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Improved Outcome in Children With Newly Diagnosed High-Risk Neuroblastoma Treated With Chemoimmunotherapy: Updated Results of a Phase II Study Using hu14.18K322A

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abstract

PURPOSE We evaluated whether combining a humanized antidisialoganglioside monoclonal antibody (hu14.18K322A) throughout therapy improves early response and outcomes in children with newly diagnosed high-risk neuroblastoma.

PATIENTS AND METHODS We conducted a prospective, single-arm, three-stage, phase II clinical trial. Six cycles of induction chemotherapy were coadministered with hu14.18K322A, granulocyte-macrophage colony-stimulating factor (GM-CSF), and low-dose interleukin-2 (IL-2). The consolidation regimen included busulfan and melphalan. When available, an additional cycle of parent-derived natural killer cells with hu14.18K322A was administered during consolidation (n = 31). Radiation therapy was administered at the end of consolidation. Postconsolidation treatment included hu14.18K322A, GM-CSF, IL-2, and isotretinoin. Early response was assessed after the first two cycles of induction therapy. End-of-induction response, event-free survival (EFS), and overall survival (OS) were evaluated.

RESULTS Sixty-four patients received hu14.18K322A with induction chemotherapy. This regimen was well tolerated, with continuous infusion narcotics. Partial responses (PRs) or better after the first two chemoimmunotherapy cycles occurred in 42 of 63 evaluable patients (66.7%; 95% CI, 55.0 to 78.3). Primary tumor volume decreased by a median of 75% (range, 100% [complete disappearance]-5% growth). Median peak hu14.18K322A serum levels in cycle one correlated with early response to therapy ($P = .0154$, one-sided t-test). Sixty of 62 patients (97%) had an end-of-induction partial response or better. No patients experienced progressive disease during induction. The 3-year EFS was 73.7% (95% CI, 60.0 to 83.4), and the OS was 86.0% (95% CI, 73.8 to 92.8), respectively.

CONCLUSION Adding hu14.18K322A to induction chemotherapy improved early objective responses, significantly reduced tumor volumes in most patients, improved end-of-induction response rates, and yielded an encouraging 3-year EFS. These results, if validated in a larger study, may be practice changing.

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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

Treatment for high-risk (HR) neuroblastoma includes induction therapy (chemotherapy and surgery), consolidation with high-dose chemotherapy followed by autologous hematopoietic stem-cell transplant (ASCT), radiotherapy, and postconsolidation treatment with isotretinoin and a monoclonal antibody (mAb) that targets the disialoganglioside GD2. Administration of a chimeric anti-GD2 mAb (dinutuximab) with granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 (IL-2), and isotretinoin in the postconsolidation setting significantly improved 2- and 5-year event-free survival (EFS; 66% v 46%, $P = .01$[1]; 56.6 ± 4.7% v 46.1 ± 5.1%, $P = .042$,[2] respectively). However, nearly half of all patients experienced relapse and succumb to disease.

Dinutuximab has been administered in postconsolidation to avoid chemotherapy-induced immunosuppression since it can adversely affect antibody-dependent cell-mediated cytotoxicity (ADCC). However, preclinical studies in neuroblastoma models and adult clinical studies demonstrated that concurrent chemotherapy with various monoclonal antibodies provides additive or synergistic benefits.[3-10] We postulated that incorporating an anti-GD2 mAb throughout therapy for the treatment of HR neuroblastoma would improve clinical responses

CONTEXT

Key Objective

Despite aggressive multimodal treatment, nearly half of all patients with newly diagnosed high-risk (HR) neuroblastoma still succumb to their disease. Will the combination of a humanized antidisialoganglioside monoclonal antibody (hu14.18K322A) with induction chemotherapy improve early response rates after two cycles and improve outcomes in children with newly diagnosed HR neuroblastoma?

Knowledge Generated

Early responses were significantly improved when compared with a historical group of children treated with identical chemotherapy, without hu14.18K322A. None of the patients in our trial experienced tumor progression during induction chemotherapy, and the overall outcomes are very encouraging.

Relevance

These results, if validated in a larger study, may change the standard of care for children with HR neuroblastoma.

and outcomes. We used a unique humanized anti-GD2 mAb, hu14.18K322A, that retains the binding specificity of dinutuximab, is 98% human, has a single point mutation to reduce complement-associated pain, and is produced in a YB2/0 rat myeloma cell line to reduce fucosylation and enhance ADCC.[11] We demonstrated that combining hu14.18K322A with chemotherapy is tolerable and clinically active in patients with relapsed neuroblastoma.[12] Therefore, hu14.18K322A was administered concurrently with chemotherapy during induction in this phase II (NB2012), single-institution setting to assess efficacy and determine if chemoimmunotherapy warrants further evaluation in a randomized phase III study. The results of the first 42 patients, demonstrating significant improvement in early responses, were previously published.[13] This report details the results of the entire cohort of 64 patients, their end-of-induction responses, estimates of event-free and overall survival (OS), and information on human antihuman antibody (HAHA) development.

In ANBL02P1, the Children's Oncology Group (COG) investigators reported a 40% partial response (PR) rate after two cycles of induction therapy (cyclophosphamide and topotecan) in HR patients[14]; this response was similar to the response described in ANBL0532 in which patients received the same induction regimen.[15] NB2012 used the identical chemotherapy induction regimen as ANBL02P1 and ANBL0532. The primary objectives of NB2012 were to assess response after two cycles of chemoimmunotherapy compared with a previous study with chemotherapy only and to estimate the EFS when hu14.18K322A was added to all phases of treatment. Changes in Curie scores (CSs) from diagnosis to end-of-induction chemotherapy were also assessed. Post-induction CSs have been previously validated as prognostic in two large independent cohorts of patients.[16-18]

PATIENTS AND METHODS

Study Design

This study was an open-label, single-arm phase II trial. The primary objective was to compare the response rates of the first two cycles of chemoimmunotherapy (cyclophosphamide, topotecan, and hu14.18K322A, followed by GM-CSF and IL-2) with the response rates of two cycles of cyclophosphamide and topotecan alone reported in a previous study (ANBL02P1) in children with newly diagnosed HR neuroblastoma.[14] At an interim analysis, the response rate exceeded the a priori benchmark for activity[13]; therefore, enrollment was expanded to estimate EFS as a primary objective.

Statistical Analysis Plan

In the COG phase III randomized trial ANBL0532,[15] the chemotherapy components of the induction regimen were identical to NB2012 and the 95% CI of 3-year EFS was (46.5%, 55.3%). A Kaplan-Meier estimate-based design was used to test the null hypothesis of a 46% 3-year EFS rate, against the desired alternative 3-year EFS rate of 62%. A total of 61 patients provided 80% power to detect a 16% increase of 3-year EFS with a one-sided type I error rate of 5%. Analysis of EFS was performed in the intention-to-treat population, defined as all enrolled patients, and the efficacy-evaluable population, defined as all patients who had received at least one dose of study treatment and had at least one available postbaseline tumor assessment. EFS and OS were analyzed using the Kaplan-Meier method. ORR and 95% CIs were calculated using the Clopper-Pearson method. Summary statistics were provided for clinical and demographic characteristics and for AEs. The association between the EFS and the peak and trough level of hu14.18K322A were explored. We performed all statistical tests using SAS (version 9.4).

Patients

Children (< 19 years) with newly diagnosed HR neuroblastoma were eligible for enrollment. Diagnosis, staging, and response assessments were performed according to the 1993 International Criteria for Neuroblastoma Response,[19] and HR neuroblastoma was defined by the criteria used by the COG.[20]

This trial (NCT01857934) was approved by our institutional review board and opened in May 2013. The last patient

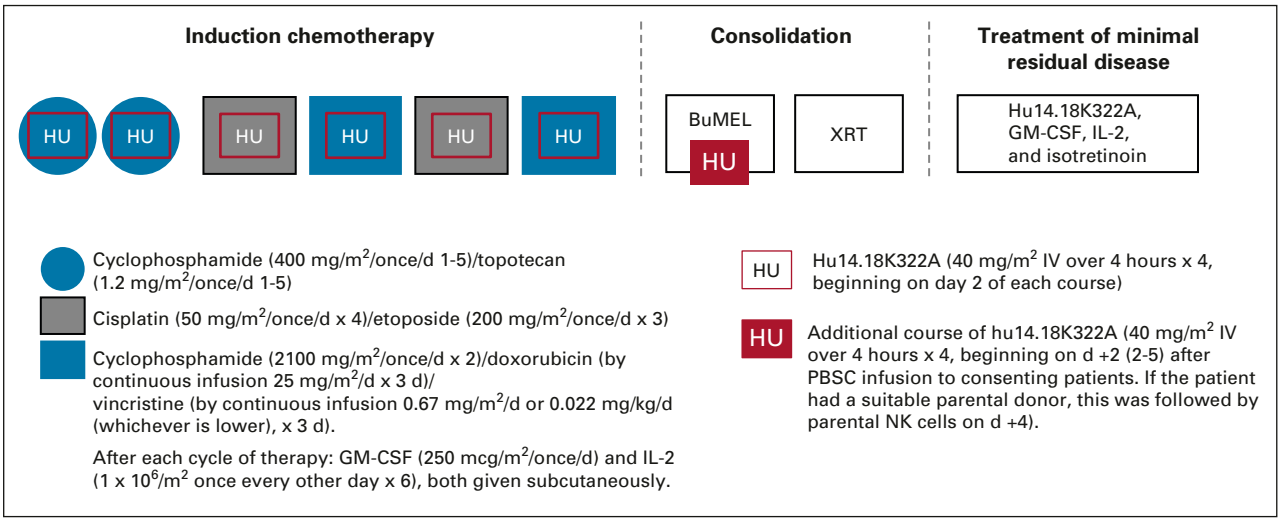

FIG 1. Study schema. BuMel, busulfan and melphalan; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-2, interleukin-2; NK, natural killer; PBSC, peripheral blood stem cell; XRT, radiation therapy.

completed therapy in July 2019. Written informed consent was obtained from participants in accordance with institutional guidelines (Protocol, online only).

Hu14.18K322A

The hu14.18K322A production cell line was provided by Merck Serono (Darmstadt, Germany) and manufactured for clinical use by the Children's GMP, LLC (Memphis, TN). With each cycle of therapy, hu14.18 K322A levels were measured by ELISA in serum obtained 1 hour after the first antibody infusion, as previously described.[12,21] Patients were monitored for the development of HAHA before first dose of hu14.18K322A with every treatment cycle (induction, consolidation, and postconsolidation phase) with an ELISA as previously described.[22]

Treatment

The schedule and dosages of induction chemotherapy agents cyclophosphamide, topotecan, cisplatin, etoposide, doxorubicin, and vincristine were identical to those used in ANBL02P1 and ANBL0532.[14,15] Four daily doses of hu14.18K322A (days 2-5; administered over 4 hours, when possible) at 40 mg/m^2/dose (once daily) were given with each cycle of induction chemotherapy. All patients received continuous infusions of opioids at standard dosages, approximately 30 minutes before antibody infusions. Systemic steroids were not allowed. Each cycle was followed by daily subcutaneous GM-CSF (250 μg/m^2 once per day) through the nadir until an absolute neutrophil count of ≥ 2,000/mm^3 and subcutaneous IL-2 (10^6 units/m^2), once every other day for six doses (Fig 1).

Hematopoietic progenitor cells were collected after induction cycle 2 or 4. Primary tumors were resected at any time point after induction cycle 2, when the surgeon determined that a maximum resection could be safely achieved. Consolidation therapy included ASCT with a Busulfan and Melphalan (BuMel)–conditioning regimen[23] and, for consenting patients, experimental therapy with an additional cycle of daily hu14.18K322A administration beginning 2 days (days 2-5) after ASCT (given on day 0) and parental natural killer (NK) cell infusions (when available), derived as previously described.[23] Tolerability of hu14.18K322A during recovery from consolidation therapy was evaluated in two dosage cohorts (25 mg/m^2 once per day for 4 days, n = 16 [with NK cells, n = 10]; 40 mg/m^2 once per day for 4 days, n = 26; with NK cells [n = 21]). The NK cells were infused 4 days after ASCT. When two of the first 42 patients developed a hemophagocytic lymphohistiocytosis-like syndrome,[24] the additional cycle of hu14.18K322A during consolidation was eliminated in the subsequent cohort (n = 17). After recovering from ASCT (typically within 43 days of stem-cell reinfusion), patients received intensity-modulated radiation therapy or scanned proton beam radiation therapy (2,340 cGy in 180 cGy fractions). Those with macroscopic residual disease after induction chemotherapy received an additional 720 cGy to those sites.

Radiation therapy was delivered to the presurgical gross tumor volume and sites of residual disease on the basis of imaging performed at the end of induction. Postconsolidation therapy with hu14.18K322A, GM-CSF, IL-2, and isotretinoin (doses and schedules identical to those reported by Yu et al[1]) was started within 100 days from ASCT. Hu14.18K322A (40 mg/m^2 once per day for 4 days) was administered in place of dinutuximab.[1] Adverse events were assessed by the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0). If narcotic infusions required increased dosages, pain was designated grade 3. Diagnosis, staging, and response assessments were performed according to the 1993 International Criteria for Neuroblastoma Response.[19] Metastatic complete response was defined as no skeletal uptake

TABLE 1. Patient Characteristics at Diagnosis

Characteristic	NB2012 (N = 64), No. (%)
Age, months	
< 18	5 (8)[a]
≥ 18	59 (92)
Sex	
Female	27 (42)
Male	37 (58)
Race	
White	43 (67)
Black	18 (28)
Others	3 (5)
Unreported	—
INSS stage	
IIb	1 (2)
III	7 (11)[b]
IV	56 (87)
INRG stage	
L1	0
L2	8 (13)[c]
M	56 (87)
MYCN status	
Not amplified	43 (67)
Amplified	21 (33)
Shimada histology	
Favorable	6 (9)
Unfavorable	41 (64)
Not performed	17 (27)

Abbreviations: INRG, International Neuroblastoma Risk Group; INSS, International Neuroblastoma Staging System; UH, unfavorable histology.

[a]Three with *MYCN* amplification and two without *MYCN* amplification but DNA index = 1.

[b]Five with *MYCN* amplification and two without *MYCN* amplification but with UH.

[c]6 of 8 *MYCN* amplified; 8 of 8 with unfavorable histology.

on nuclear imaging and complete response in bone marrow and other metastatic sites. Responses were evaluated after induction cycles 2 and 6, after consolidation, and at the end of therapy. Anatomic imaging was assessed by one study radiologist (M.B.M.). Semiquantitative MIBG scoring (ie, CS) was assessed by one nuclear medicine physician (B.S.) at diagnosis and after cycles 2 and 6 of induction chemotherapy as described by Yanik et al.[18]

RESULTS

Patient Characteristics

Sixty-three of the 64 patients had measurable or evaluable disease at enrollment. Patient characteristics are described in Table 1. Most patients were male (n = 37), White (n = 43), and ≥ 18 months (n = 59), with a median age of 3.1 years (range, 0.5-15.2 years), and had International Neuroblastoma Staging System (INSS) stage IV or International Neuroblastoma Risk Group stage M disease (n = 56). These characteristics were similar to patients enrolled on COG studies ANBL02P1,[14] ANBL0532,[15] and ANBL12P1[25] (Appendix Table A1, online only). During consolidation, 25 patients received proton beam radiation therapy and 34 patients received intensity-modulated radiation therapy.

Safety and Toxicity

The addition of hu14.18K322A to induction chemotherapy was well tolerated, with continuous infusion narcotics adjusted to patient tolerance. Therapy-related grade 3 of 4 toxicities during induction chemotherapy-hu14.18K322A coadministration are described in Appendix Table A2 (online only). Toxicities attributed to antibody infusion included pain (10%; 38 patient episodes in 379 cycles), hypotension (3%; 12 of 379), cough (1%; 4 of 379), hypoxia (6%; 21 of 379), and infusion-related reactions (4%; 14 of 379).[1,26] Events related to myelosuppression, liver function abnormalities, enterocolitis, and fever with

TABLE 2. Responses and Curie Scores During Induction Chemoimmunotherapy

	Responses					
Response	After Two Cycles (N = 64)	Metastatic Responses After Two Cycles		After Six Cycles (N = 62 evaluable[c])	Metastatic Responses after Six Cycles	
CR	2 (3%)	mCR	3/56	21 (34%)	mCR	40 (65%)
VGPR	8 (13%)	< mCR	53/56	21 (34%)	< mCR	22 (35%)
PR	32 (51%)			18 (29%)		
NR	21 (33%)			2 (3%)		
PD	0	0		0	0	
NE	1[a]	8/64[b]		2[c]	2[c]	

CSs			
CS at Diagnosis (N = 54[d])		CS After Six Cycles (N = 54[d])	
0-12	20 (37%)	0-2	47 (87%)
13-25	23 (43%)	3-6	3 (5.6%)
> 25	11 (20%)	≥ 13	4 (7.4%)

Abbreviations: CR, complete response; CS, Curie score; INSS, International Neuroblastoma Staging System; mCR, metastatic complete response; NE, non evaluable; NR, no response; PD, progressive disease; PR, partial response; VGPR, very good partial response.

[a]One patient with MYCN amplification and localized tumor had resection of her primary tumor prior to beginning chemotherapy.

[b]Eight patients had no metastases at diagnosis.

[c]One patient was taken off study prior to completing induction chemotherapy because of severe diarrhea from a vasoactive intestinal peptide secreting tumor and one patient withdrew electively for parental preference.

[d]Fifty-six patients had INSS IV disease, two withdrew prior to completing induction chemotherapy.

or without infection were similar to those reported previously.[13,14]

Tumor Response

Responses were evaluated using the International Neuroblastoma Response Criteria (INRC)[19] after two cycles of therapy (early response) and at the end of induction (Table 2). Two patients had resection of their primary tumors before beginning therapy but had other sites of evaluable disease. Of the 63 children evaluable for early response, 42 (66.7%; 95% CI, 55.0 to 78.3%) experienced PR or better. Figure 2 demonstrates the INRC components of response for individual patients after induction cycle two and the end of induction. All but one patient had a decrease in their primary tumor volume at cycle 2 with 42 of 63 achieving reductions of at least 50%. Fourteen had a metastatic very good partial response after induction cycle 2, and 3 had metastatic site complete responses. At the end of induction, 60 of 62 evaluable patients had PRs or better (96.8%; 95% CI, 88.8.0 to 99.6) including 40 with mCRs (65%).

MIBG scoring (CS) was performed at every evaluation time point. CSs at diagnosis for the 56 patients with INSS stage IV disease ranged from 1 to 28 (median, 15) and from 0 to 23

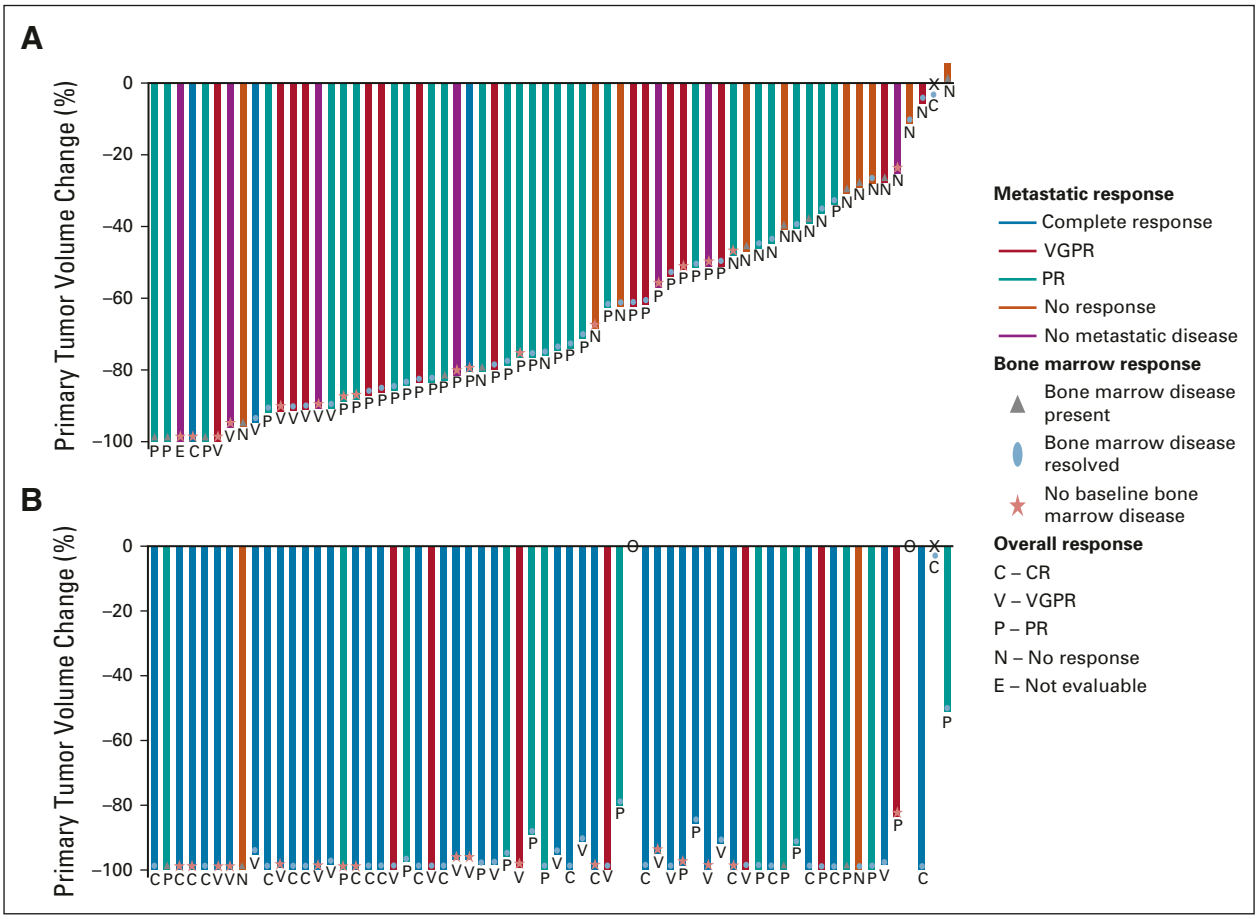

FIG 2. Disease response assessed by INRC for patients (A) after induction cycle 2 and (B) at the end of induction. Each bar represents an individual patient. The primary tumor response is demonstrated as a percentile change in volume of the primary tumor compared with the primary tumor volume at baseline. The color of the individual bars signifies the metastatic response including anatomic imaging, functional imaging (MIBG or PET), and bone marrow assessment. The blue bar represents a complete metastatic response. The red bar represents a metastatic VGPR. The teal bar represents a metastatic partial response. The orange bar represents no metastatic response. The purple bar signifies patients without metastatic disease at diagnosis. Bone marrow response is depicted by the following shapes: gray triangle represents the presence of bone marrow disease at the time of the assessment, blue oval represents bone marrow disease present at baseline that resolved at the time of the assessment, and the red star represents no bone marrow disease at baseline. Together, the primary tumor response, metastatic response, and bone marrow response are used to inform the INRC overall response. The overall response of the individual patient is presented by the letter below each bar. The letter C signifies a CR; V is a VGPR; P is a PR; N is a no response; and E means that the patient was not evaluable at that time point because of the primary tumor resection performed before therapy. X represents a stage IV patient who did not have a primary tumor at diagnosis. O represents two patients who discontinued protocol therapy before completing induction. CR, complete response; INRC, International Neuroblastoma Response Criteria; MIBG, [^{125}I]metaiodobenzylguanidine; PET, positron emission tomography; PR, partial response; VGPR, very good partial response.

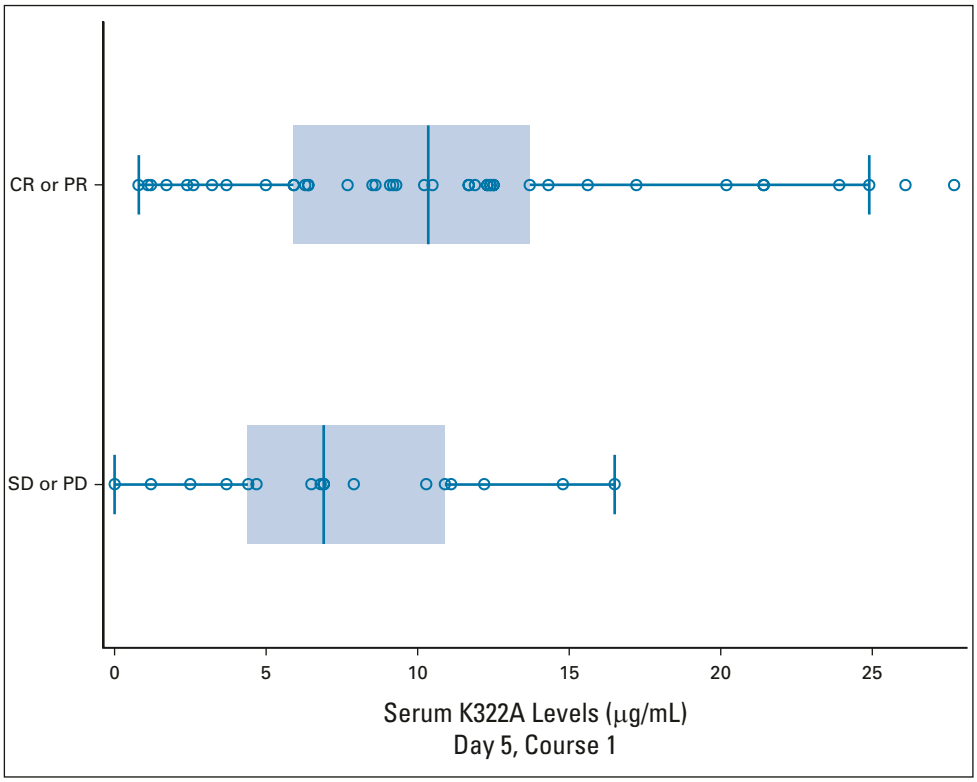

FIG 3. Association of hu14.18K322A peak serum levels (μg/mL) and response after two cycles of chemoimmunotherapy. Relationship between response (CR or PR [n = 42] *v* SD or PD [n = 17]) and peak hu14.18K322A levels, measured on first day of mAb infusion, 1 hour after a 4-hour infusion. $P = .0154$ (one-tailed *t*-test). CR, complete response; mAb, monoclonal antibody; PD, progressive disease; PR, partial response; SD, stable disease.

(median, 0) at the end-of-induction chemotherapy. For the 54 patients with INSS stage IV disease who completed induction, 47 (87%) had end-of-induction CSs of two or less, as compared with a median CS of 15 at diagnosis (Table 2).

Quantitative and qualitative differences in NK cells on a subset of these children were also examined and previously reported.[27]

Hu14.18K322A Levels and HAHA Response

Median peak hu14.18K322A serum levels on cycle 1 of induction correlated with the clinical responses that were performed after the first two cycles of induction chemoimmunotherapy (PR or greater; n = 42) versus those who had stable disease (n = 17; $P = .054$, one-sided Wilcoxon rank-sum test; Fig 3). There was no association of EFS with the peak level of hu14.18K322A in cycle 1 (peak levels were dichotomized, above median or below median, and compared using the log-rank test [$P = .682$], and there was no association of early response after two cycles [PR or greater] with the trough level of hu14.18K322 A at the start of cycle 2 [$P = .336$, two-sided Wilcoxon rank-sum test]).

Five of 64 evaluable patients (7.8%) developed significant HAHA responses to hu14.18K322A after chemoimmunotherapy. None had a HAHA response associated with a drop in the peak hu14.18K322A level in cycles after the HAHA development (Appendix Table A3, online only). The five HAHA+ patients did not show any significant difference in EFS ($P = .75$, log-rank test) or noticeable differences in tolerance to antibody than the 59 HAHA– patients.

Effect of an Additional Cycle of hu14.18K322A With or Without Parentally Derived NK Cells During Consolidation

Fifty-nine of 64 patients received consolidation with BuMel (Fig 4) as previously described.[23] Forty-two of these received an additional cycle of hu14.18K322A (25 mg/m^2 once per day × 4 [n = 16] or 40 mg/m^2 once per day × 4 [n = 26]), and 31 also received parentally derived NK cells. Seventeen patients received BuMel without the additional cycle of hu14.18K322A with or without NK cells. There was no difference in EFS between patients who received ASCT with BuMel and those who received ASCT with BuMel or hu14.18K322A with or without NK cells (data not shown).

Survival Outcomes

As of November 4, 2020, 54 of the 64 patients were alive, with a median follow-up time for the 48 patients who did not experience an event of 4.3 years (range, 1.1-6.7 years). Two patients died of complications from therapy without

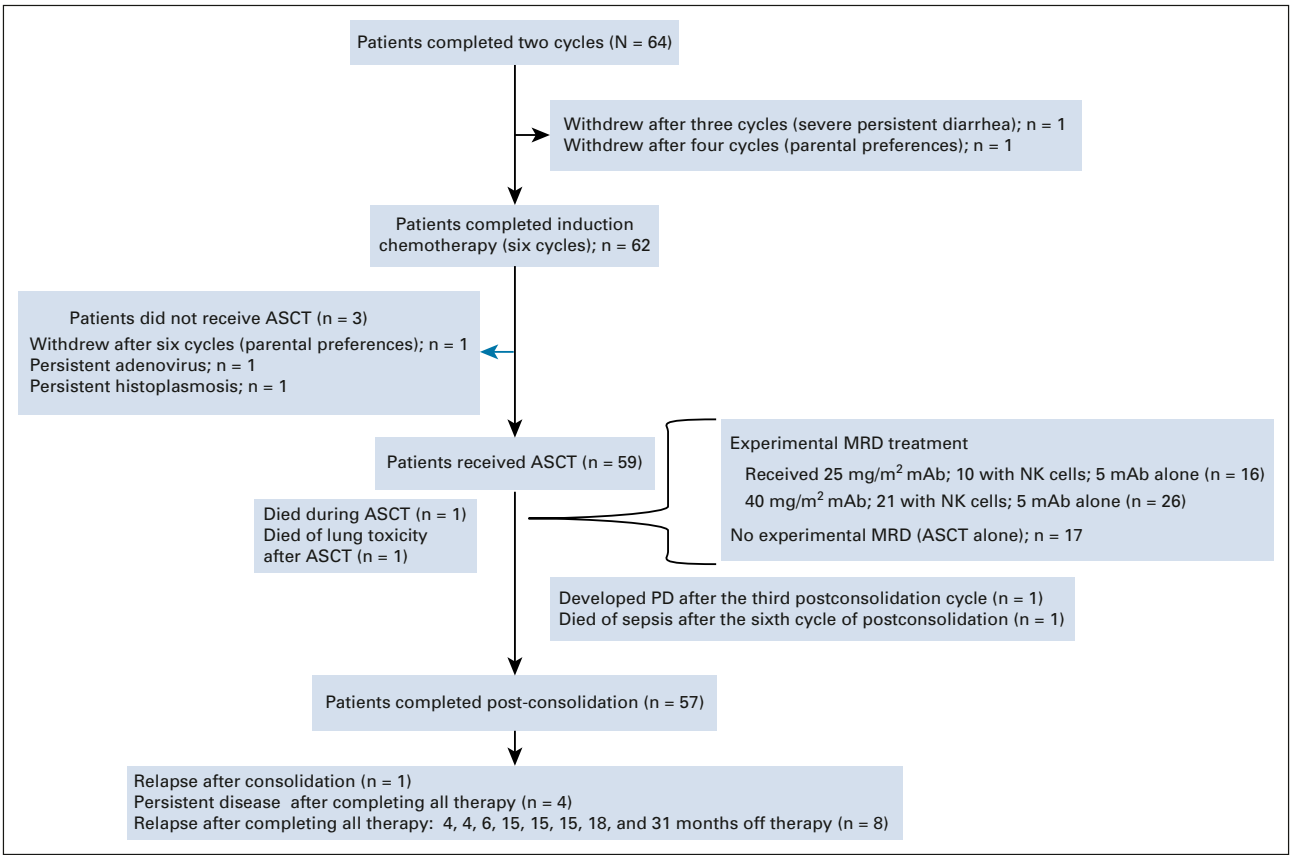

FIG 4. Flow diagram. ASCT, autologous hematopoietic stem-cell transplant; mAb, monoclonal antibody; MRD, minimal residual disease; PD, progressive disease.

evidence of disease (one because of transplant-related complications and the other because of non-neutropenic sepsis); one progressed after consolidation, four had persistent disease after completing all therapy; one patient experienced disease recurrence after postconsolidation minimal residual disease cycle 3; and eight patients experienced relapse at 4, 4, 6, 15, 15, 15, 18, and 31 months, respectively, after completing all therapy. Four of these patients, one with persistent disease and three with relapsed disease, also died of disease progression. The overall 3-year EFS and OS rates were 73.7% (95% CI, 60.0 to 83.4) and 86% (95% CI, 73.8 to 92.8), respectively (Fig 5).

DISCUSSION

We have demonstrated that the addition of hu14.18K322A to induction chemotherapy significantly improves early responses in patients with newly diagnosed HR neuroblastoma (66.7% *v* 39.1%; 95% CI 55.0 to 78.3 *v* 35.3 to 43.0; $P < .0001$) compared with ANBL0532.[15] Additionally, our chemoimmunotherapy induction regimen resulted in CSs ≤ 2 in 47 of 54 patients with INSS IV disease (87%), a finding that has been correlated with improved outcomes in both COG and SIOPEN trials.[17,18] The significant improvement in early responses and postinduction CSs suggests that hu14.18K322A, GM-CSF, and IL-2 added to induction chemotherapy could significantly affect outcome. Although our sample size was small, our encouraging 3-year EFS of 73.7% (95% CI, 60 to 83.4) compares favorably with recent COG, GPOH, and SIOPEN trials (Appendix Table A4, online only).[15,25,28,29] Park et al[15] reported an end-of-induction (EoI) Objective Response (≥ PR) of 75.2% and a 3-year EFS from the time of random assignment for tandem transplant of 51.1% (95% CI, 47.1 to 55.0) for all 652 eligible patients.[15] In our trial, the EoI Objective Response was 97%. More recently, Granger et al[25] evaluated BuMel ASCT after a five-cycle COG induction regimen and reported an EoI Objective Response of 80% and a 3-year EFS of 55.6% ± 4.2% for all 146 patients enrolled (Appendix Table A4).[25] Comparing NB2012 results with ANBL0032,[1,15,25] ANBL0532[15] and ANBL12P1[25] are complicated by the facts that in the study by Yu et al,[1] only patients who achieved a PR or better to induction therapy were included, Park et al[15] only randomly assigned patients at end of induction and only 355 of 652 patients originally enrolled were randomly assigned, and in Granger's report, 39 of 146 patients (26.7%) discontinued treatment early.[25] The NB2012 EFS estimate begins at the time of enrollment and includes all patients. Importantly, we did not observe disease progression in any patients during induction,

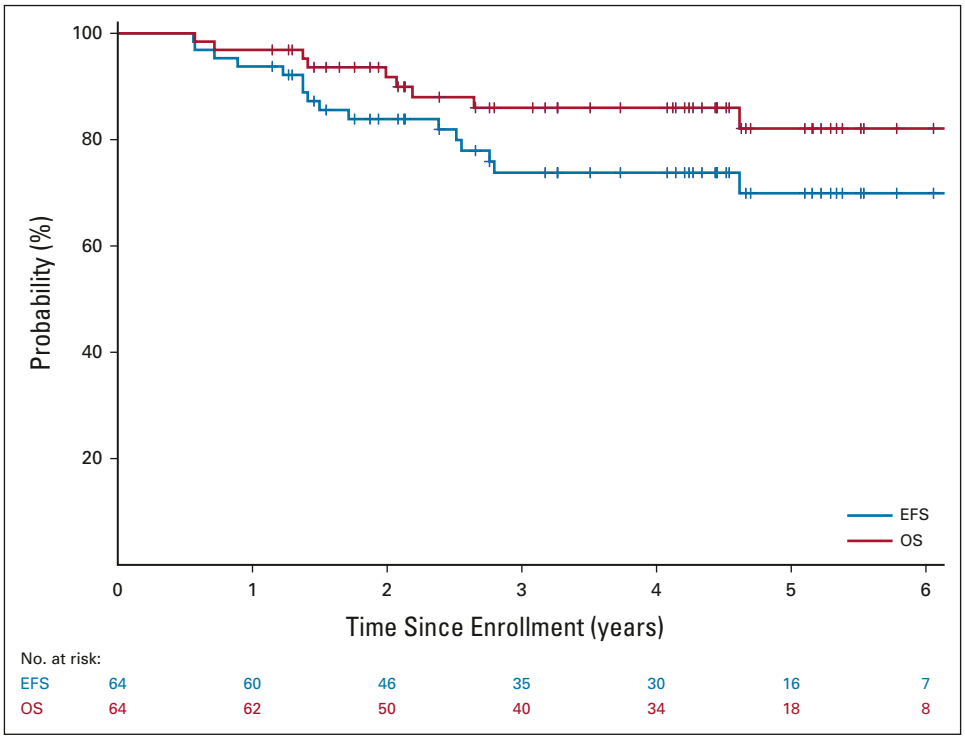

FIG 5. EFS and OS for all patients enrolled. EFS, event-free survival; OS, overall survival.

whereas 7%-14% of patients enrolled in previous COG trials experienced disease progression before receiving ASCT.[15,30]

Peak serum levels of hu14.18K322A during the induction and postconsolidation cycles generally exceeded the level of antibody expected to facilitate ADCC in vivo.[31] Higher levels of serum hu14.18K322A in cycle 1 were associated with more rapid response to treatment (PR or better after two cycles), consistent with dinutuximab plus temozolomide and irinotecan in relapsed neuroblastoma.[32] These findings may suggest that greater in vivo exposure to an anti-GD2 mAb might be associated with improved outcomes. Although there was no significant association of hu14.18K322A levels with EFS, this might reflect the overall favorable EFS for the patients in this trial, which limits the statistical power to associate differences in hu14.18K322A levels with favorable versus unfavorable outcomes. The incidence of HAHA was substantially less than that seen in our phase I trial (5 of 64 *v* 15 of 37 patients [40.5%; $P < .0001$, Fisher's exact test]).[11] Furthermore, the HAHA response here seemed weaker even in the five patients who developed HAHA (on the basis of the absence of any drop in peak hu14.18K322A levels for these five HAHA+ patients) compared with that observed in our previous phase I trial.[11] We think that this difference likely reflects the immunosuppressive effect of concurrent chemotherapy on the induction of an antidrug-antibody response (like HAHA or HACA), as also seen recently in a COG trial of dinutuximab plus temozolomide and irinotecan.[32]

Although promising, these results must be interpreted within the context of a single-institution phase II study. Although the chemotherapy backbone was identical and the major prognostic factors of our patient populations were comparable with those of ANBL02P1,[14] ANBL0532,[15] and ANBL12P1[25] (Appendix Table A1), several other differences in therapy might have contributed to the excellent NB2012 response rates. For example, GM-CSF was used during induction chemoimmunotherapy, rather than filgrastim, both for its ability to enhance ADCC[33,34] and for primary prophylaxis of febrile neutropenia.[35] Additionally, low-dose IL-2 was used for its ability to enhance ADCC.[36] The BuMel preparative regimen and additional cycle of hu14.18K322A (n = 42) with parental-derived NK cells (n = 31 of 42) during consolidation might have also affected EFS. Although BuMel was found to be a superior consolidation regimen within the context of rapid COJEC induction of SIOPEN,[37] an arm evaluating its efficacy within the COG induction on the current open COG HR trial ANBL1531 was recently closed because of inferior outcomes. NB2012 was not designed to evaluate the effects of these modifications on the overall outcome.

In conclusion, the addition of hu14.18K322A to induction chemotherapy resulted in a near doubling of early responses, major reductions in tumor volume, improved responses, and a very encouraging 3-year EFS of 73.7% (95% CI, 60 to 83.4). These results, if validated in a larger study, may change the standard of care for children with HR neuroblastoma.

AFFILIATIONS

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APPENDIX

TABLE A1. Comparison of Patient Characteristics

Characteristic	NB2012 (n = 64) (Reference level), No. (%)	ANBL02P1 (n = 31), No. (%)	*P*	ANBL0532 (n = 652), No. (%)	*P*	ANBL12P1 (n = 146), No. (%)	*P*
Age, months							
< 18	5 (8)	5 (16)	NS	Not reported		22 (15)	NS
≥ 18	59 (92)	26 (84)				124 (85)	
Median, months	37.5	30		37.2		37.3	
Sex							
Female	27 (42)	6 (19)	.028	286 (44)	NS	Not reported	
Male	37 (58)	25 (81)		366 (56)			
INSS stage							
IIb	1 (2)	Non stage IV: 4 (13)	NS	7 (1)	NS	Non stage IV: 20 (14)	NS
III	7 (11)			68 (10)			
IV	56 (87)	27 (87)		577 (89)		126 (86)	
MYCN status							
Not amplified	43 (67)	15 (60)	NS	327 (57)	NS	75 (56)	NS
Amplified	21 (33)	10 (40)		249 (43)		58 (44)	
Unreported	0	6		76		13	
Shimada histology							
Favorable	6 (9)	1 (3)	NS	23 (3)	.013	7 (5)	NS
Unfavorable	41 (64)	21 (68)		588 (86)		119 (82)	
Not performed	17 (27)	9 (29)		71 (10)		20 (14)	

Abbreviations: INSS, International Neuroblastoma Staging System; NS, not significant.

TABLE A2. Treatment-Related Grade 3/4 Toxicities Reported During the First Six Cycles of Induction Therapy in at Least 5% of Patients Plus Toxicities of Interest

Toxic Effect	Cycle 1 (n = 64)	Cycle 2 (n = 64)	Cycle 3 (n = 64)	Cycle 4 (n = 63)	Cycle 5 (n = 62)	Cycle 6 (n = 62)
Allergic						
Allergic reaction	—	—	—	—	1	—
Anaphylaxis	1	—	—	—	—	—
Cardiac						
Hypertension	5	2	1	—	2	2
Hypotension	1	—	2	3	2	4
Febrile neutropenia	30	12	4	35	12	30
Fever	15	2	—	5	4	6
GI						
Abdominal pain	3	2	2	2	3	7
Abnormal alkaline aminotransferase	3	4	5	1	1	4
Abnormal AST	4	1	1	1	1	1
Abnormal bilirubin	4	1	—	2	1	4
Diarrhea	2	2	4	1	3	1
GGT elevated	5	5	1	1	2	6
Nausea	—	—	4	3	4	3
Vomiting	—	1	7	8	2	6
Infection						
Catheter-related infection	2	4	2	6	4	9
Enterocolitis infectious	9	4	5	13	7	8
Sepsis	—	—	1	1	1	4
Upper respiratory infection	13	10	3	11	8	19
Urinary tract infection	3	—	6	5	2	8
Metabolic						
Acidosis	5	7	9	5	4	2
Hyperglycemia	3	4	9	7	9	3
Hypocalcemia	5	1	5	4	3	5
Hypokalemia	16	5	24	11	19	12
Hyponatremia	7	1	13	7	9	5
Hypophosphatemia	7	—	22	1	24	6
Mucosal						
Esophagitis	1	—	—	2	—	7
Mucositis	1	—	—	21	1	21
Pain	18	8	5	3	2	2
Respiratory						
Apnea	—	1[a]	—	1	—	—
Bronchospasm	2	—	—	—	1	—
Cough	1	—	2	1	—	—
Hypoxia	14	3	1	—	1	2
Pleural effusion	6	1	1	—	—	—
Respiratory failure[b]	1	—	—	—	—	—
Others						
Hearing impaired	—	—	2	4	2	9
Infusion-related reaction	6	1	4	2	1	—

Abbreviation: GGT, Gamma Glutamyl Transferase.

[a]Episode of apnea was related to opioid administration.

[b]Respiratory failure was related to significant thoracic disease burden.

TABLE A3. Serum Antibody Levels in Three Representative HAHA– Patients and All Five Patients Who Developed HAHA During Therapy

Pt-ID	HAHA[a]	Antibody	C1	C2	C3	C4	C5	C6	Int	M1	M2	M3	M4	M5	M6
9	–	K322A[b]	15.6	16.7	17.0	15.7	14.5	14.2	NA	12.9	10.5	11.8	13.7	13.9	NA
		HAHA	0	0	0	0	0	0	0	0	0	0	0	0	0
12	–	K322A	27.7	16.1	17.9	26.7	15.3	14.4	3.5	9.4	12.0	11.8	8.8	9.8	NA
		HAHA	0	0	0	0	0	0	0	0	0	0	0	0	0
13	–	K322A	12.2	23.2	21.0	23.2	20.6	17.9	NA	11.4	10.7	13.3	9.0	13.9	NA
		HAHA	0	0	0	0	0	0	NA	0	0	0	0	0	0
23	+	K322A	1.2	13.9	17.7	22.2	15.5	15.5	8.0	7.4	NA	9.5	NA	12	NA
		HAHA	0	0	0.15	0.71	NA	0.40	0	0	0	NA	0	0	0
66	+	K322A	12.4	NA	7.4	15.1	13.1	13.3	12.5	12.4	11.0	14.1	11.2	18.6	NA
		HAHA	0	1.3	0	0	0	0	0	0	0	0	0.89	0	0
69	+	K322A	5.9	3.8	17.0	16.2	18.3	15.4	10.3	12.7	11.5	12.1	15.8	16.8	NA
		HAHA	0	0.78	0	0	0	0	0	0	0	0	0	0	0
112	+	K322A	12.4	13.9	12.8	10.1	10.8	7.6	10.5	9.3	13.3	12.1	8.4	6.2	NA
		HAHA	0.72	3.2	1.7	0.84	0.46	0.93	0.82	0	0	0	0	0	0
145	+	K322A	5	16.8	10.6	9.5	12.6	15	NA	13	20	21.1	33.1	19.5	NA
		HAHA	0	0	0	0	0	0	0.82	0	0	0	0	0	0

Abbreviations: C, induction cycle; HAHA, human antihuman antibody; Int, intensification phase; K322A, hu14.18K322A; M, maintenance cycle; Pt-ID, patient identification number.

[a]HAHA levels, measured as optical density (OD) units, detected in a bridging ELISA assay with serum obtained at the initiation of the indicated cycle, before therapy began in that cycle. HAHA+ indicates patients with ≥ 0.7 OD unit increase at any time, as compared with the baseline C1 sample. The five patients who developed evidence of a HAHA response at any time are shown. Three representative patients (of 40) without a HAHA response are also shown.

[b]K322A concentration (μg/mL) for each indicated cycle, obtained 1 hour after completion of the K322A infusion on day 1 of that cycle.

TABLE A4. Comparison of End of Induction Objective Responses, EFS and OS of ANBL12P1, ANBL0532, HR-NBL1.5, NB2004-HR and NB2012

Response	ANBL12P1[25] (N = 146; 125 evaluable for response)	P[a]	ANBL0532[15] (N = 652)	P[a]	HR-NBL1.5[29] (N = 566)[b]		P[a]	NB2004-HR[29] (N = 389)[c]		P[a]	NB2012 (N = 64)
CR	28/125 (22.4%)	.0896	277 (42.5%)	< .0001[d]	16 (3%)		< .0001[d]	109 (28%)		.4010	21 (34%)
VGPR	30/125 (24%)	.1485			99 (17%)		.0011[d]	59 (15%)		≤ .0001[d]	21 (34%)
PR	42/125 (33.6%)	.5258	213 (32.7%)	.5519	295 (52%)		.0006[d]	177 (46%)		.0105[d]	18 (29%)
≥PR	80%	.0018[d]	75.2%	< .0001[d]	72%		< .0001[d]	89%		.0483	97%
MR	10/125 (8%)	NA	79 (12.1%)	.0510[d]	87 (15%)		NA				0
NR	9/125 (7.2%)	.41			50 (9%)		.1691	3 (1%)		.4475	2 (3%)
PD	6/146 (4.1%)	NA	46 (7.1%)	NA	12 (2%)		NA	40 (10%)		NA	0
D/C early	39/146 (26.7%)	< .0001[d]	NE 37 (5.7%)	.7788	Death before response evaluation 7 (1%)		.1546				3 (4%)
					rCOJEC (N = 313)[e]	N5 (N = 313)[e]		STD (N = 211)	EXP (N = 211)		
Mean 3-year EFS	55.6% ± 4.2%	.0131[d]	51.1% ± 3.95%	.0005[d]	44% (38%-50%)	47% (41%-53%)	< .0001[d] < .0001[d]	34% (28%-40%)	32% (26%-38%)	< .0001[d] < .0001[d]	73.7% ± 11.7%
Mean 3-year OS	74.5% ± 3.7%	.0640	Reported in graph only		60% (54%-66%)	65% (58%-70%)	< .0001[d] .0010[d]	54% (46%-62%)	48% (40%-56%)	< .0001[d] < .0001[d]	86.0% ± 9.5%

Abbreviations: CR, complete response; EFS, event free survival; MR, mixed response; NA, not applicable; NR, no response; OS, overall survival; PD, progressive disease; PR, partial response; VGPR, very good partial response.

[a]Compared to NB2012.

[b]Responses in HRNB1.5 were assessed prior to surgical resection of primary tumor.[28]

[c]NB2004-HR was a randomized comparison between six courses of standard (STD) induction chemotherapy versus an experimental (EXP) induction regimen with two additional courses of topotecan, cyclophosphamide and etoposide.[29]

[d]*P* values from two proportions Z-test with significance level ≤ 0.05.

[e]HR-NBL1.5 was a randomized comparison of two different induction regimens: rCOJEC (comprised eight cycles within 70 days alternating vincristine and carboplatin [course A], vincristine and cisplatin [course B], and vincristine, etoposide, and cyclophosphamide [course C] in the sequence ABCBABCB. The interval between courses was 10 days irrespective of hematologic recovery.) and MSKCC N5 (comprised high-dose cyclophosphamide, doxorubicin with vincristine [CAV] alternating with high-dose cisplatin, etoposide [PE] for a total of five courses [CAV, PE, CAV, PE, and CAV] within 110 days.)[28]