rate following romidepsin infusions [30]. Taken together, these data suggest that the cardiac endothelium depends more heavily on ABCB1 to exclude romidepsin and prevent romidepsin-induced ECG changes.

ABCB1 variant diplotypes are frequently inherited among many different populations [31], and a large subset of the population receiving HDIs is likely subjected to an increased risk of QTc prolongation. However, given that careful monitoring of potassium and magnesium in patients with cardiac disease limits the clinical utility of these findings vis-à-vis romidepsin. Nevertheless, these results may have implications for other ABCB1 substrates that cause cardiac effects.

Vorinostat

The activity of several hepatic glucuronidases was tested in human liver microsomes (HLMs) coincubated with vorinostat. Several glucuronidases were found to have detectable activity in this assay: UGTs 1A3, 1A7, 1A8, 1A9, 1A10 and 2B17. Of these, UGT2B17 was found to have the third highest half-maximal reaction velocity (V_{MAX}/K_M; 16 ± 6.5), and the lowest K_M (300 µmol/l); and HLMs with homozygous *UGT2B17* deletions (*UGT2B17*2/*2*) had a 75% increase K_M with no change in V_{MAX} [32]. These results were confirmed in a subsequent study, which also showed that heterozygous deletions of *UGT2B17* did not result in a decrease in enzyme activity and that a *UGT2B7* SNP (802C>T; rs7439366) slowed the metabolic rate in HLMs [33]. In patients, homozygous carriers of the *UGT2B17*2* null alleles metabolized vorinostat less efficiently (by ~30%) and had longer

progression-free survival than UGT2B17*1 carriers; albeit, this observation only approached statistical significance due to low statistical power [21]. It therefore appears that the *UGT2B17*2/*2* genotype could be a major determinate of vorinostat efficacy and/or toxicity. The *UGT2B17*2/*2* genotype is most frequent in Asian patients (~60% in Asians vs ~10–20% in Caucasians and African–Americans); however, future studies are required to validate the phenotypic importance of this genotype. Asian patients may benefit more from PG testing [21,34,35]. However, this fact should not preclude genotyping all patients since genotyping is an excellent and inexpensive way to determine UGT2B17 activity and expression status [32,33,36], and self-reported race is unlikely to reflect a true genetic background in increasingly heterogeneous patient populations.

Although *UGT2B17*2* variant alleles appear to impact the PK of vorinostat, not all clinical reports are consistent. For example, among patients with advanced cancers receiving both vorinostat and vinorelbine, *UGT2B17*1/*2* (n = 4) carriers did not have a different PK profile than *UGT2B17*1/*1* carriers (n = 3) and no interaction between vinorelbine and vorinostat was observed [22]. Therefore, future studies are required to provide a level of evidence that is sufficient for clinical translation.

Belinostat

The FDA approved belinostat (1000 mg/m^2, 30-min intravenous infusions once daily on days 1–5 of a 21-day cycle) in 2014 for the treatment of relapsed or refractory peripheral T-cell lymphoma. Belinostat is primarily metabolized by glucuronidation via UGT1A1 [37]. Commonly reported *UGT1A1* genetic variants associated with impaired enzymatic expression or activity are *UGT1A1*6* (211G>A; rs4148323) [38,39], *UGT1A1*28* (A[TA]$_7$TAA; rs8175347) [40,41], *UGT1A1*60* (3279T>G; rs4124874) [42] and *UGT1A1*93* (1791C>T; rs10929302) [42]. The phenotypic consequences of *UGT1A1* polymorphisms have already been demonstrated preclinically in HLMs harboring *UGT1A1*28*, which glucuronidated belinostat at a slower rate than did WT microsomes [37]. A 25% lower belinostat dose (750 mg/m^2) was then recommended by the FDA in patients homozygous for *UGT1A1*28* [43].

The clinical relevance of *UGT1A1* genetic variants on belinostat dosing was confirmed in a retrospective analysis in patients with solid tumors receiving a 48-h continuous intravenous infusion (CIVI) with belinostat (400–800 mg/m^2 per 24 h, n = 23) in combination with cisplatin and etoposide (BPE trial) (Table 1) [23]. The effects of *UGT1A1* polymorphisms on belinostat PK, pharmacodynamics and toxicities were then evaluated. Instead of the approved 30-min infusion on days 1–5 of a 21-day cycle, belinostat was dosed as a 48-h infusion in the BPE trial to enhance cytotoxicity [44,45]. An increased number of *UGT1A1*28* and especially *UGT1A1*60* variant alleles were significantly associated with increased belinostat plasma concentrations and an increased risk of thrombocytopenia and neutropenia [23]. This gene–drug interaction was more profound at higher belinostat doses, and these data were consistent with other UGT1A-mediated PG effects, such as that on SN38, the active metabolite of irinotecan [46,47]. These findings underline the importance of including *UGT1A1*60* genotyping besides *UGT1A1*28*, since both *UGT1A1*28* and **60* predicted belinostat-related toxicities. Coadministered etoposide has an overlapping toxicity profile with belinostat, is also metabolized by UGT1A1 and could therefore contribute to the increased risk for thrombocytopenia in carriers of *UGT1A1*28* and *UGT1A1*60* variant alleles. However, etoposide is also metabolized by CYP3A4 and CYP3A5, and glucuronidation by UGT1A1 is a minor metabolic route. The increased incidence of thrombocytopenia in *UGT1A1* variant carriers was therefore most likely attributable to the administration of belinostat.

Based on the 48-h CIVI dosage regimen used in the BPE trial, a two-compartment population pharmacokinetic model utilizing nonlinear-mixed effects modeling was developed to optimize belinostat dose adjustments leading to equivalent belinostat exposure in patients carrying *UGT1A1*28* and **60* genetic variants [44]. The final model included the covariates that significantly affected belinostat clearance (*UGT1A1* genotype status [**28* and **60*], serum albumin concentration, creatinine clearance) and volume of distribution (body weight). Using simulations via the final model, equivalent AUCs were achieved when a dose of 600 mg/m^2 per 24 h was simulated in patients WT for both *UGT1A1*28* and **60 or* heterozygous for **28* ('extensive metabolizers'), while patients homozygous for *UGT1A1*28* or patients heterozygous or homozygous for *UGT1A1*60* ('impaired metabolizers') received a reduced simulated dose of 400 mg/m^2 per 24 h. At the time of writing, these recommended doses are prospectively investigated in a genotype-directed expansion of the BPE trial at the National Cancer Institute.

Panobinostat

Panobinostat (LBH589) is a potent (nanomolar) cinnamic hydroxamic acid HDI of class I, II and IV HDACs (a pan-DAC inhibitor) [48]. Panobinostat is metabolized by numerous routes, including oxidation, reduction and hydrolysis, the former two largely mediated by CYP3A4 (70–98%) with minor contributions from CYPs 2D6 and 2C19 [24]. All metabolites were

much less potent HDIs than parent panobinostat [49]. Because CYP3A metabolizes a plethora of other compounds, the potential for DDIs between panobinostat and CYP3A substrates was assessed. When panobinostat was coadministered clinically with the common CYP3A4 inhibitor ketoconazole, patients experienced a 1.6-fold and 1.8-fold increase in C_{MAX} and AUC, respectively, while half-life remained the same [24]. However, the fraction of panobinostat cleared by CYP3A was 0.4, suggesting that the oxidative metabolic pathway is not the predominant route of metabolism, and that a roughly 1.7-fold increase in exposure was not clinically relevant [24]. This was supported by the large interpatient variability (60%) in panobinostat exposure that is comparable in magnitude with the ketoconazole effect on panobinostat exposure [24]. While panobinostat was determined to have no clinically relevant DDIs, the fact that patients coadministered both ketoconazole and panobinostat had greater incidence (36%, n = 5) of QTc prolongation (>30 ms) compared with patients on panobinostat alone (29%, n = 4), clinicians still suggest monitoring patients given panobinostat and other CYP3A-mediated compounds.

Although panobinostat is not metabolized by UGTs, pharmacogenomic analyses were performed using *CYP3A* genotype status from 14 Caucasian patients genotyped for *CYP3A4*1B* (rs2740574), *CYP3A5*2* (rs28365083), **3* (rs776746), **6* (rs10264272) and **7* (rs76293380) (Table 1) [24]. These patients received panobinostat alone on day 1, the CYP3A4 inhibitor ketoconazole on days 5–9 and the second administration of panobinostat on day 8. All 14 patients were homozygous WT *CYP3A4*1A*; 11 patients were homozygous for *CYP3A5*3*; three patients were heterozygous for *CYP3A5*1/*3*. There were no differences in panobinostat PK based on *CYP3A5*3* genotype status (heterozygous [n = 3] vs homozygous [n = 11]) [24]. Since all patients in this study were homozygous WT for *CYP3A4*1A*, no conclusions pertaining to *CYP3A4* genotype can be drawn from this study. Furthermore, coadministration of ketoconazole caused an increase of panobinostat plasma concentrations, however, this increase was not considered clinically relevant.

Novel HDIs currently being tested in Phase I & II trials

There are several novel HDIs currently under clinical development in either Phase I (HBI-8000 [chidamide], kevetrin, CUDC-101, AR-42, CHR-2845, 4SC-202, CG200745, ricolinostat [ACY-1215], ME-344) or Phase II (mocetinostat, abexinostat, entinostat, SB939, resminostat, givinostat, quisinostat) trials. Additionally, chidamide is already approved in China. Unfortunately, none of these HDIs, first, are metabolized by UGTs and/or, second, there is no relevant pharmacogenomic data available.

Conclusion & future perspective

Patients treated with the HDIs belinostat, romidepsin and VPA may benefit from upfront genotyping. However, before genotype-directed dosing guidelines are being implemented in drug labels confirmation of the reported associations between genotype and PK, efficacy or toxicity is necessary in prospective clinical trials with larger numbers of patients.

UGT1A1-directed genotyping could be useful for patients undergoing therapy with belinostat, since *UGT1A1*28* and **60* genetic variants affect belinostat clearance and the risk for hematological toxicities. Also for romidepsin, there is evidence that genetic polymorphisms could have an impact on drug toxicity. *ABCB1* polymorphisms associated with cardiac *ABCB1* gene expression have been shown to affect intracardiac romidepsin concentrations and therefore also the risk for QT prolongation both preclinically and clinically. For patients receiving vorinostat therapy, *UGT2B17*2* could be useful, since this polymorphism is associated with decreased vorinostat glucuronidation possibly leading to increased efficacy and/or toxicity. Furthermore, treatment with VPA could be improved by *UGT1A6* genotyping to identify patients requiring higher VPA doses.

In contrast, limited pharmacogenomic data on *CYP3A5* polymorphisms suggest no clinically relevant role of genotype-directed dosing in patients receiving panobinostat. At last, future studies should reveal the relevance of genotype on therapy outcome

Executive summary

- HDACs induce chromatin unwinding allowing gene transcription to occur.
- HDAC inhibitors (HDIs) as a class have been demonstrated to be cytotoxic to various cancer types.
- Several HDIs are metabolized by polymorphic UGT enzymes.
- This article reviews the pharmacogenomic impacts of UGT enzymes on the PK, efficacy and toxicity of their HDI substrates (UGT1A1 for belinostat, UGT1A6 for valproic acid, UGT2B17 for vorinostat).
- Romidepsin has been shown to be susceptible to pharmacokinetics, efficacy and toxicity differences based on the genotype of a drug transporter, ABCB1.
- Prospective genotype for these genes with respect to their HDI substrates to maximize therapeutic benefit while minimizing toxicity.

with novel HDIs currently tested in Phase I and II trials.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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References

1 Eyal S, Yagen B, Shimshoni J, Bialer M. Histone deacetylases inhibition and tumor cells cytotoxicity by CNS-active VPA constitutional isomers and derivatives. *Biochem. Pharmacol.* 69(10), 1501–1508 (2005).

2 Argikar UA, Remmel RP. Effect of aging on glucuronidation of valproic acid in human liver microsomes and the role of UDP-glucuronosyltransferase *UGT1A4*, *UGT1A8*, and *UGT1A10*. *Drug Metab. Dispos.* 37(1), 229–236 (2009).

3 Ito M, Ikeda Y, Arnez JG, Finocchiaro G, Tanaka K. The enzymatic basis for the metabolism and inhibitory effects of valproic acid: dehydrogenation of valproyl-CoA by 2-methyl-branched-chain acyl-CoA dehydrogenase. *Biochim. Biophys. Acta* 1034(2), 213–218 (1990).

4 Ghodke-Puranik Y, Thorn CF, Lamba JK *et al.* Valproic acid pathway: pharmacokinetics and pharmacodynamics. *Pharmacogenet. Genomics* 23(4), 236–241 (2013).

5 Sakaguchi K, Green M, Stock N, Reger TS, Zunic J, King C. Glucuronidation of carboxylic acid containing compounds by UDP-glucuronosyltransferase isoforms. *Arch. Biochem. Biophys.* 424(2), 219–225 (2004).

6 Kiang TK, Ho PC, Anari MR, Tong V, Abbott FS, Chang TK. Contribution of *CYP2C9*, *CYP2A6*, and *CYP2B6* to valproic acid metabolism in hepatic microsomes from individuals with the *CYP2C9*1/*1* genotype. *Toxicol. Sci.* 94(2), 261–271 (2006).

7 Chatzistefanidis D, Georgiou I, Kyritsis AP, Markoula S. Functional impact and prevalence of polymorphisms involved in the hepatic glucuronidation of valproic acid. *Pharmacogenomics* 13(9), 1055–1071 (2012).

8 Krishnaswamy S, Hao Q, Al-Rohaimi A *et al.* UDP glucuronosyltransferase (UGT) 1A6 pharmacogenetics: II. Functional impact of the three most common nonsynonymous *UGT1A6* polymorphisms (S7A, T181A, and R184S). *J. Pharmacol. Exp. Ther.* 313(3), 1340–1346 (2005).

9 Guo Y, Hu C, He X, Qiu F, Zhao L. Effects of *UGT1A6*, *UGT2B7*, and *CYP2C9* genotypes on plasma concentrations of valproic acid in Chinese children with epilepsy. *Drug Metab. Pharmacokinet.* 27(5), 536–542 (2012).

10 Hung CC, Ho JL, Chang WL *et al.* Association of genetic variants in six candidate genes with valproic acid therapy optimization. *Pharmacogenomics* 12(8), 1107–1117 (2011).

11 Sun YP, Tan L, Wang Y, Song JH. Effect of *UGT1A6* genetic polymorphisms on the metabolism of sodium valproate. *Zhonghua Yi Xue Za Zhi* 87(29), 2033–2035 (2007).

12 Chu XM, Zhang LF, Wang GJ, Zhang SN, Zhou JH, Hao HP. Influence of UDP-glucuronosyltransferase polymorphisms on valproic acid pharmacokinetics in Chinese epilepsy patients. *Eur. J. Clin. Pharmacol.* 68(10), 1395–1401 (2012).

13 Wang Y, Gao L, Liu YP, Huang NN, Xu SJ, Ma DJ. Effect of *UGT1A6* A541G genetic polymorphism on the metabolism of valproic acid in Han epileptic children from Henan. *Zhongguo Dang Dai Er Ke Za Zhi* 12(6), 429–432 (2010).

14 Chung JY, Cho JY, Yu KS *et al.* Pharmacokinetic and pharmacodynamic interaction of lorazepam and valproic acid in relation to *UGT2B7* genetic polymorphism in healthy subjects. *Clin. Pharmacol. Ther.* 83(4), 595–600 (2008).

15 Sun YX, Zhuo WY, Lin H *et al.* The influence of *UGT2B7* genotype on valproic acid pharmacokinetics in Chinese epilepsy patients. *Epilepsy Res.* 114, 78–80 (2015).

16 Wang Q, Zhao L, Liang M *et al.* Effects of *UGT2B7* genetic polymorphisms on serum concentrations of valproic acid in Chinese children with epilepsy comedicated with lamotrigine. *Ther. Drug Monit.* 38(3), 343–349 (2016).

17 Inoue K, Suzuki E, Yazawa R *et al.* Influence of uridine diphosphate glucuronosyltransferase 2B7 -161C>T polymorphism on the concentration of valproic acid in pediatric epilepsy patients. *Ther. Drug Monit.* 36(3), 406–409 (2014).

18 Ma H, Zhang T, Gong Z *et al.* Effect of *UGT2B7* genetic variants on serum valproic acid concentration. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 38(8), 766–772 (2013).

19 Woo S, Gardner ER, Chen X *et al.* Population pharmacokinetics of romidepsin in patients with cutaneous T-cell lymphoma and relapsed peripheral T-cell lymphoma. *Clin. Cancer Res.* 15(4), 1496–1503 (2009).

20 Sissung TM, Gardner ER, Piekarz RL *et al.* Impact of *ABCB1* allelic variants on QTc interval prolongation. *Clin. Cancer Res.* 17(4), 937–946 (2011).

21 Wong NS, Seah E, Wang LZ *et al.* Impact of UDP-gluconoryltransferase 2B17 genotype on vorinostat metabolism and clinical outcomes in Asian women with breast cancer. *Pharmacogenet. Genomics* 21(11), 760–768 (2011).

22 Gandia P, Arellano C, Chalret Du Rieu Q *et al.* Unexpected high levels of vorinostat when combined with vinorelbine in patients with advanced cancer. *Curr. Clin. Pharmacol.* 6(4), 274–279 (2011).

23 Goey AK, Sissung TM, Peer CJ *et al.* Effects of *UGT1A1* genotype on the pharmacokinetics, pharmacodynamics and toxicities of belinostat administered by 48 h continuous infusion in patients with cancer. *J. Clin. Pharmacol.* 56(4), 461–473 (2015).

24 Hamberg P, Woo MM, Chen LC *et al.* Effect of ketoconazole-mediated CYP3A4 inhibition on clinical pharmacokinetics of panobinostat (LBH589), an orally active histone deacetylase inhibitor. *Cancer Chemother. Pharmacol.* 68(3), 805–813 (2011).

25 Fatima N, Cohen DC, Sukumar G *et al.* Histone deacetylase inhibitors modulate KATP subunit transcription in HL-1

cardiomyocytes through effects on cholesterol homeostasis. *Front. Pharmacol.* 6, 168 (2015).

26 Cho YS, Whitehead L, Li J *et al.* Conformational refinement of hydroxamate-based histone deacetylase inhibitors and exploration of 3-piperidin-3-ylindole analogues of dacinostat (LAQ824). *J. Med. Chem.* 53(7), 2952–2963 (2010).

27 Shultz MD, Cao X, Chen CH *et al.* Optimization of the *in vitro* cardiac safety of hydroxamate-based histone deacetylase inhibitors. *J. Med. Chem.* 54(13), 4752–4772 (2011).

28 Song P, Lamba JK, Zhang L *et al.* G2677T and C3435T genotype and haplotype are associated with hepatic *ABCB1* (*MDR1*) expression. *J. Clin. Pharmacol.* 46(3), 373–379 (2006).

29 Meissner K, Jedlitschky G, Meyer Zu Schwabedissen H *et al.* Modulation of multidrug resistance P-glycoprotein 1 (*ABCB1*) expression in human heart by hereditary polymorphisms. *Pharmacogenetics* 14(6), 381–385 (2004).

30 Noonan AM, Eisch RA, Liewehr DJ *et al.* Electrocardiographic studies of romidepsin demonstrate its safety and identify a potential role for K(ATP) channel. *Clin. Cancer Res.* 19(11), 3095–3104 (2013).

31 Kim RB, Leake BF, Choo EF *et al.* Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin. Pharmacol. Ther.* 70(2), 189–199 (2001).

32 Balliet RM, Chen G, Gallagher CJ, Dellinger RW, Sun D, Lazarus P. Characterization of UGTs active against SAHA and association between SAHA glucuronidation activity phenotype with UGT genotype. *Cancer Res.* 69(7), 2981–2989 (2009).

33 Kang SP, Ramirez J, House L *et al.* A pharmacogenetic study of vorinostat glucuronidation. *Pharmacogenet. Genomics* 20(10), 638–641 (2010).

34 Park J, Chen L, Ratnashinge L *et al.* Deletion polymorphism of UDP-glucuronosyltransferase 2B17 and risk of prostate cancer in African American and Caucasian men. *Cancer Epidemiol. Biomarkers Prev.* 15(8), 1473–1478 (2006).

35 Gruber M, Le T, Filipits M *et al.* UDP-glucuronosyltransferase 2B17 genotype and the risk of lung cancer among Austrian Caucasians. *Cancer Epidemiol.* 37(5), 625–628 (2013).

36 Gruber M, Bellemare J, Hoermann G *et al.* Overexpression of uridine diphospho glucuronosyltransferase 2B17 in high-risk chronic lymphocytic leukemia. *Blood* 121(7), 1175–1183 (2013).

37 Wang LZ, Ramirez J, Yeo W *et al.* Glucuronidation by UGT1A1 is the dominant pathway of the metabolic disposition of belinostat in liver cancer patients. *PLoS ONE* 8(1), e54522 (2013).

38 Akaba K, Kimura T, Sasaki A *et al.* Neonatal hyperbilirubinemia and mutation of the bilirubin uridine diphosphate-glucuronosyltransferase gene: a common missense mutation among Japanese, Koreans and Chinese. *Biochem. Mol. Biol. Int.* 46(1), 21–26 (1998).

39 Minami H, Sai K, Saeki M *et al.* Irinotecan pharmacokinetics/pharmacodynamics and *UGT1A* genetic polymorphisms in Japanese: roles of *UGT1A1*6* and **28*. *Pharmacogenet. Genomics* 17(7), 497–504 (2007).

40 Hall D, Ybazeta G, Destro-Bisol G, Petzl-Erler ML, Di Rienzo A. Variability at the uridine diphosphate glucuronosyltransferase 1A1 promoter in human populations and primates. *Pharmacogenetics* 9(5), 591–599 (1999).

41 Beutler E, Gelbart T, Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? *Proc. Natl Acad. Sci. USA* 95(14), 8170–8174 (1998).

42 Innocenti F, Grimsley C, Das S *et al.* Haplotype structure of the UDP-glucuronosyltransferase 1A1 promoter in different ethnic groups. *Pharmacogenetics* 12(9), 725–733 (2002).

43 FDA. Full prescribing information Beleodaq. www.accessdata.fda.gov/

44 Peer CJ, Goey AKL, Sissung TM *et al. UGT1A1* genotype-dependent dose adjustment of belinostat using population pharmacokinetic modeling and simulation in patients with small cell lung cancer and other advanced cancers. *Clin. Pharmacol. Ther.* 56(4), 450–460 (2015).

45 Thomas A, Rajan A, Szabo E *et al.* A Phase I/II trial of belinostat in combination with cisplatin, doxorubicin and cyclophosphamide in thymic epithelial tumors: a clinical and translational study. *Clin. Cancer Res.* 20(21), 5392–5402 (2014).

46 Hu ZY, Yu Q, Pei Q, Guo C. Dose-dependent association between *UGT1A1*28* genotype and irinotecan-induced neutropenia: low doses also increase risk. *Clin. Cancer Res.* 16(15), 3832–3842 (2010).

47 Hoskins JM, Goldberg RM, Qu P, Ibrahim JG, Mcleod HL. *UGT1A1*28* genotype and irinotecan-induced neutropenia: dose matters. *J. Natl Cancer Inst.* 99(17), 1290–1295 (2007).

48 Atadja P. Development of the pan-DAC inhibitor panobinostat (LBH589): successes and challenges. *Cancer Lett.* 280(2), 233–241 (2009).

49 Fredenhagen A, Kittelmann M, Oberer L *et al.* Biocatalytic synthesis and structure elucidation of cyclized metabolites of the deacetylase inhibitor panobinostat (LBH589). *Drug Metab. Dispos.* 40(5), 1041–1050 (2012).