TREATMENT OF HIGH-RISK NEUROBLASTOMA WITH INTENSIVE CHEMOTHERAPY, RADIOTHERAPY, AUTOLOGOUS BONE MARROW TRANSPLANTATION, AND 13-*CIS*-RETINOIC ACID

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ABSTRACT

Background   Children with high-risk neuroblastoma have a poor outcome. In this study, we assessed whether myeloablative therapy in conjunction with transplantation of autologous bone marrow improved event-free survival as compared with chemotherapy alone, and whether subsequent treatment with 13-*cis*-retinoic acid (isotretinoin) further improves event-free survival.

Methods   All patients were treated with the same initial regimen of chemotherapy, and those without disease progression were then randomly assigned to receive continued treatment with myeloablative chemotherapy, total-body irradiation, and transplantation of autologous bone marrow purged of neuroblastoma cells or to receive three cycles of intensive chemotherapy alone. All patients who completed cytotoxic therapy without disease progression were then randomly assigned to receive no further therapy or treatment with 13-*cis*-retinoic acid for six months.

Results   The mean (±SE) event-free survival rate three years after the first randomization was significantly better among the 189 patients who were assigned to undergo transplantation than among the 190 patients assigned to receive continuation chemotherapy (34±4 percent vs. 22±4 percent, P=0.034). The event-free survival rate three years after the second randomization was significantly better among the 130 patients who were assigned to receive 13-*cis*-retinoic acid than among the 128 patients assigned to receive no further therapy (46±6 percent vs. 29±5 percent, P=0.027).

Conclusions   Treatment with myeloablative therapy and autologous bone marrow transplantation improved event-free survival among children with high-risk neuroblastoma. In addition, treatment with 13-*cis*-retinoic acid was beneficial for patients without progressive disease when it was administered after chemotherapy or transplantation. (N Engl J Med 1999; 341:1165-73.)

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NEUROBLASTOMA, the most common extracranial solid tumor of childhood, has a long-term survival rate of only 15 percent.[1] At diagnosis the defining characteristics of high-risk neuroblastoma include an age of more than one year, metastases, amplification of the *MYCN* oncogene, and histologic findings.[2-4] Recent progress in the treatment of high-risk neuroblastoma may be due to the use of higher doses of chemotherapy[5] and improved supportive care. Some studies have attributed the improvement to the use of myeloablative doses of cytotoxic therapy in conjunction with autologous bone marrow transplantation.[6-15] However, since none of these studies involved a randomized comparison, selection bias may have influenced the results.

Relapse remains common, despite the achievement of a complete clinical remission with myeloablative therapy and marrow transplantation, suggesting that minimal residual disease is an important cause of recurrence.[11] All-*trans*-retinoic acid (tretinoin) and 13-*cis*-retinoic acid (isotretinoin) decrease proliferation and induce differentiation in neuroblastoma cell

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lines, including some established from refractory tumors, and may be effective against residual tumor cells that are resistant to cytotoxic agents.[16-20] A phase 1 study of children with high-risk neuroblastoma found that an intermittent schedule of high-dose 13-*cis*-retinoic acid after bone marrow transplantation had minimal toxicity, achieved levels that were effective against neuroblastoma cell lines in vitro, and resulted in the clearing of tumor cells in bone marrow, as determined by morphologic assessment, in 3 of 10 patients.[21]

These promising results led to the present prospective, randomized study in which a combination of myeloablative chemotherapy, total-body irradiation, and transplantation of autologous bone marrow purged of cancer cells was compared with intensive nonmyeloablative chemotherapy in children and young adults with high-risk neuroblastoma. The study design also included a second randomization to determine whether treatment with 13-*cis*-retinoic acid after transplantation or chemotherapy further improves event-free survival.

METHODS

Patients

Eligible patients included children and adolescents with newly diagnosed high-risk neuroblastoma who were 1 to 18 years of age. A total of 434 patients had Evans stage IV neuroblastoma; 72 had stage III disease with one or more of the following: amplification of the *MYCN* oncogene,[2] a serum ferritin level of at least 143 ng per milliliter,[22] and unfavorable histopathological findings[23,24]; 1 had stage II disease with amplification of *MYCN* (age, >1 year); 13 had stage I or II disease with bone metastases before therapy other than surgery; and 19 had had stage IV disease with *MYCN* amplification for less than one year. Parents or guardians provided written informed consent, and the study was approved by the appropriate local institutional review boards. Enrollment began in January 1991 and ended in April 1996.

Serum ferritin was measured by radioimmunoassay; levels of at least 143 ng per milliliter were designated as unfavorable, and levels below 143 ng per milliliter were designated as favorable.[22] Biologic features of the tumor including histologic findings and the number of *MYCN* genes were determined centrally in the neuroblastoma reference laboratory of the Children's Cancer Group. Amplification of *MYCN* was measured by Southern blotting (from 1991 to 1993)[25] or on the basis of the pattern and intensity of the *MYCN* protein on immunohistochemical and semiquantitative polymerase-chain-reaction assays (from 1994 to 1996).[26] One investigator reviewed all tumors and classified the histopathological findings as favorable or unfavorable according to the method of Shimada et al.[24]

Treatment

All patients received initial therapy with cisplatin (60 mg per square meter of body-surface area administered intravenously over a period of six hours on day 0), doxorubicin (30 mg per square meter intravenously on day 2), etoposide (100 mg per square meter intravenously on days 2 and 5), and cyclophosphamide (1000 mg per square meter intravenously on days 3 and 4) for five cycles at 28-day intervals, plus surgery and radiotherapy for gross residual disease (Fig. 1). The requirements for the local control of disease were identical for all patients. For the transplantation group, the conditioning regimen consisted of carboplatin (1000 mg per square meter) and etoposide (640 mg per square meter) administered by continuous infusion over a period of 96 hours beginning eight days before transplantation; melphalan (a bolus infusion of 140 mg per square meter seven days before transplantation and a bolus infusion of 70 mg per square meter six days before transplantation); and total-body irradiation (333 cGy daily for the three days before transplantation), followed by an infusion of purged bone marrow (median dose, 2×10^8 mononuclear cells per kilogram of body weight) on day 0 and then by granulocyte–macrophage colony-stimulating factor (250 μg per square meter per day intravenously). The continuation-chemotherapy group received three cycles of cisplatin (160 mg per square meter), etoposide (500 mg per square meter), and doxorubicin (40 mg per square meter), administered as a continuous infusion over a period of 96 hours and given simultaneously with a bolus injection of ifosfamide (2500 mg per square meter on days 0 to 3) and mesna (600 mg per square meter per dose every 3 hours for five doses), followed by granulocyte colony-stimulating factor (5 μg per kilogram subcutaneously). The first randomization was performed just before the third cycle of initial therapy, at week 8 of the protocol (median, 60 days after diagnosis), and included patients
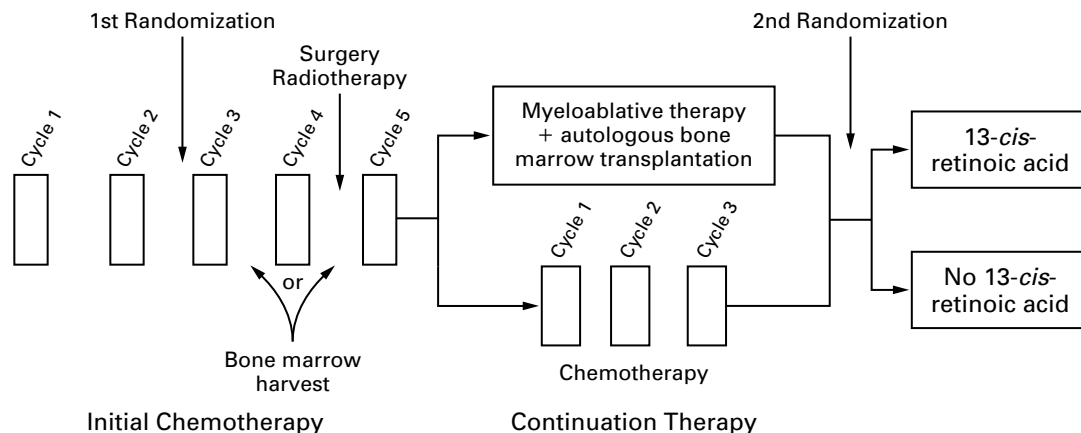

Figure 1. Treatment Regimens.
The conditioning regimen for autologous bone marrow transplantation consisted of carboplatin, etoposide, melphalan, and total-body irradiation. Details of the chemotherapy regimens are given in the Methods section.

without disease progression. After transplantation or the end of continuation therapy (week 34 of the protocol; median, 288 days after diagnosis), patients without disease progression were randomly assigned to receive six cycles of 13-*cis*-retinoic acid (160 mg per square meter per day administered orally in two divided doses for 14 consecutive days in a 28-day cycle) or no further therapy. Clinical evaluations were performed at diagnosis and at the end of induction therapy, continuation chemotherapy or transplantation, and 13-*cis*-retinoic acid therapy. Responses were assessed with use of the international criteria for the response to treatment of neuroblastoma.[27,28]

Harvesting and Purging of Bone Marrow

Harvesting of bone marrow and purging were done at the neuroblastoma purging center of the Children's Cancer Group just before the fourth or fifth cycle of initial therapy, if a bone marrow aspirate obtained one week earlier had less than 1 percent tumor cells on morphologic and immunocytologic analysis (sensitivity of assay, 1 tumor cell per 10^5 nucleated bone marrow cells).[29] Bone marrow was purged with the use of sedimentation, filtration, and two cycles of immunomagnetic separation of neuroblastoma cells.[10,30,31] None of the infused marrow preparations had detectable tumor cells on immunocytologic analysis.

Statistical Analysis

The design of the study called for two separate sequential randomizations. Patients who had progressive disease before week 8 of the protocol were ineligible for the trial. Patients who had progressive or histologically confirmed disease at the completion of cytotoxic therapy were ineligible for the second randomization. Some patients who were ineligible for the first randomization because they were ineligible for transplantation were assigned in a nonrandom fashion to receive continuation chemotherapy. If these patients were free of detectable disease after chemotherapy, they were eligible for the second randomization. They were not considered in the intention-to-treat analysis of the first randomization.

A permuted-block design was used for the random assignment of approximately equal numbers of patients from each of two strata (those with and those without metastatic disease) to transplantation or continuation chemotherapy. The second randomization was similarly balanced with respect to the numbers of patients from each group of the first randomization and nonrandomized patients who were ineligible for transplantation.

The study committee and investigators were unaware of patients' treatment assignments, and the study was monitored by an independent committee according to a group sequential monitoring plan.[32] Interim analyses were performed after the fourth, fifth, and sixth years of enrollment. The study continued to completion, because predefined early stopping criteria were not met. The analysis described here was performed on February 4, 1999. Similarities between patients in the two groups were assessed with chi-square tests for homogeneity of proportions. Outcome analyses used life-table methods and associated statistics.[33]

Life-table estimates were calculated according to the Kaplan–Meier procedure. The standard errors of the life-table estimates of event-free survival and overall survival after three years of follow-up were calculated according to the method described by Peto et al.[34] A two-sided test of proportions was performed by dividing the difference in event-free survival by its standard error and comparing this value with the percentiles for a standard normal random variable. The primary end point, prespecified by the protocol, was event-free survival calculated from the time of randomization. The events considered were disease progression, death from any cause, and a second neoplasm, whichever occurred first. A test of the significance of the differences in event-free survival at three years was chosen over the customary log-rank statistic because there was a priori concern that the assumption of proportional hazards underlying the log-rank statistic might be invalid for two reasons. First, the initial randomization preceded transplantation by a median of 4.3 months. During this interval, patients

in both groups were treated identically and thus had identical risks. Second, it was anticipated that patients in the transplantation group would have a high risk of early, treatment-related death, even if event-free survival was ultimately better in this group than in the group assigned to receive continuation chemotherapy. The comparison of treatment regimens was performed according to the intention to treat.

RESULTS

Characteristics of the Patients

Of 560 patients who were assessed for the study, 539 were found to be eligible; of these, 379 patients participated in the first randomization, and 258 participated in the second randomization. Ineligible patients included 2 patients with incorrect diagnoses, 10 who did not meet the criteria for high-risk neuroblastoma, 1 with high bilirubin levels, and 8 for whom the procedures of the local institutional review board were inadequate. The stage of disease, age of the patients, sites of metastases, biologic features, and response to initial therapy were not significantly different between the group of patients assigned to transplantation and the group assigned to continuation chemotherapy in the first randomization or between the group assigned to 13-*cis*-retinoic acid and the group assigned to no further therapy in the second randomization (Table 1). The group that was nonrandomly assigned to chemotherapy differed from the randomized population only in the higher percentage of patients with stage III disease (21 percent vs. 12 percent, P=0.02) and the lower median age (2.6 vs. 3.1 years, P=0.01).

Randomization and Actual Treatment Received

Of 539 eligible patients, 190 were randomly assigned to continuation chemotherapy and 189 to transplantation, and 118 were nonrandomly assigned to continuation chemotherapy. The remaining 42 patients never underwent randomization, because of disease progression (16 patients), death (3), withdrawal of parental consent (8), withdrawal from the study at the request of their physicians (4), or substantial deviation from treatment (11). Of the 189 patients who were assigned to transplantation, 129 actually received a bone marrow transplant according to the protocol and before disease progression (median interval from study entry to transplantation, 190 days; range, 154 to 264). Of the 190 patients who were assigned to continuation chemotherapy, 150 actually received chemotherapy according to the protocol (median time from study entry to the start of chemotherapy, 178 days; range, 143 to 261). Of the 100 patients who did not receive the treatment to which they were randomly assigned, progressive disease developed in 52 before they could receive the assigned therapy (28 in the chemotherapy group and 24 in the transplantation group). Two patients died after randomization but before receiving the assigned treatment. The remaining 46 patients had a substan-

TABLE 1. CHARACTERISTICS OF THE PATIENTS.*

CHARACTERISTIC	FIRST RANDOMIZATION		SECOND RANDOMIZATION	
	TRANSPLAN-TATION (N=189)	CONTINUATION CHEMOTHERAPY (N=190)	13-*cis*-RETINOIC ACID (N=130)	NO THERAPY (N=128)
	number of patients			
Age at diagnosis				
<1 yr	5	4	2	4
1–2 yr	44	45	29	28
>2 yr	140	141	99	96
Stage of disease				
III	21†	24	23	17†
IV‡	168	166	107	111
Serum ferritin level				
<143 ng/ml	66	66	50	49
≥143 ng/ml	102	109	66	66
Unknown	21	15	14	13
Pathological findings on tumor analysis				
Favorable	6	9	8	4
Unfavorable	120	128	81	82
Unknown	63	53	41	42
Amplification of *MYCN*				
No	85	94	58	59
Yes	49	55	34	29
Unknown	55	41	38	40
Bone metastases				
Absent	65	84	57	50
Present	124	106	73	78
Immunocytologic analysis of bone marrow at diagnosis§				
Negative	28	38	27	20
Positive	92	92	55	64
Unknown	69	60	48	44
Initial response				
Complete	60	57	58	59
Very good partial response	38	35	28	28
Partial response	37	37	21	21
Stable disease or mixed response	14	20	13	7
Progressive disease	28	24	0	0
Unknown	12	17	10	13

*There were no significant differences between groups with respect to any characteristic after the first or second randomization (P>0.05).

†This number also includes the one patient who was more than one year of age who had stage II neuroblastoma with amplification of *MYCN*.
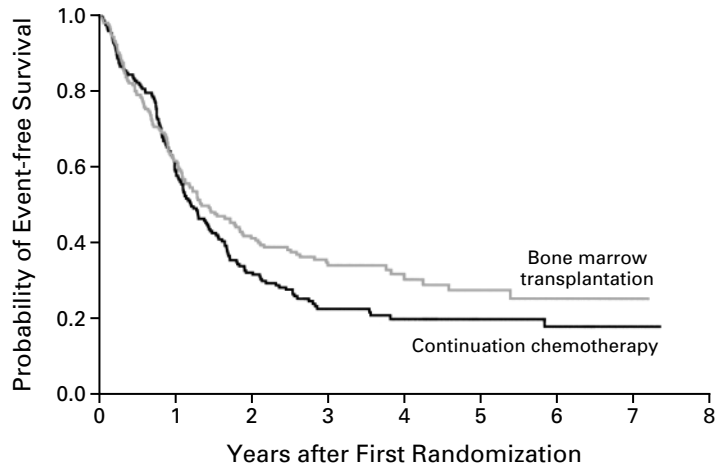
‡Included in the group with stage IV neuroblastoma are nine randomized patients (five in the transplantation group and four in the continuation-chemotherapy group) who were initially in stage I or II but in whom bone metastases developed before therapy other than surgery.

§The assay had a sensitivity of 1 tumor cell per 10^5 nucleated bone marrow cells.

tial deviation from the protocol before disease progression as a result of a decision by their parents or physicians (14 were assigned to chemotherapy and 32 to transplantation). Compliance with the protocols of the first randomization among patients without progressive disease was 86 percent. Regardless of the actual subsequent treatment, all patients who underwent randomization were included in all analyses according to their assigned treatment.

A total of 319 patients completed cytotoxic therapy without disease progression. Of this group, 130 were randomly assigned at week 34 to receive 13-*cis*-retinoic acid and 128 to receive no further therapy. Fifty-two percent of the patients who were randomly assigned to receive transplantation underwent the second randomization; 55 percent of those who were randomly assigned to chemotherapy underwent the second randomization. The median times from transplantation or the beginning of continuation chemotherapy to the beginning of 13-*cis*-retinoic acid therapy were 97 and 115 days, respectively. In addition, 37 patients were nonrandomly assigned to receive 13-*cis*-retinoic acid because of residual disease. The parents of 24 patients declined to enroll their children in the second phase, and these patients were excluded from the analysis. Compliance with the protocol of the second randomization was 98 percent; two patients in the 13-*cis*-retinoic acid group did not receive

Figure 2. Probability of Event-free Survival among Patients Assigned to Bone Marrow Transplantation or Continuation Chemotherapy.

Follow-up began at the time of the first randomization (eight weeks after diagnosis). The difference in survival between the two groups was significant at three years (P=0.034).

treatment according to the protocol, and four patients in the group assigned to no further treatment received 13-*cis*-retinoic acid.
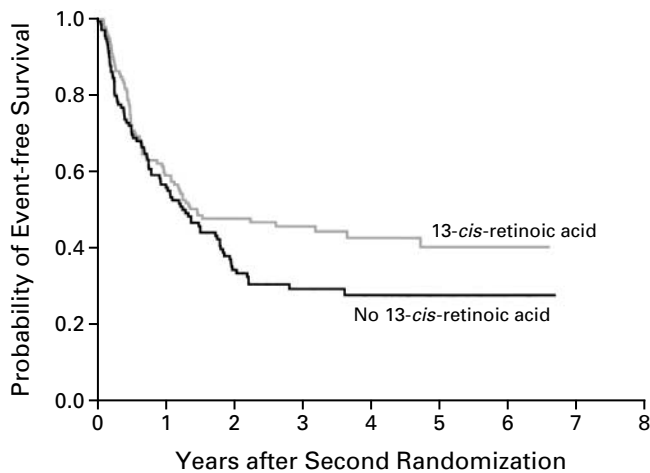
Outcomes of Autologous Bone Marrow Transplantation and Continuation Chemotherapy

The mean (±SE) event-free survival rate three years after diagnosis was 30±2 percent, and the overall survival rate during this period was 45±2 percent for all 539 eligible patients. The median duration of follow-up was 43 months (range, 2 to 89). For the 379 patients who underwent the first randomization, the three-year event-free survival rate was 28±3 percent, which was not significantly different from that for all patients, nor from that for the 118 patients who were nonrandomly assigned to chemotherapy (33±5 percent). The three-year event-free survival rate from the time of the first randomization was 34±4 percent among patients assigned to transplantation, a value that was significantly higher than the rate of 22±4 percent among those assigned to continuation chemotherapy (P=0.034) (Fig. 2). Overall survival in the two groups was not significantly different, with three-year estimates of 43±4 percent for patients assigned to transplantation and 44±4 percent for those assigned to chemotherapy (P=0.87). If event-free survival is analyzed only for the patients who actually received their assigned treatment, the three-year survival rate for the 129 patients who underwent transplantation was 43±6 percent, as compared with a rate of 27±5 percent for the patients who received continuation chemotherapy.

Effect of Subsequent Therapy with 13-*cis*-Retinoic Acid

The 3-year event-free survival rate (Fig. 3) of 46±6 percent for patients assigned to receive 13-*cis*-retinoic acid was significantly better than the rate of 29±5 percent for those assigned to no further therapy (P=0.027) (median follow-up, 36 months; range, 5 to 80). Overall survival was not significantly different between the two groups, with three-year estimates for patients assigned to 13-*cis*-retinoic acid and no further therapy of 56±6 percent and 50±6 percent, respectively (P=0.45).
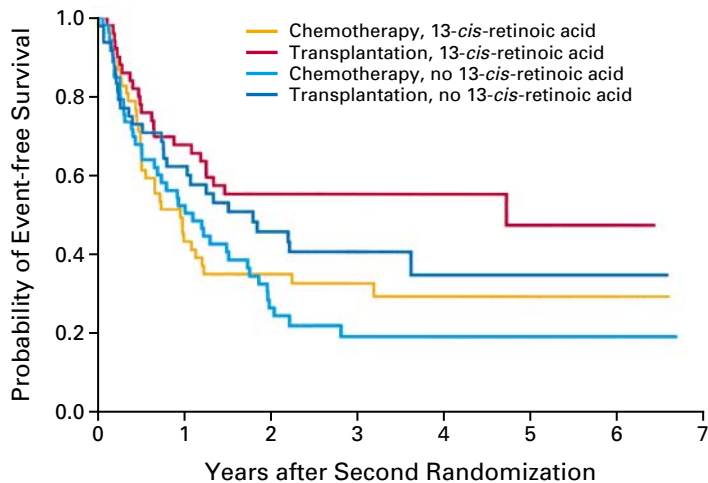
Treatment with 13-*cis*-retinoic acid appeared to benefit patients who received either a bone marrow transplant or continuation chemotherapy (Fig. 4). There was no evidence of an interaction between the second-randomization treatment and the first-randomization treatment (P=0.4). The estimated event-free survival three years after the second randomization for the patients assigned to receive transplantation followed by 13-*cis*-retinoic acid was 55±10 percent, as compared with 18±6 percent for those assigned to chemotherapy only. Since these event-free survival curves can only be shown from the time of the second randomization, not all patients from the first randomization were included. Caution in the interpretation of these results is therefore necessary. For the patients who were nonrandomly assigned to chemotherapy (data not shown) and then randomly assigned to receive 13-*cis*-retinoic acid, the three-year event-free survival rate was 53±11 percent and was not significantly different from the rate among those who were randomly assigned to no further therapy

Figure 3. Probability of Event-free Survival among Patients Assigned to Receive 13-*cis*-Retinoic Acid or No Further Treatment.

Follow-up began at the time of the second randomization (34 weeks after diagnosis). The difference in survival between the two groups was significant at three years (P=0.027).

Figure 4. Probability of Event-free Survival among Patients Who Entered Both Phases of the Study and Who Were Randomly Assigned to Receive a Bone Marrow Transplant plus 13-*cis*-Retinoic Acid, Transplant without 13-*cis*-Retinoic Acid, Continuation Chemotherapy plus 13-*cis*-Retinoic Acid, or Continuation Chemotherapy without 13-*cis*-Retinoic Acid.

Follow-up began at the time of the second randomization (34 weeks after diagnosis). Overall event-free survival was significantly better in the group treated with transplantation plus 13-*cis*-retinoic acid than in the group assigned to continuation chemotherapy without 13-*cis*-retinoic acid (P=0.02).

after nonrandom assignment to chemotherapy (31±11 percent, P=0.13).

Prognostic Factors

For all eligible patients, univariate analysis revealed these adverse prognostic factors: stage IV disease (relative risk of death as compared with patients with stage I, II, or III disease, 2.4; 95 percent confidence interval, 1.7 to 3.4), amplification of *MYCN* (relative risk as compared with patients without *MYCN* amplification, 1.3; 95 percent confidence interval, 1.2 to 1.5), unfavorable histopathological findings (relative risk as compared with patients with no unfavorable findings, 1.2; 95 percent confidence interval, 1.1 to 1.4), a serum ferritin level of at least 143 ng per milliliter (relative risk as compared with patients with a serum ferritin level of less than 143 ng per milliliter, 1.4; 95 percent confidence interval, 1.2 to 1.6), and a partial response to initial chemotherapy (relative risk as compared with patients with a complete or nearly complete response, 1.3; 95 percent confidence interval, 1.02 to 1.7). Among patients with stage IV neuroblastoma, bone metastases at diagnosis (relative risk, 1.3; 95 percent confidence interval, 1.04 to 1.6) and the presence at diagnosis of more than 100 tumor cells per 10^5 normal nucleated bone marrow cells (relative risk, 1.2; 95 percent confidence interval, 1.1 to 1.4) were also adverse factors. The event-free survival among patients assigned to transplantation was greater in every subanalysis than that among patients assigned to chemotherapy, but it was most pronounced among the subgroup of patients who were older than two years at diagnosis (P=0.01) and among the subgroup of patients with amplification of *MYCN* (P=0.03). The improved outcome among patients with stage IV neuroblastoma who were assigned to receive 13-*cis*-retinoic acid was significant among the subgroup of patients in complete remission at the end of initial chemotherapy (P=0.03), but not among those in partial remission. For the subgroup of patients with stage IV neuroblastoma, the three-year event-free survival rate was 30±4 percent among those assigned to transplantation and 20±4 percent among those assigned to continuation chemotherapy (P=0.07); for those assigned to 13-*cis*-retinoic acid the rate was 40±6 percent, as compared with a rate of 25±5 percent for those assigned to no further therapy (P=0.09).

Treatment-Related Toxicity and Deaths

During initial therapy, grade 3 or 4 toxic effects (according to the common toxicity criteria of the National Cancer Institute), most frequently hematologic effects, occurred in 71 percent of patients. Sepsis occurred in 17 percent of patients. During continuation chemotherapy, grade 3 or 4 hematologic effects occurred in all patients. For patients who underwent bone marrow transplantation, the median time to hematologic recovery (as defined by an absolute neutrophil count of more than 500 per cubic millimeter) was 17 days. Serious infections and sepsis occurred in 52 percent and 28 percent of patients assigned to chemotherapy, respectively, and in 53 percent and 26 percent of patients assigned to transplantation, respectively. Grade 3 or 4 renal effects occurred in 8 percent of patients assigned to continuation chemotherapy, as compared with 18 percent of patients assigned to transplantation. Patients assigned to bone marrow transplantation had a higher incidence of interstitial pneumonitis (10 percent, as compared with 1 percent among those assigned to chemotherapy) and veno-occlusive disease (9 percent vs. 0 percent). The mean duration of hospitalization was 45 days during the 12-week regimen of continuation chemotherapy and 47 days in the group assigned to transplantation.

Grade 3 or 4 toxic effects, including elevations in aminotransferase levels (2 percent of patients), renal effects (2 percent), gastrointestinal effects (2 percent), skin effects (cheilitis, dryness, and rash; 2 percent), infection (12 percent), and hypercalcemia (1 percent), occurred in a total of 17 percent of patients who were randomly assigned to 13-*cis*-retinoic acid. The hematologic effects (9 percent of patients) lessened over time after the end of cytotoxic therapy and probably reflected preexisting myelosuppression. Hematuria, proteinuria, hypertension, and an elevated serum creatinine level (maximum, 1.8 mg per deciliter [159 μmol per liter]) developed in five patients, all of whom had undergone transplantation and had received 13-*cis*-retinoic acid. A syndrome of hypertension, hematuria, and proteinuria has been reported after autologous bone marrow transplantation without 13-*cis*-retinoic acid.[35-37]

Second malignant neoplasms (two leukemias and one clear-cell carcinoma) have occurred in three patients (one randomly assigned to transplantation, one randomly assigned to continuation chemotherapy, and one nonrandomly assigned to chemotherapy). None of these patients had received 13-*cis*-retinoic acid. Of the 323 patients who died, 301 had progressive disease and 22 died solely of treatment-related causes. The median time from relapse to death was shorter for the patients who were assigned to transplantation than for those assigned to chemotherapy (137±21 days vs. 255±49 days, P=0.005). Only 8 percent of patients survived more than three years after relapse. For the patients who underwent the first-randomization treatment, there were more treatment-related deaths from the transplantation therapy (9 of 129) than from the continuation chemotherapy (1 of 150, P=0.013). For the entire study period, however, the numbers of treatment-related deaths were not different statistically (transplantation group, 6 percent; chemotherapy group, 3 percent; P=0.32).

DISCUSSION

In this randomized study of the treatment of patients with high-risk neuroblastoma, we found that transplantation of purged autologous bone marrow resulted in a significant improvement in event-free survival as compared with intensive chemotherapy. Moreover, as compared with no further therapy, subsequent treatment with high-dose, pulsed 13-*cis*-retinoic acid also improved event-free survival. Eighty-five percent of the patients we studied had stage IV disease and were older than one year of age at diagnosis. The group of 129 children who actually underwent bone marrow transplantation had a significantly higher three-year event-free survival rate than the 150 patients who actually received continuation chemotherapy (P=0.027). Although analysis according to the treatment received is potentially biased because of self-selection, the results of this analysis and of the intention-to-treat analysis were generally consistent with each other and with the results of our previous nonrandomized study.[14]

Other reports of concomitant but not randomized comparisons of transplantation and chemotherapy have yielded mixed results.[12,38] A randomized study found an advantage for autologous transplantation, but it was limited by small numbers and the method of selection, since randomization did not occur until the end of induction chemotherapy.[39] In our study, significant adverse prognostic factors were similar to those previously reported.[2,14,22,24,40] The improvement in event-free survival among patients in the transplantation group was also observed in the subgroup of patients whose tumors had *MYCN* amplification or who were over two years of age.[14] The increased intensity of cytotoxic therapy beyond the usual nonmyeloablative dose may account for the better event-free survival in the transplantation group than in the continuation-chemotherapy group, which received repeated, lower doses of chemotherapy.

Although the three-year event-free survival rate was significantly improved by autologous bone marrow transplantation, overall survival was similar for both regimens. There was a significantly shorter interval between relapse and death among patients who relapsed after bone marrow transplantation than among those who relapsed after chemotherapy (P= 0.005). However, virtually all relapses in either group resulted in death. Therefore, overall survival in this study may ultimately parallel event-free survival.

In the second portion of the study, we examined the effect of treatment with 13-*cis*-retinoic acid after maximal reduction of the tumor with the use of chemotherapy, radiotherapy, and surgery, with or without transplantation. There was a significant improvement in event-free survival among children who were given 13-*cis*-retinoic acid, regardless of the type of prior therapy. Our results suggest that 13-*cis*-retinoic acid is most effective in patients with minimal resid-

ual disease, because it did not appear to be effective in patients with proven residual disease who were nonrandomly assigned to receive 13-*cis*-retinoic acid. The greatest effect of 13-*cis*-retinoic acid in patients with stage IV neuroblastoma was found among those who had an initial complete response.

To estimate conservatively the effect of combining transplantation and 13-*cis*-retinoic acid, we multiplied the 3-year event-free survival rate after the second randomization (approximately 3.7 years after diagnosis) by the fraction of randomized patients who reached the second randomization (55 percent in the chemotherapy group and 52 percent in the transplantation group). The resulting event-free survival rate 3.7 years after the first randomization would be 29±7 percent in the group that underwent transplantation and received 13-*cis*-retinoic acid therapy and 11±4 percent in the group that received chemotherapy alone (P=0.019). This estimate is conservative, because it ignores the possibility of continued event-free survival among patients who underwent the first but not the second randomization. If these patients are included (with the conservative assumption that treatment with 13-*cis*-retinoic acid would not have affected their outcome), the estimate of the 3.7-year event-free survival rate would increase to 38±6 percent in the group that underwent transplantation and received 13-*cis*-retinoic acid and to 17±4 percent in the group that received chemotherapy alone.

In conclusion, event-free survival among patients with high-risk neuroblastoma was significantly better with high-dose chemotherapy and radiotherapy followed by transplantation of purged autologous bone marrow than with chemotherapy alone. In addition, treatment with 13-*cis*-retinoic acid further improved the outcome among patients without progressive disease. These therapeutic approaches should form the basis for the treatment of patients with high-risk neuroblastoma.

APPENDIX

The following institutions and principal investigators participated in the study: *Group Operations Center, Arcadia, Calif.* — W.A. Bleyer, A. Khayat, H. Sather, M. Krailo, J. Buckley, D. Stram, and R. Sposto; *University of Michigan Medical Center, Ann Arbor* — R. Hutchinson; *University of California Medical Center, San Francisco* — K. Matthay; *University of Wisconsin*

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