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A phase I trial of panobinostat and epirubicin in solid tumors with a dose expansion in patients with sarcoma

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Background: Treatment options for sarcoma are limited. Histone deacetylase inhibitors increase the efficacy of topoisomerase II inhibitors by promoting access to chromatin and by down-regulating DNA repair. Thus, combined panobinostat and epirubicin therapy was evaluated to treat refractory sarcoma.

Patients and methods: Patients with advanced solid tumors were enrolled in a 3 + 3 dose-escalation phase I trial of panobinostat given on days 1, 3, and 5 followed by 75 mg/m² of epirubicin on day 5 in 21-day cycles, with a dose expansion at maximum tolerated dose (MTD) in 20 sarcoma patients. Peripheral blood mononucleocyte histone acetylation was also evaluated.

Results: Forty patients received 20–60 mg panobinostat. Dose-limiting toxicities included thrombocytopenia, febrile neutropenia, and fatigue at 60 mg, defining a panobinostat MTD at 50 mg. Four responses were seen in 37 assessable patients, all after progression on prior topoisomerase II inhibitors. For those with sarcoma, 12 of 20 derived clinical benefit (1 partial response and 11 stable disease, median overall survival 8.3 months), including 8 of 14 previously progressed on topoisomerase II therapy. Treatment benefits correlated with increased histone acetylation and decreased neutrophil count on day 5.

Conclusions: Panobinostat and epirubicin treatment is well tolerated and may reverse anthracycline resistance. Changes in histone acetylation and associated decrease in neutrophil count correlated with clinical benefit and warrant investigation as predictive biomarkers.

Clinical trial: This trial is registered at www.Clinicaltrials.gov, Identifier: NCT00878904.

Key words: histone deacetylase, panobinostat, sarcoma, epirubicin, topoisomerase

introduction

More than half of patients treated for localized soft tissue sarcoma will experience relapse. Anthracycline-based chemotherapy remains the standard of care in the first-line setting [1], including topoisomerase II inhibitors doxorubicin and epirubicin that act by increasing DNA damage and promoting apoptosis [2]. Response rates are low and resistance to doxorubicin is common [3, 4]. Treatment with pazopanib modestly improves

progression-free survival (PFS) from 1.6 to 4.6 months, but not overall survival (OS) 12.5 versus 10.7 months [5]. Trabectedin was recently approved with similar benefit for liposarcoma and leiomyosarcoma; eribulin showed PFS of 2.6 months and OS of 13.5 months [6].

Histone deacetylases (HDACs) modulate gene expression and protein activity by regulating protein acetylation. HDAC inhibitors, vorinostat and romidepsin, have been approved for T-cell lymphoma and panobinostat for myeloma [7]. Preclinical studies have shown that HDAC inhibitors potentiate DNA-damaging chemotherapeutics in various cancer types, including sarcoma [8–11]. Prior clinical studies evaluating HDAC inhibitors in combination with anthracyclines demonstrated efficacy [12, 13]. Supportive preclinical studies suggest a role for

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epigenetic regulation in sarcoma tumorigenesis [14]. In particular, HDAC2 is highly expressed in sarcomas [15]. HDAC inhibition was shown to reverse repression of gene expression by translocation products such as PAX3/FKHR in alveolar rhabdomyosarcoma, EWS/FLI in Ewing's sarcoma, and SS18/SSX in synovial sarcoma [16]. Furthermore, HDAC inhibition was shown to promote differentiation and apoptosis in sarcoma [17–19].

The primary objective of this study was to determine safety, tolerability, and the recommended phase II dose (RPTD) of panobinostat in combination with epirubicin in patients with advanced solid tumors and in an expansion cohort with sarcoma.

patients and methods

Eligible patients had metastatic solid tumor malignancies and any number of prior therapies with adequate organ function and normal cardiac output [left ventricular ejection fraction (LVEF) >55%]. Prior anthracycline exposure was limited to 300 mg/m² of doxorubicin and 480 mg/m² of epirubicin (refer to supplementary Data, available at *Annals of Oncology* online for further criteria).

study treatment

Consent was received from patients after federal and institutional reviews. In a single-institution phase I study with a 3 + 3 dose-escalation design, patients received panobinostat at escalating doses (20, 30, 40, 50, and 60 mg) orally once daily on days 1, 3, and 5, followed by epirubicin (75 mg/m²) on day 5, every 21 days. Dose escalation included patients with any solid tumor and restricted to 20 patients with sarcoma at the maximum tolerated dose (MTD). The primary end points were safety and toxicity evaluation and determination of a phase II recommended dose. Secondary end points included time to progression, objective response, and correlative pharmacokinetic and peripheral blood mononucleocyte (PBMC) pharmacodynamics studies.

Cumulative epirubicin exposure was restricted to 750 mg/m² of epirubicin (~1.8-fold doxorubicin-equivalent). Exceptions were allowable for patients with documented tumor response and approval by the safety review board.

treatment assessment

Toxicities were assessed by CTCAE 4.0 criteria weekly in cycle 1. Dose-limiting toxicities (DLTs) were defined as grade 3 or 4 non-hematological toxicity, and grade 4 hematological toxicity other than grade 4 neutropenia <8 days, or toxicities reducing dose delivery in cycle 1 to <75% of planned dose. Disease restaging by RECIST criteria v1.1 and LVEF assessment by ECHO or MUGA were carried out for every two cycles.

pharmacokinetic studies

Panobinostat levels were determined from plasma on day 5, 2 h post-panobinostat administration, using a validated LC-MS/MS method.

correlative studies

Whole blood was collected pretreatment on day 1 and on days 3 and 5, 2 h post-panobinostat treatment, and PBMCs were evaluated for histone acetylation as previously described [20].

statistical methods

Descriptive statistics were used to summarize patient results. Clinical benefit was defined as complete or partial response or stable disease for >3 months. A two-sided *t*-test and Pearson's correlation coefficient method were used to evaluate correlations between two variables (SigmaPlot, Systat, Inc.).

results

patient characteristics

Twenty patients with metastatic solid tumors were enrolled in five dose-escalation cohorts, and 20 patients with advanced sarcoma in the dose expansion cohort at the MTD. Of these, 17 (43%) had received prior topoisomerase II inhibitor-based chemotherapy (e.g. doxorubicin and etoposide) and a median of three prior systemic regimens (Table 1).

patient disposition, DLT, safety, and tolerability

Panobinostat was escalated from 20–60 mg/day on days 1, 3, and 5. All patients received 75 mg/m² of epirubicin. At 50 mg panobinostat, one patient experienced atrial fibrillation (AFIB) with a rapid ventricular response, which was considered a DLT

Table 1. Patient characteristics (N = 40)

Gender, n (%)	
Female	27 (67)
Male	13 (33)
Age, median (range), years	49 (22–79)
≤64	32 (80)
≥65	8 (20)
Race, n (%)	
Caucasian	31 (77)
Asian	9 (23)
African-American	0 (0)
Ethnicity, n (%)	
Non-Hispanic	32 (80)
Hispanic/Latino	8 (20)
ECOG performance status, median (range)	1 (0–2)
Tumor histology, n (%)	
Melanoma	6 (15)
Breast	5 (13)
Ovarian	2 (5)
Lung	2 (5)
Other (neuroblastoma, testicular, colon, and pancreas)	4 (10)
Sarcoma	21 (52)
Leiomyosarcoma	5 (12)
Chondrosarcoma	4 (10)
Liposarcoma	3 (8)
Phyllodes	2 (5)
Other ^a	7 (17)
Number of prior systemic regimens, median (range)	3 (0–8)
Number of regimens, n (%)	
0	3 (8)
1	4 (10)
2	12 (30)
3+	21 (53)
Number of prior systemic regimens for sarcoma cohort, median (range)	2 (0–5)
Number of patients receiving prior topoisomerase II inhibitors, n (%)	17 (40)
Number of patient receiving prior radiation therapy, n (%)	19 (48)

^aOne each: peripheral nerve sheath, fibrosarcoma, epithelioid heman-giosarcoma, alveolar soft part, synovial, pleiomorphic and sarcomatoid carcinoma.

Table 2. DLT and grade 3/4 toxicities of dose-escalation and expansion cohorts

Cohort	Panobinostat days 1, 3, and 5 ^a (mg)	N	DLTs	Grade (N): toxicity	Responses (N)
1	20	3	0	3 (2): neutropenia, WBC	SD: melanoma, neuroblastoma
2	30	3	0	3 (1): neutropenia 3 (1): fatigue	SD: ovarian
3	40	3	0	3 (1): thrombocytopenia 3 (2): neutropenia, WBC 4 (1): neutropenia, WBC	
4	50	7	Atrial fibrillation	3 (1): neutropenia, WBC 4 (2): neutropenia, WBC	PR: breast (2) SD: NSCLC
5	60	4	Febrile neutropenia Fatigue Thrombocytopenia	3 (4): neutropenia, WBC 3 (2): fatigue	PR: SCLC
MTD	50	20	Febrile neutropenia Neutropenia	(see supplementary Table S1, available at <i>Annals of Oncology</i> online)	PR: liposarcoma SD: LMS, aveolar soft part, nerve sheath, chondrosarcoma, hemangioepithelioma, sarcamoid, synovial, liposarcoma (2), phyllodes (2)

^aEpirubicin (75 mg/m²) administered on day 5 every 21 days.
SD, stable disease ≥12 weeks; PR, partial response; NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer; LMS, leiomyosarcoma; WBC, leukocytopenia.

due to its temporal relationship with study drug. At 60 mg panobinostat, two of four patients experienced a DLT (1: grade 4 thrombocytopenia and febrile neutropenia and 2: grade 3 fatigue; Table 2). Hence, the RPTD was set as 50 mg/day panobinostat on days 1, 3, and 5, and 75 mg/m² of epirubicin on day 5. Twenty patients with metastatic sarcoma were treated at this dose.

Forty patients were evaluable for treatment-emergent toxicity. A summary of grade ≥2 adverse events (AEs) is shown in supplementary Table S1, available at *Annals of Oncology* online. Twenty-four patients (60%) experienced at least one grade 3/4 treatment-related AE, including neutropenia (45%), leukopenia (35%), lymphopenia (22.5%), thrombocytopenia (17.5%), anemia (15%), and febrile neutropenia (7.5%). The most common grade 3 non-hematologic toxicities included fatigue (15%), vomiting (5%), hepatic dysfunction (5%), and elevated blood glucose levels (2.5%). One patient was hospitalized for AFIB with a rapid ventricular response, which was asymptomatic and resolved spontaneously within hours. Due to the timing of the event, a causal relationship to panobinostat could not be excluded.

Three patients had asymptomatic prolonged QTc-interval of grade 1 or 2. No significant declines in LVEF were observed on study. All patients with a partial response received more than the planned doses of epirubicin (up to 975 mg/m²) without observing any cardiac determinant.

response and clinical benefits

Overall, 37 of 40 patients were evaluable for response. The patient with AFIB was withdrawn from study without receiving epirubicin. A breast cancer patient with extensive chest wall

disease withdrew for personal reasons after being hospitalized for chest wall bleeding following initiation of panobinostat. This serious AE was attributed to her treatment response resulting in a near complete response. Subsequently, this patient experienced grade 4 thrombocytopenia and withdrew consent. One patient withdrew from study for febrile neutropenia and infection and was not evaluable for response. In 37 assessable patients, 4 (11%) achieved a partial response and 17 (46%) had a stable disease (Figure 1). The median time to progression and median overall survival for the overall study cohort were 3.1 (95% CI: [1.8–4.6]) and 7.3 (95% CI: [5.9–10.3]) months, respectively. All four patients with objective partial response had progressed on prior topoisomerase II inhibitors. Reduced tumor burden was seen in two additional patients with prior exposure to topoisomerase II inhibitors. In total, 17 patients had received prior topoisomerase II inhibitors (e.g. 16 anthracycline and 1 etoposide), with 14 experiencing disease progression while on this regimen. Of these 14 patients, 8 (57%) benefited from panobinostat and epirubicin, including 3 with partial response.

In the sarcoma dose expansion cohort ($n = 20$), 1 partial response (myxoid liposarcoma) and 11 additional patients with stable disease were observed for >3 months. The median PFS and median overall survival was 3.4 (95% CI: [2.6–5.2]) and 8.3 (95% CI: [7.1–10.6]) months, respectively.

correlative studies

There was a weak correlation for a dose-dependent increase in panobinostat plasma levels ($R = 0.361$, $P = 0.023$) with significant interpatient variability at the 50-mg dose (Figure 2A). Patients with higher plasma panobinostat levels were more likely to experience grade 3 or 4 toxicity ($P = 0.034$; Figure 2B).

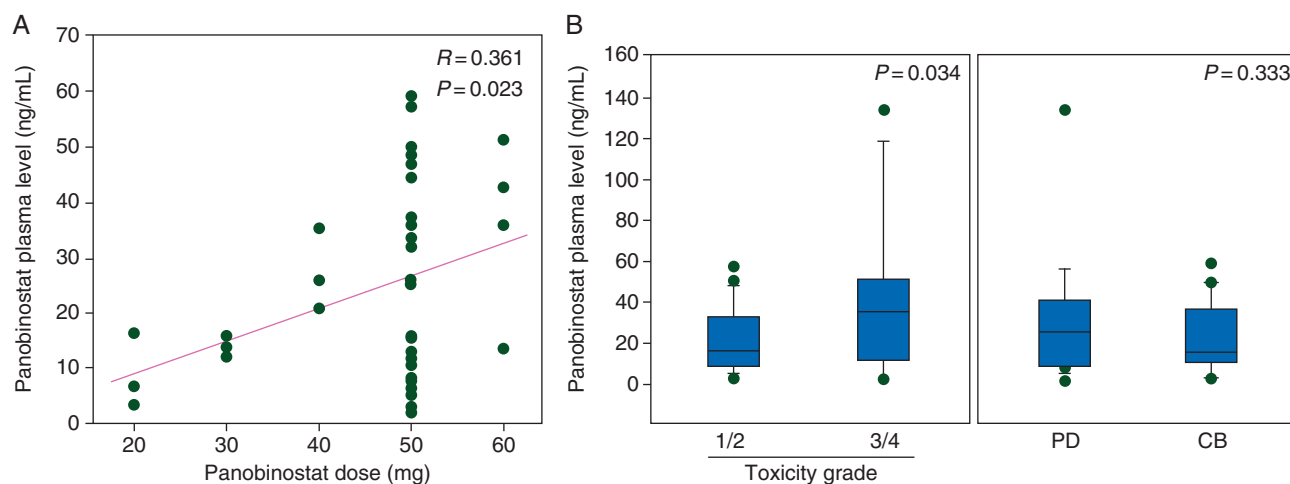


Figure 2. Relationship between panobinostat plasma concentration and dose, response, and toxicity. (A) Panobinostat plasma concentration (ng/ml) exhibits a positive correlation with dose (mg). (B) Box plots of panobinostat plasma concentrations (ng/ml) in patients who immediately progressed (PD) versus those who experienced clinical benefit (CB) and in patients who experienced grade 1/2 toxicities versus those who experienced grade 3/4 toxicities. Correlations were conducted using Pearson's correlation coefficient method.

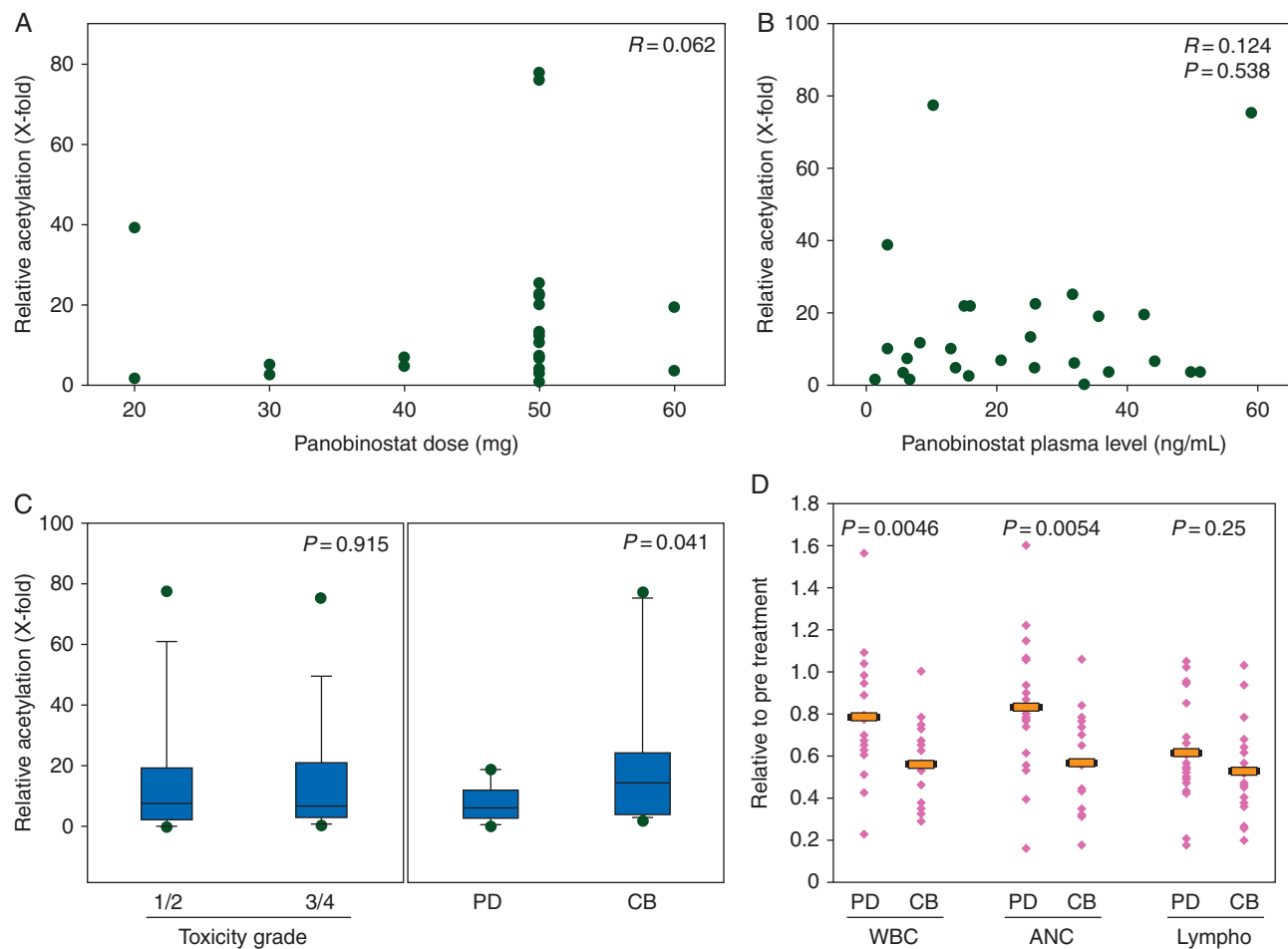


Figure 3. Peripheral blood mononucleocyte histone acetylation relationship to panobinostat dose and plasma concentration, response, toxicity, and white cell count. Histone acetylation neither correlates with panobinostat dose (A) nor plasma concentration (ng/ml, B). (C) Box plots of histone acetylation in patients who did (CB) versus those who did not (PD) benefit from treatment and in patients who experienced grade 1/2 versus grade 3/4 toxicity. (D) Dot plots of all leukocytes (WBC), neutrophils (ANC), and lymphocytes (Lympho) on day 5 of treatment normalized to pretreatment on day 1 of cycle 1 comparing patients who did (CB) and did not (PD) benefit from treatment. The bar indicates the mean. Correlations were conducted using Pearson's correlation coefficient method.

and induction of neutropenia were not correlated with panobinostat plasma levels. Panobinostat-induced PBMC histone acetylation and neutropenia are host-specific biomarkers of therapeutic effect linked to the host's ability to respond to epigenetic modification, rather than a dose-dependent pharmacodynamic effect. We found that increasing doses of the HDAC inhibitor do not result in increased hyperacetylation, but are associated with increased toxicity such as fatigue, nausea, and diarrhea, without better treatment effect. Determining a patient's response to HDAC inhibition *ex vivo*, before treatment, may allow for the enrichment of patients most likely to benefit from HDAC inhibitor-based treatment. Thus, prospective studies evaluating an *ex vivo* assay of HDAC inhibitor-induced PBMC histone acetylation could present a new means for identifying patient's likely to benefit.

funding

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disclosure

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