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All-*trans* retinoic acid (ATRA) in patients with chronic myeloid leukemia in the chronic phase

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Since *in vitro* observations indicated that all-*trans* retinoic acid (ATRA), especially in combination with IFNα, can exert significant suppressive effects on Ph+ cells, we investigated the effects and the pharmacokinetic profile of ATRA in a selected cohort of patients with Ph+ chronic myeloid leukemia (CML) in chronic phase. Eighteen patients were treated with ATRA at a dose of 80 mg/m²/day (p.o.), divided into two equal doses after meals, for 7 consecutive days every other week for a maximum of 12 courses (1 course = 1 week on and 1 week off). Pharmacokinetic profiles of ATRA were evaluated during intermittent therapy on days 1 and 7 of course 1; on day 1 of course 2; on day 1 of course 6. Out of the 18 patients treated with ATRA, 11 (61%) went off study before the sixth course of treatment because of progressive hyperleukocytosis (seven cases), or thrombocytosis (one case), or refusal (three cases). Seven (39%) patients completed the first six courses (12 weeks) of treatment with ATRA and two of them (11%) maintained a white blood cell (WBC) $<10\times10^9$/l which was induced by the pretreatment with hydroxyurea. One patient completed the 12th course of ATRA maintaining WBC $<10\times10^9$/l, platelets $<500\times10^9$/l and spleen not palpable. The treatment with ATRA was well tolerated and only one patient discontinued the therapy because of non-hematological side-effects. The area under the concentration-time curve (AUC) decreased significantly ($P<0.001$) during the first week of therapy. By adopting an intermittent dosing regimen, 1 week on/ 1 week off (1 course), at the start of courses 2 and 6, we obtained the ATRA AUCs equivalent to the ones achieved on day 1 of course 1. In conclusion, our results showed that ATRA alone appeared to be unable to control the WBC expansion in the CML patients in chronic phase. Moreover, it did not induce any remarkable cytoreductive effects on the platelet count and on the hemoglobin level. The major interest of ATRA would be in combination with other therapies. If ATRA was given in combination with IFNα or other agents, dose reduction of these would not be planned. On the basis of the pharmacokinetic profile, ATRA should be administered intermittently rather than continuously.
Keywords: ATRA; CML; pharmacokinetics; therapy

Introduction

Due to the remarkable activity of all-*trans* retinoic acid (ATRA) in patients with acute promyelocytic leukemia (APL),[1–6] increasing attention has recently been focused on the use of ATRA as a potential antileukemic agent in other hematological diseases. For this reason, ATRA alone or in combination with other agents has been tested in myelodysplastic syndromes,[7–9] in cutaneous T cell lymphomas[10,11] and in multiple myeloma.[12,13] The clinical use of ATRA in chronic myeloid leukemia (CML) was first reported by Wiernik *et al*,[14] who described a partial remission in a patient with CML in the blastic phase (BP) after treatment with ATRA. Although this patient lacked the t(15;17) translocation and the PML-RARα rearrangement, he was selected for treatment with ATRA because many of his blast cells resembled the leukemic promyelocytes observed in APL; moreover the CML colony-forming cells (CML-CFCs) were strongly inhibited by ATRA in the semisolid culture.[15] This *in vitro* observation appears to be in accordance with other preclinical data showing that the ATRA sensitivity of progenitors from CML patients in the advanced phase[16,17] was stronger than that of progenitors from patients in the chronic one.[18–20] This finding is as yet unexplained and is apparently in contrast, not only with the knowledge that retinoids are more active in the early stage of tumorigenesis,[21,22] but also with the more recent *in vitro* data indicating that ATRA, especially in combination with IFNα, can exert significant suppressive effects on Ph+ cells from patients in the chronic phase by either inducing growth inhibition[17,23] or apoptosis.[24] These preclinical observations suggested that ATRA could be clinically exploitable *in vivo*. A transient antileukemic ATRA effect was recently described in CML patients in the accelerated blastic phase[25] but, at the present time, the effects of ATRA in CML patients in the chronic phase are unknown. We therefore investigated the effects of an intermittent treatment with ATRA in a selected cohort of patients with Ph+ CML in the chronic phase. Since pharmacokinetic ATRA data in CML is not available and an intermittent schedule has never been tested for this disease, we also evaluated the pharmacokinetic profile of ATRA with the aim of achieving useful information to design the dosing regimen of future therapeutic trials with ATRA alone or in combination with other drugs.

Materials and methods

Patients

From January 1995 to June 1996, 18 patients with Ph+ CML in chronic phase were included in the study. The patients were recruited by seven hematological centers and were considered eligible if: (1) they had Ph+ CML in the first chronic phase; (2) they were older than 18 and younger than 70 years of age; (3) their ECOG performance status was 0–2; (4) they were either untreated or treated with hydroxyurea (HU) or busulfan (BUS); (5) they showed no indication for IFNα therapy or had discontinued IFNα because of toxicity, refusal or no hematological response; (6) there was no indication for bone marrow transplantation (BMT); (7) there was no associated disorder that could markedly influence the evolution of treatment or its toxicity (ie liver, renal, cardiac or psychiatric disease, diabetes, concomitant neoplasia, alcoholism); and (8) informed consent was given. As ATRA is teratogenetic a pregnancy test

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was mandatory for fertile women. Pregnancy, lactation or refusal to use an accepted method of birth control were formal exclusion criteria. The experimental characteristics of the trial as well as the need for more frequent clinical and laboratory evaluations than are usually required, including pharmacokinetic studies, were pointed out.

Of the 18 patients, nine were male and nine female, with a median age of 58 years (range 33–70). The median time from diagnosis was 21 months (range 0–129). Three patients were untreated, while the other 15 patients had been treated with IFNα (one case) or HU alone (four cases), or IFNα and other cytotoxic agents (10 cases) including HU, BUS or cytarabine (AC). Before therapy with ATRA, 14/18 patients had received a pretreatment with HU at a dose ranging from 1 to 2 g/day for a median of 22 days (range 10–63) in order to achieve a WBC less than 10×10^9/l. The patients' characteristics are reported in Table 1.

Treatment plan

Pretreatment with HU: Patients with WBC $> 10 \times 10^9$/l first received treatment with HU up to 2 g/day p.o.; as soon as WBC $< 10 \times 10^9$/l, HU was withdrawn and treatment with ATRA was immediately started.

Treatment with ATRA: All-*trans* retinoic acid (Ro 01-5488, ATRA, Vesanoid), a synthetic stereo-isomer of isotretinoin, was authorized for experimental clinical use in Ph+ CML. It was supplied free of charges from Roche SpA (Milan, Italy) in bottles containing 100 capsules (1 cp = 10 mg gelatin) for oral administration.

ATRA was given at a dose of 80 mg/m^2/day (p.o.), divided into two equal doses after meals, for 7 consecutive days every other week for a total of 12 courses (1 course = 1 week on and 1 week off). After each course, continuation of treatment was based on WBC and/or platelet count (PLT) which were checked weekly. If WBC was $<50 \times 10^9$/l and/or PLT was $<1500 \times 10^9$/l, treatment with ATRA was continued; if WBC was $>50 \times 10^9$/l and/or PLT was $>1500 \times 10^9$/l, ATRA was stopped and the patients went off the study. At the end of the sixth course (12th week) of therapy the patients with WBC $< 10 \times 10^9$/l, PLT $< 500 \times 10^9$/l, Hb > 11 g/dl and spleen not palpable were scheduled to continue ATRA at the same dose and schedule for a further six courses. The patients who did not meet these criteria were removed from the study. At the end of the 12th course (24th week) the treatment with ATRA was discontinued and cytogenetics was checked only if WBC $< 10 \times 10^9$/l, PLT $< 500 \times 10^9$/l, Hb > 11 g/dl and spleen not palpable were maintained.

ATRA dose adjustment: The ATRA dose was adjusted according to hematological and/or extra-hematological toxicity as follows: in the case of Hb 85–75 g/l, and/or WBC 4.0–2.0×10^9/l, and/or PLT 99–50×10^9/l, and/or extra-hematological toxicity grade II (WHO), ATRA was continued at 50% of the dose without stopping until recovery; in the case of Hb $<$ 75 g/l, WBC $< 2.0 \times 10^9$/l, and/or PLT $< 50 \times 10^9$/l, and/or extra-hematological toxicity grade III (WHO), ATRA was immediately stopped until complete recovery and then it was started again at 50% of the dose (ie 40 mg/m^2/day); in the case of extra-hematological toxicity grade IV (WHO), ATRA was permanently discontinued.

Table 1 Characteristics of the 18 Ph+ CML patients in first chronic phase before therapy with ATRA

Case No.	*Sex/Age*	*Time from diagnosis (months)*	*Previous therapy*	*Pretreatment with HU (days)*
1	M/33	4	HU	28
2	F/68	12	HU	10
3	F/54	76	IFNα, HU	63
4	M/70	22	IFNα, AC, HU	27
5	F/42	64	IFNα, HU	21
6	M/39	1	HU	23
7	F/68	20	IFNα	14
8	M/60	6	HU	60
9	M/68	69	IFNα, HU	20
10	M/56	0	—	—
11	F/51	26	IFNα, AC, HU	—
12	F/48	49	HU, IFNα	14
13	F/62	16	HU, IFNα	63
14	F/50	106	HU, IFNα	13
15	F/67	1	—	54
16	M/58	129	HU, IFNα, BUS	—
17	M/58	1	—	59
18	M/69	24	HU, IFNα	—
Median	58	21		22
Range	33–70	0–129		10–63

IFNα, interferonα; HU, hydroxyurea; BUS, busulfan; AC, cytosine arabinoside.

Pharmacokinetics of ATRA

Pharmacokinetic profiles of ATRA were evaluated during intermittent therapy according to the following schedule:

Profile 1: on day 1, of course 1 (1st week of therapy).
Profile 2: on day 7, of course 1 (1st week of therapy).
Profile 3: on day 1, of course 2 (3rd week of therapy).
Profile 4: on day 1, of course 6 (11th week of therapy).

Each profile consisted of seven time-points, starting immediately before the ATRA morning dose and continuing for 1, 2, 3, 4, 6 and 8 h after drug administration. Plasma samples obtained by centrifugation at 3200 *g* for 10 min, were protected from light to prevent ATRA photoisomerization and stored at –20°C until assayed. Plasma ATRA concentrations were determined by a modification of a previously described, high performance liquid chromatography (HPLC) method.[26]

Briefly, plasma (1 ml) was treated with ethanol (2 ml) and ethylacetate (2 ml). After brief centrifugation, the supernatant was kept at 4°C. The pellet was broken and washed with ethylacetate (2 ml), and the washing was added to the supernatant. The solvent was removed under a stream of nitrogen at 35°C. The residue was dissolved in 120 μl of methanol and 50 μl injected into the HPLC system. The detection was done at 345 nm, with a spherical C 18 column (25 cm $\times$ 4.6 nm, 5 μ) and a mobile phase of ammonium acetate buffer acetonitrile (34:66) at a flow rate of 1.8 ml/min. The limit of quantitation of our method was 5 ng/ml. The inter–intra day precision of tretinoin standards was less than 10%.

In each case, plasma concentration-time curves were fitted by means of a proper analysis program (Siphar-Kinetics;

Simed, Paris, France). The time to reach peak plasma concentration (t_{max}), the maximum observed plasma concentration (C max), the elimination half-life ($t_{\frac{1}{2}}$) and the area under the plasma drug concentration-time curve (AUC) were the pharmacokinetic parameters analyzed. The AUCs calculated by the trapezoidal rule from t_0 to t_∞ correspond to the AUCτ during the dosing interval, since 10–12 h after administration, plasma ATRA concentrations were below the sensitivity limit of our method.

The pharmacokinetic parameters obtained in each patient were analyzed by means of the analysis of variance (ANOVA) and the Turkey test. A P value <0.05 was considered significant.

Results

Hematological effects of ATRA

Of the 18 patients treated with ATRA, 11 (61%) went off the study before the sixth course of treatment because WBC progressively increased to more than 50×10^9/l (seven cases), platelet count augmented to more than 1500×10^9/l (one case), and three patients refused to continue the therapy. Five (39%) patients completed the first six courses (12 weeks) of treatment with ATRA but they were withdrawn from the study because the WBCs increased to more than 10×10^9/l; the remaining two patients who had maintained WBC less than 10×10^9/l and had PLT $< 500 \times 10^9$/l and spleen not palpable were selected to continue the ATRA treatment at the same dose and schedule for the further six courses. However, one of the two refused to continue ATRA (case No. 7), the other one (case No. 1) continued the treatment with ATRA and completed the 12th course maintaining WBC $< 10 \times 10^9$/l, PLT $< 500 \times 10^9$/l and spleen not palpable (Table 2). At the end of the 12th course, cytogenetics was checked in this patient but the karyotypic response was absent (Ph negative metaphases = 0%). An increasing proportion of granulocytic precursors (PMC + MC%) and peripheral blasts (MB%) in the differential was observed in all 16 (89%) patients who were not able to maintain a WBC $< 10 \times 10^9$/l (Table 2). However, none of them progressed towards the accelerated blastic phase. The platelet counts doubled or increased by more than 25% in comparison with the baseline values, in six of the 16 (37.5%) patients. No significant variations of hemoglobin level were observed during the treatment in any of the patients who were treated with ATRA (Table 2).

ATRA toxicity

The treatment with ATRA was well tolerated and only one patient discontinued the therapy because of non-hematological side-effects. Headache, xerostomia and dry skin frequently occurred, but their grade (WHO) was mild (Table 3). In only two cases (Nos 5 and 9) was the ATRA dose reduced by 50% because of headaches (WHO grade II) (Table 2). Four (22%) patients refused to continue the treatment with ATRA. Of them three patients decided to stop therapy because of the low efficiency of ATRA in controlling the WBC. The last patient (case No. 7) decided to discontinue ATRA at the sixth course of therapy because of the persistence of several side-effects consisting of headache (WHO grade II), xerostomia (WHO grade II) and pain (WHO grade II).

ATRA pharmacokinetic results

Body systemic exposure to ATRA, as determined by the area under the plasma concentration-time curve (AUC), decreased significantly during the first course of drug administration from 678.3 ± 498.1 ng.h/ml on day 1 to 258.7 ± 272.4 ng.h/ml on day 7, with a mean decrease of 49.1%. After one week without ATRA administration the mean plasma AUC on day 1 of course 2 of therapy was not statistically different from the corresponding value observed on day 1 of course 1 (560.6 ± 390.2 *vs* 678.3 ± 498.1 ng·h/ml). Mean (±s.d.) C_{max} observed were 231.3 (±143.0) ng/ml on day 1 of course 1, 96.0 (± 90.1) ng/ml on day 7 of course 1 and 232.5 (± 160.4) ng/ml on day 1 of course 2, with a statistically significant difference between day 1 and day 7 of course 1. No statistically significant differences were found in other pharmacokinetic parameters evaluated during intermittent therapy. The ATRA pharmacokinetic parameters are shown in Table 4. An addition ATRA pharmacokinetic profile was evaluated on day 1 of course 6, in seven patients who completed the first six courses of therapy. The AUC values obtained were compared with the ones observed during course 1 on days 1 and 7 and during course 2 on day 1. A statistically significant difference was noticed by comparing the AUC value calculated on day 7 of course 1 with the AUC values obtained on days 1 of courses 1 (week 1), 2 (week 3) and 6 (week 11) of intermittent ATRA administration. The data are reported in Table 5. We did not observe any statistically significant difference between the ATRA AUCs of the patients who had completed the first six courses of therapy and the ATRA AUCs of the patients who went off the study before the sixth course (422.2 ± 177.5 ng·h/ml *vs* 442.2 ± 247.2 ng·h/ml, $P > 0.05$).

Discussion

At present, the effects of ATRA in CML patients in the chronic phase are substantially unknown. In fact, information on the hematological and non-hematological effects of ATRA is insufficient and data on the dosing regimen is completely lacking. To answer these questions we treated 18 CML patients in the first chronic phase with ATRA at a dose of 80 mg/m^2/day for 7 consecutive days every other week. Before starting ATRA, the majority of patients (14/18) underwent HU pretreatment with the aim of achieving WBC $< 10 \times 10^9$/l and reducing the potential risk of the ATRA syndrome. The choice of using a dose of ATRA higher than the one usually given in APL was arbitrary and only in part due to the *in vitro* observations which suggested that ATRA effects on CML progenitors were dose-dependent. Furthermore, it has to be considered that when we started the study no comparison could be made between our dosing regimen and others. The intermittent schedule of ATRA administration was chosen in an attempt to prevent the induction of pharmacokinetic resistance to ATRA, as indicated by previous observations in solid tumors.[27] As far as the hematological effects are concerned, we observed that ATRA, used as a single agent, was not able to control WBC in the majority of patients. Furthermore, it did not induce any substantial cytoreductive effects either on the platelet count or on the hemoglobin level. An increasing proportion of granulocytic precursors in the differential was observed in all of the patients who did not maintain WBC $< 10 \times 10^9$/l. It was impossible to establish whether that increase was spontaneous or was triggered by ATRA. The poor cytoreductive effects of ATRA observed in our patients could be influenced by the fact

Table 2 Response to intermittent therapy with ATRA in the 18 Ph+ CML patients in first chronic phase

Case No.	Pre-treatment hematological values					ATRA therapy No. courses	Post-treatment hematological values					Causes of Early ATRA discontinuation
	WBC $\times 10^9$/l	PMC + MC %	MB %	PLT $\times 10^9$/l	Hgb g/dl		WBC $\times 10^9$/l	PMC + MC %	MB %	PLT $\times 10^9$/l	Hgb g/dl	
1	8.9	0	0	125	12.2	12	9	0	0	116	11.9	—
2	6	0	0	272	11.6	6	37.5	19	0	183	10.5	—
3	8.4	1	0	374	11.6	6	32.3	10	0	387	13.1	—
4	10	0	0	339	13.5	6	20.8	6	0	373	13.1	—
5	2.7	1	0	139	12.1	6[a]	15.5	4	4	219	11.6	—
6	3	0	0	150	15.3	6	11.3	2	0	223	15.1	—
7	7.3	2	0	270	12.0	6	9.4	0	0	330	12.9	Refusal
8	7.3	0	0	82	12.8	5	24.3	4	3	47	10.9	Refusal
9	8.5	3	0	394	12.4	3[a]	84	21	2	900	13.0	WBC > 50×10^9/l
10	6.8	2	0	182	13.2	2	72.9	16	0	432	14.1	WBC > 50×10^9/l
11	8.6	2	0	882	11.9	2	73.9	12	0	626	11.8	WBC > 50×10^9/l
12	7.1	0	0	456	10.2	2	91	10	1	609	10.8	WBC > 50×10^9/l
13	5.5	0	0	53	10.3	2	72.9	16	1	123	10.7	WBC > 50×10^9/l
14	4.6	0	0	422	11.1	2	47	23	0	662	12.9	Refusal
15	6.6	0	0	259	13.4	2	22.1	11	0	413	14.3	Refusal
16	8.1	0	0	225	16.4	1	72.3	19	0	235	16.2	WBC > 50×10^9/l
17	6.3	0	0	1360	11.7	1	15.7	0	0	1550	12.4	PLT > 1500×10^9/l
18	8.2	1	0	973	11.6	1	51	19	0	1380	12.0	WBC > 50×10^9/l
Median	7.2	0	0	271	12.0		34.9	10.5	0	380	12.6	
Range	2.7–10	0–3		53–1360	10.2–16.4		9–91	0–23	0–4	47–1550	10.5–16.2	

[a]50% reduction of ATRA dose.

Table 3 Non-hematological toxicity during intermittent treatment with ATRA

Side-effects	Grade – WHO	Frequency (%)
Headache	I–II	10/18 (55)
Xerostomia	I–II	9/18 (50)
Dry skin	I–II	4/18 (22)
Xerophtalmia	I	3/18 (16)
Erythema	I	3/18 (16)
Pain	I–II	2/18 (11)
Constipation	I	2/18 (11)
Pruritus	I	1/18 (5)
Nausea	I	1/18 (5)
Hepatic toxicity	I	1/18 (5)

that most patients had a lengthy chronic phase (median time from diagnosis: 21 months). However, it cannot be excluded that the cytotoxic activity was poor because the ATRA plasma levels were lower than the ones required to control *in vivo* the expansion of the Ph+ clone. Our pharmacokinetic results did not show any apparent relationships between ATRA plasma levels and the time of WBC progression. In fact, we did not observe any differences between ATRA AUCs in the patients who had completed the first six courses of therapy and ATRA AUCs of the patients who went off the study before the sixth course (422.2 ± 177.5 ng·h/ml *vs* 442.2 ± 247.2 ng·h/ml, $P > 0.05$). The results of our pharmacokinetic study confirmed the reversibility of the ATRA accelerated clearance and the possibility of modulating ATRA pharmacok-

Table 4 Pharmacokinetic parameters during intermittent ATRA therapy

	AUC_∞ (ng·h/ml)	C_{max} (ng/ml)	t_{max} (h)	$t_{½}$ (h)
Day 1, Course 1	678.3 ± 498.1	231.3 ± 143.0	2.7 ± 0.8	0.9 ± 0.3
Day 7, Course 1	258.7 ± 272.4[a]	96.0 ± 90.1[b]	2.7 ± 1.6	1.0 ± 0.6
Day 1, Course 2	560.6 ± 390.2	232.5 ± 160.4	2.6 ± 0.7	0.8 ± 0.2

[a]Statistically significant difference compared to day 1 course 1 ($P < 0.001$) and to day 1 course 2 ($P < 0.05$).
[b]Statistically significant difference compared to day 1 course 1 ($P < 0.001$) and to day 1 course 2 ($P < 0.01$).

Table 5 Mean ATRA AUCs in seven patients completing the first six courses of intermittent ATRA therapy

	Day 1 Course 1	Day 7 Course 1	Day 1 Course 2	Day 1 Course 6
AUC_∞ (ng·h/ml)	634.7 ± 255.0	87.4 ± 63.3[a]	494.2 ± 347.7	435.6 ± 207.6

[a]Statistically significant difference compared to day 1 course 1 ($P < 0.01$), to day 1 course 2 and to day 1 course 6 ($P < 0.05$).

inetics by altering the schedule of drug administration.[28] In our patients, the area under the concentration-time curve (AUC) decreased significantly ($P < 0.001$) during the first week of therapy (mean percentage of reduction in the AUC value was 49%). By adopting an intermittent dosing regimen, 1 week on/1 week off (1 course), at the start of courses 2 and 6, we obtained the ATRA AUCs equivalent to the ones achieved on day 1 of course 1. A marked inter-individual variability in the main ATRA pharmacokinetic parameters was observed in all of the patients studied and the extent of plasma increase in AUCs after 1 week without the drug administration was unpredictable. It is still not certain whether the higher plasma concentrations of ATRA, achieved by means of an alternate schedule, should be more effective than the ones of a chronic daily schedule. However, the clinical relevance of maintaining therapeutic ATRA plasma concentrations seems to be confirmed by the assessment of low plasma ATRA AUCs in APL patients at relapse when the drug was administered as maintenance therapy. These observations suggested that the acquired ATRA resistance could be related to the inability to sustain the intranuclear ATRA concentrations required to initiate differentiation.

In conclusion, our results first showed the effects of ATRA in Ph+ CML patients in chronic phase and provided useful information for planning future therapeutic trials with ATRA alone or in combinatiion with other agents including IFNα. Although ATRA, as a single agent, appeared to be unable to induce *in vivo* relevant cytoreductive effects on the Ph+ clone, this observation does not rule out the possibility that it can induce differentiating effects and play a useful role in CML therapy when combined with other therapies. Since it has been noticed that in different leukemia cell lines ATRA can induce a remarkable upregulation of two IFNα-responsive genes, interferon responsive factor 1 (IRF1) and 2′-5′ oligoadenylate synthetase,[29] the potential interactive effects of ATRA in combination with IFNα could be worth evaluating in CML. Due to the low cytotoxic activity, if ATRA was given in combination with IFNα and/or other cytotoxic agents, dose reduction of these would not be planned. However, on the basis of our pharmacokinetics results, ATRA should be administered intermittently rather than continuously.

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References

1 Castaigne S, Chomienne C, Daniel MT, Ballerini P, Berger R, Fenaux P, Degos L. All-*trans* retinoic acid as a differentiation therapy for acute promyelocytic leukemia: I Clinical results. *Blood* 1990; **76**: 1704–1709.
2 Warrel RP, Frankel SR, Miller WH Jr, Scheinberg DA, Itri LM, Hittelman WN, Vyas R, Andreef M, Tafuri A, Jakubiwski A, Gabrilove J, Gordon MS, Dimitrowski E. Differentiation therapy of acute promyelocytic leukiemia with tretinoin (all-*trans* retinoic acid). *New Engl J Med* 1991; **324**: 1385–1393.
3 Chen ZX, Xue YQ, Ahang R, Tao RF, Xia XM, Li C, Wang W, Zu WY, Yao XZ, Ling BJ. A clinical and experimental study on all-*trans* retinoic acid treated acute promyelocytic leukemia patients. *Blood* 1991; **78**: 1413–1419.
4 Ohno R, Yoshida H, Fukutani H, Naoe T, Oshima T, Kyo T, Endoh N, Fujimoto T, Kobayashi T, Hiraoka A, Mizoguchi H, Kodera Y, Suzuki H, Hirano M, Akiyama H, Aoki N, Shindo H, Yokomaku S, the leukaemia Study Group of the Ministry of Health and Welfare. Multi-institutional study of all-*trans* retinoic acid as a differentiation therapy of refractory acute promyelocytic leukemia. *Leukemia* 1993; **7**: 1722–1727.
5 Frankel SR, Eardley A, Heller G, Berman E, Miller WH, Dmitrovsky E, Warrel RP. All-*trans* retinoic acid for acute promyelocytic leukemia. Results of the New York study. *Ann Intern Med* 1994; **120**: 278–286.
6 Avvisati G, Lo Coco F, Diverio D, Falda M, Ferrara F, Lazzarino M, Russo D, Petti MC, Mandelli F. AIDA (all-*trans* retinoic acid + idarubicin) in newly diagnosed acute promyelocytic leukemia: a Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) pilot study. *Blood* 1996; **88**: 1390–1398.
7 Kurzrock R, Estey E, Talpaz M. All-*trans* retinoic acid tolerance and biologic effects in myelodisplastic syndrome. *J Clin Oncol* 1993, **11**: 1489–1495.
8 Ohno R, Naoe T, Hirano M, Kobayashi M, Hirai H, Tubai K, Oh H, and the Leukemia Study Group of the Ministry of Health and Welfare. Treatment of myelodisplastic syndromes with all-*trans* retinoic acid. *Blood* 1993; **81**: 1152–1154.
9 Visani G, Tosi P, Manfroi S. All-*trans* retinoic acid in the treatment of myelodysplastic syndromes. *Leuk Lymphoma* 1993; **19**: 277–280.
10 Kuzel T, Roenig H Jr, Samuelson E, Rosen S. The treatment of mycosis fungoides (MF) with all-*trans* retinoic acid (ATRA). *Blood* 1993; **82** (Suppl. 1): 570a.
11 Esteey E, Cabanillas F, Rosenblum M, Kurzrock R, Bole J, Andreeff M, Keating M, Kantarjian H, Lopez-Berestein G. Phase I and pharmacology study of liposomal all-*trans* retinoic acid. *Blood* 1994; **84** (Suppl. 1): 380a.
12 Niesvizky R, Siegel D, Strauss D, Nichols G, Muindi J, Young C, Warrel RP Jr, Michaeli J. Hypercalcemia and increased serum interleukin-6 (sIL-6) levels induced by all-*trans* retinoic acid (ATRA) in patients with multiple myeloma (MM). *Proc Am Soc Clin Oncol* 1993; **12**: 1395a.
13 Musto P, Falcone A, Sajeva MR, D'Arena G, Bonini A, Carotenuto M. All-*trans* retinoic acid for advanced multiple myeloma. *Blood* 1995; **85**: 3769–3770.
14 Wiernik PH, Dutcher JP, Paietta E, Hittelman WN, Vyas R, Strack M, Castaigne S, Degos L, Gallagher RE. Treatment of promyelocytic blast crisis of chronic myelogenous leukemia with all-*trans*-retinoic acid. *Leukemia* 1991; **5**: 504–509.
15 Gallagher R, Ahearn G, Thompson D, Streck M, Paietta E, Dutcher J, Wiernik P. Retinoic acid enhanced neutrophilic differentiation of granulocyte macrophage progenitors in blastic crisis chronic myelogenous leukemia. *Blood* 1990; **76**: 272a.
16 Januszewicz E, Rabizadah E, Maimon Z, Zaizof R, Shaklai M. Inhibition by retinoic acid of myeloid progenitors in chronic myeloid leukemia and myeloproliferative disease: increased sensitivity in blast phase of chronic myeloid leukemia. *Pathology* 1988; **20**: 1–6.
17 Sagayadan GE, Peter H, Wiernik, Sun N, Ahearn G, Thompson D, Hallam SJ, Hu XP, Dutcher JP, Gallagher RE. Effect of retinoic acid and interferon alpha on granulocyte–macrophage colony forming cells in chronic myeloid leukemia: increased inhibition by all-*trans* and 13-*cis*-retinoic acid in advanced stage disease. *Leukemia Res* 1994; **18**: 741–748.
18 Aglietta M, Piacibello W, Stacchini A, Sanavio E, Gavosto F. *In vitro* effect of retinoic acid on normal and chronic myeloid leukemia granulopoiesis. *Leukemia Res* 1985; **9**: 879–883.
19 Nilsson B, Olofsson T, Olsson I. Myeloid differentiation in liquid cultures of cells from patients with chronic myeloid leukemia: effects of retinoic acid and indomethacin. *Exp Hematol* 1984; **12**: 91–99.
20 Moore M, Mertelsmann R, Pelus I. Phenotypic evaluation of chronic myeloid leukemia. *Blood Cells* 1981; **7**: 217–236.
21 Sporn M, Roberts AB. Role of retinoids in differentiation and carcinogenesis. *Cancer Res* 1983; **43**: 3034–3040.
22 Warrel RP Jr. Applications for retinoids in cancer therapy. *Semin Hematol* 1994; **31** (Suppl. 5): 1–13.
23 Zheng A, Savolainen ER, Koistinen P. All-*trans* retinoic acid combined with interferon-alpha effectively inhibits granulocyte–macrophage colony formation in chronic myeloid leukemia. *Leukemia Res* 1996; **20**: 243–248.
24 Guglielmo P, Stagno F, Cacciola E Jr, Cacciola RR, Impera S, Gius-

tolisi R, Cacciola E. All-*trans* retinoic acid (ATRA) induced apoptosis in peripheral freshly isolated chronic myeloid leukemia cells. Proc. ASH, Seattle, 1995. *Blood* 1995; **86** (Suppl. 1): 253a.
25 Cortes J, Kantarjian H, O'Brien S, Berann M, Estey E, Keating M, Talpaz M. A pilot study of all-*trans* retinoic acid in patients with Philadelphia chromosome-positive chronic myelogenous leukemia. *Leukemia* 1997; **11**: 929–932.
26 Barua AB, Olson JA. Retinoyl β-glucuronide: an endogenous compound of human blood. *Am J Clin Nutrit* 1986; **43**: 481–485.
27 Adamson PC, Bailey J, Pluda J, Poplack DG, Bauza S, Murphy RF, Yarchoan R, Balis FM. Pharmacokinetics of all-*trans* retinoic acid administered on an intermittent schedule. *J Clin Oncol* 1995; **13**: 1238–1240.
28 Russo D, Iacona I, Sacchi S, Avvisati G, Lazzarino M, Pellicci PG, Visani G, Regazzi MB for GER (Gruppo Ematologico Retinoidi). Pharmacokinetic profile of all-*trans* retinoic acid in patients with Ph^+ chronic myeloid leukemia in chronic phase treated with an intermittent schedule. Proc. Sixth International Congress Anti-Cancer Treatment, Paris, 1996, 289a.
29 Garattini E, Rambaldi A, Terao M, Rortino I, Gianni M. STAT1 is induced and activated by all-*trans* retinoic acid in acute promyelocytic leukemia cells. Proc. IV Congresso Nazionale Societa Italiana Ematologia Sperimentale, Rome 1996, 88a.