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Anti-GD2 Antibody with GM-CSF, Interleukin-2, and Isotretinoin for Neuroblastoma

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Abstract

BACKGROUND—Preclinical and preliminary clinical data indicate that ch14.18, a monoclonal antibody against the tumor-associated disialoganglioside GD2, has activity against neuroblastoma and that such activity is enhanced when ch14.18 is combined with granulocyte–macrophage colony-stimulating factor (GM-CSF) or interleukin-2. We conducted a study to determine whether adding ch14.18, GM-CSF, and interleukin-2 to standard isotretinoin therapy after intensive multimodal therapy would improve outcomes in high-risk neuroblastoma.

METHODS—Patients with high-risk neuroblastoma who had a response to induction therapy and stem-cell transplantation were randomly assigned, in a 1:1 ratio, to receive standard therapy (six cycles of isotretinoin) or immunotherapy (six cycles of isotretinoin and five concomitant cycles of ch14.18 in combination with alternating GM-CSF and interleukin-2). Event-free survival and overall survival were compared between the immunotherapy group and the standard-therapy group, on an intention-to-treat basis.

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RESULTS—A total of 226 eligible patients were randomly assigned to a treatment group. In the immunotherapy group, a total of 52% of patients had pain of grade 3, 4, or 5, and 23% and 25% of patients had capillary leak syndrome and hypersensitivity reactions, respectively. With 61% of the number of expected events observed, the study met the criteria for early stopping owing to efficacy. The median duration of follow-up was 2.1 years. Immunotherapy was superior to standard therapy with regard to rates of event-free survival (66±5% vs. 46±5% at 2 years, P = 0.01) and overall survival (86±4% vs. 75±5% at 2 years, P = 0.02 without adjustment for interim analyses).

CONCLUSIONS—Immunotherapy with ch14.18, GM-CSF, and interleukin-2 was associated with a significantly improved outcome as compared with standard therapy in patients with high-risk neuroblastoma.

Neuroblastoma, a cancer of the sympathetic nervous system responsible for 12% of deaths associated with cancer in children under 15 years of age,[1] is a heterogeneous disease, with nearly 50% of patients having a high-risk phenotype characterized by widespread dissemination of the cancer and poor long-term survival, even if intensive multimodal treatments are used.[2] The initial results of the last randomized, controlled trial showing a significant improvement in outcomes were published over a decade ago[3,4] and established the standard therapy for high-risk neuroblastoma: myeloablative therapy with stem-cell rescue, followed by the treatment of minimal residual disease with isotretinoin. However, more than half the patients receiving standard therapy have a relapse and ultimately die from the tumor. Thus, once remission is achieved, the major obstacle to a cure is residual chemotherapy-refractory disease that eludes current methods of detection.

A promising approach to treating minimal residual disease is immunotherapy targeting a tumor-associated antigen, the disialoganglioside GD2, which is uniformly expressed by neuroblastomas, most melanomas, and some other tumors.[5,6] In normal human tissues, GD2 expression is restricted to neurons, skin melanocytes, and peripheral sensory nerve fibers.[7] The high expression of GD2 in neuroblastomas and its restricted distribution in normal tissues make anti-GD2 monoclonal antibodies potentially suitable for immunotherapy. A chimeric human–murine anti-GD2 monoclonal antibody[8] called ch14.18 has shown activity against neuroblastoma in preclinical studies[9] and early-phase clinical trials[10,11]; this activity could be enhanced when ch14.18 is used in combination with granulocyte–macrophage colony-stimulating factor (GM-CSF)[12,13] or interleukin-2[14–16] to augment antibody-dependent cell-mediated cytotoxicity. The feasibility of administering ch14.18 in combination with GM-CSF, interleukin-2, and isotretinoin during the early post-transplantation period has been shown in two sequential pilot phase 1 studies.[17,18] These paved the way for our study, the Children's Oncology Group (COG) ANBL0032 randomized phase 3 study, in which we tested whether adding immunotherapy (consisting of ch14.18 with GM-CSF and interleukin-2) to isotretinoin therapy, as compared with the use of isotretinoin alone, improves the survival of children with high-risk neuroblastoma that is in remission after myeloablative therapy and stem-cell rescue.

METHODS

STUDY DESIGN AND ENROLLMENT

The National Cancer Institute (NCI) was the sponsor of the study and also provided the ch14.18 monoclonal antibody. Bayer provided the GM-CSF. Neither the NCI nor Bayer had a role in the study design or analysis. The academic authors designed the study, collected and interpreted the data, prepared the manuscript, made the decision to submit the manuscript for publication, and vouch for the completeness and accuracy of the reported

data and analyses. All data were maintained by the COG Statistics and Data Center and were reviewed by the COG data and safety monitoring committee.

Patients were enrolled at COG institutions (listed in Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org) after approval by the local institutional review board and after the patients provided written informed consent or assent, when applicable. Randomized enrollment began on October 18, 2001, and ended on January 13, 2009. The study was performed in accordance with the study protocol.

PATIENTS

Eligible patients had high-risk neuroblastoma, defined strictly by the COG[2] and confirmed by means of review of clinical, pathological, and biologic features by the COG Neuroblastoma Biology Study Committee and local institutions, before study enrollment. Other eligibility requirements were an age at diagnosis of under 31 years; completion of induction therapy, autologous stem-cell transplantation, and radiotherapy; achievement of at least a partial response at the time of evaluation before autologous stem-cell transplantation; autologous stem-cell transplantation performed within 9 months after the initiation of induction therapy; enrollment between day 50 and day 100 after the final autologous stem-cell transplantation; absence of progressive disease; and adequate organ function and a life expectancy of at least 2 months. An additional eligibility criterion enforced early on in the study was the requirement for enrollment in the COG biology study (ANBL00B1).

Patients with biopsy-proven residual disease after autologous stem-cell transplantation were eligible for enrollment but not for randomization and were nonrandomly assigned to receive immunotherapy. They were excluded from the primary efficacy analysis. Previous data indicate that patients with residual disease have a poorer prognosis than those without residual disease.[4]

TREATMENT

Standard Therapy—Patients in the standard-therapy group received isotretinoin given at a dose of 160 mg per square meter of body-surface area per day, divided into two daily doses, for 14 consecutive days within each of six consecutive 28-day cycles.

Immunotherapy—Patients received ch14.18 at a dose of 25 mg per square meter per day for 4 consecutive days during each of five consecutive 4-week cycles. During the last 2 weeks in each of the five cycles, they also received isotretinoin at a dose of 160 mg per square meter per day (see the immunotherapy schedule in Fig. S1A in the Supplementary Appendix); this dose of isotretinoin was also given by itself during a final sixth cycle. During cycles 1, 3, and 5, GM-CSF (Leukine, Berlex) was given daily at a dose of 250 μg per square meter per day for 14 days, starting 3 days before ch14.18 was started (Fig. S1B in the Supplementary Appendix). During cycles 2 and 4, interleukin-2 (Proleukin, Chiron) was given, by means of continuous infusion, for 4 days during week 1 at a dose of 3.0×10^6 IU per square meter per day, as well as for 4 days during week 2 at a dose of 4.5×10^6 IU per square meter per day, concurrent with ch14.18 (Fig. S1C in the Supplementary Appendix).

STATISTICAL ANALYSIS

The primary analysis was an intention-to-treat comparison of event-free survival in the two treatment groups. The study was designed to enroll 386 randomly assigned patients, for a statistical power of 80% with a two-sided log-rank test at a level of 0.05 (or a one-sided test at a level of 0.025) to detect an absolute difference of 15 percentage points between the two groups in the 3-year estimate of event-free survival (50% in the standard-therapy group vs. 65% in the immunotherapy group). Sequential monitoring of the intention-to-treat

population was performed, and early stopping was considered if a significant difference between the two groups was found[19] (Fig. S2 in the Supplementary Appendix) or if the conditional power fell below 20%. The relative risk of an event was calculated for standard therapy as compared with immunotherapy on the basis of the 3-year estimate of event-free survival; under the alternative hypothesis, the relative risk is equal to 1.6. The Lan–DeMets[19] upper (efficacy) boundary was calculated with the spending function alpha×time^2, for a cumulative alpha level of 0.025. A total of 137 events was expected to be reported. A secondary analysis of overall survival in the intention-to-treat population, according to treatment group, was to be performed only if the two groups were found to differ significantly with regard to event-free survival.

For event-free survival, the time to an event was defined as the time from study enrollment (which occurred after transplantation) until the first occurrence of relapse, progressive disease, secondary cancer, or death or, if none of these events occurred, until the last contact with the patient. Overall survival was defined as the time from study enrollment until death or the last contact with the patient, if death did not occur during the study. Kaplan–Meier survival curves[20] were generated. Point estimates are reported as the estimate ±SE.[21]

Randomization occurred at the time of enrollment and was stratified on the basis of factors thought to potentially affect the post-transplantation outcome: the response before autologous stem-cell transplantation, induction-therapy protocol, number of transplantations of autologous stem cells, and purged versus nonpurged stem-cell infusion. Patients with biopsy-proven persistent disease after autologous stem-cell transplantation and radiotherapy were nonrandomly assigned to the immunotherapy group and were excluded from the primary outcome analyses.

We tested the comparability of the two treatment groups in terms of their known prognostic factors and stratification factors at the time of study enrollment by using a chi-square test. P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

CHARACTERISTICS OF STUDY PATIENTS

Of the 252 patients enrolled (Fig. 1), 1 patient was ineligible (the patient did not enroll in the COG biology study), and 25 patients with biopsy-proven persistent disease after autologous stem-cell transplantation were nonrandomly assigned to immunotherapy.

The remaining 226 patients were randomly assigned to receive immunotherapy (113 patients) or standard therapy (113 patients) and were included in the primary analysis. All 251 eligible patients were analyzed for toxic effects. The median duration of follow-up after randomization for patients who were alive and had not had a study event was 2.1 years (range, 4 days to 6.9 years; see the Supplementary Appendix for details). There were no significant differences between the two groups with respect to baseline characteristics (Table 1).

PRIMARY ANALYSIS ACCORDING TO RANDOMIZED TREATMENT GROUP

As of January 13, 2009, with 226 eligible patients enrolled and randomly assigned to a treatment group (of 386 anticipated) and 83 of the expected 137 events reported (61%), the COG data and safety monitoring committee determined that the study met the criteria for early stopping of the randomization, on the basis of the superiority of immunotherapy over standard therapy with regard to event-free survival (Fig. S2 in the Supplementary Appendix). The 2-year estimate for event-free survival was 66±5% in the immunotherapy group and 46±5% in the standard-therapy group (P = 0.01) (Fig. 2A). Immunotherapy was

also superior to standard therapy with regard to the estimated rate of overall survival (86±4% vs. 75±5% at 2 years, P = 0.02 without adjustment for interim analyses) (Fig. 2B).

The effect of immunotherapy on the subgroup of patients 1 year of age or older who had stage 4 disease, according to the International Neuroblastoma Staging System (INSS), at the time of diagnosis was also analyzed, since this subgroup accounts for the majority of high-risk cases (179 of 226 randomized patients). The rate of event-free survival was significantly greater in the immunotherapy group (63±6% at 2 years) than in the standard-therapy group (42±6% at 2 years, P = 0.02) (Fig. 2C). There was also a trend toward improved overall survival with immunotherapy (84±4% at 2 years) as compared with standard therapy (76±5% at 2 years, P = 0.10) (Fig. 2D).

PATIENTS NONRANDOMLY ASSIGNED TO RECEIVE IMMUNOTHERAPY

Twenty-five patients were nonrandomly assigned to undergo the immunotherapy regimen because of biopsy-proven residual disease after autologous stem-cell transplantation. The 2-year estimates for event-free survival and overall survival were 36±10% (16 events) and 76±9% (10 deaths, all disease-related), respectively (Fig. S3 in the Supplementary Appendix). The median duration of follow-up among the patients who did not have an event was 3.6 years (range, 1.0 to 6.7). All 25 patients were over 18 months of age at diagnosis, and 23 had INSS stage 4 disease; 6 tumors showed *MYCN* amplification, 16 had unfavorable histologic features, and 12 were diploid (see Table S2 in the Supplementary Appendix). A total of 21 of the 25 patients had a partial response before autologous stem-cell transplantation; only 1 of the 25 had undergone two autologous stem-cell transplantations (rather than one).

PROGNOSTIC FACTORS

Survival rates were compared between the two treatment groups on the basis of nine prognostic factors (Table 1). The event-free survival was worse in patients with disease of INSS stage 4 than in patients with disease of INSS stage 2, 3, or 4S (P = 0.003). Diploidy, representing normal tumor-cell DNA index, was predictive of worse overall survival than hyperdiploidy (P = 0.007). A complete or very good partial response, as compared with a partial response, before autologous stem-cell transplantation was predictive of improved event-free survival (P = 0.04) and overall survival (P=0.02). No other factors were significantly predictive of the outcome. Although randomization was not stratified according to INSS stage or tumor ploidy, the two treatment groups were balanced with respect to the number of patients with stage 4 disease (P = 0.93), diploid tumors (P = 0.33), and a complete or very good partial response before transplantation (P = 0.96) (Table 1); therefore, the treatment-group comparisons were not influenced by these factors.

TREATMENT-RELATED TOXIC EFFECTS AND DEATH

The immunotherapy regimen was associated with important treatment-related clinical toxic effects. The effects of most interest reported in the immunotherapy group were pain, hypotension, capillary leak syndrome, and hypersensitivity reactions (Tables 2 and 3), with relatively few toxic effects in the standardtherapy group. Pain of grade 3 or 4 was observed in 52% of patients (during 25% of 598 cycles of immunotherapy). Pain reactions in the immunotherapy group were most frequent during cycle 1, occurring in 37% of patients, and decreasing to 14% during cycle 5 (P<0.001) (Table 3). The most common site of pain was the abdomen. The capillary leak syndrome was reported in a total of 23% of patients, during 8% of immunotherapy cycles. It occurred more frequently during cycles 2 and 4, which involved interleukin-2, with incidences of 11% and 13%, respectively, as compared with 3 to 7% during courses involving GM-CSF (cycles 1, 3, and 5) (P = 0.06). Grade 3 or 4 hypersensitivity reactions were reported in 25% of patients, during 15% of immunotherapy

cycles. Hypersensitivity reactions were more frequent during the two cycles involving interleukin-2, with incidences of 26% and 25%, as compared with 5 to 12% during the three cycles involving GM-CSF (P = 0.001). Such reactions may be attributable to symptoms and signs that reflect both toxic effects of interleukin-2 and antibody-related hypersensitivity.

Other toxic effects that were common during immunotherapy cycles included fever (in 39% of patients), hypokalemia (35%), hyponatremia (23%), liver dysfunction (abnormal alanine aminotransferase level, 23%), hypotension (18%), diarrhea (13%), urticaria (13%), and hypoxia (13%). Early in the study, two patients were inadvertently given an overdose of the scheduled interleukin-2 (i.e., a dose >20 times the scheduled dose) due to a medication error; one of these patients died of interleukin-2–related capillary leak and pulmonary edema. No other treatment-related deaths were reported. All other toxic effects were self-limited and resolved soon after the cessation of treatment and well before the beginning of the subsequent treatment.

DISCUSSION

This randomized clinical trial tested the use of an immunotherapy regimen administered after autologous stem-cell transplantation, in order to enhance antibody-dependent cell-mediated cytotoxicity to GD2-positive tumor cells. The results indicate that the inclusion of the immunotherapy resulted in significantly superior event-free and overall survival. The rate of event-free survival during this study was superior in the immunotherapy group as compared with the standardtherapy group (66% vs. 46% at 2 years). The rate of overall survival was also superior with immunotherapy (85% at 2 years). At the time of this report, the data for overall survival had not yet met the stringent statistical criteria for early stopping that the data for event-free survival did, and the results are extremely unlikely to differ from those showing a benefit in event-free survival, though this is admittedly not out of the realm of statistical possibility. Even so, the 2-year estimate of event-free survival of 66% indicates that a substantial proportion of the 113 patients in the immunotherapy group had events (1 died from an interleukin-2 overdose, and 32 had a relapse, 18 of whom died after the relapse). Regarding the patients who were still alive after relapse, previous studies indicate that children with recurrent or progressive disease are rarely cured.[22]

Not surprisingly, immunotherapy was more effective in patients with minimal, rather than substantial, residual disease: the outcome was superior among patients who had been randomly assigned to a treatment group than among those nonrandomly assigned to receive immunotherapy for residual disease. Thus, despite the significant improvement in the rates of event-free survival and overall survival with this immunotherapy regimen, there is need for further improvement in treatment.

Though the use of the ch14.18 monoclonal antibody in combination with cytokines is associated with important toxic effects, these effects differ in scope, type, and duration from the myelosuppressive, renal, and gastrointestinal toxic effects of chemotherapy regimens used during the induction and consolidation phases of treatment.[10,11] The toxic effects seen with the immunotherapy regimen used in our study were expected and were primarily attributable to antibody binding to GD2 expressed on normal nerve cells,[23,24] to cytokine-mediated capillary leak,[25] or to hypersensitivity reactions associated with the ch14.18 antibody or cytokines. These toxic effects may also reflect the proposed mechanism of action of this combination: effector functions induced by the monoclonal antibody, including complement activation, and distinct pathways of antibody-dependent cell-mediated cytotoxicity mediated by natural killer cells,[16] neutrophils[26,27] and monocytes.[28]

The immunotherapy regimen tested in this study was based on several considerations and preclinical data. Antibody-dependent cell-mediated cytotoxicity is often depressed in patients with cancer,[29] and antibody-dependent cell-mediated cytotoxicity modulated by various effector cells can be augmented by independent cytokines, namely GM-CSF and interleukin-2. These cytokines increase the number of granulocytes or macrophages and natural killer cells, respectively, and enhance their ch14.18-directed antibody-dependent cell-mediated cytotoxicity.[13] The feasibility of combining anti-GD2 monoclonal antibodies with cytokines was shown in a Pediatric Oncology Group phase 2 trial of ch14.18 and GM-CSF[12] and a Children's Cancer Group phase 1 study of 14.G2a and interleukin-2.[30] Another consideration was that greater clinical effects would be seen if immunotherapy was given in patients with minimal residual disease.[31] This hypothesis is consistent with the relatively small number of complete or partial responses to anti-GD2 monoclonal antibodies (administered with or without cytokines) in children who have a relapse of neuroblastoma and adults who have melanoma with bulky disease.[10–12,14]

In patients with newly diagnosed high-risk neuroblastoma, we chose to achieve minimal residual disease through the use of conventional induction therapy and intensive consolidation therapy with autologous stem-cell transplantation. Providing anti-GD2 antibody with cytokines after autologous stem-cell transplantation may also promote immune-cell activation and elimination of immunosuppression, a concept being tested in separate ongoing studies of cell-mediated cancer immunotherapy.[32] Our two small, sequential pilot phase 1 studies of ch14.18 in combination with GM-CSF or with GM-CSF and interleukin-2[17,18] showed the feasibility of giving ch14.18 with these cytokines during the early post-transplantation period. The second of these studies showed a 3-year estimate of overall survival of 78%, reflecting a benefit in a comparison with historical controls,[17] a benefit now confirmed in the current randomized trial.

In a separate, nonrandomized study, Simon and colleagues performed a retrospective analysis involving 334 children with high-risk neuroblastoma.[33] All the children had completed initial induction therapy (with or without autologous stem-cell transplantation), and 166 received ch14.18 at doses similar to the dose used in the current study. In contrast to our results, there was no significant improvement in the rate of event-free survival or overall survival among children receiving ch14.18 as compared with those not receiving the antibody, although an updated analysis with a median follow-up period of 10.3 years (range, 2.3 to 17.7) indicated that ch14.18 may prevent late relapse.[34] Although our study differs from the study by Simon and colleagues with respect to the dosing schedule (six cycles of immunotherapy, with cycles every other month, vs. five cycles, with cycles every month) and the timing of the start immunotherapy (within 100 days after autologous stem-cell transplantation vs. a range of 39.5 to 343 days [median, 65.5 days]), the primary difference may be that our study included treatment with GM-CSF and interleukin-2 to activate antibody-dependent cell-mediated cytotoxicity and treatment with isotretinoin. The difference in outcome between the study by Simon and coworkers and our study therefore suggests, though does not prove, that the addition of GM-CSF and interleukin-2 augments the antibody-dependent cell-mediated cytotoxicity in vivo conferred by the ch14.18 monoclonal antibody and improves survival. Although we cannot entirely rule out the possibility that the observed therapeutic benefit was due to the cytokines alone, clinical studies showing the efficacy of interleukin-2 or GM-CSF monotherapy in patients with neuroblastoma are lacking.

Other tumor-reactive monoclonal antibodies being used or tested as cancer treatment can induce antibody-dependent cell-mediated cytotoxicity; these include rituximab, trastuzumab, and cetuximab.[35–37] To date, published clinical trials of regimens in which interleukin-2 or GM-CSF was added to these other monoclonal antibodies have not shown any benefit over

treatment with the monoclonal antibody alone.[38,39] However, these published studies have focused on treatment for refractory or relapsed disease. The results from our study suggest that the efficacy of ch14.18 used in combination with GM-CSF and interleukin-2 may be detected more readily when tested as adjuvant therapy or in patients with minimal residual disease. Our findings also suggest that protocol designs similar to that used in the COG study may be appropriate for testing of other monoclonal antibodies that mediate antibody-dependent cell-mediated cytotoxicity.

In summary, the addition of ch14.18, GM-CSF, and interleukin-2 to isotretinoin therapy was associated with improved event-free and overall survival among children with high-risk neuroblastoma who had a response to initial chemotherapy and received immunotherapy within 100 days after autologous stem-cell transplantation. Our data suggest that more routine use of this immunotherapy regimen for such patients may be beneficial. Future avenues of investigation include developing more effective and less toxic ways to stimulate ch14.18-mediated antibody-dependent cell-mediated cytotoxicity and identifying more efficacious GD2-targeted monoclonal antibodies[40] or genetically modified constructs targeting GD2.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Enrollment, Randomization, and Follow-up of the Study Patients
Patients receiving protocol therapy were still being treated with isotretinoin, with or without immunotherapy, at the time the data were analyzed.

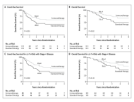

Figure 2. Kaplan–Meier Estimates of Survival among the 226 Study Patients Who Had Been Randomly Assigned, According to Treatment Group

Data are shown for event-free survival (Panel A) and overall survival (Panel B) for all 226 patients and for event-free survival (Panel C) and overall survival (Panel D) for the 179 patients 1 year of age or older at enrollment. The estimated survival (±SE) at 2 years is indicated in each plot.

Table 1

Characteristics of the 226 Study Patients at Baseline, According to Treatment Group, and Results of Analyses According to Treatment Group and Characteristics.*

Characteristic	Baseline Comparability			Outcomes				
	Standard Therapy (N = 113)	Immunotherapy (N = 113)	P Value	No. of Patients (N = 226)	2-Yr Event-free Survival	P Value	2-Yr Overall Survival	P Value
	no. (%)			*no. (%)*	*%*		*%*	
Treatment group						0.01		0.02
Immunotherapy				113 (50)	66±5		86±4	
Standard therapy				113 (50)	46±5		75±5	
Age			1.00			0.34		0.89
<18 Mo	4 (4)	4 (4)		8 (4)	73±17		73±17	
≥18 Mo	109 (96)	109 (96)		218 (96)	55±4		81±3	
INSS stage†			0.93			0.003		0.12
2	0	4 (4)		4 (2)	87±7		85±8	
3	16 (15)	10 (10)		26 (12)				
4S‡	0	2 (2)		2 (1)				
4	92 (85)	89 (85)		181 (85)	52±4		80±3	
Unknown	5	8		13				
Tumor *MYCN* status			0.42			0.29		0.19
Not amplified	51 (53)	52 (59)		103 (56)	63±6		86±4	
Amplified	45 (47)	36 (41)		81 (44)	53±6		73±6	
Unknown§	17	25		42				
Tumor histologic features			0.94			0.10		0.13

Characteristic	Baseline Comparability			Outcomes			
	Standard Therapy (N = 113)	Immunotherapy (N = 113)	P Value	No. of Patients (N = 226)	2-Yr Event-free Survival	P Value	2-Yr Overall Survival	P Value
	no. (%)			*no. (%)*	*%*		*%*	
Favorable	5 (6)	4 (6)		9 (6)	89±10		100	
Unfavorable	81 (94)	68 (94)		149 (94)	56±5		81±4	
Unknown	27	41		68				
Tumor ploidy			0.33			0.16		0.007
Hyperdiploid	48 (51)	49 (58)		97 (54)	62±6		85±4	
Diploid	46 (49)	35 (42)		81 (46)	48±6		72±5	
Unknown	19	29		48				
Response before ASCT¶			0.96			0.04		0.02
Complete response	38 (34)	40 (35)		78 (35)	61±7		86±5	
Very good partial response	49 (43)	47 (42)		96 (42)	59±6		83±4	
Partial response	26 (23)	26 (23)		52 (23)	45±8		67±8	
No. of ASCTs			0.31			0.80		0.62
1	102 (90)	107 (95)		209 (92)	57±4		80±3	
2‖	11 (10)	6 (5)		17 (8)	83±11		75±22	
No. of purged infusions			0.79			0.34		0.91
≥1	29 (33)	28 (31)		57 (32)	65±7		83±5	
0	58 (67)	61 (69)		119 (68)	56±5		81±4	
Unknown	26	24		50				

*Plus–minus values are survival estimates ±SE. Percentages and P values were calculated on the basis of patients with data for the given characteristic (with patients with “unknown” status not included). P values were calculated with the use of the chi-square test for the baseline characteristics and the log-rank test for the analyses of survival.

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[†] All P values for International Neuroblastoma Staging System (INSS) stage are reported for stage 4 versus stage 2, 3, or 4S.

[‡] The two patients with an INSS stage of 4S had neuroblastoma considered to be high risk because of *MYCN* amplification.

[§] Since obtaining a tumor specimen for purposes of ascertaining *MYCN* status was not an eligibility requirement, this information was unavailable for some patients.

[¶] All P values for response before autologous stem-cell transplantation (ACST) are reported for complete response or very good partial response versus partial response.

[||] For patients who underwent two ASCTs, the maximum duration of follow-up with regard to the rates of survival was 1.5 years.

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Table 2

Toxic Effects of Grade 3 or 4, According to Treatment Group.*

Toxic Effect	Immunotherapy (N = 137)	Standard Therapy (N = 108)
	number of patients (percent)	
Neuropathic pain	71 (52)	6 (6)
Hypotension	24 (18)	0
Hypoxia	18 (13)	2 (2)
Fever without neutropenia	53 (39)	6 (6)
Acute capillary leak syndrome	31 (23)	0
Hypersensitivity reaction	34 (25)	1 (1)
Urticaria	18 (13)	0
Infection (any)	54 (39)	24 (22)
Infection, catheter related	18 (13)	7 (7)
Nausea	4 (3)	1 (1)
Vomiting	8 (6)	3 (3)
Diarrhea	18 (13)	1 (1)
Hyponatremia	31 (23)	4 (4)
Hypokalemia	48 (35)	2 (2)
Abnormal ALT†	31 (23)	3 (3)
Abnormal AST†	14 (10)	0
Hypercalcemia	7 (5)	6 (6)
Serum sickness	1 (1)	0
Ocular symptoms	0	1 (1)
Seizure	1 (1)	1 (1)
CNS cortical symptom‡	5 (4)	0
None	8 (6)	40 (37)

*Six patients (one in the immunotherapy group and five in the standard-therapy group) could not be evaluated for toxic effects: four withdrew consent before the start of treatment, and two did not report any data. Although a patient may have reported a given toxic effect multiple times, only the worst grade of toxic effect per patient per type is given. Grade 5 toxic effects occurred in one patient only; the patient died from capillary leak syndrome owing to an interleukin-2 overdose. Grade 3 pain refers to pain or severe pain or the use of analgesics severely interfering with the activities of daily living; grade 4 pain refers to disabling pain.

† Grade 3 elevations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were defined as levels that were 5 to 20 times the upper limit of the normal range, and grade 4 elevations as levels that were more than 20 times the upper limit of the normal range. Toxicities were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (CTCAE V3).

‡ Central nervous system (CNS) cortical symptoms were encephalopathy, confusion, and psychosis.

Table 3

Toxic Effects of Grade 3 or 4 in Patients Randomly or Nonrandomly Assigned to Receive Immunotherapy, According to Immunotherapy Cycle.*

Toxic Effect	Cycle 1 (N = 137)	Cycle 2 (N = 127)	Cycle 3 (N = 121)	Cycle 4 (N = 114)	Cycle 5 (N = 107)	Cycle 6 (N = 104)
	number of patients (percent)					
Pain	50 (37)	30 (24)	23 (19)	33 (29)	15 (14)	4 (4)
Hypersensitivity reaction	14 (10)	33 (26)	6 (5)	29 (25)	13 (12)	3 (3)
Capillary leak syndrome	9 (7)	14 (11)	8 (7)	15 (13)	3 (3)	0

*The monoclonal antibody ch14.18 was given in cycles 1 through 5; granulocyte–macrophage colony-stimulating factor was given in cycles 1, 3, and 5; interleukin-2 was given in cycles 2 and 4; and isotretinoin was given in all six cycles. Grade 5 toxic effects occurred in one patient only; the patient died from capillary leak syndrome owing to an interleukin-2 overdose. Grade 3 pain refers to pain or severe pain or the use of analgesics severely interfering with the activities of daily living; grade 4 pain refers to disabling pain. For details about the doses and agents given in each cycle, see Figure S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org.