

Phase I Trial of 13-*cis*-Retinoic Acid in Children With Neuroblastoma Following Bone Marrow Transplantation

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Purpose: Treatment of neuroblastoma cell lines with 13-*cis*-retinoic acid (*cis*-RA) can cause sustained inhibition of proliferation. Since *cis*-RA has demonstrated clinical responses in neuroblastoma patients, it may be effective in preventing relapse after cytotoxic therapy. This phase I trial was designed to determine the maximal-tolerated dosage (MTD), toxicities, and pharmacokinetics of *cis*-RA administered on an intermittent schedule in children with neuroblastoma following bone marrow transplantation (BMT).

Patients and Methods: Fifty-one assessable patients, 2 to 12 years of age, were treated with oral *cis*-RA administered in two equally divided doses daily for 2 weeks, followed by a 2-week rest period, for up to 12 courses. The dose was escalated from 100 to 200 mg/m²/d until dose-limiting toxicity (DLT) was observed. A single inpatient dose escalation was permitted.

Results: The MTD of *cis*-RA was 160 mg/m²/d. Dose-

limiting toxicities in six of nine patients at 200 mg/m²/d included hypercalcemia (n = 3), rash (n = 2), and anemia/thrombocytopenia/emesis/rash (n = 1). All toxicities resolved after *cis*-RA was discontinued. Three complete responses were observed in marrow metastases. Serum levels of 7.4 ± 3.0 μ mol/L (peak) and 4.0 ± 2.8 μ mol/L (trough) at the MTD were maintained during 14 days of therapy. The DLT correlated with serum levels ≥ 10 μ mol/L.

Conclusion: The MTD of *cis*-RA given on this intermittent schedule was 160 mg/m²/d. Serum levels known to be effective against neuroblastoma in vitro were achieved at this dose. The DLT included hypercalcemia, and may be predicted by serum *cis*-RA levels. Monitoring of serum calcium and *cis*-RA levels is indicated in future trials.

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DESPITE a high complete response rate following intensive chemoradiotherapy and bone marrow transplantation (BMT), approximately 50% of patients with high-risk neuroblastoma subsequently develop tumor recurrence.¹ Alternative therapeutic strategies are needed to eradicate minimal residual disease that remains after BMT that is resistant to cytotoxic agents.

The synthetic retinoid 13-*cis*-retinoic acid (*cis*-RA), or isotretinoin, is derived from naturally occurring all-*trans*-retinoic acid (*trans*-RA) by modification of the terminal carboxyl group. When neuroblastoma cell lines are exposed to *trans*-RA or *cis*-RA in vitro, they exhibit de-

creased proliferation, decreased expression of the N-*myc* oncogene, and morphologic differentiation.²⁻⁵ Growth arrest and differentiation in response to *cis*-RA have been observed in neuroblastoma cell lines initiated from tumors at the time of progression after chemoradiotherapy,^{3,5} which suggests that resistance to cytotoxic chemotherapy does not induce resistance to *cis*-RA. Most neuroblastoma cell lines, both with and without amplification of N-*myc*, show expression of the retinoic acid receptors α and γ and induction of the β -retinoic acid receptor in response to RA.⁶

cis-RA has been shown to be effective as a single agent in the treatment of squamous cell carcinomas of the skin⁷ and mycosis fungoides,⁸ and in combination with interferon alfa-2a for squamous cell carcinoma of the cervix.^{9,10} The development of skin cancer in patients with xeroderma pigmentosum¹¹ and secondary cancers in patients with previous head and neck squamous cell carcinomas¹² can also be prevented by *cis*-RA.

Although responses to *cis*-RA have been reported in patients with neuroblastoma treated with minimal or stable residual disease,³ a phase II trial using *cis*-RA for children with recurrent neuroblastoma found objective responses in only two of 22 patients.¹³ Thus, we hypothesized that *cis*-RA would be most effective against neuroblastoma in vivo against limited residual disease that may remain following maximal reduction of tumor burden with BMT. We expected that *cis*-RA was likely to be well tolerated post-BMT, since its reported toxicities are generally mild, consisting primarily of cheilitis, dry skin, conjunctivitis, and hypertriglyceridemia.¹⁴

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Since the clinical toxicities previously reported with *cis*-RA tend to increase with repetitive daily dosage, we chose to use an intermittent schedule (2 weeks of treatment, followed by 2 weeks of rest), which could potentially allow tolerance of higher doses of *cis*-RA. We hypothesized that this would achieve higher serum levels of *cis*-RA, comparable to those required for the in vitro responses observed in neuroblastoma cell lines. The dosing schedule was based on an in vitro model in which 2 weeks of 5 $\mu\text{mol/L}$ of *cis*-RA exposure per month for 2 consecutive months produced complete growth arrest of a neuroblastoma cell line for 120 days.⁵ A phase I trial was therefore designed to determine the maximal-tolerated dosage (MTD), toxicity, and pharmacokinetics of *cis*-RA in children with neuroblastoma following BMT.

PATIENTS AND METHODS

Patient Eligibility

Patients from birth to 21 years of age who had undergone allogeneic or autologous BMT for high-risk neuroblastoma were eligible for this study. Staging was performed according to the International Neuroblastoma Staging System.¹⁵ Patients with any disease status, including no measurable disease, were eligible. A neutrophil count greater than 500/ μL was required for at least 1 week before study entry. Patients who still required RBC and/or platelet transfusions were eligible; however, their hematopoietic toxicity was defined as an increase in transfusion requirements after study entry. Other eligibility requirements included serum transaminase levels less than twice normal, serum bilirubin level less than 2.0 mg/dL, and serum creatinine concentration ≤ 1.2 mg/dL. Informed consent was obtained from the patient and/or parents. The study was approved by the human investigations committee at each institution where patients were entered the study, and was performed under Food and Drug Administration Investigational New Drug Application #36,781.

Study Design and Analysis

Four consecutive groups of patients were treated with oral *cis*-RA at dosages of 100, 125, 160, or 200 mg/m²/d administered in two equally divided doses for 2 weeks, followed by a 2-week rest period. The *cis*-RA (Accutane; Hoffman-LaRoche, Nutley, NJ) was given as 10-, 20-, or 40-mg capsules provided by the manufacturer. The capsules were either swallowed whole, chewed, or the contents squeezed into a small amount of soft food. A single inpatient 25% dose escalation was allowed after a minimum of two courses were completed with no grade 3/4 toxicity. Dose escalation occurred in 16 of 51 patients after the second ($n = 10$), third ($n = 3$), fourth ($n = 1$), fifth ($n = 1$), or eighth ($n = 1$) course. The remainder of the patients either required dose reductions due to toxicity, remained at their entry level due to physician discretion, or were entered at the determined MTD to obtain additional pharmacokinetic data and were ineligible for dose escalation. A maximum of 12 courses were given according to the protocol. However, five patients continued therapy beyond 12 courses at the parent's and/or referring physician's preference for a total of 13 ($n = 2$), 14 ($n = 2$), or 17 courses ($n = 1$).

A minimum of three patients were entered and required to complete two courses at a given dose level without dose-limiting toxicity

(DLT) before a subsequent cohort was entered at the next highest dose level. Toxicity for each level was evaluated by combining patients who entered or were escalated/deescalated to a given dose. Toxicities were graded according to the National Cancer Institute common toxicity criteria. Required monitoring included weekly physical examination, complete blood cell count with differential, and levels of serum albumin, total protein, transaminases, alkaline phosphatase, lactate dehydrogenase (LDH), cholesterol, uric acid, bilirubin, glucose, and triglycerides. In addition, serum calcium, phosphorus, blood urea nitrogen (BUN), creatinine, creatinine clearance, and urinalysis measurements were required at study entry. An amendment to require serum calcium and phosphorus measurement on days 0, 7, and 14 was added after hypercalcemia was observed in several patients. Laboratory requirements and physical examination were decreased to a monthly basis after two to three courses had been tolerated at the dose level to be used for the remainder of therapy. The DLT was defined as grade 3 toxicity not returning to less than grade 2 before the next course of therapy, or grade 4 toxicity. The MTD was defined as one dose level below the level at which at least 50% of patients experienced DLT. The drug was discontinued temporarily in patients with DLT until resolution of the toxicity, after which the patient was restarted on *cis*-RA with a 25% dose reduction.

Tumor response was assessed by the International Response Criteria for Neuroblastoma.¹⁵ Bilateral bone marrow aspirates were required before study entry, with immunocytologic analysis performed when possible according to previously described methods.^{16,17} Patients with evidence of marrow tumor cells before therapy were monitored monthly. Disease status was also assessed by physical examination, computed tomographic (CT)/magnetic resonance imaging scans, bone scans, and urine catecholamine measurements every 3 to 6 months, as specified by the BMT protocol for each patient.

Pharmacokinetics

When possible, based on venous access and patient ability to comply with clinic visit requirements, serum *cis*-RA levels before, and 2, 4, 6, and 8 hours after the morning dose on days 1 and 14 were performed during the first course. The serum levels were determined by a modified high-performance liquid chromatography (HPLC) assay.¹⁸ After blood samples were drawn, they were wrapped immediately in aluminum foil. Samples were centrifuged in dim yellow light and the serum stored at -70°C protected from light. Aliquots of 0.2 mL of serum thawed in the dark to 0°C were added to 0.8 mL of extraction solvent (100% methanol with 0.1 mg/mL butylated hydroxytoluene [BHT] as an antioxidant) in amber centrifuge tubes. The mixture was immediately vortexed and placed in an ice bath (0°C) for 5 minutes in the dark. The tubes were then centrifuged at 2,000 rpm for 10 minutes at 5°C . Aliquots of 0.05 to 0.2 mL of the supernatant (methanol phase) were injected onto a reverse-phase $\mu\text{C}18$ column and assayed using a Waters Associates HPLC system (Milford, MA). The mobile phase was methanol/water 80:20, which was run isocratically for 20 minutes. The eluents were followed at 350 nm with a Waters 484 UV/VIS detector, using the Maxima 820 software (Waters Associates). Mean peak serum levels were calculated using the 4-hour postdose level. Area under the concentration-versus-time curve (AUC) values were calculated using software developed by the University of Southern California School of Pharmacy. AUC values were derived from 12-hour pharmacokinetic data due to the need to administer drug every 12 hours. *cis*-RA levels in serum and plasma drawn at the same time were identical.

Table 1. Patient Characteristics

No. entered	52
No. assessable	51
No. of assessable courses	407
Age (years)	
Median	4
Range	2-12
Male/female	26:25
Stage	
3	6
4	45
N-myc gene copy no.	
≥ 10 (amplified)	6
< 10 (nonamplified)	28
Unknown	17
Type of BMT	
Allogeneic	2
Autologous	49
Months post-BMT at study entry	
Median	3
Range	1-10

RESULTS

Patient Characteristics

A total of 52 patients were entered onto the study, of which 51 were assessable. A single patient had no copy of the study consent form submitted to the study chair after verbal consent was obtained by the treating physician, and was excluded from the study analysis. However, this patient did not experience any unique toxicities.

Patient characteristics are listed in Table 1. All patients underwent BMT on Childrens Cancer Group (CCG) or Childrens Hospital Los Angeles (CHLA) protocols. The two allogeneic BMT patients received cisplatin/etoposide/melphalan (PEM) and total-body irradiation (TBI) before BMT (per CCG 321P1 protocol).^{19,20} The majority of the autologous BMT patients were treated according to the CCG 321P3 protocol with PEM/TBI ($n = 18$), carboplatin/etoposide/melphalan (CEM)/TBI ($n = 22$), or cisplatin/teniposide/melphalan/doxorubicin (VAMP)/TBI ($n = 2$).^{1,19,20} Another seven patients received CEM without TBI, according to CHLA protocol 91LA6. Forty-three patients began therapy from 2 to 4 months following BMT, when they were able to tolerate an oral agent. One patient began therapy following disease recurrence at 10 months following BMT, and the remaining seven patients began therapy at 1 ($n = 1$), 5 ($n = 2$), 7 ($n = 2$), and 8 ($n = 2$) months following BMT, with no unique toxicities. No patient received other therapy in the interval between BMT and the start of *cis*-RA therapy. The two allogeneic patients had no evidence of graft-versus-host disease (GVHD) at study entry, were no longer on immunosup-

pressive drugs, and did not experience any unique toxicity or flare of GVHD while on therapy. Nine patients had only a single kidney due to either renal infarction or nephrectomy at the time of tumor resection. There was no significant increase in grade 3/4 toxicities in this patient group.

Toxicity

Because of DLT in six of nine assessable patients treated at 200 mg/m²/d, the MTD was determined to be 160 mg/m²/d (Table 2). The DLTs observed included hypercalcemia, rash, and emesis with anemia plus thrombocytopenia in order of frequency of occurrence. All toxicities resolved after discontinuation of the drug.

There were three cases of grade 4 hypercalcemia, one at 160 mg/m²/d and two at 200 mg/m²/d, and one case of grade 3 hypercalcemia at 200 mg/m²/d. Only one of these patients was symptomatic, with myalgias and arthralgias. The incidence of all grades of hypercalcemia was 13 of 41 patients (32%) during 29 of 335 courses (9%) in which serum calcium level was evaluated.

Hematopoietic toxicity occurred in eight of 51 patients (16%) and 10 of 407 courses (3%). Only four patients experienced grade 3 hematopoietic toxicity (absolute neutrophil count, 500 to 999/ μ L; hemoglobin level, 5 to 7 mg/dL; and/or platelet count 25 to 49,000/ μ L). These included two patients with neutropenia, one with anemia and thrombocytopenia, and one with thrombocytopenia. Isolated grade 2 anemia (hemoglobin level, 7.1 to 9.0 mg/dL) occurred in one patient at 100 mg/m²/d and in two patients at 160 mg/m²/d. In addition, a single patient had grade 1 thrombocytopenia (platelet count 75,000 to 120,000/ μ L) with grade 2 anemia at 160 mg/m²/d.

Some type of grade 1 or 2 toxicity was observed in the majority of patients at each dose level (Table 3). Skin toxicity, which was the most frequent grade 1 or 2 toxicity, was observed at each dose level in 58% to 89% of patients. This consisted of dry and/or peeling skin, cheilitis, conjunctivitis, and erythematous maculopapular or bullous eruptions. Grade 1 and 2 asymptomatic hypercalcemia, hepatic toxicity (elevated transaminases and/or alkaline phosphatase levels), and gastrointestinal complaints (diarrhea, emesis, nausea, abdominal pain/distension, and anorexia) occurred at all dose levels. Mild elevation of serum triglyceride levels (165 to 494 mg/dL) was also noted at all dose levels. The frequency of hypercalcemia and hypertriglyceridemia increased with the dose level (Table 3). Other grade 1 to 2 clinical toxicities included myalgias, arthralgias, dysuria, and fatigue. Mild reversible elevations of BUN, creatinine, and uric acid were also observed.

Table 2. DLTs

Daily Dose (mg/m ² /d)	No. of Assessable Patients/Courses	Grade 3 (no. of patients)	Grade 4 (no. of patients)
100	23/103	1: neutropenia	1: hepatic (AST/ALT)*
125	24/112	3: skin† 1: hepatic (AST/ALT) 1: neutropenia	1: hepatic (AST/ALT) + grade 3 skin None
160	23/144	3: skin 1: hepatic (AST/ALT) 1: emesis/skin	1: hypercalcemia + grade 3 hepatic (AST/ALT)
200	9/48	2: skin 1: anemia/thrombocytopenia/emesis/skin 1: hypercalcemia	2: hypercalcemia

*Patient also had positive serology for hepatitis C.

†Grade 3 skin toxicity was concurrent with varicella zoster infection in one patient.

There were no cases of pseudotumor cerebri. One patient each at 125, 160, and 200 mg/m²/d developed grade 1 headache, associated with mild somnolence in the patient treated at 125 mg/m²/d. The neurologic and fundoscopic examinations, as well as head CT scan, were normal in all three patients. Vertigo, unilateral otalgia, and tinnitus were noted in a fourth patient treated at 125 mg/m²/d, which improved while continuing therapy at the same dosage. A single patient was also reported to have unilateral bone marrow necrosis, associated with severe hip pain, 2 years after completing seven courses of *cis*-RA.

Bilateral pulmonary nodules (largest, 1.5 cm) were noted on a routine follow-up chest CT scan in a single

patient after completion of 10 courses of therapy. This patient received courses 1 and 2 at 125 mg/m²/d, courses 3 to 7 at 100 mg/m²/d due to grade 3 skin toxicity during course 2, and courses 8 to 10 at 125 mg/m²/d after no significant toxicity occurred during courses 3 to 7. The nodules were not consistent with disease recurrence, since a complete disease reevaluation showed no other evidence of active tumor. The patient was asymptomatic and receiving no other medication. The nodules resolved completely following discontinuation of *cis*-RA. This patient is now 3 years postcompletion of *cis*-RA therapy and has no evidence of tumor recurrence or recurrence of the nodules.

The five patients who received more than 12 courses of therapy (13, 13, 14, 14, and 17 total courses) experienced only grade 1 to 2 skin and gastrointestinal toxicities, with no unique toxicities. The dosages for these extra courses were 100 mg/m²/d (n = 1), 125 mg/m²/d (n = 3), and 160 mg/m²/d (n = 1).

Clinical Responses

Table 4 lists the clinical responses observed in the 10 patients with assessable disease, based on serial radiologic studies and/or routine bone marrow morphology. Three patients had a complete response in marrow metastases by routine morphology. The two responses based on serial bone scans are difficult to ascribe to *cis*-RA therapy, since gradual improvement in bone scan abnormalities is common in patients who receive no further therapy following autologous BMT.

Immunocytologic analysis of bilateral bone marrow aspirates was performed before and after study entry in 31 patients. This included the allogeneic patient described earlier whose marrow immunocytology was positive at study entry and increased with further disease progres-

Table 3. Common Grade 1 and 2 Toxicities

Daily Dose (mg/m ² /d)	Toxicity*	No. of Patients With Toxicity/No. Assessable	% Patients With Toxicity
100	Skin	15/23	65
	Hepatic	6/23	26
	Hypertriglyceridemia	1/23	4
	Hypercalcemia	1/18	6
	Hematopoietic	1/23	4
125	Skin	14/24	58
	Hepatic	8/24	33
	Hypertriglyceridemia	4/24	17
	Hypercalcemia	1/19	5
160	Skin	17/23	74
	Hepatic	7/23	30
	Hypertriglyceridemia	5/23	22
	Hypercalcemia	6/21	29
	Hematopoietic	3/23	13
200	Skin	8/9	87
	Hepatic	4/9	44
	Hypertriglyceridemia	3/9	33
	Hypercalcemia	2/7	29

*See Results for detailed description of toxicities.

Table 4. Clinical Responses

Disease Status at Study Entry	Clinical Response	No. of Courses Before Response	Months Post-BMT	Site(s) of Response	Total No. of Courses Received	Follow-Up
PR	CR	2	5	Marrow	12	CR, 33 months off therapy
PR	CR	7	15	Marrow	7	PD, 9 months off therapy
PR	CR	17	22	Marrow/bone	17	CR, 23 months off therapy
VGPR	CR	3	6	Bone	6	PD, after 6 courses
PR	SD	12	14	Primary	12	SD, 32 months off therapy
PR	PD	1	2	Marrow	1	
VGPR	PD	1	4	Primary	1	
VGPR	PD	4	8	Primary/bone/marrow	4	
VGPR	PD	14	17	Lymph nodes	14	
PD	PD	1	10	Marrow	1	

Abbreviations: PD, progressive disease; PR, partial response; VGPR, very good partial response; CR, complete response; SD, stable disease.

sion. Sixteen patients remained negative for tumor cells before and after study entry. Thirteen patients developed positive marrow immunocytology after study entry, which was associated with clinical disease progression at other sites in addition to the bone marrow. Two patients had 0.001% tumor cells in the marrow by immunocytology before initiation of *cis*-RA. One of these patients had negative marrow immunocytology after three and nine courses of therapy, and then developed progressive disease with 7% tumor cells in the marrow and bone metastases 6 months after completion of 12 courses of *cis*-RA. The second patient's marrow had no detectable tumor cells after two courses of *cis*-RA, 3% tumor cells after five courses, and 40% tumor cells after seven courses associated with new bone metastases.

Of 50 patients entered onto the study before disease progression, 27 (54%) developed disease progression after a median of five courses of therapy (range, one to 14). Relapse occurred in the primary tumor site only ($n = 2$), primary tumor plus distant sites ($n = 8$), or distant sites only ($n = 17$). Of 25 cases in which the marrow was evaluated at the time of relapse, tumor metastases were found in 13 patients. No significant difference in the rate of relapse was seen based on the entry dose level or the maximum dose level received, although only small numbers of patients were evaluated at each dose level in this study.

Pharmacokinetics

Pharmacokinetic studies were performed in 30 of 51 patients and have been reported separately.²¹ The mean serum level of *cis*-RA ranged from 4.9 to 8.9 $\mu\text{mol/L}$ at doses of 100 to 200 $\text{mg/m}^2/\text{d}$. Both the mean peak serum level and the AUC increased with dose escalation (Fig 1). Trough levels did not increase significantly with increasing dose, which suggests increased clearance of *cis*-RA at higher doses. Significant interpatient and inpatient

tient variability in peak serum levels was observed. A mean peak serum concentration of $7.4 \pm 3.0 \mu\text{mol/L}$ and trough of $4.0 \pm 2.8 \mu\text{mol/L}$ were attained at the MTD of 160 $\text{mg/m}^2/\text{d}$. Peak serum levels of *cis*-RA $\geq 10 \mu\text{mol/L}$ were correlated with a higher incidence of grade 3 and 4 toxicities (eight of 16 courses [50%]) versus levels less than 10 $\mu\text{mol/L}$ (11 of 92 courses [12%]) ($P = .001$, Fischer's exact test). There was no statistically significant difference in the AUC or peak serum levels from days 1 to 14 of each course. The AUC was also maintained during repetitive cycles evaluated in one patient over 204 days of therapy.

DISCUSSION

The MTD determined in this trial for *cis*-RA given on an intermittent schedule in pediatric patients was 160 $\text{mg/m}^2/\text{d}$ divided into two equal doses. The DLTs consisted of hypercalcemia, and combinations of skin, gastrointestinal, and hematopoietic toxicities. This study demonstrates that

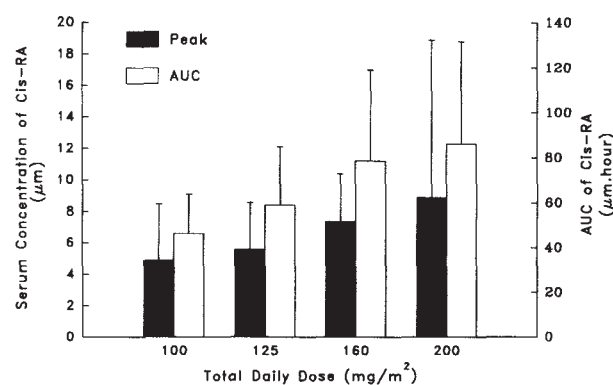


Fig 1. Peak serum concentrations and AUC for *cis*-RA at different total daily doses. The 4-hour postdose level was used as the peak level for patients with limited time points. Although there is marked patient-to-patient variability, both the peak levels and AUC increase with each dose level.

higher doses of *cis*-RA are well tolerated using an intermittent rather than a daily schedule, and can achieve serum levels equivalent to those effective in vitro against neuroblastoma cell lines.⁵ Furthermore, *cis*-RA therapy was well tolerated by patients who recently received intensive myeloablative chemoradiotherapy.

Hypercalcemia is a novel DLT for *cis*-RA, and the details of these patients have been reported separately.²² Only one previous trial with *cis*-RA²³ has reported hypercalcemia, using 15 to 240 mg/m²/d of *cis*-RA administered daily to patients with metastatic breast cancer, with an incidence of 22% (four of 18 patients). This was not a DLT and did not require dose reduction. A pediatric phase I trial of *trans*-RA for refractory cancer noted asymptomatic grade 2 hypercalcemia in only two of 17 patients (12%), but this resolved with dose modification.²⁴ Adult trials with *trans*-RA have not reported hypercalcemia.²⁵⁻²⁷ The higher incidence of severe hypercalcemia in our trial may be related to the patient population being pediatric rather than adult, or to the higher doses of *cis*-RA used. However, serum levels of *cis*-RA did not correlate with hypercalcemia based on the limited pharmacokinetic data obtained in five patients during courses when this toxicity occurred.²² The etiology of hypercalcemia is not established for *cis*-RA or *trans*-RA, but multiple laboratory and clinical studies with vitamin A suggest that the mechanism involves increased bone reabsorption.²⁸⁻³⁴ Hypercalcemia was considered a DLT, even though only one patient was symptomatic, since significant clinical bone abnormalities have occurred with chronic hypervitaminosis A.^{28,31,32,35} This must be avoided in actively growing children treated with *cis*-RA for 1 year.

Hematopoietic toxicity is of special concern in therapies used after BMT. Only 3% of assessable courses had hematopoietic toxicity affecting either WBCs, RBCs, or platelets, which resolved after *cis*-RA was discontinued. The low incidence of hematopoietic toxicity indicates that *cis*-RA is well tolerated following BMT. The other toxicities observed were known to be associated with *cis*-RA, and included skin dryness/eruptions, cheilitis, conjunctivitis, gastrointestinal symptoms, dysuria, fatigue, and anorexia.^{36,37} Laboratory abnormalities included elevations in transaminases, alkaline phosphatase, triglycerides, BUN, creatinine, and uric acid levels, all of which were asymptomatic and resolved after discontinuation of *cis*-RA therapy. Single cases of asymptomatic and transient pulmonary nodules and bone marrow necrosis were reported, which cannot be proven to be caused by *cis*-RA, although no other etiology was found.

Of 10 patients assessable for response by radiologic scans or morphologic bone marrow analysis, four

achieved a complete response during *cis*-RA therapy based on the resolution of bone marrow metastases in three patients and bone scan abnormalities in two. However, since bone scan abnormalities may persist for some time after the resolution of active disease, it is difficult to assess the role of *cis*-RA in bone metastases measured by bone scan only. Two of four patients who showed a bone marrow response remain in remission at 23 and 33 months after completion of *cis*-RA therapy, while the other two patients developed progressive disease 3 to 9 months following the complete response. One patient who entered the study in partial remission (based on a residual abdominal mass) remains with stable disease 32 months after completion of therapy. In addition, two patients with 0.001% tumor cells in the bone marrow by immunocytologic analysis before *cis*-RA therapy showed resolution of marrow metastases after two and three courses of *cis*-RA, but both patients subsequently relapsed with bone marrow involvement. The responses in bone marrow metastases observed in this study are consistent with those reported previously.³

No response was seen in the single patient entered onto the study with progressive disease. Only two of 22 patients (9%) with advanced recurrent neuroblastoma responded to *cis*-RA in the study reported by Finklestein et al,¹³ which used a dosage of 100 mg/m²/d administered daily on a continuous schedule. These data suggest that *cis*-RA has little efficacy against advanced recurrent disease, but is able to eliminate limited residual disease in some patients with neuroblastoma. Using the regimen determined in this phase I study, the effect of *cis*-RA on progression-free survival and relapse patterns after completion of chemoradiotherapy for high-risk neuroblastoma is currently being studied in a CCG randomized trial (CCG 3891).

This study suggests several important differences between *cis*-RA and *trans*-RA. Pseudotumor cerebri, the DLT in children for *trans*-RA,²⁴ was not observed in our study. Pharmacokinetic data show that peak plasma concentrations achieved with 60 mg/m²/d of *trans*-RA are only 0.62 ± 0.25 μ mol/L, with up to an 80% decrease in the AUC during a 28-day course.^{24,27,38} In contrast, 160 mg/m²/d of *cis*-RA achieves mean peak serum levels greater than 5 μ mol/L (7.4 ± 3.0 μ mol/L),²¹ and the AUC is maintained during repetitive courses. A longer half-life is also seen with *cis*-RA (5.1 ± 3.2 hours) compared with *trans*-RA (45 ± 14 minutes).^{21,24} Mechanisms for the decrease in *trans*-RA concentrations may include the induction of drug metabolism by a cytochrome P450 system³⁹ and/or increased expression of cellular retinoic acid-binding proteins (CRABP).^{40,41} Epidermal CRABP

levels do not increase during *cis*-RA therapy,⁴² and we did not observe increased clearance of *cis*-RA during therapy. Thus, in contrast to *trans*-RA, *cis*-RA provides higher drug levels and no decrease in serum levels during the course of therapy.

Comparisons of the relative efficacy of *cis*-RA and *trans*-RA are dependent on the drug concentrations used. For promyelocytic leukemia, *trans*-RA was more effective than *cis*-RA at 0.1- μ mol/L concentrations in inducing differentiation in vitro,⁴³ although at 10- μ mol/L concentrations, the two agents were equivalent. Clinical responses in promyelocytic leukemia were also more favorable with *trans*-RA, based on no response in two patients treated with 45 mg/m²/d of *cis*-RA compared with complete responses in both patients treated with the same dosage of *trans*-RA. However, when 5- μ mol/L concentrations of both retinoids are used against neuroblastoma cell lines, there is no advantage to *trans*-RA.⁵ Since higher *cis*-RA serum levels can be achieved and sustained in patients with minimal clinical toxicity, *cis*-RA may offer

therapeutic advantages over *trans*-RA, at least for neuroblastoma.

We conclude that the MTD of *cis*-RA in pediatric patients is 160 mg/m²/d, and the side effects are tolerable in patients with prior myeloablative therapy. The clinical responses observed in some patients in this trial suggest that *cis*-RA merits further testing in the prevention or delay of recurrence of neuroblastoma from limited residual disease after maximal cytotoxic therapy.

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