

**Keywords:** GIST; imatinib resistance; histone deacetylase inhibitors; KIT; targeted therapy; LBH589; panobinostat

# Phase I study of panobinostat and imatinib in patients with treatment-refractory metastatic gastrointestinal stromal tumors

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**Background:** Panobinostat, a pan-deacetylase inhibitor, overcomes imatinib resistance in preclinical models of gastrointestinal stromal tumours (GIST). Here we determined the maximum tolerated dose (MTD) and dose-limiting toxicities (DLT) of panobinostat in combination with imatinib (IM) for treatment of patients with refractory GIST.

**Methods:** Following a 7-day run-in phase of IM (400 mg per day), escalating doses of panobinostat were added following a '3 plus 3' design. Twelve heavily pretreated GIST patients were enrolled in two dose levels.

**Results:** Most common adverse events were thrombocytopenia, anaemia, fatigue, creatinine elevation, nausea, emesis and diarrhoea. Twenty micrograms of panobinostat and 400 mg IM were declared the MTD. Pharmacologically active concentrations of panobinostat and IM were achieved as evidenced by histone H3 acetylation in blood mononuclear cells *in vivo* and inhibition of the IM-resistant KIT (D816) mutation *in vitro*. In FDG-PET-CT scans after IM run-in and following 3 weeks panobinostat treatment, 1 out of 11 evaluable patients showed a metabolic partial response, 7 patients were metabolically stable and 3 patients progressed. Longest treatment duration was 17 weeks (median 6).

**Conclusion:** Panobinostat and IM can be administered at doses achieving target inhibition *in vivo*. Further clinical exploration of patients with treatment-refractory GIST is warranted. Correlative studies in this trial may help to optimise dosing schedules in GIST.

Activating mutations of KIT or platelet-derived growth factor receptor A (PDGFRA) represent the key oncogenic events in gastrointestinal stromal tumors (GIST) (Hirota *et al*, 1998; Heinrich *et al*, 2003). Imatinib mesylate (IM) is a small molecule inhibitor of KIT and PDGFRA that provides long-lasting responses

in most patients (82–86%), with a median overall survival of 5 years (Verweij *et al*, 2004; Blanke *et al*, 2008a, 2008b). Despite long-lasting responses, most patients eventually acquire resistance and progress. Salvage treatments such as sunitinib and regorafenib may still achieve objective remissions (7% and 4.5%), but median

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time to progression is only in the range of 6 months (Demetri *et al*, 2006, 2012). Therefore, alternative treatment strategies for IM-resistant GIST are needed.

Panobinostat (LBH589) is a novel cinnamic hydroxamic acid inhibitor of histone deacetylases (HDACi), which is active on HDAC class I and II isoforms at nanomolar concentrations (Geng *et al*, 2006). Inhibition of HDACs has been effective in several human cancers, in particular haematologic malignancies, in clinical trials (Giles *et al*, 2006). Since 2006, two HDACi, vorinostat (SAHA) and romidepsin, have received FDA approval for the treatment of advanced cutaneous T-cell lymphoma (Mann *et al*, 2007; Xu *et al*, 2007; Whittaker *et al*, 2010). We have preclinically evaluated the activity of different classes of HDACi in GIST. The pan-HDACi panobinostat (LBH589) potentially inhibited the growth of GIST cell lines *in vitro* (IC50 values between 80 and 100 nM) (Mühlenberg *et al*, 2009). Interestingly, panobinostat activity was only seen in KIT-positive GIST models indicating target specificity. This was mediated by transcriptional repression of KIT and by acetylation and subsequent inhibition of heat-shock protein 90 (HSP90), a critical chaperone protein for KIT stabilisation and non-histone client of HDAC6 (Mühlenberg *et al*, 2009). Additive antitumoral efficacy of panobinostat and IM was observed in IM-resistant GIST models *in vitro* and *in vivo* (Floris *et al*, 2009a).

Against this background, we conducted a phase I study of panobinostat in combination with IM for treatment of patients with metastatic GIST who had progressed on IM and sunitinib.

## PATIENTS AND METHODS

**Patients.** Patients with metastatic GIST (aged 18 years and older) refractory to IM and sunitinib were enrolled in the trial following written informed consent. Additional main inclusion criteria were Eastern Cooperative Oncology Group performance score 2 or lower, absolute neutrophil count 1500 per litre, platelets 100 000 per litre, serum transaminase activity  $<2.5 \times$  ULN, total bilirubin  $<1.5 \times$  ULN, serum creatinine  $<1.5 \times$  ULN or a creatinine clearance of  $\geq 60$  ml min<sup>-1</sup>, fasting serum cholesterol  $\leq 350$  mg dl<sup>-1</sup>, triglycerides  $\leq 300$  mg dl<sup>-1</sup>, proteinuria  $\leq 1$  g in 24 h, no prior thromboembolic disease, no history of haematemesis or haemoptysis. Patients with a history of cardiac disease (congestive heart failure ( $>$  New York Heart Association class 2), active coronary artery disease, cardiac arrhythmias requiring antiarrhythmic therapy other than beta blockers or digoxin, uncontrolled hypertension; myocardial infarction less than 6 month before study entry) were excluded. There was no limit on the number of previous anticancer regimens. The institutional review boards of both sites approved the study. A central pathology review that included KIT and PDGFRA mutational testing was performed for all patients from archival tissue by one of the investigators (FG).

**Study design and treatment.** WTZ-GIST-09-01 was an open-label, single-arm, bicentric phase I trial to determine the maximum-tolerated dose (MTD) and dose-limiting toxicities (DLT) of panobinostat in combination with IM. In addition, the study assessed the safety, tolerability, activity, pharmacokinetics and pharmacodynamic activity of the combination. Given a low risk of QTc prolongation, a strict ECG screen at baseline and during treatment was incorporated in the protocol. To obtain a preliminary signal for clinical activity, a prespecified expansion of the MTD cohort to a maximum of nine patients was implemented. IM was administered orally at a dose of 400 mg once daily. As patients may respond to an imatinib rechallenge (<http://meetinglibrary.asco.org/content/116140-132>), the baseline FDG-PET scan was performed following a 7-day run-in phase, and

escalating doses of panobinostat (three times per week for 3 out of 4 weeks) were added. One treatment cycle consisted of 29 days. The dose escalation for panobinostat followed the strict traditional escalation rule (STER). Using STER, patients are treated in cohorts of three, each receiving the same dose. If no DLT occurs, the next cohort of three will receive the next higher dose. If one DLT occurs, the cohort is expanded to three other patients. If two or more DLTs occur, escalation stops and the dose is de-escalated to the next lower level (Chin and Lee, 2008). We selected 20 mg as the starting dose following the discussion with the panobinostat development team from Novartis, based on the existing data on combination trials with panobinostat (Drappatz *et al*, 2012). WTZ-GIST-09-01 (EudraCT number: 2009-011417-24; German Clinical Trials Register: DRKS00000657) was approved by the institutional ethics committees of the two participating centers and the German Federal Regulatory Drug Agency (BfArM; 4035954).

**Dose-limiting toxicities.** DLTs were defined from the safety profile of cycle 1 for each dose level. At dose level 1, panobinostat 20 mg was given as a three-times-per-week (MWF schedule) oral dose for 3 out of 4 weeks. Panobinostat doses were increased by 10 mg if no DLT emerged. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC version 3.0). Haematological DLTs were defined as  $\geq$ CTCAE grade 3 neutropenia lasting for  $>7$  days, CTCAE grade 3 thrombocytopenia lasting for  $>7$  days, CTCAE grade 4 thrombocytopenia and neutropenic fever (ANC (including bands)  $<1.0 \times 10^9$  l<sup>-1</sup>, fever  $\geq 38.5$  °C). Non-haematologic DLT was defined as any grade 3 or 4 toxicity occurring during cycle 1.

**Pharmacokinetics and biomarkers.** Blood sampling for pharmacokinetic (PK) and pharmacodynamic (PD) studies was performed on day 1, 8, 15, 22 and 29. For PK analyses, blood was withdrawn into EDTA tubes and centrifuged immediately at  $2000 \times g$  for 10 min at 4 °C. For extraction of panobinostat and IM, 400  $\mu$ l plasma was mixed with 600  $\mu$ l acetonitrile. Free IM, *N*-desmethyl-IM (CGP074588) and panobinostat were measured by liquid chromatography coupled to a photodiode array (LC/PDA) spectrometer (Acquity Ultra Performance LC system, Waters GmbH, Eschborn, Germany). Pharmacokinetics were calculated using the TOPFIT 2.0 software (Thomae GmbH, Biberach, Germany). A concentration/time plot for IM and desmethyl-IM was generated. The pharmacokinetics of IM and desmethyl-IM have been described by a two-compartment distribution.

Acetylation of histone H3 in peripheral blood mononuclear cells (PBMNC) was assessed as a PD marker of panobinostat activity *in vivo*. Protein lysates were prepared from PBMNC and subjected to electrophoresis and immunoblotting as previously described (Rubin *et al*, 2001; Duensing *et al*, 2004). Changes in protein expression were visualised by chemiluminescence and captured and quantified using a FUJI LAS3000 system with Science Lab 2001 ImageGauge 4.0 software (Fujifilm Medical Systems, Stamford, CT, USA). To identify the lowest plasma level of panobinostat that would induce acetylation, PBMNC from two healthy volunteers were incubated *ex vivo* with increasing concentrations of panobinostat. In addition, IM-sensitive GIST-T1 (exon 11-mutant) cells and the highly IM-resistant subline GIST-T1-juke (exon 11 and exon 17 D816E double mutant) were incubated *in vitro* with panobinostat at concentrations that were achieved in study patients as confirmed by our PK analyses.

**Evaluation of clinical activity.** Full-dose CT scans were performed at least 14 days before randomisation and at least every 56 days. Metabolic imaging studies were conducted at baseline after the run-in period with IM (day 7) before administration of the first dose of panobinostat to exclude metabolic effects by IM rechallenge. All patients were instructed to fast for 6 h before intravenous administration of 18 F-FDG (median: 273 MBq,

range: 250–450 MBq). Patients with a blood glucose level exceeding 150 mg dl<sup>-1</sup> were not included in the study. Whole-body PET/CT scans were obtained using a PET/CT system (mCT; Siemens Molecular Imaging, Eschborn, Germany) after a median of 65 min (range: 50–80 min) post injection of 18F-FDG.

PET scans were repeated on day 29 of the first cycle. In case of clinical progression, a full-dose PET-CT was performed instead. Metabolic response was defined according to the EORTC-PET Study Group criteria; radiologic response was evaluated per RECIST. Patients with clinical, radiological and/or metabolic response or disease stabilisation were continued on trial medication until progression. In the follow-up phase of the study, CT scans were repeated every 56 days.

## RESULTS

**Patient characteristics.** In all, 12 patients (median 56 years, range 34–74) with metastatic GIST were enrolled at two sarcoma centers. All patients were eligible and evaluable for toxicity. Patients had been heavily pretreated with a median of five treatment lines (range 3–11) before study entry. Clinical and epidemiological data and treatment outcome of the study patients are summarised in Table 1.

**Safety.** All patients experienced at least one adverse event (AE), the majority of AEs were graded mild. The most common AEs were thrombocytopenia (92%), fatigue (67%), creatinine elevation (58%), nausea (50%), anaemia (42%), diarrhoea (42%) and vomiting (33%; Table 2). The majority of events (thrombocytopenia, nausea, diarrhoea) occurred in the third week of panobinostat treatment, but quickly resolved during week 4, in which no panobinostat was given. Although AE grades 3 or 4 were uncommon, the MWF schedule for 3 weeks was associated with

severe thrombocytopenia, which was the dose-limiting toxicity at dose level 2 (30 mg panobinostat). Grade 3 and 4 toxicities that were clearly attributable to study treatments were thrombocytopenia, neutropenia and nausea. After panobinostat rechallenge (3 patients at panobinostat 30 mg, 1 patient at 20 mg) at reduced dose, thrombocytopenia was <grade III except for one patient (grade 3) in cycle 2. This resolved after further dose reduction. There were no treatment-related deaths, but one patient died of progressive disease shortly after discontinuation of treatment.

**Pharmacokinetics.** Plasma concentrations of IM, desmethyl-IM (IM-metabolite) and panobinostat were determined in 14 treatment courses from 12 patients. Results are summarised in Table 3. The mean peak concentrations (C<sub>max</sub>) of panobinostat were 12.6, 16.6 and 15.6 ng ml<sup>-1</sup> (Days 1, 8 and 15) at dose level 1 (*n* = 8), which was declared the MTD (Table 3, Figure 1A). Because of low plasma concentrations of panobinostat, only the C<sub>max</sub> was calculated. The lower limit of detection for panobinostat in plasma was 2 ng ml<sup>-1</sup>, and the lower limit of quantification was 4 ng ml<sup>-1</sup>. The mean C<sub>max</sub> for IM was 2068 ng ml<sup>-1</sup> during the 7-day run-in period, which was not significantly different from the C<sub>max</sub> of 2564 ng ml<sup>-1</sup> during combination therapy (*P* = 0.185, *t*-test). Mean IM trough levels during the run-in period and during combination therapy amounted 1085 ng ml<sup>-1</sup> and 1290 ng ml<sup>-1</sup> (*P* = 0.125). The terminal half-life for IM in the presence of panobinostat was 20.5 h, whereas the terminal half-life of IM alone is approximately 18 h (Peng *et al*, 2005). Actual plasma levels for desmethyl-IM, the main metabolite of IM, were as predicted, thus making a pharmacodynamic interaction of panobinostat on IM metabolism highly unlikely (Figure 1B).

**Pharmacodynamic biomarkers for target inhibition *in vivo*.** We assessed the acetylation of histone H3 (H3) in PBMCs obtained from five patients treated at dose level 1 (MTD) as a pharmacodynamic biomarker for the activity of panobinostat

Table 1. Patient characteristics

Pat	Dose level	Age	Sex	# Pretreatments	Type of pretreatment	Gene, exon	Primary mutation	ECOG	PET-response	RECIST	Days on study treatment
101	20	34	m	11	IM, SU, other	<i>KIT</i> exon 11, Exon 17	Del E554-V559; D820Y	0	mPD	PD	25
102	20	41	m	5	IM, SU, Nilo, Sora, other	<i>PDGFR</i> Exon 12	Del V559-P573; D820G	0	mPR	SD	116
103	30	60	m	4	IM, SU, Dasa, Nilo	<i>PDGFR</i> Exon 18	D842V	0	mSD	PD	12
104	30	38	w	5	IM, SU, Nilo, Sora, other	<i>KIT</i> exon 9	duplication A502 and Y503; Y823D		mPD	SD	26
106	20 Extension	75	w	4	IM, SU, Nilo, Sora	—	NA	1	mSD	SD	35
107	20 Extension	38	m	4	IM, SU, Nilo, other	<i>KIT</i> intron 10/exon 11	K550–558del <sup>a</sup>	0	mPD	—	18
108	20 Extension	55	m	5	IM, SU, other	<i>KIT</i> , Exon 11	K558-V559insP	0	mSD	SD	
201	20	56	m	3	IM, SU, other	<i>KIT</i> , Exon 13	K642E	0	mSD	SD	74
202	30	54	w	4	IM, SU, other	c- <i>KIT</i> Exon 11	Del K558	1	mSD	SD	65
203	30	74	w	5	IM, SU, Sora, other	—	Exon 11 wt, other exons not analysed	1	—	PD	86
204	20	63	w	8	IM, SU, Nilo, Sora, other	<i>KIT</i> , Exon 9	Duplication A502 and Y503	1	mSD	SD	51
205	20	69	m	10	IM, SU, Nilo, Sora, other	<i>KIT</i> , Exon 11	Y553-K558delinsW	1	mSD	SD	72

Abbreviations: IM = imatinib; mPD = metabolic progressive disease; mPR = metabolic partial response; mSD = metabolic stable disease; NA = not applicable; Nilo = nilotinib; Sora = Sorafenib; SU = sunitinib.

<sup>a</sup>Mutational analysis kindly provided by Professor Stephan Dirnhofer, Basel.

Table 2. Summary of adverse events (&gt; 10% relative frequency)

Toxicity	Dose level panobinostat	No. of patients						Total	%
		Grade 1	Grade 2	Grade 3	Grade 4				
Thrombocytopenia	20 mg	3	1	1	1	6	11	91.7	
	30 mg	—	—	3	2	5			
Fatigue	20 mg	3	3	—	—	6	8	66.7	
	30 mg	—	2	—	—	2			
Creatinine	20 mg	2	2	1	—	5	7	58.3	
	30 mg	1	1	—	—	2			
Nausea	20 mg	1	1	—	—	2	6	50.0	
	30 mg	1	2	1	—	4			
Anaemia	20 mg	—	3	—	—	3	5	41.7	
	30 mg	—	2	—	—	2			
Diarrhoea	20 mg	3	1	—	—	4	5	41.7	
	30 mg	1	—	—	—	1			
Vomiting	20 mg	—	1	—	—	1	4	33.3	
	30 mg	2	1	—	—	3			
Dizziness	20 mg	2	—	—	—	2	3	25.0	
	30 mg	1	—	—	—	1			
Dyspepsia	20 mg	2	—	—	—	2	3	25.0	
	30 mg	1	—	—	—	1			
Hypothyroidism	20 mg	3	—	—	—	3	3	25.0	
	30 mg	—	—	—	—	0			
Muscle spasms	20 mg	1	1	—	—	2	3	25.0	
	30 mg	—	1	—	—	1			
Oedema	20 mg	2	—	—	—	2	3	25.0	
	30 mg	1	—	—	—	1			
Weight loss	20 mg	1	1	—	—	2	3	25.0	
	30 mg	—	1	—	—	1			
Chills	20 mg	1	1	—	—	2	2	16.7	
	30 mg	—	—	—	—	0			
Constipation	20 mg	1	—	—	—	1	2	16.7	
	30 mg	1	—	—	—	1			
Dry mouth	20 mg	2	—	—	—	2	2	16.7	
	30 mg	—	—	—	—	0			
Dry skin	20 mg	1	—	—	—	1	2	16.7	
	30 mg	1	—	—	—	1			
Dysgeusia	20 mg	2	—	—	—	2	2	16.7	
	30 mg	—	—	—	—	0			
Headache	20 mg	1	—	—	—	1	2	16.7	
	30 mg	—	1	—	—	1			
Lymphopenia	20 mg	—	—	1	—	1	2	16.7	
	30 mg	—	1	—	—	1			
Neutropenia	20 mg	—	—	—	1	1	2	16.7	
	30 mg	—	1	—	—	1			

The highest grade per patient that occurred within the core study (cycle 1) or consecutive cycles as graded by NCI-CTCAE toxicity criteria (version 3.0).

(Figure 1C). Peak H3 acetylation was observed between 4 and 8 h following the first oral dose of panobinostat in four of five patients. H3 acetylation was maintained for 24 h in three patients and decreased at later time points in most patients (not shown). H3 acetylation correlated with detectable plasma levels of panobinostat, although not quantitatively (Figure 1C).

To corroborate these findings, we incubated PBMNCs from healthy donors with panobinostat *ex vivo* for 8 h. At concentrations of 5 ng ml<sup>-1</sup> and above, H3 acetylation became detectable (Figure 2A). Time course experiments with GIST-T1 cells (IM-sensitive cell line) confirmed that H3 acetylation persisted

for 8 h after removal of panobinostat and completely disappeared within 24 h (Figure 2B). Incubating GIST-T1 cells and IM-resistant GIST-T1 (KIT D816E mutant) with panobinostat and IM at those concentrations clinically achieved in study patients treated at the MTD (C<sub>max</sub> and ½ C<sub>max</sub>) demonstrated complete inhibition of KIT phosphorylation (Figure 2C).

**Clinical activity.** No objective responses per RECIST were observed (8 SD, 3 PD). Most patients had extensive metastatic disease, and a decrease in size or change of density was rarely seen in single lesions. Changes in metabolic activity of tumour lesions

Table 3. Summary of pharmacokinetic analyses for IM and panobinostat

PK parameter	Concentration (ng ml <sup>-1</sup> ) (± s.e.m.)	Concentration (± s.e.m.)
<b>Panobinostat Cmax (mean) at 20 mg</b>		
D1	12.6 (± 3.6)	36.1 nM (± 10.3 nM)
D8	16.6 (± 2.3)	47.5 nM (± 16.6 nM)
D15	15.6 (± 3.8)	44.6 nM (± 10.9 nM)
IM Cmax (mean): (P=0.185)	2068 (± 310) (without pan) 2564 (± 190) (with pan)	3.5 μM (± 0.5 μM) (without pan) 4.3 μM (± 0.3 μM) (with pan)
IM terminal half-life:	20.5 h (with pan)	
IM trough levels: (mean) (P=0.125)	1085 (± 480) (without pan) 1290 (± 440) (with pan)	1.8 μM (± 0.8 μM) (without pan) 2.2 μM (± 0.7 μM) (with pan)

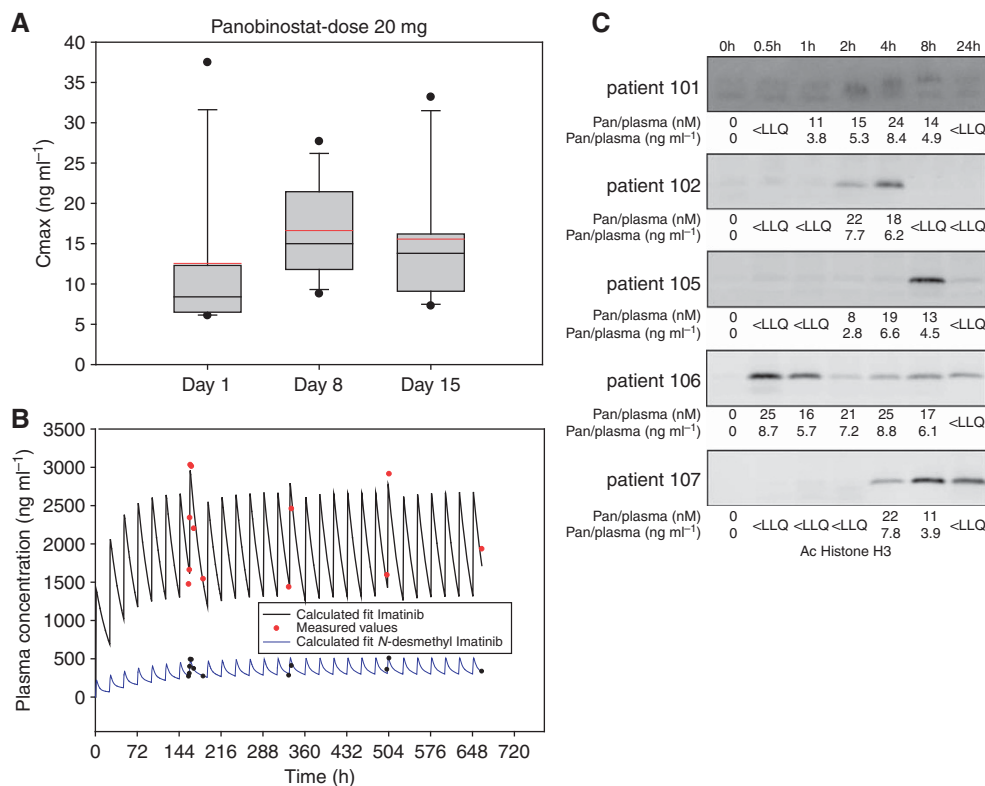


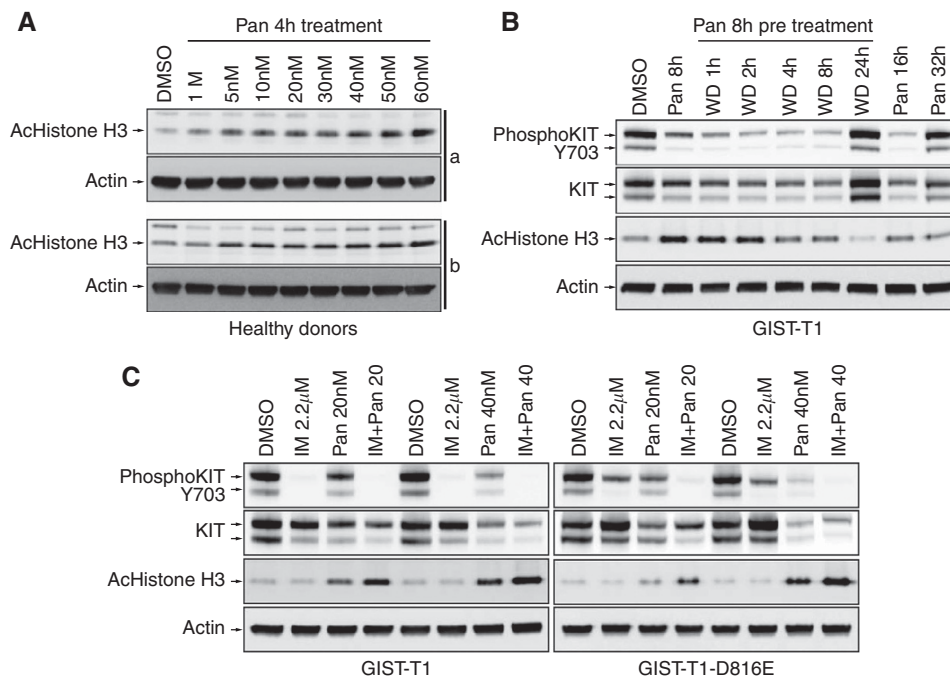
Figure 1. Pharmacokinetic studies and biomarker analyses. (A) Cmax diagram for panobinostat at day 1 (first dose panobinostat), day 8 and day 15. The black lines within the boxes indicate the median values, the red lines the mean values. (B) Calculated fit curves for IM and d-desmethyl IM. (C) Immunoblot analyses for acetylated histone H3 from lysates of PBMCs taken at various time points from selected patients with corresponding panobinostat plasma levels. LLQ = lower level of quantification.

were assessed by comparing the individual patients' <sup>18</sup>F-DG-PET scans obtained at the end of the IM run-in with those obtained on day 29 of cycle 1. Eleven patients were evaluable for PET response, and one patient treated at dose level 1 achieved a metabolic partial response (mPR) as defined by EORTC-PET Study Group criteria (Young *et al*, 1999). Another seven patients exhibited metabolic disease stabilisation, and three patients had metabolically progressive disease (Figure 3A). The median treatment duration was 6 weeks. The patient achieving a mPR was treated for 17 weeks (Table 1; Figure 3B). He had been diagnosed with metastatic disease 6 years before study enrolment and already received imatinib, sunitinib, nilotinib, imatinib rechallenge, radiation therapy to the large tumour bulk and sorafenib. We attribute his disease stabilisation to the panobinostat addition as he was treated with imatinib at 800 mg qd<sup>-1</sup> before the study and enrolled into the trial due to progressive disease at that dose.

## DISCUSSION

In spite of the vast improvement in the treatment of metastatic GIST since the introduction of IM, most patients eventually develop resistance and progress. The mortality of IM-resistant GIST patients amounts 10% per year, which highlights the need for effective salvage treatments (Blanke *et al*, 2008a). Secondary mutations of the KIT kinase domain are the major mechanism of resistance to IM and sunitinib (Heinrich *et al*, 2006), thus providing an opportunity for the development of better KIT inhibitors. However, the genomic heterogeneity of these resistance mutations poses a pharmacological challenge to develop selective KIT inhibitors that inhibit all possible mutations (Wardelmann *et al*, 2005). In contrast to ATP-competitive drugs, such as IM, sunitinib or regorafenib, allosteric KIT inhibitors exhibited an



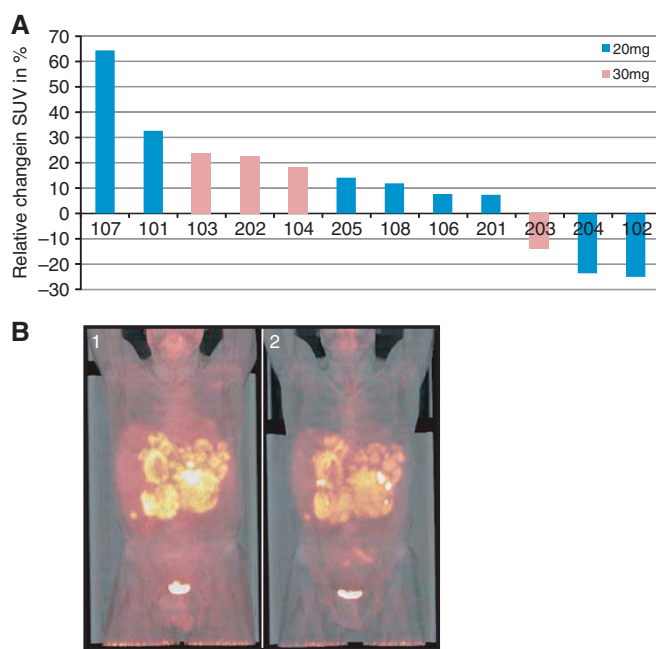


**Figure 2.** Preclinical revalidation of clinical biomarkers and tumour-specific target inhibition. **(A)** Immunoblot analyses for acetylated histone H3 from lysates of PBMNCs from healthy volunteers incubated *ex vivo* with increasing concentrations of panobinostat. **(B)** Immunoblot analysis of GIST-T1 cells treated with panobinostat (Pan, 50 nm) for 8 h following a time course after withdrawal of treatment (WD). Continuous treatment with panobinostat at 16 h and 32 h are shown in the right columns. **(C)** Immunoblot analyses of imatinib-sensitive GIST-T1 and imatinib-resistant GIST-T1R treated with IM, panobinostat or with IM and panobinostat combined.

improved capability to inhibit various secondary KIT mutations including those affecting exon 17 (for example, point mutations of D816). However, the clinical utility of these compounds still remains to be proven (Heinrich *et al*, 2010).

Modulating the KIT chaperone heat-shock protein 90 (HSP90) is an alternative strategy to overcome IM resistance in GIST. Pharmacologic HSP90 inhibitors effectively inhibit KIT *in vitro* at concentrations that can be achieved in patients regardless of secondary KIT mutations (Bauer *et al*, 2006; Wagner *et al*, 2008). Unfortunately, encouraging results from early clinical trials were followed by unexpected toxicities, which were observed in the subsequent phase III trial (Demetri *et al*, 2010). Hence, the therapeutic potential of HSP90 inhibitors in the treatment of GIST remains unclear and is subject of ongoing clinical trials. Recently, HDACi such as SAHA or panobinostat were shown to have potent antitumoral activity in GIST models *in vitro* and *in vivo* (Mühlenberg *et al*, 2009; Floris *et al*, 2009b). Mechanistically, HDACi in part act by indirect inhibition of KIT via acetylation and functional inhibition of HSP90 and by transcriptional repression of KIT itself (Mühlenberg *et al*, 2009; Floris *et al*, 2009b). Combining HDACi with IM further enhanced the activity, which was even observed in IM-resistant GIST models (Mühlenberg *et al*, 2009; Floris *et al*, 2009b). These findings provided the rationale for the present phase I study. Here we aimed to identify a feasible dose and schedule for combination therapy of GIST with panobinostat and IM. We further explored pharmacodynamic effects of plasma levels of both drugs, which could be achieved in the clinic.

Dose levels of the trial were chosen based on clinical and pharmacological considerations. Notably, the MTD had to be defined after only 2 dose levels following a classical ‘3 plus 3’ design. The dose-limiting toxicity at dose level 2 (30 mg panobinostat and 400 mg IM) was thrombocytopenia, which correlated well with DLTs seen in other phase I trials with panobinostat (Fukutomi *et al*, 2012; Morita *et al*, 2012). Although most of the latter trials targeted malignancies affecting the



**Figure 3.** Clinical activity of panobinostat and imatinib in GIST. **(A)** Water fall plot of the relative change in SUV of baseline scans compared with the scans at the end of cycle 1. **(B)** FDG-PET-CT images of a patient with advanced metastatic GIST at baseline after imatinib run-in (left) and after 3 weeks panobinostat (right) resulting in a partial metabolic remission.

bone marrow, haematotoxicity also is important in GIST, a cancer not known for common bone marrow involvement. Notably, haematologic toxicity, especially in the third week of panobinostat,

requiring dose interruptions was still observed at the MTD in subsequent cycles. In this combination trial, we did not observe additional toxicities as compared with panobinostat or IM monotherapy trials in patients with other types of cancer (Fukutomi *et al*, 2012). Common toxicities included fatigue, nausea, changes in appetite or dysgeusia. These toxicities peaked during the third week of combination therapy. Notably, severe hepatotoxicity, as seen with ansamycin-based HSP90 inhibitors in GIST, did not occur. On the basis of the literature, panobinostat and IM could have a two-way pharmacokinetic interaction. CYP3A4 appears to be the main enzyme responsible for the oxidative metabolism of panobinostat in human liver microsomes. Panobinostat itself has been found to be a weak inhibitor of CYP2D6 and a weak inhibitor of CYP3A4/5. In contrast, IM is a potent inhibitor of CYP3A4 and may increase substrate levels 2- to 3.5-fold (Hamberg *et al*, 2011; Filppula *et al*, 2012). In the present trial, we observed an increase of the mean C<sub>max</sub> of IM by 23%. This is suggestive of an interaction. However, because of the limited number of patients, these findings did not reach statistical significance. Fukutomi *et al* (2012) recently reported C<sub>max</sub> plasma levels for panobinostat monotherapy of 10 ng/ml<sup>-1</sup> when given at a dose of 20 mg thrice weekly. Panobinostat C<sub>max</sub> levels in our combination study ranged from 13 to 16 ng ml<sup>-1</sup>, which may hint at an increase in exposure of panobinostat resulting from the coadministration of IM. This could not be formally proven as the study design did not provide a period of panobinostat monotherapy for comparison. Nonetheless, we did not find evidence for a disproportionately strong interaction.

With regard to clinical activity, we did not observe objective radiographic responses but did observe evidence of metabolic inhibition and prolonged disease stabilisation in some patients, which is noteworthy in the light of a median of five previous treatment lines. Importantly, *in vivo* inhibition of HDAC in PBMNCs from study patients was demonstrated using histone H3 acetylation as a pharmacodynamic biomarker. Moreover, our correlative *ex vivo* and *in vitro* studies confirmed that target inhibition of HDACs and KIT can be achieved at plasma levels of panobinostat and IM (C<sub>max</sub> and ½ C<sub>max</sub>), which were observed in the present trial. Notably, at these concentrations, panobinostat and IM inhibited the notorious KIT D816E mutation, which is known to confer cross-resistance to IM, sunitinib, sorafenib and regorafenib in GIST (Heinrich *et al*, 2012; and data not shown).

In the present trial, panobinostat plasma levels were below the lowest level of quantification 24 h after the first dose. This situation was recapitulated in our preclinical models by removing panobinostat from the media from responding cells, resulting in complete reactivation of KIT signalling within 24 h. As KIT activation drives the disease, these findings would beg for a continuous treatment schedule, as used with direct KIT inhibitors such as IM. Notably, continuous panobinostat treatment for 32 h resulted in reactivation of KIT phosphorylation (Figure 2B).

We believe that these data are particularly relevant, as it shows that HDAC inhibitors may require disease-specific dosing schedules. In GIST, HDACi exert the antitumoral effect via HSP90 acetylation rather than on a complex epigenetic 'reprogramming' as in lymphomas. Dosing intervals should therefore be short enough to prevent the GIST cell to fully reactivate its KIT-driven cell cycle machinery.

With regard to the optimal duration of panobinostat treatment, the experience from the PANORAMA-1 trial in multiple myeloma has shown that panobinostat for three consecutive weeks is associated with unacceptable toxicity, as we have seen in our trial. Panobinostat 20 mg (thrice weekly) in a 2 week on ora 1 week off schedule has substantially reduced the rate of grade 3 thrombocytopenia in the combination trial in

myeloma (personal communication Novartis) and improved the tolerability, which would be the blueprint for future studies in GIST.

In conclusion, the present schedule of panobinostat in combination with IM is tolerated in heavily pretreated patients with metastatic GIST but future studies should use a 2 week on or 1 week off schedule to improve the tolerability. Haematological toxicity represents the dose-limiting toxicity. Target inhibition was achieved at the MTD, and limited evidence for metabolic tumour inhibition was observed. Acetylation of histone H3 in PBMNCs correlated with measurable panobinostat levels and seems to be a valuable biomarker. On the basis of our correlative studies, additive effects can be expected with IM and panobinostat, and KIT inhibition may even be achieved in exon 17-mutant GIST. These findings warrant further clinical exploration of HDAC inhibitors in combination with KIT inhibitors.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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