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A phase II study of vorinostat (MK-0683) in patients with polycythaemia vera and essential thrombocythaemia

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Summary

Inhibition of histone deacetylases may be an important target in patients with myeloproliferative neoplasms. This investigator-initiated, non-randomized, open-label phase II multi-centre study included 63 patients (19 essential thrombocythaemia, 44 polycythaemia vera) from 15 centres. The primary objective was to evaluate if vorinostat was followed by a decline in clonal myeloproliferation as defined by European Leukaemia Net. Thirty patients (48%) completed the intervention period (24 weeks of therapy). An intention-to-treat response rate of 35% was identified. Pruritus was resolved [19% to 0% ($P = 0{\cdot}06$)] and the prevalence of splenomegaly was lowered from 50% to 27% ($P = 0{\cdot}03$). Sixty-five per cent of the patients experienced a decrease in *JAK2* V617F allele burden ($P = 0{\cdot}006$). Thirty-three patients (52% of patients) discontinued study drug before end of intervention due to adverse events (28 patients) or lack of response (5 patients). In conclusion, vorinostat showed effectiveness by normalizing elevated leucocyte and platelet counts, resolving pruritus and significantly reducing splenomegaly. However, vorinostat was associated with significant side effects resulting in a high discontinuation rate. A lower dose of vorinostat in combination with conventional and/or novel targeted therapies may be warranted in future studies.

Keywords: essential thrombocythaemia, polycythaemia vera, phase II study, histone deacetylase inhibition, vorinostat.

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The Philadelphia-negative myeloproliferative neoplasms (MPN), essential thrombocythaemia (ET) polycythaemia vera (PV) and primary myelofibrosis (PMF), arise due to acquired stem cell defects resulting in accumulation and proliferation of myeloid cells (Campbell & Green, 2006). Major determinants of morbidity and mortality are thromboembolic or haemorrhagic complications, bone marrow failure and leukaemic transformation (Elliott & Tefferi, 2005). An important breakthrough in the understanding of the pathogenesis of these disorders occurred in 2005, when the *JAK2* V617F mutation was discovered in the large majority of patients with PV (about 97%) and in 50% of those with ET and PMF (Baxter *et al*, 2005; James *et al*, 2005; Kralovics *et al*, 2005; Levine *et al*, 2005). This mutation in the pseudokinase-autoinhibitory domain results in constitutive kinase activity and induces hypersensitivity to a number of growth factors.

Recently, histone deacetylase inhibitors (HDACi) have been demonstrated to induce tumour cells to undergo growth arrest, differentiation and/or apoptotic cell death (Butler *et al*, 2002; Deroanne *et al*, 2002; Catley *et al*, 2003; de Ruijter *et al*, 2003; Atadja *et al*, 2004; Bradbury *et al*, 2005) and also to inhibit endothelial cell proliferation and angiogenesis *in vivo* (Deroanne *et al*, 2002; Rossig *et al*, 2002; Qian *et al*, 2004). Among these agents ZOLINZA® (Merck Sharp & Dohme Corp., Whitehouse Station, NJ, USA) [Vorinostat; suberoylanilidehydroxamic acid (SAHA)] has been approved by the US Food and Drug Administration for treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma (CTCL) with progressive, persistent or recurrent disease on or following two systemic therapies (Thompson, 2006). Vorinostat inhibits the enzymatic activity of histone deacetylases HDAC1, HDAC2 and HDAC3 (Class I) and HDAC6 (Class II).

In patients with MPN a pronounced deregulation of HDAC genes, involving significant up-regulation of *HDAC6*, *HDAC9* and *HDAC11* has recently been described (Skov *et al*, 2012). HDACi have been shown to have potent inhibitory activity on the autonomous proliferation of haematopoietic cells of PV and ET patients carrying the *JAK2* V617F mutation (Guerini *et al*, 2008). Haematological responses have been recorded during treatment with the HDACi, givinostat, in patients with PV and myelofibrosis (Rambaldi *et al*, 2010) and the combined use of givinostat and hydroxycarbamide (HC) have recently shown encouraging results (Rambaldi *et al*, 2011). Most recently, vorinostat has been shown to normalize the peripheral blood counts and markedly reduce splenomegaly in *JAK2* V617F knock-in mice, and to decrease the mutant allele burden (Akada *et al*, 2012). Furthermore, vorinostat restored physiological levels of histone H3 acetylation, decreased NF-E2 expression, and normalized platelet numbers in NF-E2 transgenic mice with hypoacetylation of histone H3 (Kaufmann *et al*, 2012).

Hence, HDACi may represent important epigenetic therapy in the treatment of patients with MPN and emphasize the need to characterize the efficacy and safety of this novel class of cytoreductive agents (Vannucchi & Biamonte, 2011). Therefore we conducted a phase II study of vorinostat in patients with ET and PV.

Material and methods

Study design and success criteria

This was an investigator-initiated, non-randomized, open-label phase II multicentre study (EudraCT No. 2007-005306-49). The study period was 2 years. Patients were included from 15 centres in Denmark, Sweden, The Netherlands and UK. We intended to determine if vorinostat influenced any of the efficacy variables as noted below. If the study were to show that vorinostat indeed had a positive effect upon any of the parameters–defined by a favourable impact upon any of the efficacy parameters in at least 25% of the patients and with acceptable side effects–a new protocol including a larger number of patients with PV, ET and PMF would be developed focusing on the efficacy and safety of long-term treatment.

Objectives and endpoints

The primary objective of this study was to investigate whether vorinostat, as monotherapy in patients with PV and ET, could induce a clinicohaematological response per the European Leukaemia Net (ELN) response criteria (Barosi *et al*, 2009). Secondly, to investigate whether treatment with vorinostat influenced the *JAK2* V617F mutant allele burden.

Response and safety assessment

Clinicohaematological response was assessed after 24 weeks of therapy (end of intervention) and again after 12 weeks of observation (Fig 1). Patients who obtained partial (PR) – or complete responses (CR) on vorinostat as monotherapy were defined as 'responders'. Response rates were analysed on an intention to treat (ITT) basis where the denominator included all the eligible patients at baseline ($n = 63$). Assessment of splenomegaly was clinical by palpation and both splenomegaly and pruritus were based on dichotomy statistics as 'present or absent'. Toxicities were graded at every visit by the use of the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf), physical examination and relevant laboratory analysis.

Patient selection and eligibility criteria

Sixty-three patients (44 PV and 19 ET) were included after giving written informed consent. The study population included newly diagnosed or previously treated patients in

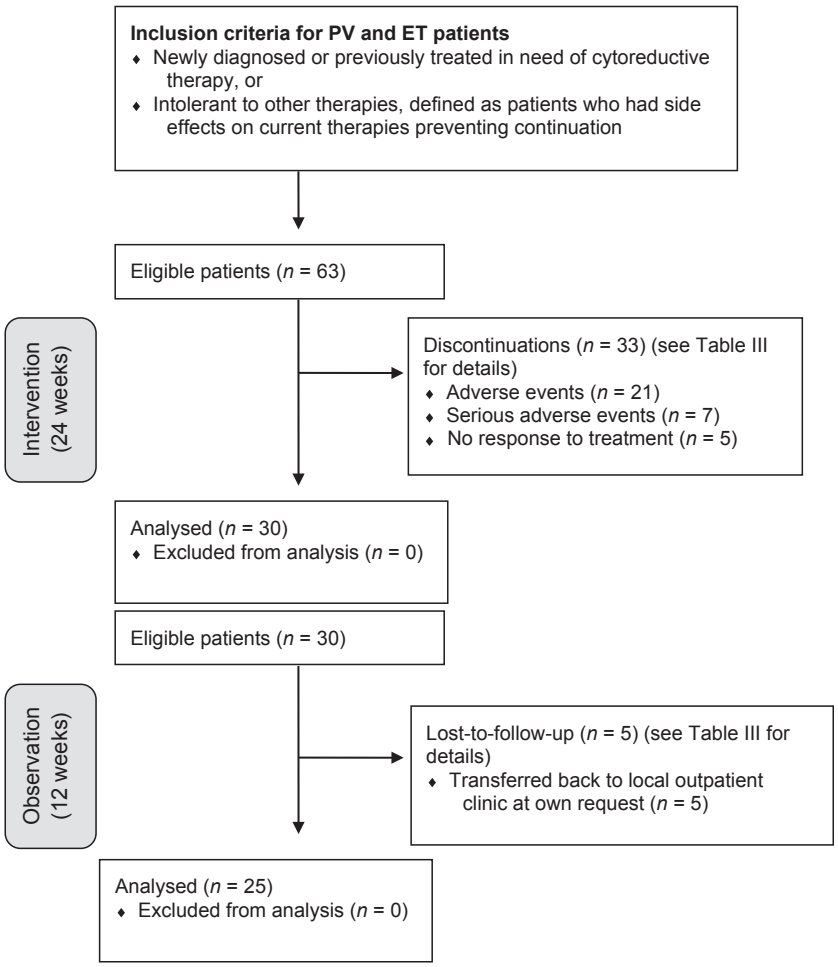

Fig 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram of patients through each stage of the study

need of cytoreductive therapy, or intolerant to other therapies, defined as patients with PV or ET who had side effects on current therapies preventing continuation (leg ulcers on HC, unacceptable fatigue etc. on interferon). Patients with post-ET myelofibrosis, post-PV myelofibrosis and primary myelofibrosis were not included in this study. Diagnostic criteria were according to World Health Organization criteria (Tefferi & Vardiman, 2008).

Patients were not eligible for inclusion in case of marked thrombocytosis (>1500 $\times$ 10^9/l), blast cells in peripheral blood >1%, a Eastern Cooperative Oncology Group (ECOG) performance score $\geq$3 or any serious concomitant disease or circumstances that could limit compliance with the study, including CTCAE version 3.0 grade 3–4 'Cardiac general and arrhythmia', evidence of impaired renal function [serum creatinine >2 $\times$ upper normal limit (UNL)] or liver function (total serum bilirubin >1·5 $\times$ UNL, serum aspartate transaminase/alanine transaminase >3 $\times$ UNL), or psychiatric or social conditions interfering with patient compliance. Furthermore, patients were not included if they were receiving interferon alpha, anagrelide or HC within 1 week of day 1 or valproic acid within 28 d, had active viral infections, were pregnant/lactating or had prior malignancies with the exception of localized malignancies that had undergone curative therapy.

Vorinostat therapy

Vorinostat, 400 mg, was administered once daily for 24 weeks after which patients were observed for an additional 12 weeks without vorinostat (Fig 1). In the observation period patients were followed monthly. The patients were monitored for relapse at which point they were offered reinstatement of active cytoreductive treatment, which could differ from vorinostat.

Dose-limiting toxicities, interruption and resuming

Vorinostat was discontinued in the presence of grade 3 or 4 adverse events (AEs) and treatment was resumed on resolution to grade 1 or baseline. In the presence of grade 3 or 4 drug-related non-haematological toxicity, vorinostat was paused until the toxicity resolved to grade 1 or baseline. For toxicities that could not be treated symptomatically or medically, such as thrombocytopenia, liver dysfunction or renal dysfunction, vorinostat was discontinued and upon recovery re-instituted at 300 mg daily. For both haematological and non-haematological toxicities, dose modifications were undertaken for grade 3 or 4 events. Patients who required dose modification to lower than a dose level of 300 mg were

withdrawn from the study. If treatment was withheld for longer than 14 d due to toxicity or any other reason the patients were withdrawn from the study.

Concomitant therapy

Concurrent administration of HC was permitted during the first 12 weeks of treatment to maintain the platelet count $<1000 \times 10^9$/l. In addition, HC was permitted to control the platelet count if the patient developed ischaemic symptoms (e.g. transitory cerebral ischaemia or completed stroke) or progression of microcirculatory ischaemic symptoms at any platelet level. Treatment with allopurinol and aspirin was allowed.

Quantitative JAK2 analysis

DNA purification. Blood samples for *JAK2* analyses were collected at baseline, and after 12, 24 and 36 weeks. After red cell lysis with ammonium chloride lysis buffer, DNA was extracted from unfractionated leucocytes using a MagnaPure Robot (Roche Diagnostics, Mannheim, Germany; Larsen *et al*, 2007a).

Real-time quantitative polymerase chain reaction. We utilized two qPCR assays with a common forward primer 5′-CTTTCTTTGAAGCAGCAAGTATGA-3′ and a common forward probe 6-FAM-TGAGCAAGCTTTCTCACAAGCAT TTGGTTT-TAMRA. The reverse primers were designed as a wild type-specific primer 5′-GTAGTTTTACTTACTCTCG TCTCCACAtAC-3′ or a *JAK2* V617F mutation-specific primer 5′-GTAGTTTTACTTACTCTCGTCTCCACAtAA-3′, both with an intended mismatch at the 3′-minus 2-position. In order to determine the sensitivity of the mutation-specific primer set, a standard curve was created by a five-fold dilution series of homozygous *JAK2* V617F mutated DNA into donor wild type DNA. From standard curves the slope was calculated for both the wild type-specific and mutation-specific primer-probe sets. The assay sensitivity was calculated to 1:10 000. However we defined a 10-fold higher cut-off limit, corresponding to 1:1000, to be significant regarding detection of *JAK2* V617F mutated alleles. The qPCR reaction volume was 25 μl and primer-concentrations were 300 nmol/l, whereas the concentration of the probe was 200 nmol/l. The PCR amplification conditions were: An initial enzyme activation step of 10 min at 95°C, followed by 50 cycles of 15 s at 95°C and 60 s at 60°C. All qPCR reactions were performed in triplicates on an ABI Prism7900HT (Applied Biosystems, Foster City, CA, USA; Larsen *et al*, 2007b).

Statistics. Haematological parameters (haematocrit, leucocyte and platelet counts, before and during treatment) were inspected for normality of distribution. Skewed variables were log transformed. Two-sample *t*-test was used to compare differences between groups of patients and paired *t*-test was used to compare differences in patients at different time points. Comparisons between responses in *JAK2*-status groups were performed by the use of Fisher′s exact test. Comparisons of disease-specific symptoms during treatment were analysed by the exact McNemar test. Comparisons of *JAK2* V617F tumour allele burdens during treatment were performed by Wilcoxon-signed-rank test because changes in *JAK2* V617F allele burden were not normal distributed, but symmetrical. All *P*-values given were two-sided and *P*-values below 0·05 were considered significant. IBM SPSS Statistics version 19.0.0 (2010; IBM, Somer, NY, USA) or R Statistical Software version 2.14.1 (2011; R Foundation for Statistical Computing, Vienna, Austria) were used.

Ethics. The study was carried out in accordance with Good Clinical Practice as defined by the International Conference on Harmonization. The research was approved by all local institutional review boards and ethics committees.

Results

Sixty-three patients (ET = 19, PV = 44) were enrolled between September 2008 and June 2010. A significant percentage of patients had palpable splenomegaly (48%) and with regard to symptoms 38% reported fatigue, 19% pruritus, 16% weight loss and 14% headache. Patient characteristics and laboratory data at baseline are listed in Table I.

Efficacy – clinicohaematological responses

Intervention period

Thirty patients (48%) completed the intervention period; 27 of these had been treated with vorinostat as monotherapy and the other three had also received concomitant HC. Thirty-three patients discontinued in the intervention period due to ‘AEs’ (21 patients), ‘Serious AEs’ (SAEs – seven patients) or ‘no response’ (NR) (five patients). Please refer to ‘Adverse effects’ and Tables II and III for details on these patients. A response rate of 35%, comprising three patients with CR, 19 with PR and five with NR, was identified. Patients treated with both vorinostat and HC are depicted in detail in Table SI. In patients completing the intervention period on vorinostat only, pruritus resolved [19% to 0% ($P = 0{\cdot}06$)] and the prevalence of splenomegaly was lowered from 50% to 27% ($P = 0{\cdot}03$).

Observation period

Twenty-five patients (40% of total) completed the study (completion of intervention and observation period). Five patients were lost-to-follow-up in the observation period as they were transferred back to their local outpatient clinics at their own request. One of these was in CR, two in PR and two with NR at the time of discontinuation. Of patients completing study and only having been treated with vorinostat in the

Table I. Baseline data for included patients.

Patients	All	ET	PV
Number	63	19 (30%)	44 (70%)
Age, years (median, range)	64 (29–82)	62 (50–77)	65 (29–82)
Gender (male/female)	31/32 (49%/51%)	9/10 (47%/53%)	22/22 (50%/50%)
Time from diagnosis to inclusion, years (median, range)	2·8 (0–27·4)	2·9 (0–22·6)	2·8 (0–27·4)
JAK2 V617F-status			
Positive (%)	50 (79%)	7 (37%)	43 (98%)
JAK2 V617F burden (median, range)	68 (0–99)	0 (0–82)	83 (0–99)
Laboratory work-up at baseline (median, range)			
Haemoglobin (g/l)	142 (89–266)	137 (101–193)	146 (89–266)
Haematocrit (%)	42 (27–58)	37 (31–46)	43 (27–58)
Leucocytes ($\times 10^9$/l)	9·8 (1·0–56·3)	7·4 (3·0–22·1)	13·1 (1·0–56·3)
Monocytes ($\times 10^9$/l)	0·53 (0·01–5·05)	0·53 (0·01–1·20)	0·55 (0·01–5·05)
Lymphocytes ($\times 10^9$/l)	1·4 (0·24–4·61)	1·4 (0·89–3·19)	1·4 (0·24–4·61)
Eosinophils ($\times 10^9$/l)	0·2 (0·005–6·04)	0·09 (0·005–0·6)	0·3 (0·005–6·04)
Platelets ($\times 10^9$/l)	526 (33–1279)	593 (317–1210)	454 (33–1279)
Alkaline phosphatase (u/l)	100 (36–383)	73 (36–369)	112 (58–383)
Lactate Dehydrogenase (u/l)	428 (132–2462)	388 (177–2462)	481 (132–2206)
Creatinine (μmol/l)	74 (40–151)	76 (51–151)	73 (40–145)
Treatment prior to vorinostat			
Hydroxycarbamide	35 (56%)	13 (68%)	22 (50%)
Alpha-interferon	8 (13%)	2 (13%)	6 (14%)
Anagrelide	11 (18%)	7 (39%)	4 (10%)
Busulfan	3 (5%)	1 (6%)	2 (5%)

No. treatments prior to vorinostat irrespective of diagnosis	Frequency (*N*)	Percent
0	21	33
1	29	46
2 or more	13	21
Total	63	100

ET, essential thrombocythaemia; PV, polycythaemia vera.

Table II. All reported adverse events (AEs) in the intervention period not causing discontinuation.

	None *n* (%)	Grade 1 *n* (%)	Grade 2 *n* (%)	Grade 3 *n* (%)	Grade 4 *n* (%)	NS* *n* (%)
Anorexia	44 (70)	15 (24)	1 (2)	2 (3)	1 (2)	
Bleeding	61 (97)	1 (2)	0 (0)	1 (2)	0 (0)	
Chills	60 (95)	3 (5)	0 (0)	0 (0)	0 (0)	
Constipation	55 (87)	8 (13)	0 (0)	0 (0)	0 (0)	
Diarrhoea	33 (52)	17 (27)	10 (16)	3 (5)	0 (0)	
Dry mouth	38 (60)	18 (29)	4 (6)	3 (5)	0 (0)	
Fatigue	13 (21)	22 (35)	20 (32)	7 (11)	1 (2)	
Hair loss	32 (50)	6 (10)	3 (5)	0 (0)	0 (0)	22 (35)
Hyperglycaemia	54 (86)	5 (8)	4 (6)	0 (0)	0 (0)	
Nausea	35 (56)	16 (25)	9 (14)	3 (5)	0 (0)	
Other gastrointestinal	19 (30)	18 (29)	19 (30)	7 (11)	0 (0)	
Vomiting	54 (86)	2 (3)	7 (11)	0 (0)	0 (0)	
Weight loss	35 (56)	14 (22)	13 (20)	1 (2)	0 (0)	

*Not specified.

intervention period, two were still in CR and four in PR after the 12 weeks of observation, giving a response rate of 9·5%. Six patients recommenced vorinostat in the observation period: two without any change in response (PR), three with CR and one with a PR. Two patients recommenced vorinostat at the last visit.

Table III. Causes of discontinuation in the study.

	Number of patients	Time to discontinuation, weeks. Median (range)
Full follow-up	25	
Discontinuation – observation period	5	32 (24–32)
Lost-to-follow-up*	5	
Discontinuation – intervention period		
SAE†	7	8 (4–20)
Diarrhoea	2	
AML	1	
Deep vein thrombosis	1	
Headache	1	
Palpitations	1	
Neuropathy	1	
AE‡	21	12 (2–20)
Diarrhoea	5	
Fatigue	5	
Renal impairment	5	
Hair loss	3	
Nausea	3	
Headache	1	
Unspecified pain	1	
Leg ulcers	1	
Weight loss	1	
Thrombocytopenia	1	
NR	5	8 (2–20)

SAE, serious adverse event; AE, adverse event; NR, no response; AML, acute myeloid leukaemia.

*The five patients who were lost to follow-up were all transferred back to their referring hospital before the end of the observation period at their own request.

†Other SAE's not causing discontinuation were: One patient [Electronic case report form (eCRF) allocation = 4] experienced a speech disorder, tearfulness and visual impairments of ungraded severity. None of the SAE's were considered causal to therapy and all resolved. One patient (eCRF allocation = 161) experienced an ulcer, arterial stenosis, elevation of transaminases, grade III nausea and unspecified pain. Elevation of transaminases (ungraded) was not considered causal and was not resolved at discontinuation. Nausea was considered causal and resolved. The remaining AEs were considered non-causal and resolved. One patient (eCRF allocation = 2) experienced a grade III diarrhoea that was considered causal and resolved. One patient (eCRF allocation = 43) experienced thrombocytopenia of unspecified severity, which was not considered causal and which was not resolved at discontinuation. One patient (eCRF allocation = 115) experienced heart palpitations, which were considered causal and resolved. One patient (eCRF allocation = 41) experienced cystitis, pyrexia and pneumonia. All were considered non-causal and resolved. One patient (eCRF allocation = 162) experienced anorexia of unspecified grade, which was considered non-causal and which resolved. One patient (eCRF allocation = 131) experienced cholecystitis with was considered non-causal and resolved. One patient (eCRF allocation = 62) experienced a high International Normalized Ratio (INR) on warfarin therapy, which was considered non-causal and resolved.

‡The sum of individual AEs exceeds 21 because some patients reported more than one AE.

Six patients were started on HC in the observation period: four patients without any change in response (NR = 3, PR = 1), one with a CR and one discontinued therapy. Two patients were started on human leucocyte interferon (Multiferon) and busulfan, respectively, resulting in PR for both.

All PV patients but one were *JAK2* V617F-positive while only 37% of ET patients were *JAK2* V617F-positive. Responses were found to be independent of *JAK2* V617F-status in ET patients ($P = 0{\cdot}63$). Response rates for patients treated with vorinostat only who discontinued therapy showed that 63% had a clinicohaematological response, providing evidence that toxicity issues were of concern rather than lack of clinical effect.

Haematological parameters of relevance to response assessment are illustrated in Fig 2A–C. Results within the observation period from visit 8–11 may have been affected by reinstitution of cytoreductive therapies, i.e. vorinostat, HC, busulfan, Multiferon.

Efficacy – molecular responses

It was possible to compare quantitative *JAK2* analyses after 12 weeks of therapy with baseline data for 43 patients. Thirteen patients were negative at baseline and data was missing for seven patients. Sixty-five per cent experienced a decrease in *JAK2* V617F tumour allele burden; ($P = 0{\cdot}006$). However, the numerical median decrease in *JAK2* V617F tumour allele burden among all molecular responding patients was only 5·6%. The differences were more apparent for PV patients, with a responding group of 70% and statistically significant responses ($P = 0{\cdot}0003$, Table SII). Interestingly, a subset of patients showed an increase in the mutant allele burden, but nevertheless experienced a partial or complete clinicohaematological response (Fig 3A, B). At 24 weeks of therapy we observed a similar pattern in *JAK2* V617F mutation burden, which was statistically significant ($P = 0{\cdot}05$). Median change was a decrease of 3·4% and 71% of patients exhibited a decrease in mutant allele burden. There was no significant change in mutation burden between 12 and 24 weeks of therapy ($P = 0{\cdot}4$). At the end of the observation period we observed no difference from baseline values ($P = 0{\cdot}7$), but could only compare 17 patients. No *JAK2* V617F positive patients experienced a major molecular response, defined as undetectable *JAK2* V617F by the high-sensitivity qPCR-assay used. No significant correlation between severity of tumour allele burden at inclusion and molecular response to vorinostat was found using a Spearman correlation analysis ($\rho = 0{\cdot}16$, $P = 0{\cdot}3$).

Adverse effects

The AEs causing discontinuation were almost exclusively non-haematological and included fatigue, renal impairment, diarrhoea, hair loss, weight loss, nausea, unspecified pain, headache and leg ulcers. One patient discontinued due to

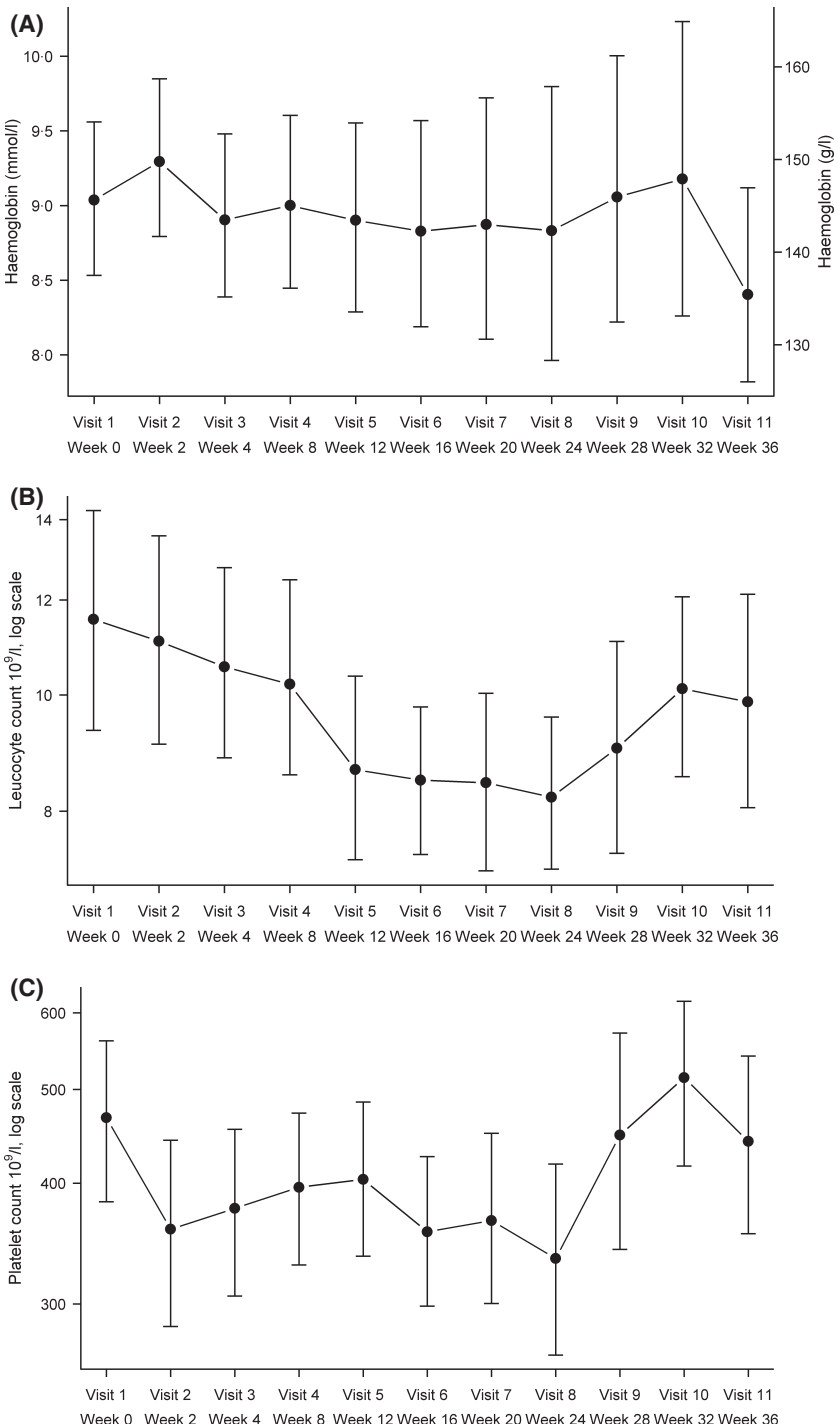

Fig 2. (A) Changes in haemoglobin concentrations for all patients in the study. Bars show 95% confidence intervals for the mean. (B) Changes in leucocyte counts for all patients in the study. (C) Changes in platelet counts for all patients in the study.

thrombocytopenia of unspecified grade. The SAEs causing discontinuation were deep vein thrombosis, diarrhoea, progression to acute myeloid leukaemia, headache, palpitations and neuropathy. An overview of reported AEs and all causes of discontinuation are given in Tables II and III respectively.

Fifty per cent of all patients experienced reversible hair loss. 14 patients (22%) completed the study without a dose reduction (Table SIII).

The most commonly reported AEs during the intervention period among patients who completed the study were fatigue and gastrointestinal (GI) (anorexia, nausea, vomiting, diarrhoea, dryness of the mouth). GI symptoms were generally manageable.

Discussion

The present investigator-initiated study has shown that clinical responses to vorinostat were obtained in a high

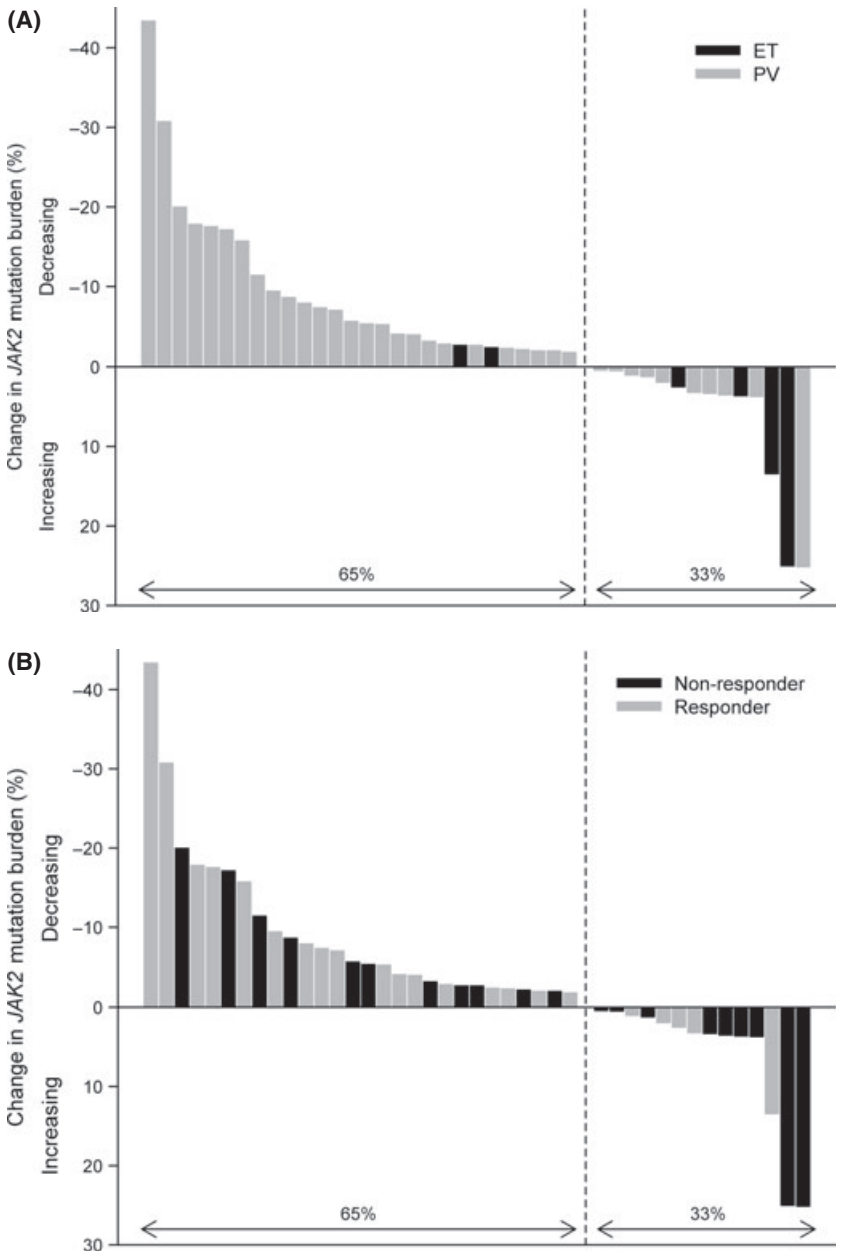

Fig 3. (A) Changes in *JAK2* V617F mutation burden in regard to diagnosis at 12 weeks of therapy. (B) Changes in *JAK2* V617F mutation burden in regard to clinicohaematological response at 12 weeks of therapy.

proportion of patients adhering to therapy and even in patients that discontinued therapy due to adverse events. However, a high early discontinuation rate draws attention to the significant side effects of vorinostat. Most of our patients exhibited moderate impairment of renal function with increases in plasma creatinine within the normal range or levels slightly above the upper normal limit. In all patients renal dysfunction was reversible upon dose reduction or discontinuation. The reasons for impaired renal function during vorinostat treatment are unknown, but have been reported previously (Mann *et al*, 2007). Additional insulin had to be administered to one patient with concomitant diabetes. Cases of hyperglycaemia have also been reported previously, but causes remain unknown (Mann *et al*, 2007). Other important side effects were varying degrees of reversible hair loss, fatigue and diarrhoea. Eleven patients were concomitantly treated with HC with very heterogeneous response patterns. Accordingly, our data did not allow firm conclusions in regard to a potential synergistic effects of vorinostat and HC which recently has been demonstrated between another HDACi – givinostat – and HC (Rambaldi *et al*, 2011).

Importantly, a marked reduction in the prevalence of splenomegaly was recorded. This effect was observed during the first weeks of therapy and very similar to the rapid resolution of large splenomegaly in patients with myelofibrosis

during treatment with JAK-inhibitors (Harrison *et al*, 2012; Verstovsek *et al*, 2012). The resolution of splenomegaly with both JAK-inhibitors and HDACi may be partly attributed to the potent anti-inflammatory effects of both agents. Furthermore, within days a rapid and sustained resolution of pruritus was recorded, whereas vorinostat had no or minor impact upon constitutional symptoms (fatigue, weight loss), probably explained by 'overriding' toxicity symptoms (e.g. loss of appetite, diarrhoea).

Molecular responses were modest (a median decrease of 5·6% after 3 months of therapy), yet statistically significant among *JAK2* V617F -positive patients, although the clinical and biological significance of this modest reduction remains to be established. Interestingly, three patients exhibited more than a 10% decrease in mutant allele burden without an accompanying clinicohaematological response. Speculatively, this discordance might reflect that vorinostat reduces the *JAK2* V617F-positive progenitor compartment without major impact upon the pre-*JAK2* V617F compartment or a competing *JAK2* V617F negative clone.

An apparent point of criticism of the study is the heterogeneous study population, which encompassed both newly diagnosed and previously treated patients. However, the included patients represent the diversity of ET and PV patients in the outpatient clinic. Overall, the patients could be defined as 'all comers in need of therapy'.

In retrospect, it is evident that a phase I/II study with identification of dose-limiting toxicities in PV and ET patients would have been timely. Likewise, it can be argued that the study could have implemented more flexible rules for decreasing dosages, which may or may not have allowed more patients to remain on treatment. However, at the time of study planning, the use of 400 mg. vorinostat daily was considered safe and tolerability, pharmacokinetics and efficacy studies of vorinostat in haematological patients with CTCL were well documented. Hence the authors assumed that a dosing regimen <300 mg. daily would be without adequate effect and furthermore did not anticipate the observed degree of toxicities.

Notably however, a subgroup of patients responded well and with a considerable improvement in overall quality of life documented by an improvement of at least 10 points in the 'Myeloproliferative Neoplasm Assessment Form (MPN-SAF) Total Symptom Score (TSS)', which constitutes a large improvement in symptom burden for these patients (Andersen *et al*, 2012).

Considering the heterogeneity and complexity of oncogenic events, involving both deregulated tyrosine kinase activity as well as epigenetic deregulation in MPN pathogenesis, a combination approach may be more efficacious than single agent therapy. This strategy might also allow for dose reduction of single agents and accordingly a decrease in toxicity, which was the major limiting factor for patients adhering to treatment in the present study.

In conclusion, the present study has demonstrated that vorinostat was effective in patients with ET and PV adhering to therapy by normalizing elevated leucocyte and platelet counts in the majority of patients as well as moderately reducing the *JAK2* V617F mutant allele burden. In addition, pruritus rapidly vanished and splenomegaly resolved in many patients. However, vorinostat therapy at the present dosage was associated with significant toxicity and consequently also a high discontinuation rate. Therefore, future studies should focus upon combination therapies with both conventional drugs (e.g. interferon-alpha2), but also with other targeted drug formulations (e.g. DNA-hypomethylating agents and JAK1–2 inhibitors). This may allow a lower dose of vorinostat to minimize side effects.

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Author contributions

Elisabeth Ejerblad, Sonja Zweegman, Claire Harrison, Savio Fernandes, David Bareford, Steven Knapper, Jan Samuelsson, Eva Löfvenberg, Olle Linder, Björn Andreasson, Erik Ahlstrand, Morten Krogh Jensen, Ole Weis Bjerrum, Hanne Vestergaard, Herdis Larsen and Torben Mourits-Andersen all collected patient-data throughout the study and revised the manuscript for important intellectual content. Tobias Klausen performed statistical analysis. Christen Lykkegaard Andersen collected data, analysed and interpreted data and wrote the manuscript. The study was designed by Mary Frances McMullin

and Hans Carl Hasselbalch. In this regard, we would like to acknowledge the valuable comments from Claire Harrison, Jan Samuelsson, Gunnar Birgegård and other colleagues participating in the study. Mary Frances McMullin and Hans Carl Hasselbalch also collected patient-data and revised the manuscript critically for important intellectual content.

Conflict of interest

The authors have no competing interests.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table SI. Characteristics of the 11 patients concomitantly treated with hydroxyurea in addition to vorinostat.

Table SII. Changes in *JAK2*V617F mutant allele burden at 12 weeks of therapy. Statistical significance was determined using the Wilcoxon signed-rank test.

Table SIII. Dose modifications in the intervention period.

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