

The Histone Deacetylase (HDAC) Inhibitor Valproic Acid as Monotherapy or in Combination with All-Trans Retinoic Acid in Patients with Acute Myeloid Leukemia

Andrea Kuendgen, M.D.¹
 Mathias Schmid, M.D.²
 Richard Schlenk, M.D.²
 Sabine Knipp, M.D.¹
 Barbara Hildebrandt, Ph.D.³
 Christian Steidl, M.D.⁴
 Ulrich Germing, M.D.¹
 Rainer Haas, M.D.¹
 Hartmut Dohner, M.D.²
 Norbert Gattermann, M.D.¹

¹ Department of Hematology, Oncology, and Clinical Immunology, Heinrich-Heine-University, Dusseldorf, Germany.

² Third Department of Internal Medicine, University of Ulm, Ulm, Germany.

³ Institute of Human Genetics, Heinrich-Heine-University, Dusseldorf, Germany.

⁴ Department of Hematology/Oncology, University of Gottingen, Gottingen, Germany

Address for reprints: Andrea Kuendgen, M.D., Department of Hematology, Oncology, and Clinical Immunology, Heinrich-Heine-University, Moorenstr. 5, D-40225 Dusseldorf, Germany; Fax: (011) 49-1-211-8118853; E-mail: kuendgen@med.uni-duesseldorf.de

Received April 7, 2005; revision received June 16, 2005; accepted July 19, 2005.

BACKGROUND. Valproic acid (VPA) inhibits histone deacetylase activity and, synergizing with all-trans retinoic acid (ATRA), achieves differentiation induction of myeloid blast cells in vitro.

METHODS. We used VPA in 58 patients with acute myeloid leukemia (AML) who were too old and/or medically unfit to receive intensive chemotherapy (32 AML secondary to myelodysplastic syndrome [MDS], 22 de novo AML, 4 AML secondary to myeloproliferative syndrome). VPA serum concentrations were 50–100 $\mu\text{g}/\text{mL}$. Thirty-one patients received VPA monotherapy. ATRA was added later in 13 patients who did not respond or who relapsed. Another 27 patients received VPA plus ATRA from the start. Median treatment duration was 93 days for VPA and 88 days for ATRA.

RESULTS. The response rate was only 5% according to International Working Group (IWG) criteria for AML but was 16% when IWG response criteria for MDS were used, which capture hematologic improvement and stabilization of the disease. These endpoints, which are not necessarily correlated with diminishing blast counts, are relevant for the patients' quality of life. Among 23 patients with a peripheral blast count $> 5\%$, 6 (26%) showed a diminishing blast count, and 5 of these had a complete peripheral blast clearance.

CONCLUSIONS. Future trials should combine VPA with chemotherapy or demethylating agents. *Cancer* 2006;106:112–9. © 2005 American Cancer Society.

KEYWORDS: valproic acid, acute myeloid leukemia, histone deacetylase inhibitors.

Valproic acid (VPA), an anticonvulsant used for over 2 decades in the treatment of seizures, has in vitro activity against a variety of malignant cells. VPA modulates tumor growth, affecting different cellular properties including proliferation,^{1,2} differentiation,^{3–6} tumor metastasis,^{7,8} tumor immunogenicity,³ and angiogenesis.^{9,10} Some of VPA's activities were shown to be attributable to inhibition of histone deacetylases (HDAC).^{6,11,12} In contrast to other HDAC inhibitors, valproic acid specifically inhibits HDAC2, which undergoes basal turnover by proteasomal degradation.¹³

Together with histone acetyltransferases (HAT), HDACs are involved in chromatin modification, which plays a crucial role in the regulation of gene transcription. Histone deacetylation restores a positive charge of lysine residues in core histones, resulting in a tight interaction of DNA and histones, and maintaining chromatin in a transcriptionally silent state. Conversely, histone acetylation neutralizes the positive charge, thereby disrupting DNA-histone interactions

and leading to a more "open" DNA conformation. This facilitates access of transcription factors. It is hypothesized that by this mechanism, HDAC inhibitors lead to derepression of silenced tumor suppressor genes.¹⁴⁻¹⁷

Acute myeloid leukemia (AML) is a disease lending itself to HDAC-targeted therapies because several leukemia-associated fusion genes inhibit gene transcription via recruitment of HDACs.¹⁸⁻²⁰ VPA and other HDAC inhibitors have also shown *in vitro* activity against AML blasts regardless of underlying genetic alterations.^{6,21-23}

In a Phase II study of VPA as monotherapy or in combination with ATRA, 23 patients with MDS and sAML/MDS were treated, and an overall response rate of 35% was observed. As a result of treatment, elevated bone marrow blast counts were diminished in 3 of 9 patients.²⁴ These effects in patients with MDS, together with published *in vitro* data supporting the use of valproic acid in AML and demonstrating synergism with ATRA, prompted us to initiate a trial in patients with AML, using VPA as monotherapy or in combination with ATRA. The study included patients with relapsed or refractory AML, as well as elderly patients whose general condition precluded intensive chemotherapy. Because VPA is generally well tolerated and can be administered orally on an outpatient basis, this compound appears to be suitable for this group of patients.

MATERIALS AND METHODS

Study Design

The study was performed to determine the response rate to VPA and to examine the tolerability and the effect of VPA + ATRA. VPA was administered to reach serum concentrations between 50 and 100 $\mu\text{g}/\text{mL}$, which is also the therapeutic range in antiepileptic treatment. Serum VPA levels were measured with a commercially available fluorescence polarization immunoassay (Abbott, Wiesbaden, Germany). There were two different treatment schedules for ATRA. At one of the participating centers (Dusseldorf), 80 mg/m^2 each day was given in 2 divided doses, Days 1-7, every other week. At the other center (Ulm), 15 mg/m^2 was given daily, starting on Day 4. Both drugs were administered orally. Treatment was continued as long as neither significant side effects nor disease progression occurred. Evaluation of treatment response included all patients who had received study medication for at least 2 weeks. This was the minimum time to response that had been observed in MDS patients treated with VPA alone.²⁴ Thirty-one patients received VPA monotherapy. The addition of ATRA was planned for patients who showed no response after 6 weeks or

relapsed after an initial response to VPA. In an attempt to enhance responses, 27 patients started with a combination of VPA + ATRA. During study treatment, other investigational drugs or cytotoxic chemotherapy were not allowed. As an exception, hydroxyurea or oral idarubicin were permitted within the first 28 days of study treatment if it was deemed necessary to control leukocytosis. During the trial, only 4 patients received erythropoetin or G-CSF, which was not considered an exclusion criterion.

Inclusion Criteria

Patients with AML diagnosed according to World Health Organization (WHO) criteria²⁵ were eligible for entering the study if they were refractory to chemotherapy, had a relapse after successful induction chemotherapy, or were unable to receive intensive cytotoxic treatment because of old age or concomitant disease. Eligibility criteria did not include a minimum performance status or maximum serum creatinine. Concomitant diseases were not considered an exclusion criterion as long as they did not represent a known contraindication for valproic acid, like liver disease. Transaminases (AST, ALT) and alkaline phosphatase were required to be $< 2.5 \times \text{ULN}$ (upper limit of normal). All patients gave written informed consent according to institutional guidelines.

Between February 2002 and November 2004, 58 patients were entered into the study. Median age was 71 years. Seventy-two percent of patients were considered medically unfit for intensive chemotherapy. These patients were not eligible for intensive treatment because of old age (> 75 yrs) ($n = 22$), concomitant disease ($n = 28$), or complex karyotype in patients > 60 years of age ($n = 11$). Twenty-eight percent of the patients had previously received one or more cycles of chemotherapy and had relapsed or were refractory. Seventeen percent had received alternative treatments like cytokines, thalidomide, interferon-alpha or low-dose chemotherapy. Among patients who were analyzed cytogenetically, only 1 showed a low-risk karyotype (inv16), 69% had an intermediate-risk and 28% a high-risk karyotype.²⁶ Clinical characteristics of the patients are summarized in Table 1.

Response Criteria

Treatment response was assessed according to revised recommendations of the international working group (IWG) for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia.²⁷ According to those criteria, morphologic complete remission (CR) requires a bone marrow blast count $< 5\%$, an absolute neutrophil count $> 1000/\mu\text{L}$, and

TABLE 1
Clinical and Laboratory Characteristics of Study Patients

Characteristics	Values
Age in yrs	
median (range)	71 (42-86)
Gender, no. (%)	
Male	33 (57)
Female	25 (43)
Diagnosis (%)	
de novo AML	22 (38)
sAML/MDS	32 (55)
sAML/MPS	4 (7)
Time from diagnosis, mos	
Median (range)	1 (< 1-72)
Pretreatment	
Previous chemotherapy cycles, no. (%)	
None	42 (72)
1-2	10 (17)
≥ 3	6 (10)
Other treatments, ^a no. (%)	10 (17)
Previous allogenic PBSCT, no. (%)	3 (5)
Interval between VPA and prior chemotherapy, days	
Median (range)	38 (21-240)
Peripheral blast count, %	
Median (range)	3 (0-90)
Bone marrow blast count, %	
Median (range)	40 (10-100)
Baseline peripheral blood counts	
WBC × 10 ⁹ /L, median (range)	1900 (400-92,000)
Platelets × 10 ⁹ /L, median (range)	53,500 (3000-540,000)
Karyotype, no. (%), Grimwade et al. ²⁶	
Low risk	1 (2)
Intermediate risk	27 (46)
High risk	11 (19)
Not done	19 (33)

AML: acute myeloid leukemia; sAML/MDS: AML secondary to myeloplastic syndrome; sAML/MPS: AML secondary to myeloproliferative syndrome; PBSCT: peripheral blood stem cell transplant.

^a Including cytokines, interferon alpha, thalidomide, low-dose Ara-C, mitoxantrone, gemcitabine.

platelets > 100,000/ μ L. Some patients fulfil criteria for CR but show residual neutropenia and/or thrombocytopenia. This is called complete remission with incomplete recovery of blood counts (CRi). The designation of PR requires ANC > 1000/ μ L, platelets > 100,000/ μ L, and a decrease in percentage of bone marrow blasts by at least 50%, to a level of 5-25%.

We also assessed responses according to IWG criteria for MDS,²⁸ which capture hematologic improvement. Here, complete remission (CR) is defined as hemoglobin > 11g/dL, platelets > 100,000/ μ L, granulocytes \geq 1500/ μ L, marrow blasts < 5%, and absence of dysplasia in bone marrow. Patients with partial remission (PR) fulfil the same criteria for blood counts but have persisting cytologic abnormalities. Hematologic improvement (HI) presenting as a major erythroid response (MaR-E) is defined as an increase in untransfused hemoglobin > 2 g/dL, or a 100% de-

crease in transfusion requirements in patients with a pretreatment hemoglobin < 11 g/dL. A minor response (MiR-E) is an increase in hemoglobin between 1 and 2 g/dL. Concerning platelets, patients with pretreatment counts < 100,000/ μ L are considered to have a major response (MaR-P) if their platelets increase by at least 30,000/ μ L or if they reach transfusion independence. A minor response (MiR-P) is equivalent to a 50% increase in platelet count, not exceeding 30,000/ μ L. A major neutrophil response (MaR-N) is defined as an increase in neutrophils by \geq 500/ μ L, a minor response (MiR-N) as an increase by < 500/ μ L. All improvements must continue for at least 2 months. Patients with stable disease (SD) show neither signs of hematologic improvement nor evidence of progression for at least 2 months.

RESULTS

Response to Treatment

For the entire group of patients, median duration of treatment was 93 days (range, 20-476 days) for VPA, and 88 days for ATRA (range, 18-476 days).

According to revised response criteria for AML, 1 patient (no. 11) with early relapse after intensive chemotherapy reached CR, lasting for 16 months. There was one CRi and one PR. Response rate was 5%. Two patients had PR, but with incomplete recovery of cell counts. Three additional patients had a complete peripheral blast clearance (starting from 54%, 40%, and 11% PB blasts), and 1 patient showed a decrease in peripheral blasts from 32% to 10%.

Among 5 patients presenting with AML-M6 (erythroleukemia), 3 had circulating red blood precursor cells (RBPC), which rapidly declined in 2 patients, starting after only 5 days of treatment, from 70% to 0%, and from 53% to 15%, respectively. One of the 2 patients did not respond with recovery of peripheral cell counts, whereas the other had significant improvement in all 3 lineages but failed to achieve a response according to IWG criteria (MDS) because the response duration was only 48 days.

According to IWG response criteria for MDS, 9 (16%) patients responded. Two patients showed a trilineage response and three showed a bilineage response. Median time to response was 70 days (range, 15-168 days). Median duration of response was 5.5 months (range, 2-20 mos) (Fig. 1). Clinical characteristics of responders are given in Tables 2 and 3. Twenty (34%) patients showed stable disease, with median duration of 4 months (range, 2-16 mos). Twenty-nine (50%) patients had progressive disease. Response rates did not differ significantly between VPA monotherapy, VPA + intermittent ATRA, and VPA + continuous ATRA.

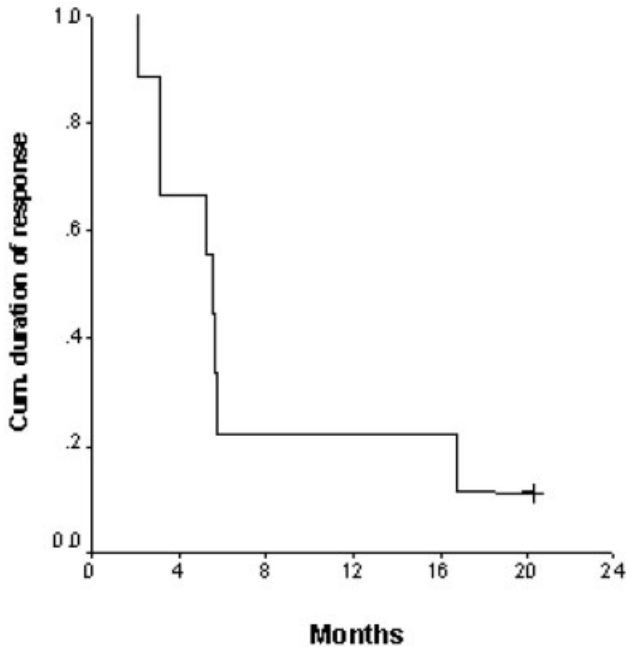


FIGURE 1. Median response duration (IWG criteria for MDS).

Median overall survival was 6.74 months. It was 11.50 months in responders and 5.65 months in non-responders (log-rank test: $P = 0.0691$; Breslow test: 0.0248) (Fig. 2).

There was no significant difference between responders and nonresponders in age, WBC, platelets, and karyotype. Responses were more frequent in males than females ($P = 0.03$), and there was a trend for a smaller percentage of marrow blasts in the responders (36% vs. 51%; $P = 0.09$).

Effect on WBC

Of 10 patients initially presenting with elevated WBC, 5 (50%) had a decrease in WBC. Only two patients discontinued study medication because of rapidly increasing blast cells. Four patients received concomitant cytoreductive medication (hydroxyurea or oral idarubicin). Among these, only one was of the responders. This was a patient who had relapsed after allogeneic bone marrow transplantation and showed an increasing WBC (from $1500/\mu\text{L}$ to $18,000/\mu\text{L}$) during the first 3 weeks of study treatment, whereas the percentage of peripheral blasts decreased from 46% to 12%. With additional hydroxyurea, he achieved a complete peripheral blast clearance lasting for 162 days. When hydroxyurea was discontinued after 4 weeks, the blast count and WBC remained low, but platelets and hemoglobin did not recover.

It is debatable whether addition of cytoreductive treatment is necessary in patients who develop an

increase in WBC during treatment with VPA. Interestingly, 4 weeks after starting VPA, a patient with AML arising from MDS/MPS had an increase in WBC up to $56,500/\mu\text{L}$. Concurrently, the blast count decreased from 27% to 3%. Leukocytosis represented neutrophils at various stages of maturation, which probably developed from differentiating blast cells. Unfortunately, there was no cytogenetic marker available for testing this hypothesis. Subsequently, the WBC rapidly decreased to $1300/\mu\text{L}$ without concomitant cytoreductive treatment, and a complete peripheral as well as bone marrow blast clearance was achieved that lasted for 6 months.

Side Effects

In general, study treatment was well tolerated. Thrombocytopenia was observed in 14 patients and was attributable to disease progression in most cases. In three patients, thrombocytopenia led to cessation of study treatment. Tremor, clearly related to therapy, occurred in seven patients, four of whom had to discontinue study medication. Fatigue is often difficult to evaluate because of disease-related symptoms. In three patients, it was most likely attributable to VPA. Side effects attributable to ATRA included Grade I/II skin toxicity in four patients, all of them receiving the higher dose on the intermittent schedule, Grade I/II gastrointestinal toxicity in three patients, and pleural effusion in one patient. In the latter patient, the pleural effusion disappeared when ATRA was discontinued.

DISCUSSION

This is the first trial to evaluate valproic acid as monotherapy and in combination with ATRA for the treatment of AML. In a previous trial of VPA and VPA + ATRA for patients with MDS and sAML/MDS, we found hematologic improvement in 7 of 23 patients, as well as 1 partial remission (35% overall response rate). Patients with low-risk MDS tended to have better responses. Nevertheless, a decrease in bone marrow blasts was achieved in three of nine patients with elevated blast count.²⁴ This prompted us to investigate the effect of VPA in AML.

Applying the revised IWG response criteria for AML²⁷ to our trial, the response rate was only 5%. However, the response rate was 16% when we used IWG response criteria for MDS, which capture hematologic improvement and stabilization of the course of disease. These endpoints, which are not necessarily correlated with diminishing blast counts, are relevant for the patients' quality of life. In our study, only four patients received cytoreductive treatment. This infrequent requirement of cytotoxic drugs may be attrib-

TABLE 2
Characteristics of Responders (IWG Criteria for MDS) after VPA Alone or in Combination with ATRA

Patient	Age (yrs)	Gender	Diagnosis	FAB subtype	Previous no. CT cycles	Cytogenetics	Schedule	Maximum dose VPA/ mg	Treatment duration (days) VPA/ATRA	Initial PB blast count %	Initial BM blast count %	Minimum PB blast count %	Minimum BM blast count %	Hematologic improvement	Time to response (days)	Duration of response (mos)	ANC before/at response	Hb before/at response	Platelets before/at response
1	74	M	sAML/MDS	M2	0	Hyperploid	V-A (int.)	1250	461/406	4	38	9	38	MIR-E	27	15 ^a	400/600	9.1/10.3	52,000/ 43,000
11	65	F	sAML/MDS	M1	1	Normal	V	1800	463/0	0	13 ^c	1	13 ^c	MaR-N, MaR-P, MaR-E	15	16 ^a	1100/ 3900	9.6/12.9	15,000/ 171,000
12	69	M	denovo AML	M0	1	46,XY,inv8	VA (int.)	2000	320/320	0	10 ^c	24	10 ^c	MaR-E	93	10	1700/ 1300	10.7/13.5	37,000/ 42,000
23	66	M	sAML/MPS	M7	0	46,XY,+1,der(1;7)	V-A (int.)	1800	151/52	54	21 ^b	n.d.	n.d.	MaR-N	17	2	200/2000	12.3/10.8	361,000/ 361,000
30	67	M	sAML/MDS	NA	0	Complex	V-A (int.)	900	404/26	2	30	30	30	MaR-N, MaR-P	96	3	300/900	7.0/7.0	72,000/ 167,000
34	72	M	sAML/MDS	M1	0	Normal	V-A (cont.)	1200	162/57	0	30	n.d.	30	MaR-N, MaR-P	49	3	200/560	9.1/9.6	62,000/ 140,000
38	47	M	denovo AML	M2	0	Normal	VA (cont.)	1200	274/274	5	60	n.d.	60	MIR-N, MaR-P, MIR-E	70	3	300/900	11.0/10.4	88,000/ 232,000
43	78	M	sAML/MDS	M1	0	Normal	VA (cont.)	1500	476/476	1	35	n.d.	35	MIR-N	168	6	50/400	11.4/9.1	83,000/ 81,000
45	72	M	denovo AML	M2	0	n.d.	VA (cont.)	1500	256/256	32	80	n.d.	80	MIR-P, MIR-E	92	5	11900/ 4800	9.0/11.5	15,000/ 38,000

IWG: international working group; MDS: myelodysplastic syndrome; VPA: valproic acid; ATRA: all-trans retinoic acid; sAML/MDS: AML secondary to myeloplastic syndrome; AML: acute myeloid leukemia; sAML/MPS: AML secondary to myeloproliferative syndrome; FAB: French-American-British classification; V-A: ATRA added in later; V: VPA monotherapy; VA: VPA + ATRA (intermittent or continuous); int: ATRA intermittent; cont: ATRA continuous; PB: peripheral blood; BM: bone marrow; MIR-E: minor erythroid response; MaR-N: minor neutrophil response; MaR-P: major platelet response; MIR-N: minor neutrophil response; MIR-P: minor platelet response.

^a Ongoing treatment.

^b Bone marrow biopsy due to advanced bone marrow fibrosis with punctio sicca.

^c Relapse after intensive chemotherapy.

TABLE 3
Characteristics of Responders (IWG Criteria for AML) after VPA Alone or in Combination with ATRA (Including 2 Patients with Reduction in Bone Marrow Blast Count but Incomplete Recovery of Peripheral Cell Counts (PRi))

Patient	Age (yrs)	Gender	Diagnosis	FAB subtype	No. previous CT cycles	Cytogenetics	Schedule	Maximum dose VPA, mg	Treatment duration (days), VPA/ATRA	Initial PB count %	Minimum PB blast count %	Initial BM blast count %	Minimum BM blast count %	Response AML criteria	Hematologic improvement	Duration of response (mos)	ANC before/at response	Hb before/at response	Platelets before/at response
1	74	M	sAML/MDS	M2	0	diploid	V-A (int.)	1250	461/406	4	0	38	9	PRi	MIR-E	15 ^a	400/600	9.1/10.3	52,000/ 43,000
2	62	M	sAML/MDS	M1	0	47,XX,+8	VA (int.)	2500	102/102	20	0	45	3	CRi	SD	6	5000/ 7000	6.7/9.4	55,000/ 10,000
8	61	F	sAML/CMML	M2	1	46,XX,inv1	V	900	63/0	3	0	15 ^b	5	PR	SD	2	4100/ 3800	10.9/10.5	540,000/ 399,000
11	65	F	sAML/MDS	M1	1	Normal	V	1800	463/0	0	0	13 ^b	1	CR	MaR-N, MaR-P, MaR-E	16 ^a	1100/ 3900	9.6/12.9	15,000/ 171,000
15	63	M	de novo AML	M1	3	Normal	V	2000	172/0	46	0	60	15	PRi	SD	5	500/2600	8.1/9.2	10,000/ 17,000

IWG: international working group; AML: acute myeloid leukemia; VPA: valproic acid; ATRA: all-trans retinoic acid; PRi: partial remission with incomplete recovery of blood counts; FAB: French-American-British classification; sAML/MDS: AML secondary to myelodysplastic syndrome; sAML/CMML: AML secondary to chronic myelomonocytic leukemia; V-A: ATRA added in later; int: ATRA intermittent; VA: VPA + ATRA (intermittent or continuous); V: VPA monotherapy; CRi: complete remission with incomplete recovery of blood counts; PR: partial remission; CR: complete remission; MIR-E: minor erythroid response; SD: stable disease; MaR-N: major neutrophil response; MaR-P: major platelet response; MaR-E: major erythroid response; ANC: absolute neutrophil count; Hb: hemoglobin.
^a Ongoing treatment.
^b Relapse after intensive chemotherapy.

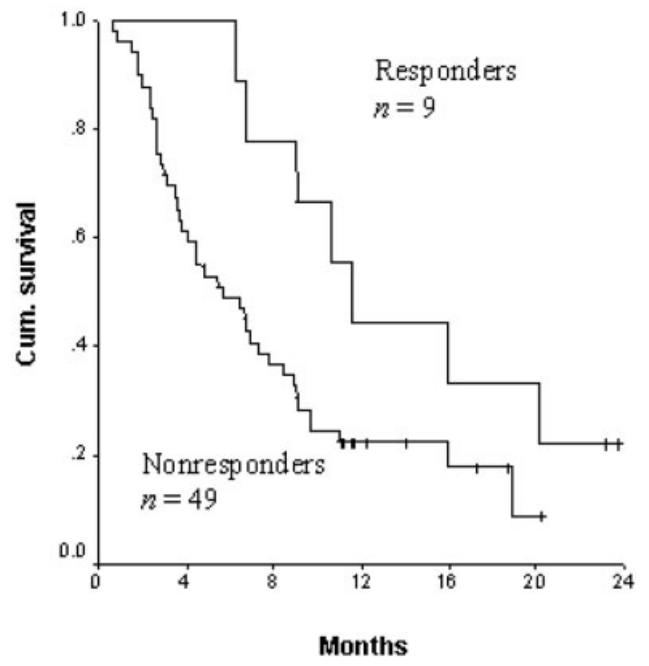


FIGURE 2. Median survival of responders versus nonresponders.

utable to the high proportion of secondary and pan-cytopenic, smoldering AML. Unlike Guel et. al.,²⁹ who treated 8 AML patients with VPA + ATRA and suggested that concomitant cytoreductive treatment was needed, we found that VPA was sufficiently active to achieve an antiproliferative effect in 50% of patients presenting with elevated WBC.

Nervi et al. also treated 8 AML patients with VPA + ATRA. They observed only one hematologic improvement. However, all patients showed morphologic, cytochemical, and immunophenotypic features of differentiation, the degree of which correlated with hyperacetylation of histones H3 and H4. Differentiation of leukemic blasts was confirmed by demonstrating cytogenetic abnormalities in maturing cells.³⁰

Diverse, structurally different HDAC inhibitors have recently entered clinical trials.^{31,32,33,34} In accord with our results, preliminary data demonstrate hematologic improvement or reduction of peripheral blast cells in a subset of AML patients. Apparently, a decrease in marrow blasts occurs less frequently. With depsipeptide,³⁵ responses occurred preferentially in patients with translocations known to recruit histone deacetylases. With butyrate, Santini et. al. reported in vitro selectivity for blasts from core binding factor AML.³⁶ Had our trial been restricted to patients with core binding factor leukemia, we might have achieved higher response rates. However, CBF-leukemias are not frequent in elderly patients with AML, and VPA was shown to be capable, at least in vitro, of inducing

AML blast cell differentiation independent of underlying genetic alterations.⁶

Remarkable responses were seen in some patients with AML-M6. A possible explanation involves transcription factors regulated by histone deacetylases. TAL1 and GATA1 have been shown to interact with HDACs. In accord with these findings, inhibitors of histone deacetylases induce differentiation of MEL cells in vitro.^{37,38}

From our results, it is hard to speculate about the additional benefit of ATRA. We found no significant differences between VPA monotherapy, on the one hand, and the two combination regimens on the other hand. Schlenk et al.³⁹ recently demonstrated an improved response rate in elderly patients treated with a combination of chemotherapy plus ATRA, so the German Leukemia Study Group I (AMLSG) is now performing a clinical trial comparing standard chemotherapy to the same regimen plus VPA and/or ATRA.

In elderly patients with AML, a reason for adding VPA to standard chemotherapy is the frequently increased expression of the multidrug resistance gene and the *p*-glycoprotein gene; both are associated with a particularly poor outcome.⁴⁰ Tang et al.²³ reported that VPA is capable of overcoming a multidrug resistance phenotype in AML cell lines, showing synergy with Ara-C. Another characteristic of secondary leukemias in the elderly is an increased proportion of CD34+ blast cells, which have a tendency to remain in G0/1 phase of the cell cycle and, thus, show resistance against chemotherapy. In vitro data indicate that HDAC inhibitors are equally effective in killing proliferating and nonproliferating tumor cells.⁴¹

According to our clinical data, VPA has beneficial effects but is not sufficiently active to be useful as single-agent therapy for AML. In elderly patients who are medically unfit for standard chemotherapy, VPA may be combined with inhibitors of important pathways involved in AML pathogenesis, like FLT3- or FTI-inhibitors, or with demethylating agents, which, based on theoretical grounds as well as in vitro findings and preliminary clinical data,⁴² should be capable of augmenting the reexpressor strategy.

REFERENCES

1. Martin ML, Regan CM. The anticonvulsant valproate teratogen restricts the glial cell cycle at a defined point in the mid-G1 phase. *Brain Res*. 1991;554:223-228.
2. Bacon CL, Gallagher HC, Haughey JC, Regan CM. Antiproliferative action of valproate is associated with aberrant expression and nuclear translocation of cyclin D3 during the C6 glioma G1 phase. *J Neurochem*. 2002;83:12-19.
3. Cinatl J Jr, Cinatl J, Driever PH, Kotchetkov R, Pouckova P, Kornhuber B, Schwabe D. Antitumor activity of sodium valproate in cultures of human neuroblastoma cells. *Anticancer Drugs*. 1996;7:766-773.
4. Werling U, Siehler S, Litfin M, Nau H, Gottlicher M. Induction of differentiation in F9 cells and activation of peroxisome proliferator-activated receptor delta by valproic acid and its teratogenic derivatives. *Mol Pharmacol*. 2001;59:1269-1276.
5. Lampen A, Carlberg C, Nau H. Peroxisome proliferator-activated receptor delta is a specific sensor for teratogenic valproic acid derivatives. *Eur J Pharmacol*. 2001;431:25-33.
6. Gottlicher M, Minucci S, Zhu P, Kramer OH, Schimpf A, Giavara S, et al. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *EMBO J*. 2001;20:6969-6978.
7. Walmod PS, Skladchikova G, Kawa A, Berezin V, Bock E. Antiepileptic teratogen valproic acid (VPA) modulates organization and dynamics of the actin cytoskeleton. *Cell Motil Cytoskeleton*. 1999;42:241-255.
8. Walmod PS, Foley A, Berezin A, Ellerbeck U, Nau H, Bock E, Berezin V. Cell motility is inhibited by the antiepileptic compound, valproic acid and its teratogenic analogues. *Cell Motil Cytoskeleton*. 1998;40:220-237.
9. Michaelis M, Michaelis UR, Fleming I, Suhan T, Cinatl J, Blaheta RA, et al. Valproic acid inhibits angiogenesis in vitro and in vivo. *Mol Pharmacol*. 2004;65:520-527.
10. Zgouras D, Becker U, Loitsch S, Stein J. Modulation of angiogenesis-related protein synthesis by valproic acid. *Biochem Biophys Res Commun*. 2004;316:693-697.
11. Phiel CJ, Zhang F, Huang EY, et al. Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. *J Biol Chem*. 2001;276:36734-36741.
12. Phiel CJ, Zhang F, Huang EY, Guenther MG, Lazar MA, Klein PS. Histone deacetylase is a target of valproic acid-mediated cellular differentiation. *Cancer Res*. 2004;64:1079-1086.
13. Kramer OH, Zhu P, Ostendorff HP, Golebiewski M, Tiefenbach J, Peters MA, et al. The histone deacetylase inhibitor valproic acid selectively induces proteasomal degradation of HDAC2. *EMBO J*. 2003;22:3411-3420.
14. Marks PA, Richon VM, Rifkin RA. Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. *J Natl Cancer Inst*. 2000;92:1210-1216.
15. Vigushin DM, Coombes RC. Histone deacetylase inhibitors in cancer treatment. *Anticancer Drugs*. 2002;13:1-13.
16. Melnick A, Licht JD. Histone deacetylases as therapeutic targets in hematologic malignancies. *Curr Opin Hematol*. 2002;9:322-332.
17. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med*. 2003;349:2042-2054.
18. Wang J, Sauntharajah Y, Redner RL, Liu JM. Inhibitors of histone deacetylase relieve ETO-mediated repression and induce differentiation of AML1-ETO leukemia cells. *Cancer Res*. 1999;59:2766-2769.
19. Redner RL, Wang J, Liu JM. Chromatin remodeling and leukemia: new therapeutic paradigms. *Blood*. 1999;94:417-428.
20. Gelmetti V, Zhang J, Fanelli M, Minucci S, Pelicci PG, Lazar MA. Aberrant recruitment of the nuclear receptor corepressor-histone deacetylase complex by the acute myeloid leukemia fusion partner ETO. *Mol Cell Biol*. 1998;18:7185-7191.
21. Ferrara FF, Fazi F, Bianchini A, Padula F, Gelmetti V, Minucci S, et al. Histone deacetylase-targeted treatment restores retinoic acid signaling and differentiation in acute myeloid leukemia. *Cancer Res*. 2001;61:2-7.

22. Kawagoe R, Kawagoe H, Sano K. Valproic acid induces apoptosis in human leukemia cells by stimulating both caspase-dependent and-independent apoptotic signaling pathways. *Leuk Res.* 2002;26:495–502.
23. Tang R, Faussat AM, Majdak P, Perrot JY, Chaoui D, Legrand O, Marie JP. Valproic acid inhibits proliferation and induces apoptosis in acute myeloid leukemia cells expressing P-gp and MRP1. *Leukemia.* 2004;18:1246–1251.
24. Kuendgen A, Strupp C, Aivado M, Bernhardt A, Hildebrandt B, Haas R, et al. Treatment of myelodysplastic syndromes with valproic acid alone or in combination with all-trans retinoic acid. *Blood.* 2004;104:1266–1269.
25. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, et al. The World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues. Report of the Clinical Advisory Committee meeting, Airlie House, Virginia, November, 1997. *Ann Oncol.* 1999;10:1419–1432.
26. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. *Blood.* 1998;92:2322–2333.
27. Cheson BD, Bennett JM, Bloomfield CD. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. *J Clin Oncol.* 2003;21:4642–4649. Erratum in: *J Clin Oncol.* 2004;22:576.
28. Cheson BD, Bennett JM, Kantarjian H, Pinto A, Schiffer CA, Nimer SD, et al. Report of an international working group to standardize response criteria for myelodysplastic syndromes. *Blood.* 2000;96:3671–3674.
29. Guel H, Wassermann B, Romanski A, et al. Effect of the histone deacetylase inhibitor valproic acid in combination with all-trans retinoic acid on normal and malignant hematopoiesis [abstract]. *Blood.* 2003;102:2313.
30. Nervi C, Lo Coco F, Pelicci PG. Valproic acid plus retinoic acid induce differentiation in chemotherapy resistant acute myeloid leucemia patients [abstract]. *Blood.* 2004;104:
31. Gore SD, Weng LJ, Zhai S, Figg WD, Donehower RC, Dover GJ, et al. Impact of the putative differentiating agent sodium phenylbutyrate on myelodysplastic syndromes and acute myeloid leukemia. *Clin Cancer Res.* 2001;7:2330–2339.
32. Byrd JC, Marcucci G, Parthun MR, Xiao JJ, Klisovic RB, Moran M, et al. A phase I and pharmacodynamic study of depsipeptide (FK228) in chronic lymphocytic leukemia and acute myeloid leukemia. *Blood.* 2005;105:959–967.
33. Gojo I, Gore SD, Jiemjiet A. Phase I study of histone deacetylase inhibitor MS-275 in adults with refractory or relapsed myeloid malignancies [abstract]. *Blood.* 2003;102:1408.
34. Garcia-Manero G, Canalli AA, Wierda W, et al. Phase I study of oral suberoylanilide hydroxamic acid (SAHA) in patients with advanced leukemias or myelodysplastic syndromes [abstract]. *Blood.* 2003;102:4742.
35. Olatoyosi MO, Alkan S, Stock W. The histone deacetylase inhibitor depsipeptide has differential activity in specific cytogenetic subsets of acute myeloid leukaemia (AML) [abstract]. *Blood.* 2004;102:
36. Gozzini A, Rovida E, Dello Sbarba P, Galimberti S, Santini V. Butyrates, as a single drug, induce histone acetylation and granulocytic maturation: possible selectivity on core binding factor-acute myeloid leukemia blasts. *Cancer Res.* 2003;63:8955–8961.
37. Huang S, Brandt SJ. mSin3A regulates murine erythroleukemia cell differentiation through association with the TAL1 (or SCL) transcription factor. *Mol Cell Biol.* 2000;20:2248–2259.
38. Watamoto K, Towatari M, Ozawa Y, Miyata Y, Okamoto M, Abe A, et al. Altered interaction of HDAC5 with GATA-1 during MEL cell differentiation. *Oncogene.* 2003;22:9176–9184.
39. Schlenk RF, Frohling S, Hartmann F, Fischer JT, Glasmacher A, del Valle F, et al. AML Study Group Ulm. Phase III study of all-trans retinoic acid in previously untreated patients 61 years or older with acute myeloid leukemia. *Leukemia.* 2004;18:1798–1803.
40. Pallis M, Turzanski J, Higashi Y, Russell N. P-glycoprotein in acute myeloid leukaemia: therapeutic implications of its association with both a multidrug-resistant and an apoptosis-resistant phenotype. *Leuk Lymphoma.* 2002;43:1221–1228.
41. Burgess A, Ruefli A, Beamish H, Warrenner R, Saunders N, Johnstone R, Gabrielli B. Histone deacetylase inhibitors specifically kill nonproliferating tumour cells. *Oncogene.* 2004;23:6693–6701.
42. Garcia-Manero G, Kantarjian H, Issa JP. Results of a phase I/II study of the combination of 5-aza-2'-deoxycytidine (DAC) and valproic acid (VPA) in patients with leukaemia [abstract]. *Blood.* 2004;104:263.