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Outcomes Following GD2-Directed Postconsolidation Therapy for Neuroblastoma After Cessation of Random Assignment on ANBL0032: A Report From the Children's Oncology Group

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abstract

PURPOSE Postconsolidation immunotherapy including dinutuximab, granulocyte-macrophage colony-stimulating factor, and interleukin-2 improved outcomes for patients with high-risk neuroblastoma enrolled on the randomized portion of Children's Oncology Group study ANBL0032. After random assignment ended, all patients were assigned to immunotherapy. Survival and toxicities were assessed.

PATIENTS AND METHODS Patients with a pre-autologous stem cell transplant (ASCT) response (excluding bone marrow) of partial response or better were eligible. Demographics, stage, tumor biology, pre-ASCT response, and adverse events were summarized using descriptive statistics. Event-free survival (EFS) and overall survival (OS) from time of enrollment (up to day +200 from last ASCT) were evaluated.

RESULTS From 2009 to 2015, 1,183 patients were treated. Five-year EFS and OS for the entire cohort were 61.1 ± 1.9% and 71.9 ± 1.7%, respectively. For patients ≥ 18 months old at diagnosis with International Neuroblastoma Staging System stage 4 disease (n = 662) 5-year EFS and OS were 57.0 ± 2.4% and 70.9 ± 2.2%, respectively. EFS was superior for patients with complete response/very good partial response pre-ASCT compared with those with PR (5-year EFS: 64.2 ± 2.2% *v* 55.4 ± 3.2%, $P = .0133$); however, OS was not significantly different. Allergic reactions, capillary leak, fever, and hypotension were more frequent during interleukin-2–containing cycles than granulocyte-macrophage colony-stimulating factor–containing cycles ($P < .0001$). EFS was superior in patients with higher peak dinutuximab levels during cycle 1 ($P = .034$) and those with a high affinity FCGR3A genotype ($P = .0418$). Human antichimeric antibody status did not correlate with survival.

CONCLUSION Analysis of a cohort assigned to immunotherapy after cessation of random assignment on ANBL0032 confirmed previously described survival and toxicity outcomes. EFS was highest among patients with end-induction complete response/very good partial response. Among patients with available data, higher dinutuximab levels and FCGR3A genotype were associated with superior EFS. These may be predictive biomarkers for dinutuximab therapy.

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ASSOCIATED CONTENT

Appendix

Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

Despite intensive therapy, outcomes for patients with high-risk neuroblastoma remain poor.[1,2] Randomized trials have shown that high-dose chemotherapy with autologous stem cell transplant (ASCT) results in improved event-free survival (EFS).[3,4] To eliminate minimal residual disease and prevent relapse following ASCT, randomized trials testing postconsolidation treatments have been conducted.[3,5,6] The Children's Oncology Group (COG) phase III trial ANBL0032 demonstrated that immunotherapy with dinutuximab, a chimeric antibody that targets the disialoganglioside GD2, in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 (IL-2), and isotretinoin in the postconsolidation setting improved EFS and overall survival (OS) for patients with high-risk neuroblastoma.[5] Two-year EFS for those randomly assigned to immunotherapy (n = 113) was 66 ± 5% compared with 46 ± 5% for those assigned to standard therapy (n = 113; $P = .01$); OS was 86 ± 4% versus 75 ± 5% ($P = .02$). These results led to early stopping of random assignment[5] and to approval of dinutuximab

CONTEXT

Key Objective

The seminal Children's Oncology Group phase III trial ANBL0032 demonstrated that the addition of immunotherapy with the anti-GD2 antibody dinutuximab with cytokines to isotretinoin in the postconsolidation setting improved event-free survival (EFS) and overall survival for patients with high-risk neuroblastoma. The immunotherapy arm remained open after cessation of random assignment to refine survival estimates and obtain additional toxicity and correlative biology data. This report describes the largest cohort of patients with high-risk neuroblastoma treated with dinutuximab therapy to date (n = 1,183).

Knowledge Generated

EFS and overall survival in this cohort were similar to those reported for the randomized cohort despite less stringent eligibility criteria, and toxicities were similar. Among the subset of patients with available data, higher dinutuximab levels and FCGR3A genotype were associated with superior EFS.

Relevance

The results of this study of GD2-directed postconsolidation therapy confirm the importance of immunotherapy for the frontline treatment of patients with high-risk neuroblastoma.

for frontline therapy.[7] After random assignment was halted, the immunotherapy arm remained open to refine survival estimates and obtain additional toxicity and correlative biology data.

PATIENTS AND METHODS

Study Design and Participants

The design of ANBL0032 has been previously described.[5,8] After halting of random assignment, patients were non-randomly assigned to immunotherapy (April 30, 2009-July 31, 2015). The Protocol (online only) was approved by the institutional review boards at participating institutions. Written informed consent was obtained. The study was conducted in accordance with Good Clinical Practice principles, and the Declaration of Helsinki.

Eligibility criteria for enrollment after random assignment ended paralleled those previously reported[5] with a few exceptions. Enrollment was permitted up to day +200 from last ASCT, and biopsy-proven residual disease was not required for assignment to immunotherapy. Patients had to achieve a pre-ASCT response of complete response (CR), very good partial response (VGPR), or partial response (PR) by 1993 International Neuroblastoma Response Criteria[9] for primary site, soft tissue metastases and bone metastases. However, patients could have ≤ 10% tumor from a bone marrow aspirate/biopsy or newly detected marrow disease if the extent of tumor involvement was ≤ 10% (ie, patients could have an overall response < PR on the basis of protocol-specified criteria for marrow response).

Procedures

The treatment schema is summarized (Appendix Fig A1, online only). Dinutuximab was initially manufactured by the National Cancer Institute and administered intravenously over 10 hours (25 mg/m^2 once per day; maximum infusion duration per dose: 20 hours) for 4 consecutive days during cycles 1-5. After January 21, 2014, dinutuximab was manufactured by United Therapeutics Corporation, and 17.5 mg/m^2 once per day was administered on the same schedule. This modification reflected the change from a theoretical extinction coefficient (1.00) to a calculated extinction coefficient (1.41). Both products contained the same amount of active protein and were comparable in a phase II bioequivalence study.[10] GM-CSF was administered during cycles 1, 3, and 5; IL-2 was administered during cycles 2 and 4.[5] During the last 2 weeks of all cycles, patients received isotretinoin orally (80 mg/m^2/dose twice daily). Cycle 6 consisted of isotretinoin alone. Disease evaluations were performed as described.[5] Response was assessed using the 1993 International Neuroblastoma Response Criteria.[9]

Outcomes

Study end points included EFS and OS and Common Terminology Criteria for Adverse Events v4.0 grade ≥ 3 adverse events.

Statistical Analyses

Demographics, International Neuroblastoma Staging System stage, tumor biology, pre-ASCT response, ASCT number, dose modifications, days from last ASCT to enrollment, and grade ≥ 3 adverse events were summarized using descriptive statistics. Patient characteristics were compared with the cohort randomly assigned to receive immunotherapy[5] using a chi-squared test or Fisher's exact test for categorical variables, and a Wilcoxon rank-sum test for continuous variables. Rates of occurrence of toxicities in GM-CSF– and IL-2–containing cycles were compared using McNemar's test for paired observations and

adjusted for multiple comparisons with Bonferroni's correction. P values $< .05$ were considered statistically significant.

EFS was measured from time of enrollment to first occurrence of relapse, disease, progression, or death, or censored at last contact if an aforementioned event did not occur. OS was measured from time of enrollment to death or censored at last contact if death did not occur. Five-year EFS and OS estimates (estimate ± SE) and Kaplan-Meier survival curves were compared with a log-rank test.

Multivariable Cox proportional hazards (PH) models were fit for EFS and OS using the Efron method of handling tied event times and included standard risk factors (age at high-risk diagnosis, stage, *MYCN* status, histology, and ploidy) and pre-ASCT response, response after consolidation, number of transplants, and whether all six cycles of ANBL0032 therapy were completed. Backward selection with a P value threshold of .05 was used to arrive at the final model.

The association between the occurrence of a dose-limiting toxicity during treatment and human antichimeric antibody (HACA) positivity was tested with a chi-square test.

Correlative Biology Methods

Dinutuximab and HACA assays. Dinutuximab peak concentrations and HACA values were provided by United Therapeutics Corporation via assays performed by Bio-Agilytix (Durham, NC) for 262/286 patients who received National Cancer Institute–produced dinutuximab and provided blood samples. Dinutuximab peak concentrations (Cmax) were assessed before starting day 4 dinutuximab infusions during cycle 1 and cycle 4 or 5. Plasma dinutuximab levels were performed using a good laboratory practice–validated Meso Scale Discovery electrochemiluminescence immunoassay; lower limit of detection was 100 ng/mL. HACA titers were measured before cycles 1, 4, and 5 using screening, confirmatory, and titer assays. To be evaluable for HACA, patients had to have ≥ 1 evaluable sample obtained following initiation of therapy. If any sample was found to be positive for HACA, the patient was designated HACA-positive (HACA+). A master mix of biotinylated dinutuximab and ruthenium-conjugated dinutuximab in assay buffer was added to dilutions of patient sera, incubated, and added to streptavidin-coated plates. Following testing for luminescence, titers were reported as the reciprocal of the last dilution above the cutpoint. Wilcoxon rank-sum tests compared median Cmax levels and Cmax ratios in HACA+ versus HACA– patients. Cox PH models tested for association between survival and cycle 1 Cmax.

Antibody-dependent cell-mediated cytotoxicity, Fc gamma receptor genotyping, and natural killer protein 30 isoform profiling. **Antibody-dependent cell-mediated cytotoxicity.** Antibody-dependent cell-mediated cytotoxicity (ADCC) of peripheral blood mononuclear cells against neuroblastoma cell line NMB-7 was assessed at baseline and before cycle 4 using a previously described chromium[51] release assay.[11] Percentage lysis is expressed by lytic unit (number of effector cells required to obtain 20% target cell lysis), determined using the exponential fit equation.[12] For survival analyses involving ADCC before cycle 4, EFS and OS time were measured from the start of cycle 4. Cox PH models tested for association between survival and ADCC levels.

FCGR **polymorphisms.** Regions surrounding the polymorphic codon 158 of FcγRIIIA (FCGR3A, rs396991) and codon 131 of FcγRIIA (FCGR2A, rs1801274) were selectively amplified, purified, and genotyped by direct sequence analysis, as described previously.[8]

NCR3 (rs986475) genotyping and isoform prediction. RNA and DNA were used to predict isoform profile of natural cytotoxicity receptors (NCRs). Isoform quantifications were performed by real-time polymerase chain reaction using natural killer protein 30 (NKp30) or beta 2 microglobulin primers,[13] with expression determined using the $2^{-\Delta\Delta Ct}$ algorithm. Relative isoform quantity was measured as a percentage of the total of A, B, and C isoforms. Patients harbored an immunosuppressive profile when isoform C (usually < 10% of total) was ≥ 25%.

NCR3 Single Nucleotide Polymorphism (SNP) rs986475 (correlates with isoform expression). Genomic DNA was amplified with: NCR3-U3F, 5′-CTGAACTTTCCCTTCCACCA-3′; NCR3-U3R, 5′-GGTCCAGCCAGTAAAAACCA-3′, and sequenced with: NCR3-U3sF, 5′-TGTCCTGAGAAATGGGAAGG-3′; NCR3-U3sR, 5′-CAGTAAAAACCATGGTCCCC-3′.

RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. After cessation of random assignment, 1,192 patients were enrolled; 1,183 eligible patients received immunotherapy (Fig 1). Most were ≥ 18 months old at diagnosis (n = 1,009; 85.3%) and had stage 4 disease (n = 765 of 921; 83.1%). Among patients with known tumor biology, 45.1% (n = 363/805) had tumors that were *MYCN*-amplified, 94.5% (n = 749/793) had tumors with unfavorable histology, and 54.9% (n = 397/723) had diploid tumors. Pre-ASCT response (excluding bone marrow) included CR (n = 352; 29.8%), VGPR (n = 418; 35.3%), or PR (n = 413; 34.9%). Most patients had undergone single (n = 1,042; 88.1%) rather than tandem (n = 141; 11.9%) ASCT. Because of protocol-specific bone marrow eligibility criteria, 29 had < PR pre-ASCT overall responses (progressive disease n = 3, no response n = 1, and mixed response n = 25).

There were no significant differences in stage ($P = .6302$), tumor *MYCN* status ($P = .4030$), or histology ($P = 1.000$) in this cohort compared with the randomized cohort that received immunotherapy; however, diploidy was more

common in those treated after random assignment ended (54.9% *v* 42.4%, *P* = .0281). End-induction response differed between the cohorts, with a lower proportion enrolling with CR/VGPR after cessation of random assignment (65.1% *v* 77.2%). More patients had undergone tandem transplant in the current cohort compared with the randomized cohort, although the difference was not significant (11.9% *v* 6.1%, *P* = .0638). Time from last ASCT was longer among patients enrolled after random assignment ended (89 days [range, 40-197 days] *v* 73.5 days [range, 59.0-124.0 days], *P* < .0001).

Treatment

In total, 84.0% (n = 994) of patients completed all six cycles of therapy (Fig 1). The remaining patients completed one (n = 38; 3.2%), two (n = 47; 4.0%), three (n = 36; 3.0%), four (n = 37; 3.1%), and five (n = 31; 2.6%) cycles. Reasons for early therapy discontinuation are shown (Fig 1). Characteristics of the 101 (8.5%) patients who relapsed on therapy are shown in Appendix Table A1 (online only). IL-2 was replaced by GM-CSF in 1.9% of patients (n = 22). Data regarding 827 planned IL-2–containing cycles were available; GM-CSF replaced IL-2 in 23 cycles (2.8%).

Survival Outcomes

Among all patients who received immunotherapy after cessation of random assignment (n = 1,183), 2-year EFS and OS from time of enrollment were 69.4 ± 1.4% and 84.4 ± 1.1%, respectively. Five-year EFS and OS from time of enrollment were 61.1 ± 1.9% and 71.9 ± 1.7%, respectively (Fig 2A). Median follow-up time was 4.1 years (range, 0.005-10.000 years). Among stage 4 patients ≥ 18 months old at diagnosis (n = 662), 5-year EFS and OS were 57.0 ± 2.4% and 70.9 ± 2.2%, respectively (Fig 2B). For stage 3 disease (n = 110), 5-year EFS and OS were 82.3 ± 4.8% and 86.7 ± 4.2%, respectively (Fig 2C). Among stage 3 patients with *MYCN*-amplified (n = 51) and *MYCN*-nonamplified (n = 55) tumors, 5-year EFS and OS were 78.1 ± 7.5% and 81.6 ± 6.9% (Fig 2D) and 86.7 ± 6.2% and 92.2 ± 4.8% (Fig 2E), respectively.

EFS and OS by prognostic factors, pre-ASCT response, response after consolidation, number of transplants, and completion of therapy are summarized (Table 2). Stage 4 patients had inferior survival compared with non–stage 4 patients (5-year EFS: 58.4 ± 2.3% *v* 79.3 ± 4.3%, *P* = .0001; OS: 70.9 ± 2.1% *v* 84.5 ± 3.8%, *P* = .0005). EFS, but not OS, was significantly higher for patients with favorable versus unfavorable histology (5-year EFS: 82.8 ± 7.3% *v* 61.1 ± 2.3%, *P* = .0116; OS: 85.5 ± 6.8% *v* 72.8 ± 2.1%, *P* = .1241) and for patients with a pre-ASCT response (excluding marrow) of CR/VGPR versus PR (5-year EFS: 64.2 ± 2.2% *v* 55.4 ± 3.2%, *P* = .0133, Fig 3A; OS: 72.7 ± 2.1% *v* 70.5 ± 2.9%, *P* = .3811, Fig 3B). EFS for patients ≥ 18 months old at diagnosis was inferior to those < 18 months old, although the difference was not significant (5-year EFS: 59.7 ± 2.0% *v* 69.8 ± 4.9%, *P* = .0831). Although EFS did not differ on the basis of number of ASCTs (Fig 3C), there was a trend toward improved OS for tandem patients (5-year OS: 76.5 ± 3.8% *v* 71.2 ± 1.9%, *P* = .0704, Fig 3D). There was no difference in EFS on the basis of *MYCN* status. However, there was a trend toward improved OS for those with *MYCN*-nonamplified versus *MYCN*-amplified tumors (5-year OS, 76.4 ± 2.6% *v* 69.2 ± 3.1%, *P* = .0631). OS, but not EFS, was significantly worse among those who did not complete protocol therapy compared with those who did (5-year EFS: 56.2 ± 6.9% *v* 66.4 ± 1.9%, *P* = .0827; 5-year OS: 64.6 ± 6.6% *v* 78.0 ± 1.7%, *P* = .0056).

In the multivariable Cox model for EFS (n = 607 with complete data; Appendix Table A2, online only), patients with stage 4 (hazard ratio [HR], 1.93, *P* = .0023), unfavorable histology (HR, 3.61, *P* = .011), and PR pre-ASCT (HR, 1.62, *P* = .0008) had statistically significantly higher risk of event. For OS, patients with stage 4 (HR, 2.06, *P* = .0071), PR pre-ASCT (HR, 1.52, *P* = .0141), and one ASCT (HR, 1.72, *P* = .0435) had statistically significantly higher risk of death.

Toxicities

Adverse events monitored during the trial are summarized (Table 3; grade ≥ 3). Pain was most frequent during cycle 1 (23.7%). In cycles 2-5, grade ≥ 3 pain was reported in a higher percentage of patients during IL-2–containing cycles than GM-CSF–containing cycles (17.9% *v* 11.7%; *P* < .0001). Other toxicities reported more commonly during IL-2–containing cycles included allergic reaction (21.0% *v* 12.4%), capillary leak (11.0% *v* 5.2%), fever (33.6% *v* 15.5%), and hypotension (13.7% *v* 9.2%; *P* < .0001). Respiratory compromise and elevated creatinine were rare (< 5%); however, elevated creatinine was more frequent during IL-2–containing cycles (1.3% *v* 0.3%, *P* = .0116). Hematologic toxicities were more frequent during IL-2–containing cycles. No treatment-related deaths were reported.

Correlative Biology Studies

HACA and dinutuximab levels. HACA data were available from BioAgilytix for 262 patients (HACA+: n = 53; HACA–: n = 209). Characteristics of these patients were similar to those for whom HACA data were unavailable (n = 921), except that a higher proportion of the latter had a pre-ASCT response of CR/VGPR rather than PR and underwent single rather than tandem ASCT. The BioAgilytix system is more sensitive than the enzyme-linked immunosorbent assay used previously.[8] To avoid focusing on low HACA values unlikely to be clinically meaningful, previous studies called specimens positive only if levels were sufficiently above background detected in pretreatment specimens.[8] Eighteen patients considered weakly HACA+ by BioAgilytix were considered negative in this analysis (HACA+: n = 35; HACA–: n = 227). As noted in other trials,[14] fewer dose-limiting toxicities were seen in HACA+ than in

TABLE 1. Patient Characteristics

Characteristic	ANBL0032 Immunotherapy Cohort After Cessation of Random Assignment (N = 1,183), No. (%)	ANBL0032 Randomized Cohort,[5] Immunotherapy Arm (N = 114), No. (%)	P[a]
Age at diagnosis, months			NA
< 18	174 (14.7)[b]	13 (11.4)[c]	
≥ 18	1,009 (85.3)[b]	101 (88.6)[c]	
INSS stage			.6302 (non–stage 4 *v* stage 4)
1	5 (0.5)	0 (0.0)	
2	31 (3.4)	4 (3.8)	
3[d]	110 (11.9)	10 (9.4)	
4S	10 (1.1)	2 (1.9)	
4	765 (83.1)	90 (84.9)	
Unknown	262	8	
MYCN status			.4030
Not amplified	442 (54.9)	53 (59.6)	
Amplified	363 (45.1)	36 (40.4)	
Unknown	378	25	
Histology			1.0000[e]
Favorable	44 (5.5)	4 (5.1)	
Unfavorable	749 (94.5)	74 (94.9)	
Unknown	390	36	
Ploidy			.0281
Hyperdiploid	326 (45.1)	49 (57.6)	
Diploid	397 (54.9)	36 (42.4)	
Unknown	460	29	
Response before ASCT			NA
CR	352 (29.8)[f]	41 (36.0)	
VGPR	418 (35.3)[f]	47 (41.2)	
PR	413 (34.9)[f]	26 (22.8)	
No. of ASCTs			.0638
1	1,042 (88.1)	107 (93.9)	
2	141 (11.9)	7 (6.1)	
Dose modification dinutuximab (cycles 1-5)			.7397
Dose reduction	318 (26.9)	29 (25.4)	
No reduction	865 (73.1)	85 (74.6)	
Dose modification IL-2 (cycles 2 or 4)			1.0000[e]
Dose reduction	422 (36.9)	3 (33.3)	
No reduction	723 (63.1)	6 (66.7)	
Missing	38	105	
Time from last ASCT to enrollment			< .0001[g]
Days, median (range)	89.0 (40.0-197.0)	73.5 (59.0-124.0)	

Abbreviations: ASCT, autologous stem cell transplant, CR, complete response; IL-2, interleukin-2; INSS, International Neuroblastoma Staging System; NA, not applicable; PR, partial response; VGPR, very good partial response.

[a]Chi-square test unless otherwise specified. Unknown categories not included in *P* value calculations.

[b]Age at high-risk diagnosis.

[c]Age at initial diagnosis.

[d]Patients with INSS stage 3 disease and *MYCN*-amplified tumors were considered to have high-risk disease regardless of age, histology, or ploidy. Patients with INSS stage 3 disease and *MYCN*-nonamplified tumors with unfavorable histology were considered high-risk if they were ≥ 12 months (eg, A3973, ANBL02P1) or ≥ 18 months (eg, ANBL0532) of age, depending on the frontline regimen used.

[e]Fisher's exact test.

[f]Response before ASCT excluding bone marrow.

[g]Wilcoxon rank-sum test.

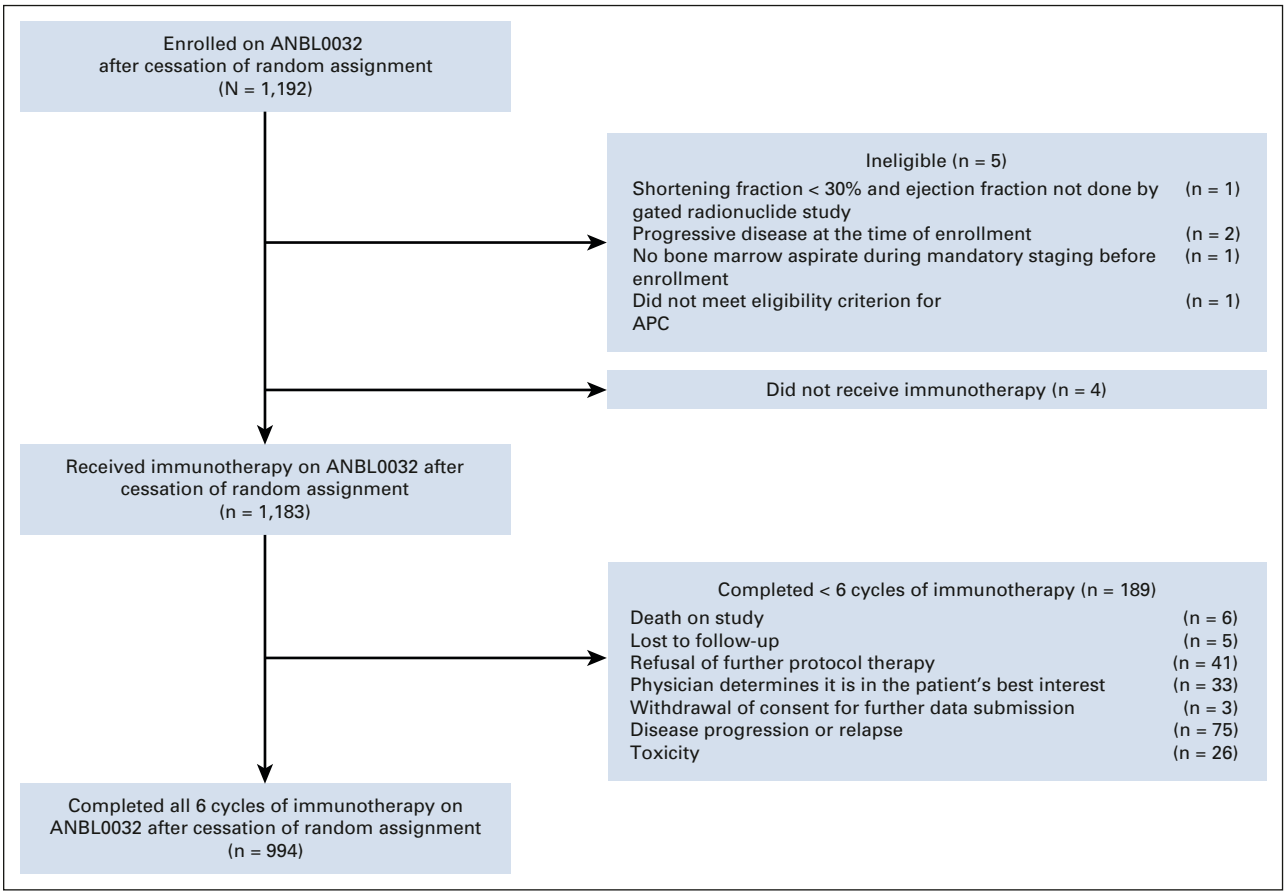

FIG 1. ANBL0032 immunotherapy cohort after cessation of random assignment. One thousand one hundred ninety-two patients enrolled and 1,183 patients nonrandomly received immunotherapy. Nine hundred ninety-four patients completed all six cycles of immunotherapy. APC, absolute phagocyte count.

HACA– patients (17.1% *v* 37.9%, $P = .0167$); however, EFS and OS did not differ ($P = .6327$ and .8531, respectively). HACA– patients showed no change in median peak dinutuximab levels from first to final cycles, whereas 34/35 HACA+ patients had detectible drops in peak dinutuximab levels ($P < .0001$), indicating an in vivo effect (Appendix Table A3, online only). Higher cycle 1 peak dinutuximab levels were associated with improved EFS ($P = .0341$) and a trend toward improved OS ($P = .0721$). A 5,000 ng/mL increase in cycle 1 Cmax was associated with a 33% lower risk of event.

Fc gamma receptor genotype, NKp30 isotype, and ADCC. In patients with genotyping data available (n = 262), frequencies of polymorphisms of FCGR2A codon 131 and FCGR3A codon 158 (Appendix Table A4, online only) are similar to those reported previously.[8,15] Among patients with high- (n = 49), low- (n = 68), or mixed- (n = 137) affinity FCGR2A genotypes, no differences in EFS or OS were identified. Although the number of patients with available samples was small, EFS was significantly better for patients with high-affinity FCGR3A genotype (n = 25) than those with low-/mixed-affinity genotypes (n = 221; $P = .0418$; Appendix Table A4, Appendix Fig A2A, online only). The difference in OS on the basis of FCGR3A genotype did not reach statistical significance ($P = .0806$; Appendix Fig A2B).

ADCC activities of peripheral blood mononuclear cells were evaluated at baseline (n = 74) and before starting cycle 4 IL-2 (n = 71). No relationship was found between ADCC and EFS/OS (Appendix Table A5, online only).

NKp30 genotype and transcript data were concordant in 37/39 (95%) patients for whom data were available. Most had an immunostimulatory NCR/NKp30 isoform (TT in SNP rs986475; Appendix Table A6, online only). Twenty-six patients had the immunosuppressive isoform on the basis of RNA isoform expression and/or by SNP (TC or CC). Only two harbored the homozygous immunosuppressive isoform (CC); these patients relapsed at 1.06 and 1.26 years after enrollment. NKp30 isoform was not correlated with EFS/OS.

DISCUSSION

This report describes the largest cohort of patients with high-risk neuroblastoma treated with anti-GD2 antibody therapy to date. Two- and 5-year EFS and OS were

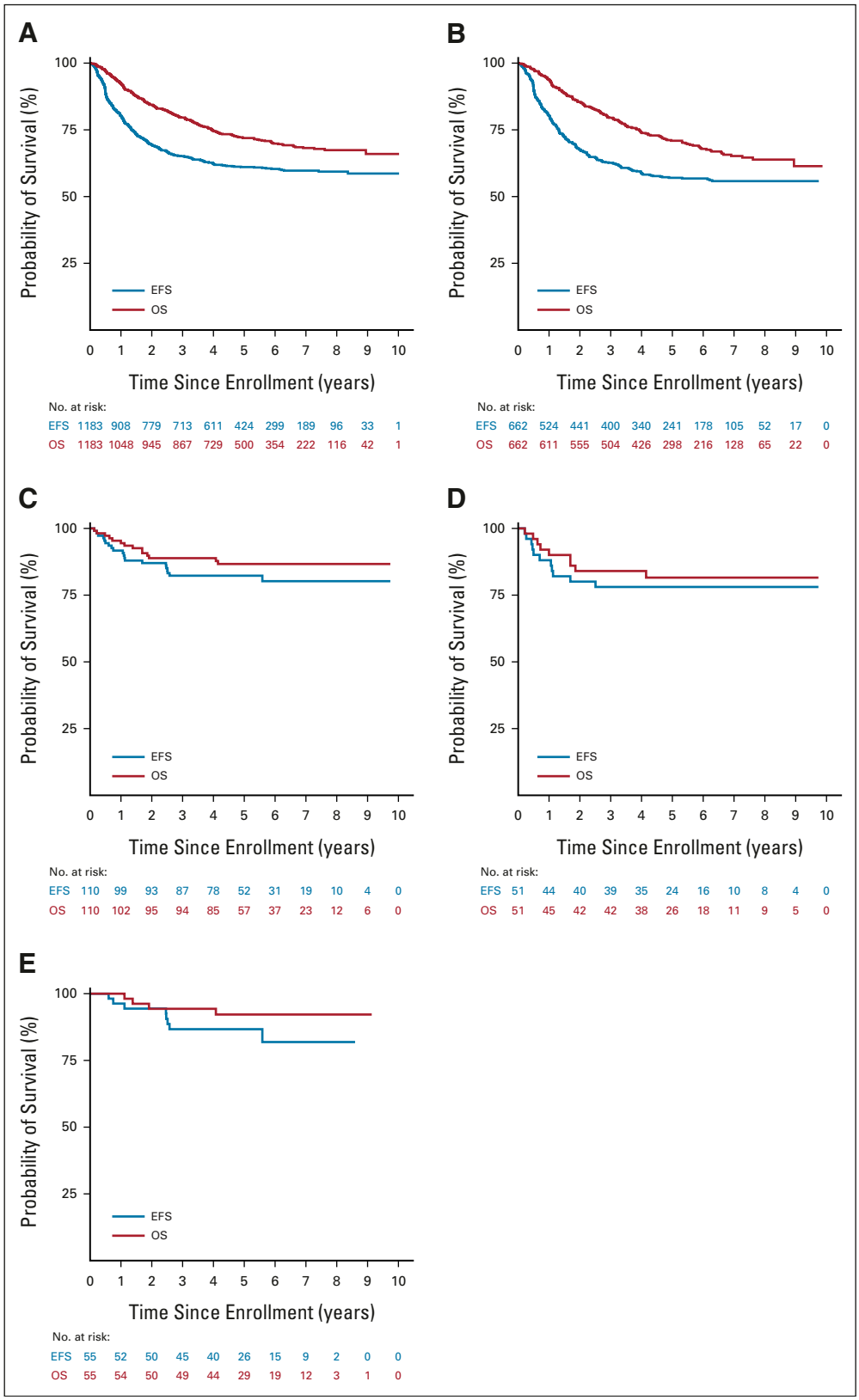

FIG 2. EFS and OS of the entire cohort and by patient characteristics and tumor biology: (A) entire cohort (n = 1,183), (B) patients with stage 4 disease and age ≥ 18 months at diagnosis (n = 662), (C) patients with stage 3 disease (n = 110), (D) patients with stage 3 disease and *MYCN*-amplified tumors (n = 51), and (E) patients with stage 3 disease and *MYCN*-nonamplified tumors (n = 55). EFS, event-free survival; OS, overall survival.

TABLE 2. EFS and OS by Patient Characteristics

Characteristic	No. (%)	5-Year EFS ± SE, %	*P*	5-Year OS ± SE, %	*P*
Age at high-risk diagnosis, months			.0831		.4307
< 18	174 (14.7)	69.8 ± 4.9		75.5 ± 4.6	
≥ 18	1,009 (85.3)	59.7 ± 2.0		71.4 ± 1.8	
INSS stage			.0001		.0005
1, 2, 3, 4S	156 (16.9)	79.3 ± 4.3		84.5 ± 3.8	
4	765 (83.1)	58.4 ± 2.3		70.9 ± 2.1	
MYCN status			.7855		.0631
Not amplified	442 (54.9)	61.1 ± 3.0		76.4 ± 2.6	
Amplified	363 (45.1)	62.0 ± 3.3		69.2 ± 3.1	
Histology			.0116		.1241
Favorable	44 (5.5)	82.8 ± 7.3		85.5 ± 6.8	
Unfavorable	749 (94.5)	61.1 ± 2.3		72.8 ± 2.1	
Ploidy			.3327		.4448
Hyperdiploid	326 (45.1)	63.2 ± 3.4		74.2 ± 3.1	
Diploid	397 (54.9)	60.3 ± 3.1		72.1 ± 2.9	
Response before ASCT[a]			.0133[b]		.3811[b]
CR/VGPR	770 (65.1)	64.2 ± 2.2		72.7 ± 2.1	
CR	352 (29.8)	69.6 ± 3.3		78.3 ± 3.0	
VGPR	418 (35.3)	59.7 ± 3.0		68.2 ± 2.9	
PR	413 (34.9)	55.4 ± 3.2		70.5 ± 2.9	
Response after consolidation			.0413[c]		.3721[c]
CR/VGPR	867 (73.3)	63.6 ± 2.1		72.7 ± 2.0	
CR	522 (44.1)	64.6 ± 2.7		74.0 ± 2.6	
VGPR	345 (29.2)	62.1 ± 3.4		70.8 ± 3.1	
PR	290 (24.5)	54.4 ± 3.9		70.2 ± 3.5	
MR/NR/PD	26 (2.2)	51.2 ± 14.6		65.1 ± 12.8	
MR	10 (0.8)	43.8 ± 23.2		60.0 ± 21.9	
NR	4 (0.3)	66.7 ± 38.5		100.0 ± 0.0	
PD	12 (1.0)	50.0 ± 20.4		58.3 ± 18.8	
No. of ASCTs			.1282		.0704
1	1,042 (88.1)	60.4 ± 2.1		71.2 ± 1.9	
2	141 (11.9)	65.9 ± 4.3		76.5 ± 3.8	
No. of cycles of therapy completed			.0827		.0056
All 6 cycles	994 (90.9)	66.4 ± 1.9		78.0 ± 1.7	
< 6 cycles[d]	100 (9.1)	56.2 ± 6.9		64.6 ± 6.6	

Abbreviations: ASCT, autologous stem cell transplant, CR, complete response; EFS, event-free survival; INSS, International Neuroblastoma Staging System; MR, mixed response; NR, no response; OS, overall survival; PD, progressive disease; PR, partial response; VGPR, very good partial response.

[a]Response before ASCT excluding bone marrow.

[b]*P* value reflects CR/VGPR versus PR.

[c]*P* value reflects CR/VGPR versus PR versus MR/NR/PD.

[d]Excludes patients who did not complete therapy because of death on study (n = 6), withdrawal of consent for further data submission (n = 3), relapse/progression (n = 75), and lost to follow-up (n = 5).

consistent with those of the randomized portion of ANBL0032[5,8] despite the less stringent eligibility criteria for the current cohort. Superior EFS and OS were observed for those with non–stage 4 disease, and superior EFS was observed for those with pre-ASCT responses of CR/VGPR compared with PR, consistent with prior results.[5,8] There

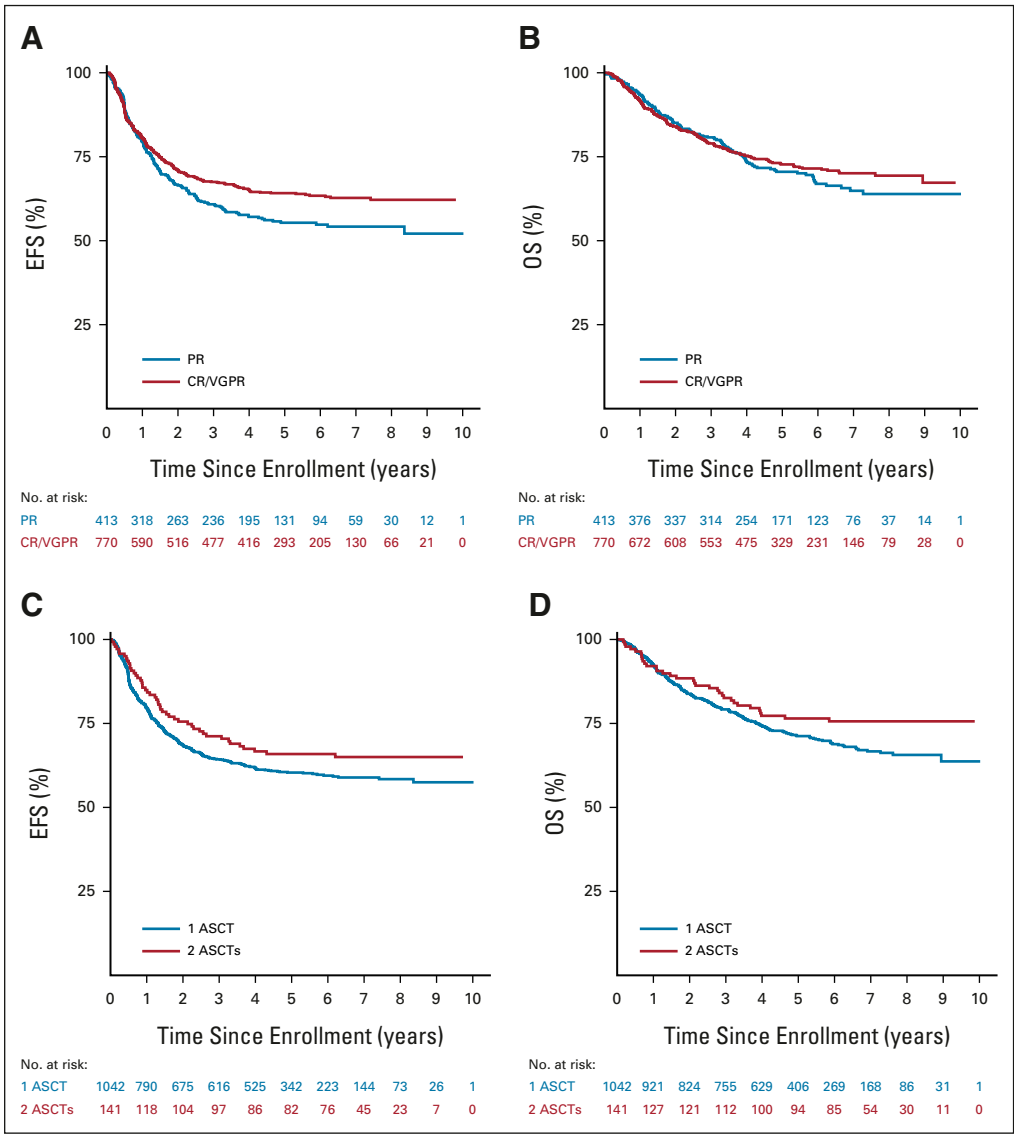

FIG 3. Survival by pre-ASCT INRC response (CR/VGPR *v* PR) and number of ASCTs: (A) EFS by pre-ASCT INRC response ($P = .0133$), (B) OS by pre-ASCT INRC response ($P = .3811$), (C) EFS by number of ASCTs ($P = .1282$), and (D) OS by number of ASCTs ($P = .0704$). ASCT, autologous stem cell transplant; CR, complete response; EFS, event-free survival; INRC, International Neuroblastoma Response Criteria; OS, overall survival; PR, partial response; VGPR, very good partial response.

was a trend toward improved OS among patients treated with tandem versus single ASCT; however, only a small proportion of patients underwent tandem ASCT. The results of COG ANBL0532 (ClinicalTrials.gov identifier: NCT00567567)[16] demonstrating superior survival in tandem ASCT were released in May 2015; thus, single ASCT was still considered standard for much of the study duration. Nevertheless, in the multivariable Cox model, there was an increased risk of death among those who underwent single transplant.

Most patients completed therapy despite treatment-related toxicities. As GD2 is expressed on nerve fibers,[17] pain is expected. More patients experienced severe pain during cycle 1 compared with subsequent cycles, as noted previously.[5] Allergic reactions and capillary leak were more frequently observed during IL-2–containing cycles, similar to prior observations[5] and consistent with established IL-2–associated toxicities.[18] GM-CSF and IL-2 were administered with dinutuximab to enhance ADCC.[19-22] However, a randomized trial conducted by the International Society of Paediatric Oncology European Neuroblastoma found added toxicity without survival benefit with addition of subcutaneous IL-2 to dinutuximab beta.[6] Long-term continuous infusion dinutuximab beta with subcutaneous IL-2 has been shown to result in elevated levels of regulatory T cells, correlating with inferior progression-free survival.[23] Together, these findings led COG to remove IL-2 from

TABLE 3. Common Terminology Criteria for Adverse Events v4.0 Grade ≥ 3 Toxicities by GM-CSF– and IL-2–Containing Cycles

Toxicity	GM-CSF Cycles[a] (1, 3, 5), No. (%)[b]	IL-2 Cycles (2, 4), No. (%)[b]	*P* for McNemar's Test
Pain[c,d]	333 (28.1)	205 (17.9)	< .0001[e]
Allergic reaction/hypersensitivity reaction[c]	147 (12.4)	240 (21.0)	< .0001[e]
Infection[c]	225 (19.0)	228 (20.0)	.5701
Capillary leak syndrome[c]	62 (5.2)	126 (11.0)	< .0001[e]
Motor neuropathy[c]	6 (0.5)	4 (0.3)	1.0000
Peripheral sensory neuropathy	5 (0.4)	2 (0.2)	.1797
Creatinine increased[c]	4 (0.3)	15 (1.3)	.0116[f]
Hypokalemia	159 (13.4)	288 (25.2)	< .0001[e]
Elevated transaminases[c]	118 (10.0)	129 (11.3)	.3830
Fever[c]	183 (15.5)	385 (33.6)	< .0001[e]
Behavioral changes[c]	7 (0.6)	4 (0.3)	.3173
Hypoxia	120 (10.1)	100 (8.7)	.2230
Respiratory compromise (other)[c]	40 (3.4)	43 (3.8)	.4126
Serum sickness	3 (0.3)	0 (0.0)	NA
Hypotension	109 (9.2)	157 (13.7)	< .0001[e]
Eye disorders	2 (0.2)	8 (0.7)	.0339[f]
Anemia	223 (18.9)	249 (21.7)	.0212[f]
Neutrophil count decreased	86 (7.3)	183 (16.0)	< .0001[e]
Platelet count decreased	165 (13.9)	199 (17.4)	.0009[e]
Lymphocyte count decreased	146 (12.3)	183 (16.0)	.0004[e]

Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-2, interleukin-2; NA, not applicable.

[a]Toxicities that occurred in cycles 2 and 4 when GM-CSF was substituted for IL-2 were included.

[b]Number (%) reflects the number/percentage of patients who experienced a toxicity during either GM-CSF–containing cycles or IL-2–containing cycles.

[c]Relevant Common Terminology Criteria for Adverse Events toxicities collapsed under listed category.

[d]Pain frequency was 23.7%, 8.5%, and 7.1% for GM-CSF–containing cycles 1, 3, and 5, respectively, and 13.4% and 10.1% for IL-2–containing cycles 2 and 4, respectively.

[e]Continued to be significant after correction for multiple comparisons.

[f]Significant at $P < .05$.

postconsolidation therapy. Strategies to potentially mitigate toxicities (including extended duration schedules and optimization of supportive care) and incorporation of patient-reported outcomes as part of future trials may improve the patient experience. Home administration may also be of interest. Development of next-generation anti-GD2 antibodies may help decrease treatment-associated pain.[24,25]

The rate of HACA positivity in this cohort was similar to that reported in the randomized cohort. The development of HACA was associated with lower dinutuximab levels and decreased toxicity, as reported previously.[14,24,26,27] However, HACA status was not associated with survival in this study. Higher cycle 1 dinutuximab levels were associated with improved EFS, consistent with the findings of two chemoimmunotherapy trials.[28,29] This suggests that greater in vivo exposure to anti-GD2 antibody could be associated with improved outcome. If alternative dosing schedules are used, the association between dinutuximab levels and survival should be evaluated.

Identification of biomarkers associated with outcome in the context of immunotherapy for neuroblastoma has long been a high priority in the field, particularly in light of the toxicity that accompanies GD2-directed therapy. The association of FCGR3A high-affinity genotype with better outcome is consistent with the trend reported in patients treated with dinutuximab beta in a European study.[30] However, no correlation between outcome and FCGR2A/FCGR3A status was detected in the randomized cohort from ANBL0032. The association of Fc gamma receptor genotype and EFS requires validation in an independent cohort.

Because natural killer (NK) cells are important for ADCC, NKp30 isoforms have been evaluated in several dinutuximab trials. The lack of association between NKp30 isoform and survival suggests that NK cell number and/or expression of other NK-activating receptors might overshadow the role of NKp30. Complete or partial loss of GD2 expression can occur during therapy or at the time of

neuroblastoma recurrence,[31] which may affect response to anti-GD2 therapy. Improved understanding of the tumor microenvironment and immune profiles of patients may also provide insight into mechanisms of resistance to GD2-directed immunotherapy.[32-36] Elucidating these mechanisms could help identify additional biomarkers to guide treatment and improve outcomes for children with high-risk neuroblastoma.

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DISCLAIMER

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Outcomes Following GD2-Directed Postconsolidation Therapy for Neuroblastoma After Cessation of Random Assignment on ANBL0032: A Report From the Children's Oncology Group

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APPENDIX

Cycles 1, 3, and 5

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14-23	24
GM-CSF	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Dinutuximab				X	X	X	X									
Isotretinoin[a]											X	X	X	X	X	

↑ Begin cycle 2 or 4

Cycles 2 and 4

Day	0	1	2	3	4-6	7	8	9	10	11-13	14	15	16	17	18-27
IL-2[b]	X	X	X	X		X	X	X	X						
Dinutuximab						X	X	X	X						
Isotretinoin											X	X	X	X	X

Cycle 6

Day	0-13	14-27
Isotretinoin		X

FIG A1. ANBL0032 immunotherapy treatment regimen. [a]Isotretinoin is given on days 11-24 during cycle 1. [b]IL-2, days 0-3: 3 MIU/m^2 daily (continuous infusion); days 7-10: 4.5 MIU/m^2 daily (continuous infusion). GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-2, interleukin-2.

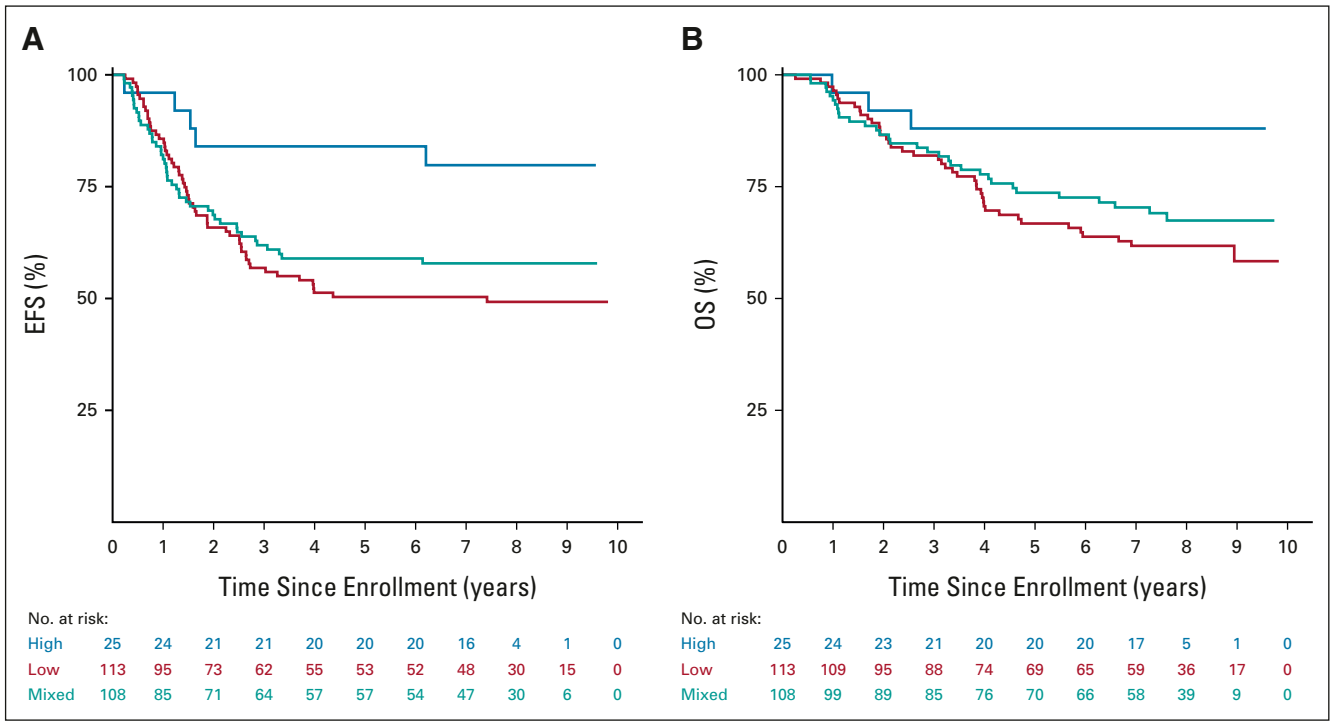

FIG A2. Survival by FCGR3A affinity: (A) EFS (P = .0418) and (B) OS (P = .0806). EFS, event-free survival; OS, overall survival.

TABLE A1. Characteristics of Patients With On-Therapy Relapse or Disease Progression

Characteristic	Patients Who Relapsed or Progressed on Therapy (N = 101), No. (%)
Age at high-risk diagnosis, months	
< 18	25 (24.8)
≥ 18	76 (75.2)
INSS stage	
1	0 (0.0)
2	1 (1.4)
3[a]	5 (7.1)
4S	1 (1.4)
4	63 (90.0)
Unknown	31
MYCN status	
Not amplified	20 (30.3)
Amplified	46 (69.7)
Unknown	35
Histology	
Favorable	2 (3.2)
Unfavorable	61 (96.8)
Unknown	38
Ploidy	
Hyperdiploid	20 (35.7)
Diploid	36 (64.3)
Unknown	45
Response before ASCT[b]	
CR	28 (27.7)
VGPR	43 (42.6)
PR	30 (29.7)
No. of ASCTs	
1	93 (92.1)
2	8 (7.9)
Dose modification dinutuximab (cycles 1-5)	
Dose reduction	18 (17.8)
No reduction	83 (82.2)
Dose modification IL-2 (cycles 2 or 4)	
Dose reduction	30 (31.6)
No reduction	65 (68.4)
Missing	6
Time from last ASCT to enrollment	
Days, median (range)	90.0 (63.0-170.0)

(continued in next column)

TABLE A1. Characteristics of Patients With On-Therapy Relapse or Disease Progression (continued)

Abbreviations: ASCT, autologous stem cell transplant, CR, complete response; IL-2, interleukin-2; INSS, International Neuroblastoma Staging System; PR, partial response; VGPR, very good partial response.

[a]Patients with INSS stage 3 disease and *MYCN*-amplified tumors were considered to have high-risk disease regardless of age, histology, or ploidy. Patients with INSS stage 3 disease and *MYCN*-nonamplified tumors with unfavorable histology were considered high-risk if they were ≥ 12 months (eg, A3973, ANBL02P1) or ≥ 18 months (eg, ANBL0532) of age, depending on the frontline regimen used.

[b]Response before ASCT excluding bone marrow.

TABLE A2. Multivariable Analysis of Features Predictive of EFS and OS

	EFS		OS	
Feature (n = 607)	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
INSS stage (1, 2, 3, 4S[a] *v* 4)	1.932 (1.266 to 2.948)	.0023	2.057 (1.217 to 3.478)	.0071
Histology (favorable[a] *v* unfavorable)	3.614 (1.342 to 9.731)	.0110	NA[b]	
Response before ASCT[c] (CR/VGPR[a] *v* PR)	1.621 (1.223 to 2.148)	.0008	1.523 (1.089 to 2.132)	.0141
No. of ASCTs (1 *v* 2[a])	NA[b]		1.716 (1.016 to 2.898)	.0435

Abbreviations: ASCT, autologous stem cell transplant; CR, complete response; EFS, event-free survival; HR, hazard ratio; INSS, International Neuroblastoma Staging System; NA, not applicable; OS, overall survival; PR, partial response; VGPR, very good partial response.

[a]The reference level for each feature. The HR is the increased risk of event/death compared with the reference level, where a HR > 1 indicates that the nonreference level has an increased risk of event/death.

[b]NA indicates that the variable was not retained in the model for the given survival outcome.

[c]Response before ASCT excluding bone marrow.

TABLE A3. HACA Status and Cmax

HACA Status	**Cmax, Median (range)**	***P***	**Cmax/Cmax – 1 Ratio, Median (range)**	***P***
HACA-negative (n = 206)	11,491.6 (1,188.4-33,970.5)	< .0001	1.0237 (0.1024-1.1523)	< .0001
HACA-positive (n = 35)	7,629.3 (6,392.4-13,741.9)		0.7570 (0.7350-1.0104)	

NOTE. Cmax values (in ng/mL) are shown for the final cycle of treatment for the 35 HACA+ patients and the 206 HACA– patients with evaluable data. The median level for the HACA+ patients is less than that for the HACA– patients. In addition, to enable each patient to be their own control, the median ratio of the Cmax values for the last cycle for each patient over that for the first cycle for that same patient is also shown. The median ratio is 1.02 for the HACA– patients, indicating no substantial change in peak value from the first to the last course. By contrast, the median ratio is 0.757 for the HACA+ patients, indicating that these patients, in general, show a drop in dinutuximab level from cycle 1 to the last cycle. Note that 21 HACA– patients were missing Cmax values.

Abbreviations: Cmax, Dinutuximab peak concentration; HACA, human antichimeric antibody.

TABLE A4. Fc Receptor Genotype and Outcomes

FCGR Genotype	No. (%)	5-Year EFS ± SE	*P*	5-Year OS ± SE	*P*
FCGR2A			.8177		.3260
Low	68 (26.8)	55.6 ± 6.3		70.3 ± 5.8	
High	49 (19.3)	59.8 ± 7.2		80.4 ± 5.9	
Mixed	137 (53.9)	57.8 ± 4.5		68.9 ± 4.2	
FCGR3A			.0418		.0806
Low	113 (45.9)	50.3 ± 4.9		66.8 ± 4.6	
High	25 (10.2)	84.0 ± 7.5		88.0 ± 6.8	
Mixed	108 (43.9)	58.9 ± 5.0		73.6 ± 4.5	

Abbreviations: EFS, event-free survival; FCGR, Fc gamma receptor; OS, overall survival.

TABLE A5. Antibody-Dependent Cell-Mediated Cytotoxicity (4-hour assay) and Survival

Sample Time Points and Change in ADCC for Paired Samples	No.	Mean ± SD	Median (min-max)	EFS HR	EFS 95% Wald Confidence Limits	EFS *P*	OS HR	OS 95% Wald Confidence Limits	OS *P*
Baseline	74	9.9 ± 8.2	7.5 (0.4 to 31.7)	1.011	0.970 to 1.054	.601	1.015	0.968 to 1.064	.534
Day 80 (before cycle 4)	71	9.5 ± 7.0	9.2 (0 to 26)	1.034	0.981 to 1.091	.215	1.041	0.981 to 1.105	.183
Change	39	1.2 ± 10.8	1.7 (–31.5 to 19.7)	1.010	0.961 to 1.061	.692	1.009	0.954 to 1.068	.754

Abbreviations: EFS, event-free survival; HR, hazard ratio; OS, overall survival; SD, standard deviation.

TABLE A6. NCR3/NKp30 Isoforms

NCR3/NKp30 Isoform	No. (%)	5-Year EFS ± SE	*P*	5-Year OS ± SE	*P*
SNP					
NCR3: CC	2 (1.6)	0	.0252	50.0 ± 35.4	.7056
NCR3: TC	23 (18.1)	59.5 ± 10.9		82.2 ± 8.4	
NCR3: TT	102 (80.3)	61.5 ± 5.0		75.3 ± 4.5	
NCR3: TC	23 (18.1)	59.5 ± 10.9	.6220[a]	82.2 ± 8.4	.6000
NCR3: TT	102 (80.3)	61.5 ± 5.0		75.3 ± 4.5	
RNA					
NKp30: stim	114 (81.4)	62.6 ± 4.8	.2302	75.9 ± 4.3	.6984
NKp30: suppressive	26 (18.6)	56.3 ± 10.3		80.3 ± 8.2	

Abbreviations: EFS, event-free survival; NCR, natural cytotoxicity receptor; NKp30, natural killer protein 30; OS, overall survival; SNP, single nucleotide polymorphism.

[a]After excluding NCR: CC (n = 2), EFS is not significantly different between the groups.