ENGAGE- 501: phase II study of entinostat (SNDX-275) in relapsed and refractory Hodgkin lymphoma

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ABSTRACT

Classical Hodgkin lymphoma treatment is evolving rapidly with high response rates from antibody-drug conjugates targeting CD30 and immune checkpoint antibodies. However, most patients do not achieve a complete response, therefore development of novel therapies is warranted to improve patient outcomes. In this phase II study, patients with relapsed or refractory Hodgkin lymphoma were treated with entinostat, an isoform selective histone deacetylase inhibitor. Forty-nine patients were enrolled: 33 patients on Schedule A (10 or 15 mg oral entinostat once every other week); 16 patients on Schedule B (15 mg oral entinostat once weekly in 3 of 4 weeks). Patients received a median of 3 prior treatments (range 1-10), with 80% of the patients receiving a prior stem cell transplant and 8% of patients receiving prior brentuximab vedotin. In the intention-to-treat analysis, the overall response rate was 12% while the disease control rate (complete response, partial response, and stable disease beyond 6 months) was 24%. Seven patients did not complete the first cycle due to progression of disease. Tumor reduction was observed in 24 of 38 (58%) evaluable patients. Median progression-free survival and overall survival was 5.5 and 25.1 months, respectively. The most frequent grade 3 or 4 adverse events were thrombocytopenia (63%), anemia (47%), neutropenia (41%), leukopenia (10%), hypokalemia (8%), and hypophosphatemia (6%). Twenty-five (51%) patients required dose reductions or delays. Pericarditis/pericardial effusion occurred in one patient after 12 cycles of therapy. Future studies are warranted to identify predictive biomarkers for treatment response and to develop mechanism-based combination strategies. (*clinicaltrials.gov identifier: 00866333*)

Introduction

While Hodgkin lymphoma (HL) is highly curable through multi-agent chemotherapy and radiation therapy, approximately 20% of patients require second-line therapy which generally includes high-dose therapy with autologous stem cell transplantation (ASCT).[1,2] Only 50% of patients undergoing ASCT are cured and the remaining patients treated with a palliative intent have a median overall survival (OS) of 2.4 years and median post progression-free survival (PFS) of 1.3 years.[2,3] In recent years, two highly effective treatments have been identified for HL. These include the antibody drug conjugate, brentuximab vedotin, and the immune checkpoint inhibitors, nivolumab and pembrolizumab.[4-6] Despite high response rates, the majority of the observed responses are partial.[4-6] In the case of brentux-

imab vedotin, patients treated in the relapsed setting have a median post progression-free survival of less than six months. For patients treated with brentuximab vedotin who achieved a complete response, approximately 50% of these patients relapse despite some receiving an allogeneic stem cell transplant for consolidation.[6,7] Re-treatment with brentuximab vedotin is feasible, offering 60% overall response rate (ORR) and 30% complete response (CR), with a potential for improvement using combination strategies.[8] Long-term results with immune checkpoint inhibitors are lacking, but to date, most responses are partial and some patients have progressed on therapy. This again highlights the need for additional drug development and identification of targeted therapy with single agent activity for combination therapy. Furthermore, the high activity of these novel targeted agents may drive the development of mechanism-based chemotherapy-free regimens with potentially less toxicity with respect to secondary cancers and cardiovascular disease. Accordingly, future treatment strategies will be aimed at developing effective new regimens that maintain a high cure rate while reducing treatment-related toxicities.

The success of brentuximab vedotin and immune checkpoint inhibitors was based on taking advantage of the unique biology of HL, restricted CD30 expression, high PD-L1 expression and large numbers of T cells in the microenvironment. In this regard, histone deacetylase (HDAC) inhibitors are ideal candidates to exploit the biology of HL by modulating tumor cell death and the tumor microenvironment *via* non-overlapping mechanisms. HDAC inhibitors directly affect proliferation by increasing expression of p21 and down-regulating STAT6, culminating in caspase-induced cell death.[9] Effects of HDAC inhibitors on the microenvironment include upregulation of OX40L (that is involved in generation of antigen specific memory T cells) and inhibition of thymus and activation regulated chemokine (TARC) (which attracts activated T-helper cells).[9-11] The effects of HDAC inhibitors on the microenvironment include downregulation of tumor suppressor T cells to aid immune-mediated response.[12] More recently, HDAC inhibitors have been shown to modulate PD-1 expression on peripheral blood T cells, suggesting synergism with immune checkpoint therapy.[13]

Several trials have studied HDAC inhibitors in HL with response rate of approximately 20%.[14-16] HDAC inhibitors vary according to their specificity for HDAC isoforms, route of administration, and schedule. Entinostat is an oral pyridylcarbamate, class I isoform selective HDACi that targets HDAC 1, 2, 3, and HDAC 11.[17,18] Compared to other HDAC inhibitors, entinostat has a unique pharmacokinetic (PK) signature with a prolonged half-life of approximately 140 hours, allowing for once or twice weekly dosing.[19] *In vitro* experiments with entinostat suggest a strong anti-proliferative and immunomodulatory signal through upregulation of p21, downregulation of anti-apoptotic proteins, and modulation of chemokines including TARC.[13,20,21] This phase II study evaluates the efficacy and safety of entinostat in patients with relapsed or refractory HL.

Methods

Patient selection

Patients with relapsed or refractory HL after an ASCT or those ineligible for ASCT were enrolled on this study. The eligibility criteria included age 18 years or over, Eastern Cooperative Oncology Group (ECOG) performance status of 0-1, and at least one site of measurable disease (≥1.5 cm). Adequate renal [serum creatinine ≤1.5xupper limit of normal (ULN)], bone marrow (absolute neutrophil count ≥1x10^9/L, and a platelet count ≥25x10^9/L in Schedule A and 50x10^9/L in Schedule B], and hepatic function (serum total bilirubin ≤1.5xULN, alanine aminotransferase and aspartate aminotransferase ≤2.5xULN) was required. Previous chemotherapy must have been completed three weeks prior to the first dose of entinostat.

Exclusion criteria included known positivity for human immunodeficiency virus, active hepatitis B or C virus, central nervous system lymphoma, pregnancy or lactation, or a history of allogeneic stem cell transplantation within three months and active immunosuppressive therapy or graft-*versus*-host disease requiring treatment. Patients with a history of pericarditis or pericardial effusion requiring medical intervention within six months were also excluded from this study. Prior HDAC inhibitor treatment was not permitted. Enrollment began in 2009, prior to approval of brentuximab vedotin for relapsed or refractory HL. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization. All patients provided written informed consent. The Institutional Review Board and Ethical Committee at each site approved the study.

Study design and treatment plan

This study was an open-label non-randomized, multicenter phase II trial of oral entinostat administered to patients with relapsed or refractory HL (*clinicaltrials.gov identifier: 00866333*) with the primary objective of assessing ORR (CR and PR). The secondary objectives included assessments of duration of response (DOR), OS, PFS, and safety and tolerability of entinostat.

Patients were enrolled on two dosing schedules, both with 1 cycle defined as 28 days. Patients on Schedule A received 10 mg entinostat administered orally (PO) once every other week on a 28-day cycle. Upon determining tolerability of the 10 mg dose, entinostat was increased to 15 mg once every other week starting in week 2. Schedule B was initiated with 15 mg entinostat administered once weekly for three weeks on a 28-day cycle to determine whether greater frequency of entinostat led to increased control of disease.

Entinostat was dose-reduced or held for grade 2 or greater non-hematologic toxicity or hematologic toxicity defined by absolute neutrophil count (ANC) less than 1x10^9/L or platelets less than 25x10^9/L. In the case of drug-associated grade 3 or 4 toxicities experienced by the patient in spite of optimal supportive care (including growth factor support and transfusion), treatment was withheld until symptoms improved to grade 1 or lower. Recurrence of grade 3 or 4 toxicities despite 2 levels of dose reduction to 10 mg and 7 mg of entinostat required treatment discontinuation. If symptoms did not resolve after four weeks of treatment interruption, the patients were removed from the study. Therapy was discontinued if there was evidence of progressive disease (PD), unacceptable toxicity, or withdrawal of consent.

Study assessments

Computed tomography (CT) of the chest, abdomen and pelvis was performed at baseline, every 2 cycles for the first 9 cycles, and every 3-4 cycles thereafter. Disease assessment by fluorodeoxyglucose (FDG) positron emission tomography (PET) was performed at the investigator's discretion. Tumor responses were based on the 2007 revised response criteria for malignant lymphoma.[22] Progression of disease was defined as the appearance of new lesions or a greater than 50% increase in the sum of the products of perpendicular lesion diameters. A patient was classified as

Table 1. Patients' characteristics (n=49, intent-to-treat population).

	Schedule A (n=33)	Schedule B (n=16)	Total (n=49)
Median age (years)	33 (19-73)	33 (20-55)	33 (19-73)
Sex			
Female	14 (42%)	10 (63%)	24 (49%)
Male	19 (58%)	6 (38%)	25 (51%)
ECOG performance status			
0	19 (58%)	11 (69%)	30 (61%)
1	14 (42%)	5 (31%)	19 (39%)
Number of previous chemotherapy treatments			
Median	3 (1-10)	3 (2-7)	3 (1-10)
< 4 lines of therapy	19 (58%)	8 (50%)	27 (55%)
≥4 prior regimens	14 (42%)	8 (50%)	22 (45%)
Prior therapy with brentuximab vedotin	3 (9%)	1 (6%)	4 (8%)
Prior bone marrow or stem cell transplant	28 (85%)	11 (69%)	39 (80%)
Prior autologous transplant	21 (64%)	9 (56%)	30 (61%)
Prior allogeneic transplant	4 (12%)	–	4 (8%)
Prior autologous & allogeneic transplant	3 (9%)	2 (13%)	5(10%)
Transplant ineligible	4 (12%)	4 (12%)	8 (16%)
Response to last treatment			
Refractory			
<50% response to last treatment	4 (12%)	4 (25%)	8 (16%)
PD within 3 months of most recent therapy	9 (27%)	4 (25%)	13 (27%)
Relapsed disease			
PD following therapy(ies) with curative intent	20 (61%)	8 (50%)	28 (57%)
Bulky disease (1 or more baseline lesions ≥ 5 cm)	28 (85%)	11 (69%)	39 (80%)
Prior radiotherapy	26 (79%)	12 (75%)	38 (78%)

ECOG: Eastern Cooperative Oncology Group; PD: progressive disease.

SD if they did not meet criteria for complete response, partial response or progressive disease. Durable stable disease was classified as stable disease lasting six months or more. Safety assessments including vital signs, complete blood cell count analysis serum chemistry analysis, and physical examination were performed every two weeks of a 4-week cycle. Adverse events (AEs) and laboratory variables were assessed using the Common Terminology Criteria for Adverse Events version 3.0.

Correlative studies

Serum cytokines including TARC were measured at baseline, after one week, and two weeks after initiation of entinostat. Sera were analyzed with commercially available enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Minneapolis, MN, USA), and Multiplex human cytokine 30-plex kits (Invitrogen Corporation, Carlsbad, CA, USA) according to the manufacturers' protocols. Entinostat plasma concentrations were measured for a subset of patients. Samples were collected on days 1, 8 and 15 of cycle 1 for PK studies using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay performed in the Analytical Pharmacology Core laboratory at the Sidney Kimmel Comprehensive Center at Johns Hopkins Medical Center.[23] Samples were collected at 0 hours (pre-dose), 0.25, 1, and 2 hours post dose as well as pre-dose on days 8 and 15.

Statistical analysis

Sample size was based on Simon's optimal 2-stage design and an ORR end point for both Schedule A and B. Response evaluable patients as defined by the *per*-protocol population were required to complete 2 cycles of entinostat and undergo response evaluation at screening and end of cycle 2. Patients who discontinued due to intolerable treatment-related AE prior to cycle 3 day 1 were included in the intent-to-treat population. Efficacy was assessed in both the intent-to-treat and *per*-protocol population. Patients were included in the safety analysis if they received at least one dose of entinostat.

Tumor response rate was estimated on the basis of the proportion of patients whose best overall response was CR or PR. Rate of disease control is defined by patients with a CR, PR, or SD lasting longer than or equal to six months. SD was measured from the start date of entinostat until the criteria for disease progression was first met. DOR was calculated for patients who achieved PR or better. For such patients, DOR represents the number of days from the start date of response to the date recurrent or progressive disease was first documented. Progression-free survival was measured from the date of the first dose of entinostat to the earlier of documented disease progression or death due to any cause. The duration of DOR and PFS was right-censored at the last disease assessment for patients alive and without documentation of PD. Patients who started a non-protocol defined anticancer therapy prior to documentation of PD were censored at the last disease assessment prior to the initiation of such therapy. OS was measured from date of first dose of entinostat to the date of death from any cause and right-censored for patients reported alive as of the date of last contact. Time to event end points (DOR, PFS and OS) were summarized descriptively using the Kaplan-Meier method.

Biomarker values were summarized in a descriptive manner at each sample collection time point [day 1 (baseline), Day 8, and Day 15]. Changes were evaluated by calculating differences within patients from baseline to each post-baseline time point. The Wilcoxon signed rank test was used to detect statistically significant ($P<0.05$) within-patient changes from baseline. The entinostat maximum plasma concentration (C_{max}) and time to maximum

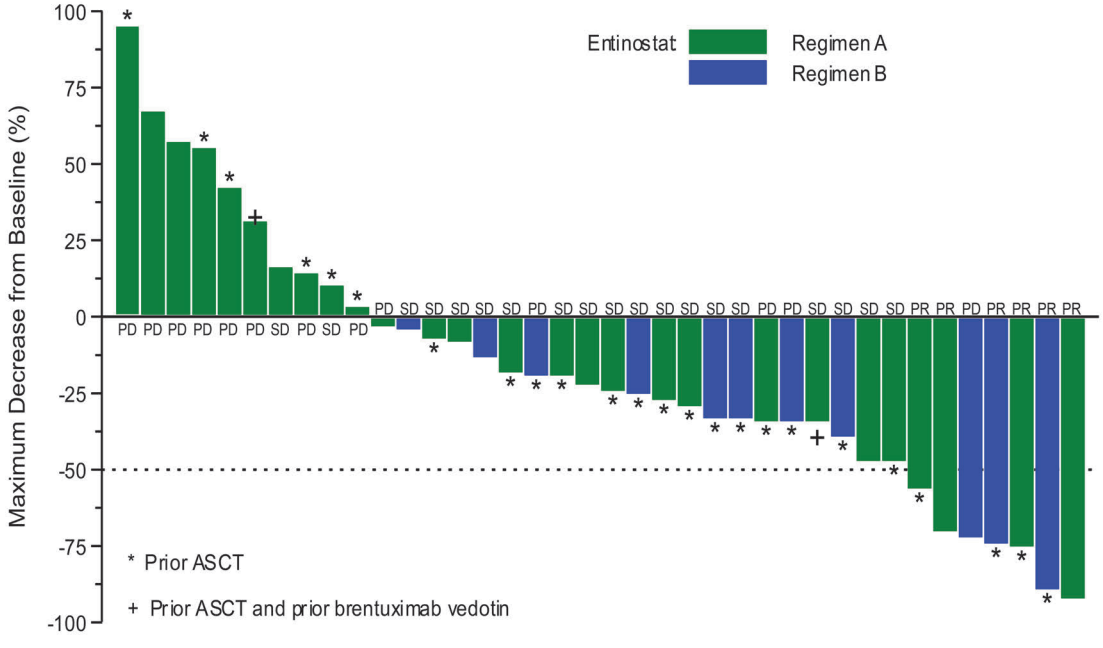

Figure 1. Waterfall plot of 38 evaluable patients (*per* protocol population) treated with entinostat. Schedule A (green) is oral entinostat at 10 or 15 mg given once every other week in a 4-week cycle. Schedule B (blue) is oral entinostat at 15 mg given once weekly three out of four weeks. Patients who have undergone autologous stem cell transplant (ASCT) and prior therapy (n=23) with brentuximab vedotin (n=4) are indicated. Approximately 40% of patients with bulky disease demonstrated tumor decrease but did not meet partial response (PR) criteria. SD: stable disease; PD: progressive disease.

Table 2. Treatment-related grade 3/4 adverse events occurring in more than 4% of patients.

	Schedule A (n=33)	Schedule B (n=16)	Total (n=49)
Thrombocytopenia	19 (58%)	12 (75%)	31 (63%)
Anemia	15 (45%)	8 (50%)	23 (47%)
Neutropenia	12 (36%)	8 (50%)	20 (41%)
Leukopenia	5 (15%)	0 (0%)	5 (10%)
Hypokalemia	1 (3%)	3 (19%)	4 (8%)
Hypophosphatemia	2 (6%)	1 (6%)	3 (6%)

plasma concentration (T_{max}) were obtained from the entinostat concentration data in the subset of patients who participated in the PK portion of the study; C_{max} and T_{max} were analyzed using descriptive statistics. Syndax Pharmaceuticals, Inc. analyzed all data and provided access to primary clinical data to all authors.

Results

Patients' characteristics

Forty-nine patients were enrolled between April 2009 and March 2011, 33 patients in Schedule A (10 or 15 mg on days 1 and 15) and 16 patients in Schedule B (15 mg on days 1, 8, and 15) (Table 1). Median age of patients was 33 years (range 19-73). Twenty-two (45%) patients had had 4 or more previous treatment regimens, and 39 (80%) patients had previously undergone one or more allogeneic or autologous hematopoietic stem cell transplants. Four patients had been previously treated with brentuximab vedotin. Eight (16%) patients were refractory to prior therapy and were never eligible for transplant. Twenty-eight (57%) patients were refractory on entry, with 8 (16%) having no response to last treatment and 13 (27%) experiencing relapse within three months of last treatment. Thirty-nine (80%) had bulky disease. Baseline characteristics were similar between patients treated in Schedule A and Schedule B.

Safety and treatment administration

All 49 patients received at least one dose of entinostat and were monitored for toxicity. Mean number of cycles of entinostat therapy was 5.7 (range 2-55). All patients have discontinued therapy. In Schedule A, 24 (73%) patients discontinued due to PD and 3 (9%) due to AEs. In Schedule B, 6 (38%) patients discontinued due to PD and 2 (13%) due to AEs. Seven patients had disease progression prior to completing one cycle of therapy. Other reasons for discontinuation were: 8 (16%) due to an adminis-

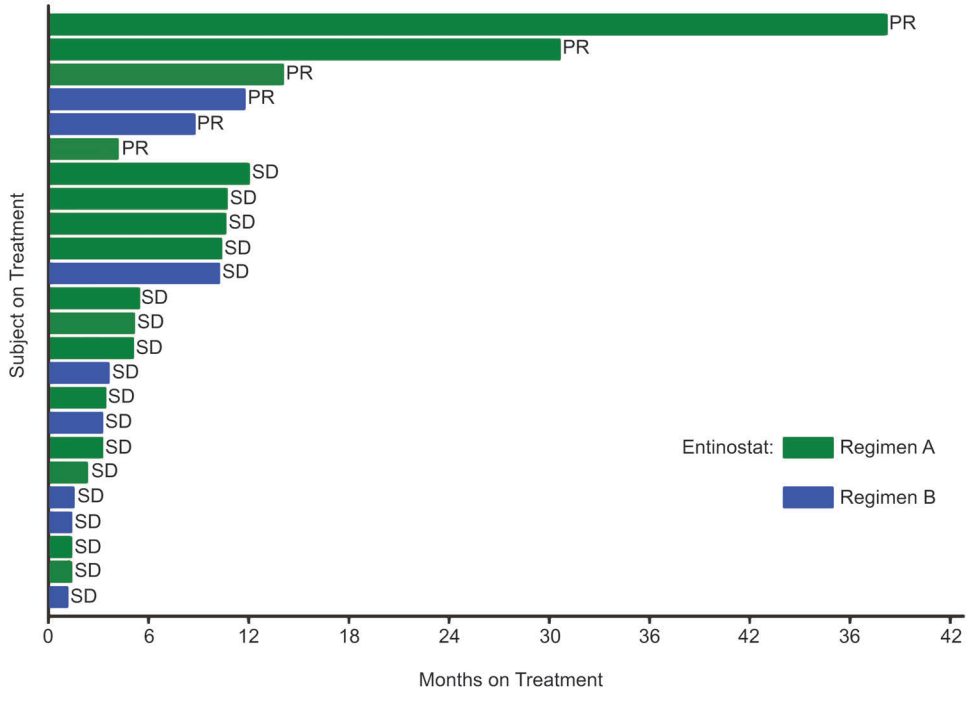

Figure 2. Swim plot of patients treated with entinostat in the *per*-protocol population who have achieved a partial response (PR) or stable disease (SD) (n=24). Median duration of response for 6 patients achieving PR is 28.5 months (range >1 day to 39.9 months; note that >1 day represents a patient who achieved PR but did not have any subsequent disease assessments that could be used for the analysis. In this case the patient was censored having undergone transplantation shortly after the response assessment that showed PR). Median duration of response for 18 patients with stable disease was 6.1 months (range >1 month to 15 months).

trative decision (most commonly for intervening therapy), 3 (6%) due to withdrawal of consent, 1 (2%) due to protocol deviation, and 2 (4%) for other reasons. Of the 49 patients, 5 (10%) experienced a treatment-related AE that required entinostat to be permanently stopped. Two patients treated in Schedule A discontinued therapy; one patient was diagnosed with pericarditis and pericardial effusion, while another patient had thrombocytopenia. Three patients treated in Schedule B discontinued therapy, one each for pulmonary embolism, spinal cord compression, and respiratory failure.

The most common grade 3 or 4 adverse events (AEs) were thrombocytopenia (31 patients, 63%), anemia (23 patients, 47%), neutropenia (20 patients, 41%), leukopenia (5 patients, 10%), hypokalemia (4 patients, 8%), hypophosphatemia (3 patients, 6%) (Table 2). Twenty-five (51%) patients had a dose decrease or dose delay. The majority (31%) of dose modifications were for hematologic toxicities, primarily neutropenia and thrombocytopenia. Fatigue was associated with dose modifications in 2 patients, one each in Schedule A and B. Overall, grade 3 or 4 non-hematologic toxicity did not exceed 10% in any system.

Twelve patients developed 26 serious adverse events (SAEs), 9 of 33 (27%) patients in Schedule A and 3 of 16 (19%) in Schedule B; multiple events were reported in these 12 patients. Treatment-related SAEs occurred in 6 patients, one each with: fever; pericarditis/pericardial effusion; renal calculi and subdural hemorrhage; dehydration; thrombocytopenia, anemia, neutropenia; and pulmonary embolism. The patient who developed pericarditis and pericardial effusion had been heavily pre-treated with 5 prior regimens, including radiation to the mediastinum; the event occurred after 12 cycles and is considered to be possibly related to entinostat. One patient on Schedule B developed a fatal respiratory failure unrelated to entinostat.

Efficacy

In the intention-to-treat analysis of efficacy, 38 of 49 patients, 27 in Schedule A and 11 in Schedule B, completed two cycles of therapy and completed radiological restaging prior to initiation of cycle 3. Eleven of 49 patients did not complete more than 1 cycle or did not undergo restaging at the required time point (7 PD, 1 AE, 2 withdrew consent, and 1 protocol violation). Six of the 49 patients (4 in Schedule A, 2 in Schedule B) treated with entinostat obtained a PR; therefore, the overall response was 12% (Table 3). Nineteen patients achieved SD with 6 patients having durable SD (defined as stable disease lasting >6 months). Disease control (CR, PR, and durable SD) was noted for 12 of 49 patients (24%): 9 of 33 patients (27%) in Schedule A and 3 of 16 patients (19%) in Schedule B.

In 38 evaluable patients who completed at least two cycles of therapy, disease was controlled in 12 of 38 patients (32%) and overall response was seen in 6 of 38 patients (16%) (Table 3). Tumor reduction, ranging between 3% to 92% as measured from baseline, was observed in 22 of 38 (58%) patients in the *per*-protocol population and 49% of intent-to-treat population (Table 3 and Figure 1). All patients treated on Schedule B demonstrated tumor reduction. In the 24 patients with clinical benefit (CR, PR and SD), 19 patients (79%) demonstrated reduction in tumor size by two months with a maximum

Table 3. Best overall response in intent-to-treat and *per*-protocol populations.

Response	Intent-to-treat population			*Per*-protocol population		
	Regimen A (n=33)	Regimen B (n=16)	Total (n=49)	Regimen A (n=27)	Regimen B (n=11)	Total (n=38)
CR	–	–	–	–	–	–
PR	4 (12%)	2 (13%)	6 (12%)	4 (15%)	2 (18%)	6 (16%)
SD (≥6 mo)	5 (15%)	1 (6%)	6 (12%)	5 (19%)	1 (9%)	6 (16%)
SD (<6 mo)	7 (21%)	6 (38%)	13 (27%)	7 (26%)	5 (45%)	12 (32%)*
Disease control (CR+PR+SD≥6 mo)	9 (27%)	3 (19%)	12 (24%)	9 (33%)	3 (27%)	12 (32%)*
Clinical benefit (CR+PR+SD)	16 (48%)	9 (56%)	25 (51%)	16 (59%)	8 (73%)	24 (63%)
Tumor reduction (CR+PR +SD with tumor reduction)			22 (49%)	14 (52%)	8 (73%)	22 (58%)
PD	11 (33%)	3 (19%)	14 (29%)	11 (41%)	3 (27%)	14 (37%)
Not assessable	6 (18%)	5 (31%)	11 (22%)			
PD < cycle 1	5 (15%)	2 (12.5%)	7 (14)	–	–	–
Other reasons	1 (3%)	3 (19%)	4 (8%)			

*CR: complete response; PR: partial response; SD: stable disease; mo: months. *Seven patients with SD (per Cheson response criteria) at end of treatment: 5 discontinued due to physician's discretion, one due to serious adverse events, and one patient decision.*

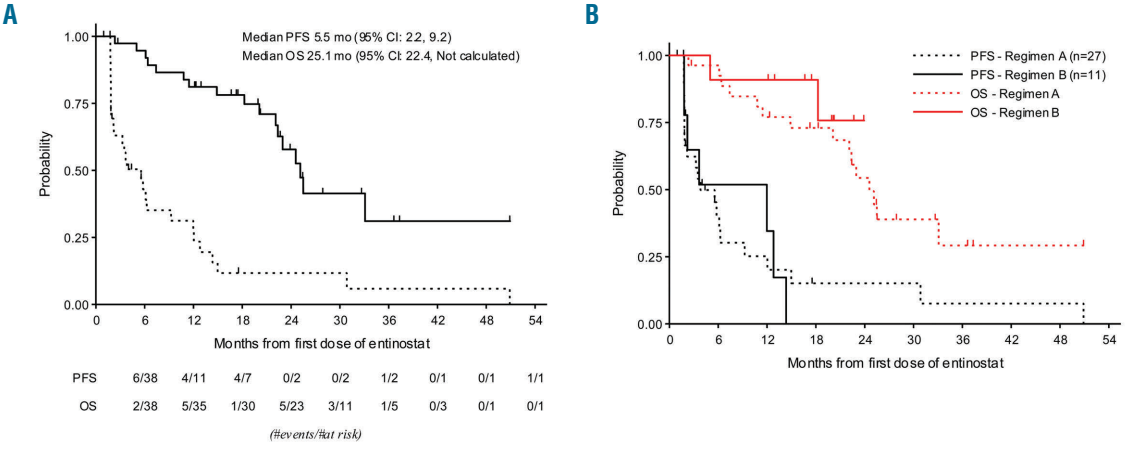

Figure 3. Kaplan-Meier estimates of progression-free survival (PFS) and overall survival (OS) in 38 evaluable patients (*per*-protocol population). (A) The median PFS is 5.5 months and median OS is 25.1 months. (B) PFS and OS for Schedule A and B. PFS is 3.8 months for Schedule A and 5.5 months for Schedule B.

response achieved after four months (Figures 1 and 2). Of 18 patients with SD, 6 patients (33%) are experiencing duration of responses lasting over six months. Two patients, both on Schedule A, have durable responses lasting longer than 32 months. Of the 6 patients with a PR, 2 patients proceeded to an allogeneic transplant and radiation therapy, respectively, for consolidation therapy, while the other patients continued on study until disease progression.

With a median follow up of 27.9 months for Schedule A and 19.9 months for Schedule B, median PFS from among the 38 evaluable patients was 3.8 months [95% confidence interval (95%CI) 1.9, 6.2 months] for Schedule A and 12 months (95%CI: 2.2, 12.8 months) for Schedule B. Median OS was 24.6 months (95%CI: 22.1, not reached) for Schedule A and was not reached for Schedule B (Figure 3).

Of the 30 patients who had previously undergone ASCT, 26 patients were evaluable for efficacy analysis with a median OS of 62.5 months when measured from the date of ASCT (*Online Supplementary Figure S1A*). Overall response was observed in 4 of 26 (15.4%) with stable disease observed in an additional 13 (50%) patients (*Online Supplementary Figure S1B*).

Entinostat induced chemokine/cytokine variations and pharmacokinetics

Changes in cytokine/chemokine levels were measured in 18 patients and TARC levels were measurable in 20 patients. Changes in TARC levels between day 1 to day 8 were measured in 20 patients and changes in TARC levels between day 1 and day 15 were measured in 18 patients (*Online Supplementary Figure S2*). The median TARC level on day 1 was 1647 pg/mL (range 259-8176 pg/mL) and reduced to 1312 pg/mL (range 275-5155 pg/mL) on day 8, 802 pg/mL (range 107-1830 pg/mL) on day 15. Comparison of within-patient changes from day 1 to day 8 and from day 1 to day 15 showed a significant reduction

Table 4. Summary of results of clinical trials with HDAC inhibitors in Hodgkin lymphoma.

Study	Phase	Drug	Isotype	Route	Schedule	N	ORR N (%)	CR	PR	SD	Tumor reduction (evaluable patients)	Median PFS
Morschhauser *et al.*, 2015[24]	I	Abexinostat	Pan	PO	Various BID dosing	11	3 (27%)	0 (0%)	3 (27%)	3 (27%)	54% (6/11)	Not reported
Younes *et al.*, 2011[16]	II	Mocetinostat	1,2,3,11	PO	85 or 110 mg 3x per week	51	14 (27%)	2 (4%)	12 (23%)	17 (33%)	81% (34/42)	10 months
Younes *et al.*, 2012[15]	II	Panobinostat	Pan	PO	40 mg 3x per week	129	35 (27%)	5 (4%)	30 (23%)	71 (55%)	74% (89/120)	6.1 months
Kirschbaum *et al.*, 2012[14]	II	Vorinostat	Pan	PO	200 mg BID, day 1-14 every 21 days	25	1 (4%)	0 (0%)	1 (4%)	12 (48%)	Not reported	4.8 months
Current study	II	Entinostat	1,2,3,11	PO	10 or 15 mg once every other week, or 15 mg once weekly in 3 of 4 weeks	49	6 (12%)	0 (0%)	6 (12%)	6 (12%)	58% (24/38)	5.5 months

N: number; ORR: overall response rate; CR: complete response; PR: partial response; SD: stable disease; PFS: progression-free survival; PO: oral administration; BID: twice daily.

in TARC levels in patients on entinostat therapy supporting an on target effect. The multiplex cytokine panel of 30 cytokines (including IL-2, IL-4-8, G-CSF, GM-CSF, RANTES, Eotaxin, EGF, HGF, VEGF, interferon alpha, interferon gamma and TNF-alpha) showed wide variability between days 1, 8 and 15, with a general reduction in cytokine levels that was not statistically significant and not associated with clinical outcome.

Entinostat systemic exposure increased with increased dose of entinostat as measured in 12 of 13 patients assessed for entinostat concentrations. Nine patients treated with 10 mg entinostat on day 1 demonstrated a mean Cmax of 85.7 ng/mL (SD±78.2 ng/mL, range 3.47-222.4 ng/mL), and a mean Tmax of 0. 44 hours (SD±0.24 h, range 0.25-1 h. Treatment with 15 mg of entinostat resulted in high serum concentrations but clinically insignificant mean maximum time of systemic exposure. Three patients who received entinostat 15 mg on day 1 demonstrated mean Cmax of 173.6 ng/mL (SD± 221.0 ng/mL, range 32.55-428.2 ng/mL), and a mean Tmax of 0.33 hours (SD±0.14 h, range 0.25-0.5 h).

Discussion

Despite high response rates seen with brentuximab vedotin and immune checkpoint antibodies, the observed responses are usually partial indicating the need for combination therapies to improve efficacy.[4-6] Immune checkpoint therapies expand cytotoxic effector T cells which are only fully functional in the context of reduced immune suppressor cells. In a murine model system, entinostat enhanced the activity of immune checkpoint blockade through potent inhibition of growth and function of myeloid-derived suppressor cells (MDSCs).[12] Combination of anti-PD-1, anti-CTLA-4 and epigenetic modifiers with DNA methyltransferase and HDAC inhibitors triggered complete regression of large orthotopic tumors. Addition of entinostat greatly reduced MDSCs directly improving cytotoxic effector T-cell activity. The ability of HDAC inhibitors to modulate PD-1 expression is of particular interest suggesting the full clinical potential of HDAC inhibitors has yet to be fully explored.[12,13] The dependence of Hodgkin Reed Sternberg cells on the microenvironment for survival suggests that combination of immune checkpoint and HDAC inhibitors may be an effective independent strategy to modulate the tumor microenvironment.

HDAC inhibitors have been studied in HL with overall response rates of 4%-27%.[14-16] While the ORR with entinostat was modest (12%), entinostat provided clinical benefit in 51% of this heavily pre-treated population. Of the 19 patients with SD, the duration of stability was over six months in 32% of these patients. The longest duration of response was greater than four years (50 months). Several HDAC inhibitors have been studied in HL and the majority demonstrate modest overall response rates ranging from 4% to 27% (Table 4[14-16,24]). Despite modest response rates, 49% of patients by intent-to-treat and 58% of evaluable patients had a reduction in tumor size (CR, PR and SD with negative tumor volume decreased from baseline) (Table 3). The median PFS of 5.5 months observed in this study is similar to HDAC inhibitors in HL (Table 4). While the median overall survival in this study was two years, the duration of clinical benefit in patients who previously underwent an ASCT is more pronounced, with a median OS of 5.2 years. This suggests that entinostat should be considered for mechanism-based combination therapy with agents such as immune checkpoint antibodies or brentuximab vedotin. In particular, recent publications show synergism between PD-1 blockade and HDAC inhibitor, possibly through the enhanced modulation of myeloid-derived suppressor cells, increased expression of PD-1 mediated by HDAC inhibitors, and alteration of the tumor immune microenvironment.[12,13,25]

Consistent with the growing experience of HDAC inhibitors in HL, entinostat demonstrates clinical activity with a well-tolerated clinical profile suitable for combination therapy. Entinostat appears well tolerated, with only

10% of patients discontinuing therapy compared to 16%-24% observed with other HDAC inhibitors.[14-16] However, similar to other HDAC inhibitors, dose reductions were necessary to mitigate hematologic toxicities. Dosing of entinostat every other week or weekly in 3 out of 4 weekly cycles showed similar toxicity. Analysis of PK data in the small number of patients for whom entinostat plasma concentrations were available was consistent with previously reported results, with a highly variable C1D1 C_{max} and a rapid T_{max}. The range of C_{max} concentrations was consistent with biologically active concentrations.

Biomarker analyses in this study were designed to confirm pre-clinical data demonstrating that entinostat could down-regulate T-helper 2-associated cytokines and growth factors while up-regulating T-helper 1-associated factors. The general trends observed in changes of cytokine levels, along with the reduction of TARC, suggest that the mechanism of action of entinostat may involve immunomodulatory effects that contribute to its anti-tumor effects. A previous study in advanced HL patients demonstrated that reduction in TARC with an HDAC inhibitor was associated with tumor response.[16] Although the majority of patients experienced reduced TARC levels on addition of entinostat, no association with tumor response was observed in this study. We will continue to evaluate TARC and additional putative biomarkers in future studies.

In conclusion, entinostat is well tolerated with demonstrable clinical activity in heavily pre-treated HL patients. The mild toxicity profile, mechanism of action and the potential synergism with immune checkpoint therapies support the further development of this therapy in combination with other novel agents, including PD-1 and PD-L1 targeted antibodies.

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