

A multicentre phase II study of vorinostat in patients with relapsed or refractory indolent B-cell non-Hodgkin lymphoma and mantle cell lymphoma

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Summary

Although initial rituximab-containing chemotherapies achieve high response rates, indolent B-cell non-Hodgkin lymphoma (B-NHL), such as follicular lymphoma (FL), is still incurable. Therefore, new effective agents with novel mechanisms are anticipated. In this multicentre phase II study, patients with relapsed/refractory indolent B-NHL and mantle cell lymphoma (MCL) received vorinostat 200 mg twice daily for 14 consecutive days in a 21-d cycle until disease progression or unacceptable toxicity occurred. The primary endpoint was overall response rate (ORR) in FL patients and safety and tolerability in all patients. Secondary endpoints included progression-free survival (PFS). Fifty-six eligible patients were enrolled; 50 patients (39 with FL, seven with other B-NHL, and four with MCL) were evaluable for ORR, and 40 patients had received rituximab-containing prior chemotherapeutic regimens. For the 39 patients with FL, the ORR was 49% [95% confidence interval (CI): 32.4, 65.2] and the median PFS was 20 months (95% CI: 11.2, 29.7). Major toxicities were manageable grade 3/4 thrombocytopenia and neutropenia. Vorinostat offers sustained antitumour activity in patients with relapsed or refractory FL with an acceptable safety profile. Further investigation of vorinostat for clinical efficacy is warranted.

Keywords: vorinostat, indolent B-cell non-Hodgkin lymphoma, follicular lymphoma, phase II trial, HAT mutation.

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Indolent B-cell non-Hodgkin lymphoma (B-NHL) comprises a heterogeneous group of lymphoid malignancies with varying degrees of aggressiveness (Swerdlow *et al*, 2008). Follicular lymphoma (FL), the most common form of the indolent B-NHL, is slow-growing and incurable in most patients. It can transform into a more aggressive histological type in some patients (Relander *et al*, 2010). Mantle cell lymphoma (MCL), which represents 3–10% of NHL, is more aggressive, with a median survival in the range of 3–5 years (Swerdlow *et al*, 2008). Rituximab in combination with cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP) chemotherapy is one of the most frequently applied first-line treatments for patients with FL and achieves a high response rate (Tobinai *et al*, 2010; Watanabe *et al*, 2011). However, most patients eventually relapse and become chemorefractory, which leads to poor outcomes in the relapsed setting, though recently developed agents, including fludarabine, bendamustine and ibritumomab tiuxetan, have demonstrated improved therapeutic outcomes (Witzig *et al*, 2002; Tobinai *et al*, 2006; Kahl *et al*, 2010; Ohmachi *et al*, 2010; Rummel *et al*, 2013). Therefore, new effective agents with novel mechanisms of action are needed to treat relapsed/refractory indolent B-NHL.

Histone deacetylase (HDAC) inhibitors, such as vorinostat (suberoylanilide hydroxamic acid; SAHA), are thought to exhibit anti-tumour activity by up-regulating tumour-suppressive genes, promoting cell differentiation and inducing cell apoptosis. In addition, several non-histone proteins [e.g. tubulin (TUBB), Hsp90 (HSP90AA1) and p53 (TP53)] are reversibly acetylated on lysine residues and undergo hyperacetylation following exposure to vorinostat (Warrener *et al*, 2003; Glaser *et al*, 2004; Bali *et al*, 2005; Carlisi *et al*, 2008). Vorinostat is an oral HDAC inhibitor approved for the treatment of refractory cutaneous T-cell lymphoma (CTCL; Olsen *et al*, 2007). In a phase I study in Japanese patients with relapsed or refractory malignant lymphoma, including FL and MCL, vorinostat (200 mg twice daily) was well-tolerated, and responses were observed in three of four treated patients with FL and one of two treated patients with MCL (Watanabe *et al*, 2010). Similarly, a single-arm, phase II study of vorinostat in the United States reported a 47% overall response rate (ORR) in 17 patients with relapsed/refractory FL and a 22% ORR in nine patients with marginal zone B-cell lymphoma (MZL) among the 35 eligible patients with relapsed/refractory indolent B-NHL and MCL dosed at 200 mg twice daily for 14 consecutive days in a 21-d cycle (Kirschbaum *et al*, 2011). The median progression-free survival (PFS) in the study was 15.6 months in patients with

relapsed/refractory FL treated with vorinostat (Kirschbaum *et al*, 2011); however, the small sample size limited the interpretation of these results.

Recently, two groups reported that frequent somatic mutations in the *CREBBP* and *EP300* genes encoding histone acetyltransferases (HATs) were present in primary tumours and acquired at relapse in several different subtypes of B-cell lymphomas (Morin *et al*, 2011; Pasqualucci *et al*, 2011). Forty-one percent of patients with FL and 39% of patients with diffuse large B-cell lymphoma (DLBCL) exhibited somatic mutations and/or genomic deletions in the *CREBBP* and *EP300* genes, as well as decreased levels of acetyltransferase activity. Therefore, it is reasonable to hypothesize that mutation-induced loss of acetylation of histones and other transcription factors caused an imbalance between acetylation and deacetylation in those patients, which partly accounted for the development of lymphomas resulting from deregulated gene expression and cell proliferation. We therefore conducted a multicentre phase II study of vorinostat in patients with relapsed/refractory FL, other subtypes of indolent B-NHL and MCL to assess the efficacy and safety. Additionally, we explored the *CREBBP* and *EP300* mutations in tumour tissues obtained from patients in the phase II study.

Patients and methods

Patient selection

Patients were eligible if they were 20–74 years of age and had histopathologically confirmed FL, MCL or other histological subtypes of indolent B-NHL, including small lymphocytic lymphoma (SLL), lymphoplasmacytic lymphoma, splenic MZL, extranodal MZL of mucosa-associated lymphoid tissue (MALT) type, or nodal MZL, diagnosed based on the 2008 World Health Organization classification (Swerdlow *et al*, 2008). Patients must have undergone ≥ 1 but ≤ 4 prior chemotherapeutic regimens, not including steroids alone or local radiation. Eligibility criteria included an Eastern Cooperative Oncology Group (ECOG) performance status score ≤ 2 , recurrent or progressive disease after the most recent therapy, the presence of a two-dimensional measurable nodal lesion or ex-nodal lesion [>1.5 cm in greatest transverse diameter by computerized tomography (CT) scan], and life expectancy >4 months. Patients had to have adequate haematological [absolute neutrophil count (ANC) $\geq 1.5 \times 10^9/l$, platelet count $\geq 100 \times 10^9/l$], hepatic (bilirubin $\leq 1.5 \times$ the upper limit of the normal range) and renal function. Patients were excluded if they had received chemotherapy within 4 weeks,

antibody therapy or radioimmunotherapy within 3 months, radiotherapy within 4 weeks, had not recovered from adverse events (AEs) from prior therapy, had received previous treatment with other HDAC inhibitors, had received allogeneic or autologous stem cell transplantation within 6 months, were pregnant, had central nervous system involvement, active systemic infection including hepatitis B, C or human immunodeficiency virus (HIV), severe hepatic dysfunction or other active malignancies.

The trial (Protocol 103) was approved by the institutional review board at each centre and complied with Good Clinical Practice guidelines, the Declaration of Helsinki and local laws. All patients provided written informed consent.

Treatment plan

This open-label, single arm phase II study was conducted at 18 centres. Enrolled patients received oral vorinostat 200 mg twice daily for 14 consecutive days followed by a 7-d break on a 21-d cycle. If patients had an ANC $\geq 0.5 \times 10^9/l$, but $< 1.0 \times 10^9/l$ (grade 3) or a platelet count of $\geq 50 \times 10^9/l$ but $< 75 \times 10^9/l$ (grade 2) at the start of a cycle, the dose was decreased by 100 mg daily from their previous dose. If step-wise dose reduction did not improve the ANC or platelet count, the patients were excluded from the study. If the ANC and platelet counts recovered to below grade 1 or the pre-dosing counts at the end of the cycle, the dose reduction was reversed. Treatment was withheld from patients who developed grade 3 or higher non-haematological toxicity or haematological toxicity of ANC $< 0.5 \times 10^9/l$ (grade 4), platelet count of $< 50 \times 10^9/l$ with blood transfusion (grade 3) or a platelet count of $< 25 \times 10^9/l$ (grade 4). Patients were restarted at a daily dose that was 100 mg less than their previous dose when the toxicity resolved to below grade 1 non-haematological toxicity or to an ANC $> 1.0 \times 10^9/l$ and a platelet count of $> 75 \times 10^9/l$. Therapy was terminated if toxicity persisted or recurred in spite of two dose reductions; otherwise, treatment was continued until unacceptable AEs, progression of primary disease, protocol violation, pregnancy, patient's request or investigator's judgment.

Patient evaluation and study design

Laboratory tests, history, physical examination and evaluation of AEs and therapeutic efficacy were conducted during study visits. Radiological assessment by CT scan was performed at baseline and on Day 21 every three cycles to assess anti-tumour efficacy. Once patients achieved complete response (CR), anti-tumour efficacy was assessed every five cycles.

The primary endpoint was ORR according to the criteria for relapsed/refractory FL patients (Cheson *et al*, 1999), as well as the safety and tolerability in indolent B-NHL and MCL. To confirm the histopathological diagnosis, an Independent Central Pathological Committee (ICPC) was formed.

When 12 months had elapsed from enrollment of the last patient, the ORR data was cut off. ORR was defined as the proportion (%) of patients with CR, complete response/unconfirmed (CRu) or partial response (PR) as best response through the study period. Secondary objectives were PFS and overall survival (OS) in relapsed/refractory FL patients. An Independent Radiology Review Committee was formed to assess the CT imaging and confirm adjudicated anti-tumour efficacy through central review meetings.

Safety was assessed by the incidence of AEs and drug-related AEs, body weight, vital signs, electrocardiogram (every three cycles) and laboratory tests. AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf). The incidence of \geq grade 3 drug-related AEs, leucopenia, neutropenia, lymphopenia, infections accompanied by a normal ANC or grade 1/2 neutropenia, febrile neutropenia and infections accompanied by grade 3/4 neutropenia, were assessed for all patients. The Efficacy and Safety Evaluation Committee evaluated the safety and efficacy results of the central efficacy assessment by the Independent Radiology Review Committee.

Statistical analysis

Referring to the phase II study design of fludarabine (Tobinai *et al*, 2006), vorinostat was considered effective in patients with FL if the ORR was $\geq 25\%$. The ORR was examined with the exact test for binomial proportion ($\alpha = 0.025$, one-sided) and the sample size was estimated based on the ORR of the FL patients. Assuming a true ORR of 50% with vorinostat and $< 25\%$ without vorinostat, 39 patients provided a 90% power to demonstrate an ORR $> 25\%$. With 39 patients with FL, the primary objective for an ORR would have been met if ≥ 16 patients demonstrated an objective response. Survival curves were plotted using the Kaplan–Meier product-limit method.

Mutation analysis of CREBBP/EP300

The formalin-fixed paraffin-embedded (FFPE) materials were obtained from the archives of each study site provided from 33 patients participating in the phase II study. Genomic DNA was extracted from the FFPE samples using the QIA-amp DNA FFPE Tissue kit (QIAGEN Inc., Valencia, CA, USA). Sequences for all annotated exons and flanking introns of CREBBP and EP300 were obtained from the NCBI 37.3 Genome database (mRNA accession numbers: NM_004380.2 and NM_001429.3, respectively). Coding sequences were obtained using polymerase chain reaction (PCR) and Sanger sequencing on genomic DNA. Primer extension sequencing was performed by GENEWIZ, Inc. (South Plainfield, NJ, USA). The sequencing data were analysed to detect any mutations compared to the genomic DNA reference

sequence. Synonymous mutations and previously reported polymorphisms (Human dbSNP Database at NCBI, Build 137) were excluded.

Results

Patient characteristics

Between February 2009 and February 2010, 56 patients from 18 centres in Japan, Korea, Taiwan and Hong Kong were enrolled in this study. Demographics and patient characteristics are listed in Table I. Of these 56 patients, six were diagnosed with non-pre-specified diseases by the ICPC (two patients with a mixture of FL and DLBCL, two with DLBCL, one with high-grade nodal MZL and one with malignant lymphoma, unclassified) were excluded from the full analysis set (FAS) population for efficacy analysis. The histologies of the 50 FAS patients were FL in 39 patients, including a patient with FL grade 3b according to the protocol definition because no diffuse area was recognized by the ICPC, other indolent B-NHL in seven patients [four patients with extranodal MZL of MALT type, two with small B-cell lymphoma

not otherwise specified (NOS), and one with SLL], and MCL in four patients. Patients with FL ($n = 39$) were categorized according to the Follicular Lymphoma International Prognostic Index (FLIPI) low ($n = 16$, 41%), intermediate ($n = 15$, 39%) and high ($n = 8$, 21%) risks. The median age of the 56 patients at the start of the protocol treatment was 60 years (range, 33–75 years). The median number of prior therapies was 2 (range, 1–4). Most of the patients (30 patients with FL, six with the other indolent B-NHL, and four with MCL) had received rituximab-containing prior chemotherapeutic regimens. Other prior regimens included alkylating agents ($n = 7$), purine analogs ($n = 5$), and radioimmunotherapy ($n = 3$) (Table I).

Treatment

The median duration of the protocol treatment was 8 months, with a maximum of 26+ months. The median ratio of the planned dosage (200 mg twice daily, orally, for 14 consecutive days in a 21-d cycle) was 86% for the 56 patients. As of the data cut-off date (February 2011), 18 patients continued to receive treatment and 38 patients had

Table I. Patient characteristics.

Characteristics	FL ($n = 39$)	Indolent B-NHL (non-FL)* ($n = 7$)	MCL ($n = 4$)	Others† ($n = 6$)	Total ($n = 56$)
Median age, years (range)	60 (33–73)	61 (44–70)	72 (60–75)	55 (46–70)	60 (33–75)
ECOG performance status, n (%)					
0	31 (80)	6 (86)	4 (100)	4 (67)	45 (80)
1	8 (21)	1 (14)	0 (0)	2 (33)	11 (20)
Number of prior therapies					
Median (range)	1 (1–4)	3 (1–4)	3 (3–4)	2 (1–2)	2 (1–4)
Prior therapy, n (%)					
R-chemotherapy	30 (77)	6 (86)	4 (100)	6 (100)	46 (82)
Alkylating agent	3 (8)	1 (14)	3 (75)	0 (0)	7 (13)
Purine analog	2 (5)	3 (43)	0 (0)	0 (0)	5 (9)
Radioimmunotherapy	2 (5)	0 (0)	0 (0)	1 (17)	3 (5)
Other	3 (8)	2 (30)	1 (25)	0 (0)	6 (11)
FLIPI, n (%)					
Low risk	16 (41)	N.A.	N.A.	N.A.	16 (29)
Intermediate risk	15 (39)	N.A.	N.A.	N.A.	15 (27)
High risk	8 (21)	N.A.	N.A.	N.A.	8 (14)
FL grade, n (%)					
1	25 (64)	N.A.	N.A.	N.A.	25 (45)
2	13 (33)	N.A.	N.A.	N.A.	13 (23)
3b‡	1 (3)	N.A.	N.A.	N.A.	1 (2)

FL, follicular lymphoma; B-NHL, B-cell non-Hodgkin lymphoma; MCL, mantle cell lymphoma; ECOG, Eastern Cooperative Oncology Group; R, rituximab; FLIPI, Follicular Lymphoma International Prognostic Index; R-chemotherapy, rituximab-containing chemotherapy; N.A., Not applicable.

*Indolent B-NHL (non-FL) included four patients with extranodal marginal zone B-cell lymphoma (MZL) of mucosa-associated lymphoid tissue type, two patients with small B-cell lymphoma not otherwise specified, and one patient with small lymphocytic lymphoma.

†Others included following diagnoses; diffuse large B-cell lymphoma (DLBCL; two patients), malignant lymphoma unclassified, follicular lymphoma (FL) grade 3a (50%) with DLBCL (50%), FL grade 3b (40%) with DLBCL (60%), and high grade nodal MZL.

‡A patient with FL grade 3b was enrolled according to the protocol definition because no diffuse area was recognized in the specimen by the Independent Central Pathological Committee.

Table II. Efficacy results.

	ORR, % (95% CI)*	Median PFS, month (95% CI)†	Survival, % ‡
FL (<i>n</i> = 39)	49 (32.4, 65.2)	20 (11.2, 29.7)	86
FLIPI low risk (<i>n</i> = 16)	44 (19.8, 70.1)	20 (8.5, 29.9)	94
FLIPI intermediate risk (<i>n</i> = 15)	53 (26.6, 78.7)	20 (7.5, N.E.)	87
FLIPI high risk (<i>n</i> = 8)	50 (15.7, 84.3)	12 (2.0, 30.1)	75
Indolent B-NHL (non-FL) § (<i>n</i> = 7)	43 (9.9, 81.6)	10 (4.1, N.E.)	86
MCL (<i>n</i> = 4)	0 (0.0, 60.2)	N.E.	50
All FAS population (<i>n</i> = 50)	44 (30.0, 58.7)	18 (10.4, 25.8)	84
All ITT population (<i>n</i> = 56)	N.A.	N.A.	81

ORR, overall response rate; CI, confidence interval; PFS, progression-free survival; FL, follicular lymphoma; FLIPI, Follicular Lymphoma International Prognostic Index; B-NHL, B-cell non-Hodgkin lymphoma; MCL, mantle cell lymphoma; FAS, full analysis set; ITT, intention to treat; N.E., not estimable; N.A., not applicable.

*ORR was analysed by the data 1 year after the last patient enrolled.

†Median PFS was analysed by the data 2 years after the last patient enrolled.

‡Survival rate was analysed at the data 2 years after the last patient enrolled.

§Indolent B-NHL (non-FL) included four patients with extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue type, two patients with small B-cell lymphoma not otherwise specified, and one patient with small lymphocytic lymphoma.

discontinued treatment due to disease progression (*n* = 25), drug-related AEs (*n* = 9) or withdrawal of consent (*n* = 4). An additional data cut-off (February 2012) was made for the PFS and OS evaluation.

Efficacy

At the data cut-off date (1 year after the last patient enrolled), a 49% ORR [95% confidence interval (CI): 32.4, 65.2] was observed among 39 assessable patients with relapsed/refractory FL (Table II), which included four patients with CR (10%), three with CRu (8%), and 12 with PR (31%). Tumour size was reduced in 92% (36/39) of FL patients, and tumour shrinkage occurred not only in the patients with *CREBBP/EP300* mutations, but also in those without the mutations (Fig 1). The relationship between clinical efficacy and *CREBBP/EP300* mutation statuses is described in more detail in the following *CREBBP and EP300 mutation analysis* section. At the additional data cut-off for PFS and OS evaluation (2 years after the last patient enrolled), the best response of one patient with FL was upgraded from stable disease (SD) to PR, the median PFS for patients with FL was 20 months (95% CI: 11.2, 29.7) (Table II, Fig 2A), and the median PFS for patients with FL who responded was 26 months. The FL patients with low, intermediate or high FLIPI scores achieved a median PFS of 20 months, 20 months or 13 months, respectively (Table II, Fig 2B). During the trial and follow-up period, two of three patients with FL refractory to rituximab obtained CRu and PR, and a 43% ORR (3/7) was observed in patients with relapsed/refractory other indolent B-NHL (two patients with small B-cell lymphoma NOS and one with extranodal MZL of MALT type) including one CR in a patient with small B-cell lymphoma NOS. None of the assessable MCL patients responded. Within the 18 patients who continued vorinostat

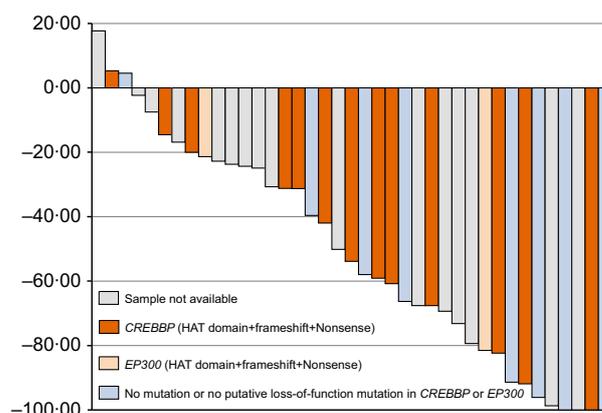


Fig 1. Waterfall plot showing percent change in tumour size for all assessable follicular lymphoma (FL) patients at the time of best response and results of histone acetyltransferase (HAT) mutation analysis.

treatment, three patients (two patients with FL and one with small B-cell lymphoma NOS) had CR and two patients with FL had CRu. Approximately 81% of the intention to treat (ITT) population (56 patients) remained alive at the additional data cut-off (February 2012) (Table II), and the median OS had not yet been reached (Fig 2C).

Safety

Forty-five patients (80%) experienced grade 3/4 AEs. The median time to onset was 15 d (range: 2–497 d). The most common drug-related AEs were thrombocytopenia, diarrhoea, neutropenia, decreased appetite, nausea, leucopenia and fatigue (Table III). Thrombocytopenia and neutropenia were the most frequently observed grade 3 or 4 AEs (Table III). All patients who developed grade 3 or 4 thrombocytopenia or neutropenia recovered after dose

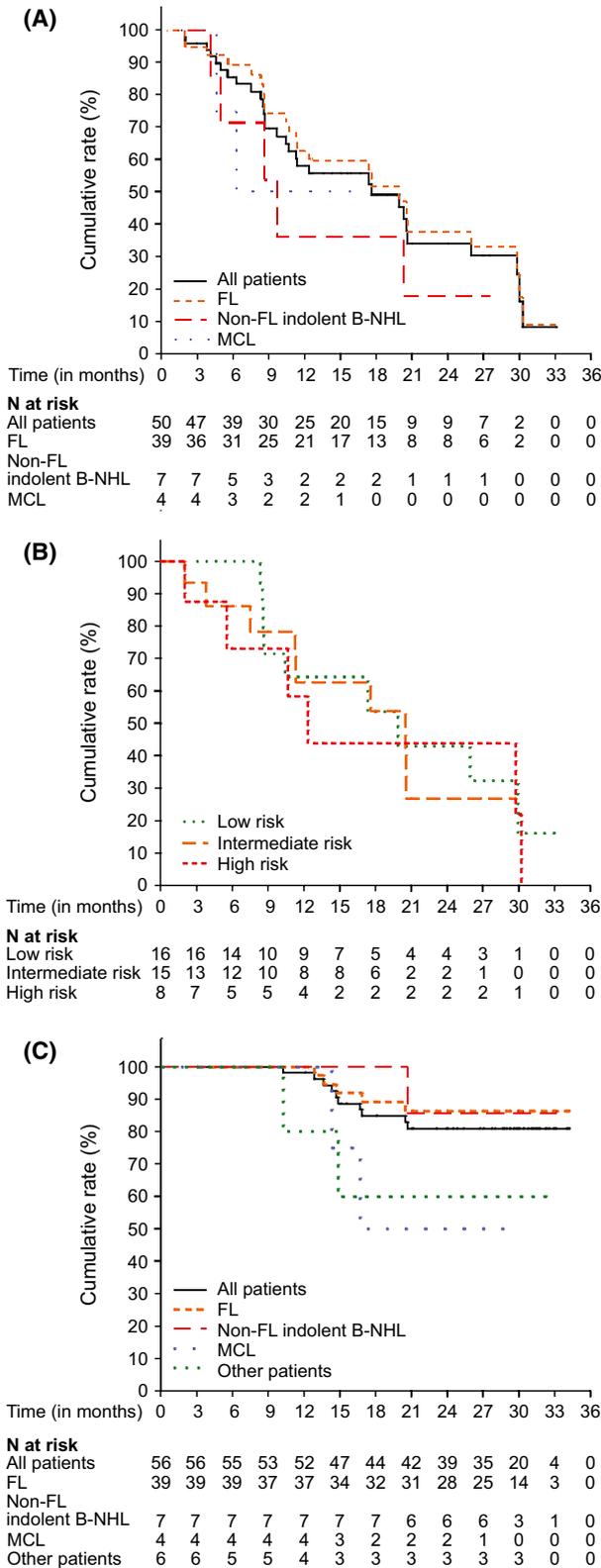


Fig 2. Kaplan–Meier estimates of progression-free survival (PFS) or overall survival (OS). (A) PFS by disease types for all assessable patients ($n = 50$), patients with FL ($n = 39$), other (non-FL) indolent B-NHL ($n = 7$) and MCL ($n = 4$). PFS was defined as the time from allocation to the first documented disease progression or death due to any cause, whichever occurred first. (B) Correlation between Follicular Lymphoma International Prognostic Index (FLIPI) scores and outcomes for PFS of the patients with FL. PFS for the patients at low ($n = 16$), intermediate ($n = 15$), or high ($n = 8$) risk according to FLIPI were calculated. (C) OS by disease types for all enrolled patients ($n = 56$), patients with FL, other (non-FL) indolent B-NHL and MCL. FL, follicular lymphoma; B-NHL, B-cell non-Hodgkin lymphoma; MCL, mantle cell lymphoma.

Table III. Drug-related adverse events.

Preferred term	Total, $n = 56$		
	All grades n (%)	Grade 3 n (%)	Grade 4 n (%)
Haematological			
Thrombocytopenia	52 (93)	13 (23)	14 (25)
Neutropenia	38 (68)	20 (36)	3 (5)
Leucopenia	31 (55)	7 (13)	0 (0)
Lymphopenia	19 (34)	7 (13)	0 (0)
Anaemia	19 (34)	2 (4)	0 (0)
Non-haematological			
Diarrhoea	38 (68)	3 (5)	0 (0)
Decreased appetite	35 (63)	4 (7)	0 (0)
Nausea	34 (61)	0 (0)	0 (0)
Fatigue	29 (52)	1 (2)	0 (0)
Weight decrease	13 (23)	1 (2)	0 (0)
Hyperglycaemia	12 (21)	2 (4)	0 (0)

grade 3 infection was observed in one patient with herpes zoster. No grade 4 infection was reported. There were no treatment-related deaths during this trial.

CREBBP and EP300 mutation analysis

Of the 33 tissue samples obtained for mutation analysis, the genomic DNA extraction or PCR failed in five samples, and sequencing succeeded in 28 samples (23 samples of FL and five of other B-NHL) (Table IV). For *CREBBP*, 19 of the 28 samples had a total of 34 mutations and 17 samples had putative loss-of-function mutations for HAT activity (total 22 mutations: 17 in the HAT domain, three frame shift and two nonsense). For *EP300*, six samples had a total of 14 mutations, and two samples had putative loss-of-function mutations (two mutations in the HAT domain) (Fig 3). Of the 23 samples from FL patients, the putative loss-of-function mutations of *CREBBP* and *EP300* were found in 13 and two samples, respectively. Of the 13 patients with *CREBBP* putative loss-of-function mutations, the ORR was 54% (one CRU, six PR and six SD) and median PFS was 11 months.

reduction, interruption or discontinuation of vorinostat and adequate supportive measures. The incidences of drug-related grade 3 leucopenia and lymphopenia were 13% each, and a

Table IV. Mutation analysis results.

	FL (<i>n</i> = 23) <i>n</i> (%)	Indolent B-NHL (non-FL)* (<i>n</i> = 3) <i>n</i> (%)	Others† (<i>n</i> = 2) <i>n</i> (%)	Total (<i>n</i> = 28) <i>n</i> (%)
<i>CREBBP</i>				
Mutation	15 (65)	2 (67)	2 (100)	19 (68)
HAT domain	10 (43)	2 (67)	2 (100)	14 (50)
HAT domain + Frameshift + Nonsense	13 (57)	2 (67)	2 (100)	17 (61)
No mutation	8 (35)	1 (33)	0 (0)	9 (32)
<i>EP300</i>				
Mutation	5 (22)	1 (33)	0 (0)	6 (21)
HAT domain	2 (9)	0 (0)	0 (0)	2 (7)
HAT domain + Frameshift + Nonsense	2 (9)	0 (0)	0 (0)	2 (7)
No mutation	18 (78)	2 (67)	2 (100)	22 (79)

Samples were not collected from patients with mantle cell lymphoma.

FL, follicular lymphoma; B-NHL, B-cell non-Hodgkin lymphoma; HAT, histone acetyltransferase.

*Indolent B-NHL (non-FL) included a patient with extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue type, and two patients with small B-cell lymphoma not otherwise specified.

†Others included a patient with diffuse large B-cell lymphoma (DLBCL), and a patient with FL grade 3b (40%) with DLBCL (60%).

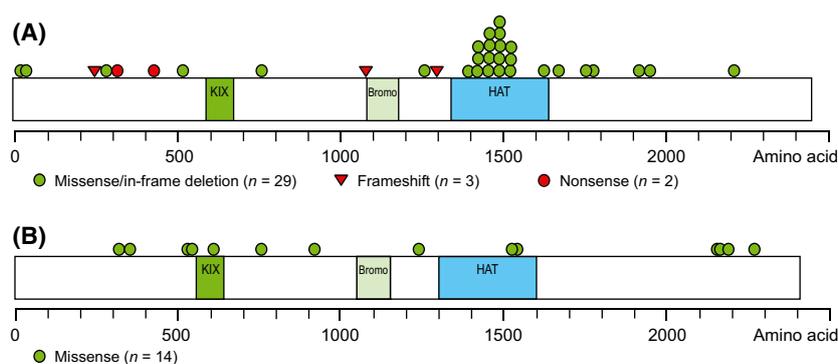


Fig 3. *CREBBP* and *EP300* mutations in patients with FL and other B-NHL. (A) Schematic diagram of the *CREBBP* protein and (B) the *EP300* protein. FL, follicular lymphoma; B-NHL, B-cell non-Hodgkin lymphoma; KIX, CREB-binding domain; bromo: bromodomain; HAT, histone acetyltransferase domain.

For the two FL patients with putative loss-of-function mutations in *EP300*, the objective responses were CR and SD, for which the PFS times were 16 and 21 months, respectively. For the six FL samples without mutations in either *CREBBP* or *EP300*, ORR and median PFS were 67% (two CRu, two PR and two SD) and 21 months, respectively.

Discussion

We conducted an open-label, single-arm phase II study of vorinostat in patients with relapsed/refractory indolent B-NHL and MCL. The ORR and median PFS values in relapsed/refractory FL (49% and 20 months respectively, *n* = 39) and the ORR in other indolent B-NHL (43%, *n* = 7) in this study are consistent with the data of a previous phase II trial of vorinostat, which reported ORR and median PFS in relapsed/refractory FL patients (*n* = 17) of 47% and 15.6 months, and a 22% ORR in other indolent B-NHL patients (*n* = 9) (Kirschbaum *et al*, 2011), which suggests there is no evidence of any ethnic difference for efficacy between Japanese and Caucasian people. Although our study had a similar single-arm design, it had a larger sample size.

Our study also showed the potential efficacy of vorinostat, such as a median PFS of 26 months, in responsive FL patients and the fact that 18 of 56 patients continued vorinostat, including three CR and two CRu patients.

The main vorinostat-related toxicities were thrombocytopenia, neutropenia, diarrhoea and decreased appetite, which appeared to be reversible and manageable. The incidences of treatment-related grade 3/4 leucopenia and lymphopenia were both 13%. In the phase II studies of purine analogs (cladribine and fludarabine) in patients with relapsed indolent B-NHL, higher frequencies of drug-related haematological toxicities were reported (Ogura *et al*, 2004; Tobinai *et al*, 2006), including reduction of white blood cells, which may increase the risk of infectious disease. Of note, grade 3 infections in 10 patients (19%) were reported in the phase II study of fludarabine (Tobinai *et al*, 2006). In contrast, in this trial, drug-related grade 3 infection was observed in one patient (2%) with herpes zoster, and no grade 4 infection was reported.

CREBBP or *EP300* mutations were identified in the HAT domain and other regions in which the mutations were predicted to cause HAT inactivation in FL patients consistent

with previous reports. However, we could not confirm the correlation between the mutations and clinical response, possibly due to the retrospective approach and small number of samples. *MEF2B* mutation is another target, but we did not analyse this because of the sample limitation. Additionally, given that HDAC inhibitors have several mechanisms of action, such as transcriptional/non-transcriptional effects, actions other than transcriptional modulation may also be candidates for predicting clinical efficacy. Further investigations are needed to find factors that predict the clinical efficacy of vorinostat, such as a prospective mutation analysis of *CREBBP* and *EP300* in randomized clinical trials.

In conclusion, this phase II study demonstrated the promising efficacy and safety of vorinostat in patients with relapsed or refractory FL. As this was a single-arm study with limited data to interpret, a comparative study to verify the efficacy of vorinostat is warranted. Given the less toxic profile of vorinostat, this drug could possibly be combined with rituximab or other molecular targeted drugs or could be used for maintenance after rituximab-containing chemotherapy in FL patients. Further clinical studies are warranted to elucidate the drug efficacy of vorinostat.

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Competing interests

M. Ogura has consultant or advisory relationships (Merck/MSD, Symbio, Zenyaku, Kyowakirin, Meijiseika Pharma) and receives research funding (Pfizer, GSK, Eisai, Chugai/Roche, Zenyaku, Symbio, Biomedics, AstraZeneca). K. Tobinai has consultant or advisory relationships (Merck/MSD, Symbio, Spectrum) and receives research funding (Pfizer, GSK, Eisai, Chugai/Roche, Symbio, Merck/MSD, Zenyaku, Biomedics). Keiko Yamamoto, M. Mori, Y. Tanaka and T. Shimamoto are employees of MSD K.K. All remaining authors have declared no conflicts of interest.

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