bjh research paper

A phase 1 clinical trial of vorinostat in combination with decitabine in patients with acute myeloid leukaemia or myelodysplastic syndrome

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Received 19 March 2014; accepted for publication 20 May 2014

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Prior presentations: This work was presented, in part, at the 50th Annual Society of Hematology (ASH) meeting, San Francisco, CA, December 6–9, 2008 and at the 51st annual ASH meeting, New Orleans, LA, December 5–8, 2009.

Summary

Patients with acute myeloid leukaemia (AML) or myelodysplastic syndrome (MDS) may respond to treatment with epigenetic-modifying agents. Histone deacetylase inhibitors may synergize with hypomethylating agents. This phase 1 dose-escalation study was designed to determine the maximum tolerated dose, recommended phase 2 dose, safety and tolerability of vorinostat plus decitabine in patients with relapsed/refractory AML, newly-diagnosed AML, or intermediate- to high-grade MDS. Thirty-four patients received concurrent therapy with decitabine plus vorinostat and 37 received sequential therapy with decitabine followed by vorinostat. Twenty-nine patients had relapsed/refractory AML, 31 had untreated AML and 11 had MDS. The target maximum administered dose (MAD) of decitabine 20 mg/m^2 daily for 5 d plus vorinostat 400 mg/d for 14 d was achieved for concurrent and sequential schedules, with one dose-limiting toxicity (Grade 3 QTc prolongation) reported in the sequential arm. Common toxicities were haematological and gastrointestinal. Responses were observed more frequently at the MAD on the concurrent schedule compared with the sequential schedule in untreated AML (46% vs. 14%), relapsed/refractory AML (15% vs. 0%) and MDS (60% vs. 0%). Decitabine plus vorinostat given concurrently or sequentially appears to be safe and well-tolerated. Concurrent therapy shows promising clinical activity in AML or MDS, warranting further investigation.

Keywords: vorinostat, decitabine, acute myeloid leukaemia, myelodysplastic syndrome, combination therapy.

First published online 8 July 2014
doi:10.1111/bjh.13016

Epigenetic-modifying agents offer a new treatment option for previously challenging disorders. The spectrum of diseases encompassed by myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML) appear to be responsive to this class of agents, with multiple trials showing clinical activity to agents that are able to induce global hypomethylation (Blum *et al*, 1997; Wijermans *et al*, 2005; Cashen *et al*, 2010; Lubbert *et al*, 2011). Hypomethylation reverses the effects of aberrant epigenetic promoter suppression, and evidence suggests that broadening the ability to inhibit repressive signals at the chromatin level might lead to enhanced normalization of regulatory protein expression and increased proapoptotic activity, leading to greater tumour cell death (Yoo & Jones, 2006; Soriano *et al*, 2007).

The nucleoside analog decitabine (Dacogen; Eisai Inc., Dublin, CA, USA), 5-aza-2′-deoxycytidine, is believed to have anticancer activity by incorporating into DNA and forming an irreversible covalent complex with DNA (cytosine-5-)-methyltransferase 1 (DNMT1) (Hurd *et al*, 1999), causing degradation and depletion of the enzyme, which leads to hypomethylation of aberrantly hypermethylated promoters. This may then allow for the reactivation of silenced tumour suppressor genes and regulatory microRNA. However, demethylation alone is not adequate to restore expression of certain genes, and broader epigenetic modulation may lead to enhanced antitumour activity (Cameron *et al*, 1999; Si *et al*, 2010). Consequently, the addition of a histone deacetylase (HDAC) inhibitor to a decitabine regimen seems to be a rational combination. Preclinical evidence supports the use of HDAC inhibitors in haematological malignancies. HDAC inhibitors as single agents have been previously shown to affect a host of cellular targets and processes that might lead to antitumour activity in myeloid malignancies (eg, angiogenesis, apoptosis, the cell cycle, and tumour immunology) and have shown *in vitro* synergy with hypomethylating agents in various tumour types (Johnstone, 2002; Fiskus *et al*, 2009).

In pilot studies, the combination of the methyltransferase inhibitor azacitidine and the HDAC inhibitor sodium phenylbutyrate led to clinical benefit or major cytogenetic responses in patients with AML or MDS (Gore *et al*, 2006; Maslak *et al*, 2006). *In vitro*, simultaneous treatment with decitabine and the investigational HDAC inhibitor MS-275 induces apoptosis in AML cell lines (Nishioka *et al*, 2011). Moreover, in a phase 1/2 trial, combined therapy with decitabine and the HDAC inhibitor valproic acid was safe and led to objective responses in patients with AML or MDS (Garcia-Manero *et al*, 2006). Vorinostat (suberoylanilide hydroxamic acid; Zolinza; Merck & Co., Inc.; Whitehouse Station, NJ, USA), an orally bioavailable synthetic hydroxamic acid class HDAC inhibitor, has both histone and protein deacetylase activity. Activity of vorinostat as single agent or in combination therapy in leukaemia has been demonstrated *in vitro* and in rodent models (Nimmanapalli *et al*, 2003; Yu *et al*, 2003; Reddy *et al*, 2004; Sanchez-Gonzalez *et al*, 2006; Shiozawa *et al*, 2009). In a phase 1 trial, treatment with vorinostat (100–300 mg administered twice or three times daily for 14 d) resulted in haematological improvement (HI) in seven patients with AML (Garcia-Manero *et al*, 2008), and therefore was a good candidate for use in combination with the hypomethylating agent decitabine. The primary objective of this phase 1 study was to determine the maximum tolerated dose (MTD), the recommended phase 2 dose (RP2D), and the safety and tolerability of vorinostat administered concurrently or sequentially with decitabine in patients with relapsed/refractory AML, newly diagnosed elderly patients with AML and patients with intermediate- to high-grade MDS.

Methods

Patients and trial design

This was a phase 1, multicentre, open-label, nonrandomized, two-cohort dose-escalating trial (ClinicalTrials.gov identifier: NCT00479232; http://clinicaltrials.gov/ct2/show/NCT00479232; Protocol 055). Patients 18 years or older with MDS (International Prognostic Scoring System intermediate 1 and above) or relapsed/refractory AML were eligible. Patients aged 60 years and older with newly diagnosed AML were also eligible if not a candidate for cytotoxic chemotherapy. Other inclusion criteria included an Eastern Cooperative Oncology Group performance status of $\leq$2, prior therapy (chemotherapy, biological therapy or investigational therapy other than hydroxycarbamide) that was completed a minimum of 4 weeks before study entry and standard criteria for organ function. There were no minimal haematological parameter requirements before enrollment; however, patients could not be refractory to platelet transfusions. Patients were excluded if they had a history of prior decitabine or azacitidine exposure or previous treatment with an HDAC inhibitor (except for valproic acid for epilepsy, in which case a 30-d washout was mandatory).

All patients received decitabine at a dose of 20 mg/m^2 infused intravenously over 1 h on days 1–5 of a 28-d cycle. Patients were simultaneously enrolled in cohorts of 3 to either concurrent or sequential treatment with decitabine as per the standard 3 + 3 phase 1 study design (Fig 1). For the concurrent vorinostat regimen, dose levels started at 400 mg administered orally (PO) daily on days 1–7 with 21 d free; if tolerable, dose level 2 added 7 d of vorinostat 400 mg PO daily on days 15–21 of a 28-d cycle. If dose level 2 was tolerated, dose level 3 was added: patients were dosed with vorinostat at 400 mg PO daily for 14 consecutive days on days 1–14. For the sequential arm, dose level 1a patients received vorinostat at 400 mg PO daily on days 6–12. If tolerable, the vorinostat dose was escalated (dose level 2a) for 10 d (days 6–15). For dose level 3a, vorinostat was administered for 14 d (days 6–19). No intrapatient dose escalation was permitted and patients did not receive additional cycles of

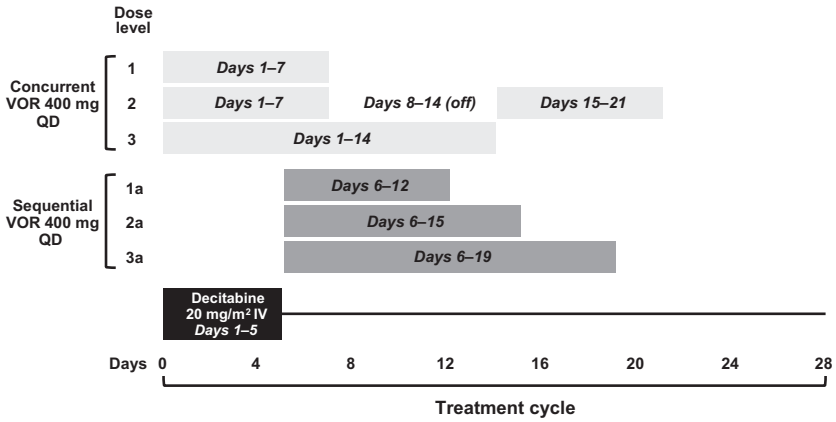

Fig 1. Concurrent and sequential dosing schedules of vorinostat in 28-d treatment cycles. Note that all patients initially received 20 mg/m^2 decitabine. VOR, vorinostat; QD, every day.

therapy earlier than 4–6 weeks from the start of the previous cycle. Grade 3–4 non-haematological toxicities according to Common Terminology Criteria for Adverse Events (CTCAE) version 3 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf) were defined as dose-limiting toxicities (DLTs), as well as prolonged myelosuppression (>42 d in absence of active disease on marrow examination), and led to defined dose modifications for future cycles. Bone marrow aspiration and biopsy was performed on patients within 14 d of commencing treatment and on days 21 and 28 of the first cycle, and aspiration on day 28 of cycle 2 and subsequently as clinically indicated. Dose escalation proceeded if none of the three patients in the cohort had a first cycle DLT. If one of the three patients in a cohort experienced a DLT, three more patients were accrued at that dose level. In the event that a second patient experienced a DLT, that is, two or more out of six patients, then patients were accrued to the next lower dose level. The MTD was defined as that at which no more than one patient out of six experienced a DLT during the first cycle of treatment. Patients with MDS and AML were enrolled to both arms initially, with an expansion at the maximum administered dose (MAD) for MDS, untreated AML or relapsed/refractory AML. With at least 14 patients accrued to the two AML groups, the upper limit of the 80% confidence interval for DLT rate excludes a rate of 33% if two or fewer patients develop DLTs.

The trial protocol was reviewed and approved by the institutional review board and ethics committee at each site before patients were allowed to enrol. This trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Study objectives and assessments

The primary objectives were to determine the MTD, RP2D and overall safety and tolerability for the combination of vorinostat with decitabine. An exploratory objective was to assess clinical activity of the combination of vorinostat and decitabine; efficacy end points included objective response rate, time to response, and progression-free survival for AML patients, and time to leukaemic transformation or death for MDS patients. Clinical responses were measured according to the International Working Group criteria established for AML and MDS, and included complete remission (CR), partial remission (PR), and HI (Cheson *et al*, 2003, 2006). Patients' cytogenetics were also assessed and categorized as poor, intermediate, or good risk according to European LeukaemiaNet criteria (Döhner *et al*, 2010). Patients received treatment until disease progression, unacceptable toxicity or withdrawal of consent up to 24 months (two patients received treatment beyond 24 months because they were benefiting from treatment).

Results

Patient disposition and baseline characteristics

Eighty-four patients were screened between June 2007 and May 2010; 71 patients received the combination of decitabine plus vorinostat – 34 patients on the concurrent arm and 37 patients on the sequential arm. Twenty-nine patients had relapsed/refractory AML, 31 patients had previously untreated AML, and 11 patients presented with MDS. Most patients with relapsed/refractory AML had received cytarabine plus idarubicin hydrochloride [11 (38%)] or single-agent cytarabine [9 (31%)]. Among the 42 patients with intermediate- to high-risk MDS or untreated AML, 38 (91%) had not received any prior therapy. The median age of the overall group was 68 years (range 18–85 years). Patient characteristics were similar in the two cohorts, other than an increased number of male patients in the concurrent group (Table I).

Safety and tolerability of vorinostat plus decitabine

Patients treated on the concurrent and sequential arm were exposed to a mean of 4·3 cycles and 2·9 cycles, respectively. The MAD of vorinostat plus decitabine in either arm was 400 mg daily PO for 14 consecutive days in a 28-d cycle. There was only one first cycle DLT (grade 3 QTc prolonga-

Table I. Patient baseline characteristics.

Patient characteristic	Vorinostat + decitabine		
	Concurrent cohort $n = 34$	Sequential cohort $n = 37$	Total $N = 71$
Gender, *n* (%)			
Male	23 (68)	19 (51)	42 (59)
Female	11 (32)	18 (49)	29 (41)
Age, years			
Mean (SD)	63·9 (13·0)	66·6 (13·8)	65·3 (13·4)
Median (range)	68·0 (33·0–80·0)	71·0 (18·0–85·0)	68·0 (18·0–85·0)
Race, *n* (%)			
White	30 (88)	34 (92)	64 (90)
Black	2 (6)	1 (3)	3 (4)
Asian	1 (3)	2 (5)	3 (4)
Other	1 (3)	0	1 (1)
ECOG performance status, *n* (%)			
0	14 (41)	8 (22)	22 (31)
1	14 (41)	24 (65)	38 (54)
2	6 (18)	5 (14)	11 (16)
Disease characteristic, *n* (%)			
Refractory or relapsed AML	14 (41)	15 (41)	29 (41)
Untreated AML	15 (44)	16 (43)	31 (44)
Intermediate or high risk MDS	5 (15)	6 (16)	11 (16)

SD, standard deviation; ECOG, Eastern Cooperative Oncology Group; AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome.

tion from 461 ms at baseline to 592 ms on day 8), observed in a patient treated at the MAD on the sequential arm. In both arms, the MAD was reached before the MTD was established. Two of 34 patients (6%) in the concurrent arm and 3 of 37 patients (8%) in the sequential arm required one dose modification due to adverse events (AEs). The number of patients requiring two or more dose modifications due to AEs was 1 (3%) in the concurrent arm and 3 (8%) in the sequential arm. Most patients in both the concurrent arm (91%) and sequential arm (84%) did not need any dose reductions. Nine patients of 61 (15%) treated at the MAD required dose reductions due to AEs; 3/31 (10%) concurrent, 6/30 (20%) sequential. Median time on therapy at the MAD, before requiring dose reduction, for patients on the concurrent and sequential regimens was 15·0 d (range: 9–67 d) and 136·5 d (range: 48–225 d), respectively.

Drug-related AEs were reported in 89% of patients (Table II). The most common drug-related AEs (all grades) reported in more than 20% of patients across both regimens were nausea (59%), diarrhoea (41%), fatigue (40%), vomiting (30%), decreased appetite (25%), thrombocytopenia (24%) and leucopenia (23%). The most common Grade 3 or higher AEs were haematological toxicities, including thrombocytopenia (21%), leucopenia (21%), neutropenia (18%) and febrile neutropenia (16%). While more haematological toxicities were reported in the concurrent arm (Table IIIA) compared with the sequential arm, fatigue was more common in the sequential arm (Table IIIB) compared with the concurrent arm. There were two cardiovascular drug-related grade 3 or above AEs reported in five patients: one Grade 4 cardiac arrest reported in Cycle 2 of sequential 14 d vorinostat + decitabine during an episode of urosepsis, and one Grade 3 tachycardia reported in Cycle 5 of concurrent 14 d vorinostat + decitabine. Serious AEs (SAEs) were reported in 78% of patients (30% of patients had drug-related SAEs). The most commonly reported drug-related SAE occurring in >10% of patients was febrile neutropenia (13%). More SAEs were reported in the concurrent arm compared with the sequential arm. AEs were the reason for discontinuation in 20% of patients; SAEs were the reason for discontinuation in 17% of patients and 6% discontinued treatment due to drug-related AEs. During treatment and protocol-defined follow-up of up to 24 months after receiving first dose of therapy, deaths were reported in 11% of patients, but none of the deaths were considered drug-related. Deaths were due to AML, pneumonia, lung infection, cerebrovascular accident, and staphylococcal bacteraemia.

Clinical activity of vorinostat plus decitabine treatment

Clinical activity with the concurrent and sequential dosing schedules was assessed among the three disease states (relapsed/refractory AML, newly diagnosed AML, and intermediate- to high-grade MDS) as an exploratory objective (Table IV). At the MAD dose level, in the concurrent arm, there were six responders (four CRs, two PRs) out of 13 patients (46%) with untreated AML, while in the sequential

Table II. Treatment-related adverse events of any grade reported in at least 10% of patients in any treatment group (all patients as treated).

Adverse event, *n* (%)	Concurrent ($n = 34$)	Sequential ($n = 37$)	Total ($N = 71$)
Nausea	19 (56)	23 (62)	42 (59)
Diarrhoea	16 (47)	13 (35)	29 (41)
Fatigue	11 (32)	17 (46)	28 (40)
Vomiting	12 (35)	9 (24)	21 (30)
Decreased appetite	9 (27)	9 (24)	18 (25)
Thrombocytopenia	11 (32)	6 (16)	17 (24)
Leucopenia	11 (32)	5 (14)	16 (23)
Neutropenia	9 (27)	4 (11)	13 (18)
Febrile neutropenia	6 (18)	5 (14)	11 (16)
Asthenia	3 (9)	5 (14)	8 (11)
Hyperglycaemia	4 (12)	5 (14)	9 (13)
Hypokalaemia	5 (15)	4 (11)	9 (13)
Dizziness	3 (9)	4 (11)	7 (10)
Hypoalbuminaemia	4 (12)	2 (5)	6 (9)
Constipation	2 (6)	4 (11)	6 (9)
Hyponatraemia	4 (12)	0	4 (6)

Table III. Grade 3–4 drug-related adverse events reported in patients treated with vorinostat and decitabine (A) concurrently (all patients as treated) and (B) sequentially (all patients as treated).

Adverse event, *n* (%)	Vorinostat 400 mg qd × 7 d/4 weeks + decitabine *n* = 3	Vorinostat 400 mg qd × 7 d/2 weeks + decitabine *n* = 3	Vorinostat 400 mg qd × 14 d/4 weeks + decitabine *n* = 28
(A)			
Anaemia	0 (0)	0 (0)	1 (3·6)
Aspartate aminotransferase increased	0 (0)	0 (0)	1 (3·6)
Cellulitis	0 (0)	0 (0)	1 (3·6)
Decreased appetite	0 (0)	0 (0)	1 (3·6)
Deep vein thrombosis	0 (0)	0 (0)	1 (3·6)
Fatigue	0 (0)	0 (0)	1 (3·6)
Febrile neutropenia	0 (0)	0 (0)	8 (28·6)
Haematmesis	0 (0)	0 (0)	1 (3·6)
Hypermagnesaemia	1 (33·3)	0 (0)	0 (0)
Hypokalaemia	0 (0)	0 (0)	2 (7·1)
Hyponatraemia	0 (0)	0 (0)	3 (10·7)
Leucopenia	0 (0)	2 (66·7)	8 (28·6)
Nausea	0 (0)	0 (0)	2 (7·1)
Neutropenia	0 (0)	1 (33·3)	8 (28·6)
Pancytopenia	0 (0)	0 (0)	1 (3·6)
Pneumonia	0 (0)	0 (0)	3 (10·7)
Pneumonia fungal	0 (0)	0 (0)	1 (3·6)
Tachycardia*	0 (0)	0 (0)	1 (3·6)
Thrombocytopenia	0 (0)	1 (33·3)	9 (39·1)
Vomiting	0 (0)	0 (0)	1 (3·6)

Adverse event, *n* (%)	Vorinostat 400 mg qd × 7 d/4 weeks + decitabine *n* = 3	Vorinostat 400 mg qd × 10 d/4 weeks + decitabine *n* = 4	Vorinostat 400 mg qd × 14 d/4 weeks + decitabine *n* = 30
(B)			
Anaemia	0 (0)	1 (25·0)	0 (0)
Aspartate aminotransferase increased	0 (0)	0 (0)	1 (3·3)
Cardiac arrest	0 (0)	0 (0)	1 (3·3)
Decreased appetite	0 (0)	0 (0)	2 (6·7)
Diarrhoea	0 (0)	0 (0)	1 (3·3)
Dizziness	0 (0)	0 (0)	1 (3·3)
ECG QT prolonged†	0 (0)	0 (0)	2 (6·7)
Fatigue	0 (0)	0 (0)	5 (16·7)
Febrile neutropenia	0 (0)	0 (0)	5 (16·7)
Hyperglycaemia	0 (0)	0 (0)	1 (3·3)
Hypertension	0 (0)	0 (0)	1 (3·3)
Hypotension	0 (0)	0 (0)	2 (6·7)
Leucopenia	1 (33·3)	1 (25·0)	3 (10·0)
Mucosal inflammation	0 (0)	0 (0)	1 (3·3)
Neutropenia	1 (33·3)	0 (0)	4 (13·3)
Renal failure, acute	0 (0)	0 (0)	1 (3·3)
Thrombocytopenia	1 (33·3)	1 (25·0)	3 (10·0)

ECG, electrocardiogram.

*Grade 3 reported in cycle 5, not treated as a dose limiting toxicity.

†One Grade 3 incident reported in cycle 1 was treated as a dose-limiting toxicity.

arm there were two CRs among 14 patients (14%). Two patients met the criteria for HI following sequential treatment at the MAD. Among patients with relapsed/refractory AML, there were two CRs out of 13 patients (15%) in the concurrent arm, while there were no responses observed on the sequential arm among the 15 patients treated at the MAD. One patient in the concurrent arm achieved HI. Among patients with MDS, three objective responses (one CR, two PRs) were observed among five patients (60%) in the concurrent arm and one patient had HI. There were no

Table IV. Objective responses at the maximum administered dose (decitabine 20 mg/m^2 for 5 d plus vorinostat 400 mg/d for 14 d*).

	Concurrent cohort		Sequential cohort	
Myeloid disease	*n*	Objective responses	*n*	Objective responses
Untreated AML	13	6 (4 CRs, 2 PRs)	14	2 (2 CRs)
Relapsed or refractory AML	13	2 (2 CRs)	10	0
MDS	5	3 (1 CR, 2 PRs)	6	0

AML, acute myeloid leukaemia; CR, complete remission; PR, partial remission; MDS, myelodysplastic syndrome.
*14 d every 4 weeks or in three cases, 7 d every 2 weeks.

responses observed among the six MDS patients treated on the sequential regimen at the MAD. There was one additional responder not treated at the MAD: a patient with untreated AML in the concurrent arm who achieved a CR. Six patients who did not have a formal response remained on treatment for more than 6 months: two patients with untreated AML on the sequential arm, three patients with MDS on the sequential arm and one patient on the concurrent arm with refractory or relapsed AML (all treated at the MAD).

The median time from treatment start to stop (treatment duration) was 70 d (range 2–815). At the MAD, the median duration was 80 d, vs. 43·5 at the lower doses (P = NS). There were 14 responders, with a median treatment duration of 224 d (range 27–815); one patient with a CR withdrew on day 27 due to AEs, and a patient with a CR went off study at 815 d but did not relapse for a total of 1465 d. Eight of 14 responders had median treatment duration of more than 6 months. Median treatment duration for the entire untreated AML cohort was 110 d (range 2–557), for relapsed or refractory AML was 46 d (range 6–349), and for MDS was 119 d (range 13–815; Fig 2). At the MAD, the concurrent arm had median treatment duration of 101 d (2–815), compared with 54 d (8–712) for the sequential arm (P = NS). The role of cytogenetic aberrations on response to treatment was also evaluated. In the untreated AML group there were three responders out of eight patients with poor risk cytogenetics, and there was one responder out of 11 patients with poor risk cytogenetics in the relapsed or refractory AML group. There were no responders in the poor risk cytogenetics group among the three patients with MDS treated with vorinostat plus decitabine (Table V).

Discussion

We studied the combination of two epigenetic agents, vorinostat and decitabine, administered either sequentially or concurrently, in older patients with untreated AML, patients with relapsed/refractory AML and intermediate- to high-grade MDS patients. Both schedules were reasonably well tolerated – only one QTc prolongation DLT was observed in the sequential arm. The MAD of 400 mg vorinostat administered PO daily for 14 consecutive days whether concurrently with or sequentially after 5 d of decitabine in a 28-d cycle was reached; therefore, the MTD was not established. The most severe AEs were haematological, which included thrombocytopenia, leucopenia and neutropenia. Based on the toxicity profile observed with vorinostat and decitabine, the combination could be considered for future investigation in patients with AML or MDS, with concurrent therapy showing the most promising clinical activity.

While the primary objective of this phase 1 study was to establish toxicity of vorinostat treatment in combination with decitabine, a secondary end point of the trial was to conduct a preliminary assessment of the treatment sequence. Epigenetic repression of transcription is a complex process that is mediated by a large number of enzymatic processes at the level of histones (acetylation, methylation, ubiquitination, etc.) and that directly affects genes via DNA methylation. A suggestion that DNA methylation was the primary effect was first demonstrated with respect to certain hypermethylated genes, which showed synergistic reversal of repression when treatment was initiated with a hypomethylating agent followed by an HDAC inhibitor, but not concurrently or with an HDAC inhibitor alone (Cameron *et al*, 1999).

In this trial, while the number of patients was small, there were more responses observed in patients treated concurrently rather than sequentially with vorinostat and decitabine in all disease states tested, with a notable increase in response among patients with untreated AML at the MAD level. This trend was also observed in the relapsed/refractory AML patient cohort and the MDS group. Responses were seen even among patients with poor risk cytogenetics – three of six in the untreated AML cohort and all three on the concurrent arm. While the clinical data stand on their own, it is possible to explain the disparity between the *in vitro* and *in vivo* results biologically as well; in the preclinical model, it is the expression of certain genes that show increased reactivation with the sequence, while other genes may require a different sequence for reactivation (Jiang *et al*, 2007). It is also possible that a significant contribution to the activity of decitabine is through a direct cytotoxic effect rather than an epigenetic one, and the effect of vorinostat is to enhance cytotoxicity via its effect upon DNA damage response elements such as TP53 or GADD45A (Kretzner *et al*, 2011).

Despite the encouraging clinical results, the optimal dose and schedule of decitabine remain unclear. A previous study in older patients with AML found a decitabine dosing schedule of 20 mg/m^2 for 5 d resulted in an overall response rate of 25% in a larger group of 55 patients (Cashen *et al*, 2010). In another study, a regimen of 15 mg/m^2 given three times daily over 3 d every 6 weeks led to an overall response rate of 26% in untreated AML patients (Lubbert *et al*, 2011), which was similar to the 20 mg/m^2 over 5 d regimen, while the use of 20 mg/m^2 administered daily to patients with untreated or relapsed AML for 10 d showed increased activity with an overall response rate of 64% (Blum *et al*, 1997).

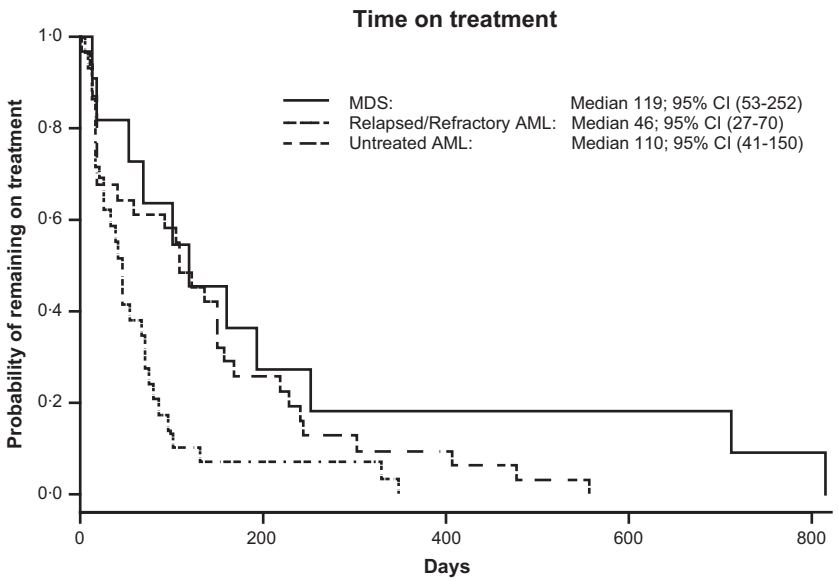

Fig 2. Median duration of treatment (days) for patients with untreated AML, relapsed or refractory AML and MDS. AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome; 95% CI, 95% confidence interval.

Table V. Cytogenetic risk and treatment response in patients with myeloid leukaemia and myelodysplastic syndrome (at any treatment dose).

Myeloid disease	Cytogenetic risk	Number of patients	Objective response
Untreated AML	Good	1	1 (CR)
	Intermediate	22	5 (4 CRs, 1 PR)
	Poor	8	3 (2 CRs, 1 PR)
Relapsed/refractory AML	Intermediate	18	1 (CR)
	Poor	11	1 (CR)
MDS	Intermediate	8	3 (1 CR, 2 PRs)
	Poor	3	0

AML, acute myeloid leukaemia; CR, complete remission; PR, partial remission; MDS, myelodysplastic syndrome.

Based on these reports, a phase 1 study combining vorinostat given concurrently with the 10-d schedule of decitabine would be of interest. Additionally, the optimum dosing schedule of vorinostat still needs to be fully elucidated. In this study, the 400 mg/d dose was used, as this is the US Food and Drug Administration–approved dose for cutaneous T cell lymphoma. However, the half-life of the drug is short, so studies were subsequently done using more frequent dosing regimens, such 200 mg PO twice a day or three times a day (Garcia-Manero *et al*, 2008; Kirschbaum *et al*, 2011). These dosing schedules demonstrated single-agent activity in indolent lymphoma, as well as leukaemia and MDS, and should be studied in combination with decitabine.

In summary, the addition of the HDAC inhibitor vorinostat at 400 mg/d for 14 d in either a concurrent or sequential dosing schedule to a 5-d regimen of the hypomethylating agent decitabine at 20 mg/m^2 was feasible and tolerable in patients with AML and MDS. Although the number of patients evaluated in the two dosing schedules was small, our study showed enhanced activity for the concurrent schedule over the sequential dosing schedule. These results support further evaluation of the concurrent schedule in a large phase 2 study, with correlative studies designed to better understand the interrelationship of hypomethylation and HDAC inhibition, two biologically and clinically important epigenetic processes.

Acknowledgements

This study was sponsored by Merck & Co., Inc., Whitehouse Station, NJ, USA. We thank Tanja Obradovic for help reviewing patient safety and vorinostat efficacy results. Mary E. Hanson, PhD, of Merck & Co., Inc., provided scientific input and editorial assistance. Editorial assistance was provided by Kristen Lewis of Merck & Co., Inc., Whitehouse Station, NJ, USA, and Jennifer Granit, PhD, of Integrus Scientific, a division of Medicus International New York (New York, NY, USA). Integrus Scientific assistance was funded by Merck & Co., Inc., Whitehouse Station, NJ, USA. The authors are fully responsible for all content and editorial decisions and received no financial support or other compensation related to the development of the manuscript.

Author Contributions

Mark Kirschbaum contributed to the design and conception; Christopher Bredeson, Joseph Eid, Ivana Gojo, Stuart Goldberg, Jean-Pierre Issa, Mark Kirschbaum, Lisa Kujawski, Peter Marks, Alessandra Tosolini and Allen Yang were involved in the collection and assembly of data; and Christopher Bredeson, Joseph Eid, Paul Frankel, Ivana Gojo, Stuart Goldberg, Jean-Pierre Issa, Mark Kirschbaum, Lisa Kujawski, Gregory Lubiniecki, Xing Sun and Alessandra Tosolini conducted data analysis and interpretation of the results.

Christopher Bredeson, Joseph Eid, Paul Frankel, Ivana Gojo, Stuart Goldberg, Jean-Pierre Issa, Mark Kirschbaum, Lisa Kujawski, Gregory Lubiniecki, Peter Marks, Alessandra Tosolini and Allen Yang wrote parts of the manuscript. All authors have approved the submission for publication and meet the International Committee of Medical Journal Editors (ICMJE) requirements for authorship.

Conflict of Interest

Mark Kirschbaum: consultancy, honoraria, research funding from Merck; Christopher Bredeson: consultancy and honoraria from Eisai Oncology; Allen Yang: employee of and owns stock in Amgen Inc.; Peter Marks: research funding from Merck; Jean-Pierre Issa: consultancy for Astex and Janssen and research funding from Astex; Joseph E. Eid and Gregory M. Lubiniecki: employees of and own stock in Merck & Co., Inc.; Ivana Gojo, Stuart L. Goldberg, Lisa A. Kujawski, Paul Frankel, Xing Sun and Alessandra Tosolini have nothing to disclose.

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