

Phase I/II Trial Outcomes of Naxitamab Combined With Stepwise Increased Dosing of Granulocyte–Macrophage Colony-Stimulating Factor in Primary Refractory High-Risk Neuroblastoma

James Anderson¹, Maria Rossi^{2*}, William Clark³

¹Department of Pediatric Oncology and Neuroblastoma Research, Faculty of Medicine, University of Manchester, Manchester, United Kingdom.

²Department of Immunotherapy and Targeted Therapy, Faculty of Medicine, University of Milan, Milan, Italy.

³Department of Clinical Trial Design and Biostatistics, Faculty of Medicine, University of Melbourne, Melbourne, Australia.

*E-mail ✉ maria.rossi@unimi.it

Abstract

In high-risk neuroblastoma, the presence of metastases in bone marrow (BM) or bones after induction therapy is linked to a worse outlook, although anti-GD2 antibodies such as naxitamab can produce responses in these sites. Here we present the outcomes from a phase I/II clinical study. Treatment cycles consisted of GM-CSF and naxitamab, given intravenously (over 30–90 minutes) on days +1, +3, and +5. During the phase I segment, naxitamab doses were increased stepwise; in the phase II expansion, it was given at the recommended phase II dose (RP2D) of 9 mg/kg/cycle (approximately 270 mg/m²/cycle). Following a major response—defined as complete response (CR) or partial response—cycles were repeated monthly for a total of 5 cycles. Of the 32 participants, 24 (75%) achieved CR, including 12 who met international criteria and 12 who showed resolution of MIBG-avid lesions, as evidenced by negative PET scans. BM CR was achieved in 22 of 23 patients with BM metastases. Among 29 individuals with abnormal ¹²³I-MIBG scans, major responses were seen in 14 of 20 with Curie scores (CS) of 10–25 and in 7 of 9 with CS of 1–9. Five of the 9 patients who had received other anti-GD2 antibodies earlier remained event-free survivors. After the protocol, 18 patients received an anti-neuroblastoma vaccine. The five-year progression-free survival and overall survival rates were 38% and 64%, respectively. Baseline CS, previous second-line treatment, and prior anti-GD2 antibody exposure did not have a statistically significant effect on survival. The combination of naxitamab plus GM-CSF represents a promising approach for primary refractory osteomedullary neuroblastoma, even in cases with substantial disease burden.

Keywords: Neuroblastoma, Naxitamab, Immunotherapy, Anti-GD2 antibody, GM-CSF

Introduction

Approximately 50% of patients with high-risk neuroblastoma (HR-NB) exhibit detectable disease in bone marrow (BM) or on ¹²³I-metaiodobenzylguanidine

(MIBG) scans following induction chemotherapy [1-3]. This form of primary refractory disease is associated with unfavorable outcomes [4]. However, chemoresistant neuroblastoma cells within the BM represent a suitable target for monoclonal antibody (mAb)-based immunotherapy, as the marrow space is highly vascularized. This facilitates ready access of residual tumor cells to both the antibody and immune effector cells (such as neutrophils and macrophages), overcoming a key barrier to immunotherapy: limited penetration into solid tumors. Earlier investigations [5] and subsequent research [6] have demonstrated the potential of anti-GD2 mAbs against BM disease, although activity against soft-

Access this article online

<https://smerpub.com/>

Received: 18 February 2024; Accepted: 25 April 2024

Copyright CC BY-NC-SA 4.0

How to cite this article: Anderson J, Rossi M, Clark W. Phase I/II Trial Outcomes of Naxitamab Combined With Stepwise Increased Dosing of Granulocyte–Macrophage Colony-Stimulating Factor in Primary Refractory High-Risk Neuroblastoma. Arch Int J Cancer Allied Sci. 2024;4(1):164-75. <https://doi.org/10.51847/od683BuFL5>

tissue neuroblastoma has been limited. The effectiveness against sizable cortical bone metastases remains unclear. Collaborative group trials have established the benefit of anti-GD2 mAbs dinutuximab [7] and dinutuximab beta [8] in HR-NB. These chimeric antibodies were combined with granulocyte-macrophage colony-stimulating factor (GM-CSF) plus interleukin-2 (IL-2) [7], IL-2 alone [8], or neither cytokine [8]. The murine anti-GD2 IgG3 mAb 3F8, administered with GM-CSF, has shown efficacy in consolidating first or subsequent remissions in HR-NB [9, 10] and in clearing primary refractory BM disease [11]. Immune responses against anti-GD2 antibodies can generate anti-drug antibodies that may diminish effectiveness by lowering circulating mAb concentrations. To mitigate this, naxitamab (formerly known as humanized-3F8), a humanized IgG1 version of 3F8, was developed [12].

Preclinical evaluations highlighted distinctions between naxitamab and other anti-GD2 mAbs [12]. Key advantages of naxitamab included enhanced antibody-dependent cellular cytotoxicity (ADCC) and preserved yet moderated complement-dependent cytotoxicity (CDC). The reduced CDC activity was expected to lessen infusion-related pain, which is often linked to complement activation [13]. We therefore postulated that naxitamab at elevated doses would be well tolerated and would elicit strong anti-neuroblastoma activity, consistent with the observed dose-dependent enhancement of ADCC in laboratory studies [14]. In the phase I portion [15], no dose-limiting toxicity was reached, and the RP2D of 9 mg/kg/cycle (≈ 270 mg/m²/cycle)—more than 2.5 times the typical dose of other anti-GD2 mAbs [7-9]—was selected based on serum level plateauing. Positive efficacy and safety data from an international phase II study [16] supported FDA approval for use in a subset of relapsed/refractory HR-NB patients with persistent bone or BM involvement who had not progressed on prior treatment.

Multiple lines of evidence justify pairing GM-CSF with anti-GD2 mAbs. The cytokine is well tolerated clinically and enhances granulocyte-mediated ADCC against neuroblastoma cells, with killing efficiency dependent on the effector-to-target cell ratio [14]. GM-CSF dosing and scheduling have differed across HR-NB trials. It was not used with dinutuximab beta. For dinutuximab, it has been given at 250 μ g/m²/day either intravenously or subcutaneously [7, 17]. In contrast, studies with 3F8 initially employed intravenous GM-CSF, followed by subcutaneous administration and always incorporated a

priming phase at 250 μ g/m²/day, followed by escalation to 500 μ g/m²/day during the week of antibody infusions [9-11]. This dose increase was intended to capitalize on the dose-response relationship of GM-CSF on ADCC [14]. Subcutaneous delivery and step-up dosing led to greater granulocyte activation, which independently predicted improved progression-free survival (PFS) relative to intravenous GM-CSF or no GM-CSF [9-11, 18]. A randomized study would be necessary to compare the higher GM-CSF regimen definitively with the standard dose.

This report summarizes findings from a phase I/II trial evaluating naxitamab combined with GM-CSF in individuals with primary refractory neuroblastoma involving bones and/or bone marrow.

Materials and Methods

The analysis includes all HR-NB patients treated under Memorial Sloan Kettering Cancer Center (MSK) protocol 12-230 (Clinicaltrials.gov NCT01757626) who had measurable chemoresistant disease confined to bones and/or BM, without soft-tissue involvement or prior disease progression. The study began as a phase I dose-escalation trial and proceeded to phase II expansion at the RP2D [15]. The expansion cohort comprised three groups: Group 1—patients with primary refractory BM disease (the focus of this report, including 8 subjects not covered in the earlier phase I publication [15]); Group 2—patients in second or later complete remission; and Group 3—patients with persistent disease after salvage therapy for relapse. Outcomes for Groups 2 and 3 will be described separately. The primary objectives for Group 1 were to evaluate treatment activity using response rates and PFS. Eligibility required organ function toxicity no worse than grade 2 per Common Toxicity Criteria for Adverse Events Version 4.0 and completion of at least 3 weeks without systemic therapy before starting. The MSK Institutional Review Board approved the protocol, and written informed consent was obtained.

Protocol treatment

Each cycle began with subcutaneous priming doses of yeast-derived recombinant GM-CSF (sargramostim; Berlex Laboratories, Seattle, WA) at 250 μ g/m²/day on days -4 to 0, followed by escalation to 500 μ g/m²/day on days +1 to +5. GM-CSF was paused if the absolute neutrophil count exceeded 20,000/ μ l. Naxitamab was administered intravenously over 30–90 minutes on days

+1, +3, and +5. Dose escalation occurred in phase I [15], establishing the RP2D of 3 mg/kg per infusion (9 mg/kg/cycle, or ~270 mg/m²/cycle) for phase II. Premedications consisted of opioids and antihistamines [19].

Cycles were given monthly and continued until disease progression, unacceptable toxicity, or completion of 5 cycles after a major response (CR or PR). Human anti-human antibody (HAHA) levels were monitored after each cycle as previously described [15]. Positive HAHA results led to treatment delays until levels normalized. Consistent with the prior 3F8 study (MSK protocol 13–260, NCT02100930), patients could receive low-dose maintenance chemotherapy, such as irinotecan-temozolomide [20] or rituximab, to reduce HAHA when needed, and resume naxitamab + GM-CSF once HAHA turned negative.

Radiotherapy to the primary tumor site, with possible treatment of metastatic sites, was permitted per standard HR-NB guidelines [21, 22].

Treatment response assessment

Disease evaluations, as previously outlined [23], were scheduled after cycles 2 and 4, then every 3 months. Assessments included ¹²³I-MIBG scans with SPECT (or ¹⁸F-FDG PET) and bone marrow histology. Responses were assessed according to the International Neuroblastoma Response Criteria (INRC) [24], with MIBG uptake quantified using the Curie score (CS). CR was defined as no detectable neuroblastoma; PR as no new lesions plus > 50% reduction in MIBG absolute bone score (relative Curie bone score ≥ 0.1 to ≤ 0.5) or $\geq 50\%$ decrease in PET-avid bone lesions, along with BM showing CR or minimal disease (> 0 to < 5% tumor cells); minor response as meeting partial criteria in one modality but stable in the other; SD as insufficient change for PR or PD; and PD as new lesions or >20% increase in existing lesions.

CR was additionally recorded for MIBG-positive sites with BM CR (assessed at 4 sites [23]), exceeding the standard 2-site INRC requirement [24], and for negative PET scans. The value of PET in distinguishing residual MIBG uptake from inactive disease has been noted in prior small series [25].

Biostatistics

This represents the final report for the primary refractory cohort; accrual is closed. Because phase I and phase II eligibility criteria overlapped, all patients from both segments are included. The study was structured as a single-stage design with two co-primary endpoints (response rate and PFS). A sample of 47 patients was calculated to test the null hypothesis that the CR rate is 67% and the 2-year PFS is 48%, with a 10% type I error and over 90% power. Success thresholds were ≥ 40 patients in CR or ≥ 28 progression-free at 2 years. Due to early closure of accrual following the opening of the international trial [16], these decision rules do not apply, and descriptive estimates of response and survival are provided. PFS was measured from study registration to progression or death, with patients alive and progression-free censored at last follow-up. Overall survival (OS) was defined as the time from registration to death, with patients censored at last follow-up. Survival probabilities were calculated via the Kaplan–Meier method. Exploratory associations between clinical variables and survival were examined using Cox proportional hazards regression. Variables included naxitamab dose level (RP2D vs. below RP2D), age at diagnosis, interval from diagnosis, baseline CS, prior second-line therapy, and prior anti-GD2 mAb exposure. Estimates include two-sided 95% confidence intervals (95% CI), using exact methods for proportions and log-survival-based intervals for time-to-event data.

Results and Discussion

Patient characteristics

Between April 23, 2014, and August 6, 2019, the 32 enrolled patients were 4.6–35.4 months (median 7.6) from initial diagnosis and ranged in age from 2.4 to 10.9 years (median 4.7) at the time of protocol entry (**Table 1**). Ages at diagnosis were 1.7–10.5 years (median 3.8). Six patients received doses below the RP2D (4.8–8.4 mg/kg/cycle) while 26 received the full RP2D of 9 mg/kg/cycle. Three patients had BM disease with negative MIBG scans. 25 patients presented with CS 1–2 or ≥ 3 , categories previously linked to poorer prognosis after induction in tandem or single myeloablative transplant settings [26, 27]. Twenty patients had high disease burden (CS 10–25).

Table 1. Characteristics of the 32 study patients.

Males: females (ratio)	18:14 (1.3)
------------------------	-------------

MYCN amplification	3 (9%)
Age at initial diagnosis: median (range)	3.8 (1.7–10.5) years
Age at 1st dose of naxitamab: median (range)	4.7 (2.4–10.9) years
Time from diagnosis to naxitamab: median (range)	7.6 (4.6–35.4) months
Curie score at enrollment median (range)	13 (0.25)
Curie 0	3 ^a
Curie 1 or 2	4
Curie 3–9	5
Curie 10–19	14
Curie 20–25	6
Naxitamab dosage	
Suboptimal/< RP2D	6 (19%)
Optimal/RP2D	26 (81%)
Prior treatment	
Induction chemotherapy	
COG	18 (56%)
MSK	12 (38%)
Other	2 (6%)
Second-line chemotherapy ^b	23 (72%)
¹³¹ I-MIBG therapy	10 (31%)
Anti-GD2 monoclonal antibody ^b	9 (28%)
Autologous stem-cell transplantation	1 (3%)

1. RP2D recommended phase II dosage

2. ^aAll had metastatic involvement of bone marrow

3. ^bUsed for refractory disease

All patients had previously undergone high-risk induction chemotherapy, consisting of the ANBL0532 regimen in 18 cases [21], an MSK-specific regimen in 12 cases [28], or alternative protocols in 2 cases. Twenty-three patients (72%) had also received second-line therapy. Twelve patients (37%) had been treated with ¹³¹I-MIBG therapy, and nine patients (28%) had prior exposure to anti-GD2 monoclonal antibodies (dinutuximab or murine 3F8), four of whom received these antibodies combined with irinotecan-temozolomide (chemoimmunotherapy) [29] (**Table 1**). Only one patient had undergone myeloablative therapy (MAT) followed by stem cell transplantation.

Responses

Among the 32 patients, the best overall response was complete response (CR) in 24 individuals (75%; 95% CI:

57%–89%). These CRs included 12 cases that fulfilled the International Neuroblastoma Response Criteria (INRC) [24] and 12 additional cases characterized by persistent MIBG positivity, bone marrow CR, and negative PET scans [23]. Partial response (PR) occurred in 4 patients (12%, 95% CI: 4%–29%), stable disease (SD) in 3 patients (9%, 95% CI: 2%–25%), and progressive disease (PD) in 1 patient (3%, 95% CI: 0%–16%) (**Table 2**).

Among the six phase I participants treated at doses below the RP2D, responses included 4 CR, 1 PR, and 1 PD. Major responses (CR or PR) were first observed after cycle 2 in 19 patients, after cycle 3 in 3 patients, after cycle 4 in 2 patients, and after cycles 5 and 6 in one patient each. The median time to achieving a major response was 1.5 months (at the evaluation after cycle 2), with a range of 1.5 to 5 months.

Table 2. Responses^a.

Sites of disease at enrollment	#patients	CR		PR	SD	PD	RT/1 or 2 bony site(s)
		INRC	PET (-)				
BM only	3	3	n/a	0	0	0	0
MIBG only							
Curie 1–9	3	1	1	0	0	1	0
Curie 10–24	6	1	2	1	2	0	2
MIBG & BM							

Curie 1–9	6	3	1	1	1 ^b	0	4
Curie 11–25	14	4	8	2	0	0	6
Total	32	12	12	4	3	1	12
Response rate (95% CI)		37% (21–56%)	37% (21–56%)	12% (4–29%)	9% (2–25%)	3% (0–16%)	

BM= bone marrow, CI = confidence interval, CR = complete remission, INRC = International Neuroblastoma Response Criteria, n/a = not applicable, PET = positron emission tomography, PD = progressive disease, PR = partial remission, and SD = stable disease.

^a No patient had a minor response

^b Came off study after 1 cycle because of HAHA-positivity

At the time of study entry, neuroblastoma was detected in the bone marrow of 23 out of 32 enrolled patients (**Table 2**). One individual was withdrawn from the trial following the initial cycle owing to HAHA positivity and exhibited persistent bone marrow involvement. All 22 other patients with baseline bone marrow disease achieved complete marrow remission (22/23 = 96%, 95% CI: 78%–100%), and 21 of these clearances were confirmed after only 2 treatment cycles.

Osteomedullary disease was visible on MIBG scans in 29 of the 32 patients (**Table 2**), with patient-specific outcomes displayed in **Figure 1**. In the subset of 20 patients who had widespread MIBG uptake (Curie scores ranging from 10 to 25), a major response (either CR or PR) was recorded in 14 cases (14/20 = 70%, 95% CI: 46%–88%), 11 of which occurred after the first two cycles. Among the 9 patients with more limited MIBG involvement (Curie scores 1–9), 7 experienced a major response (7/9 = 78%, 95% CI: 40%–97%), 6 of them after just two cycles.

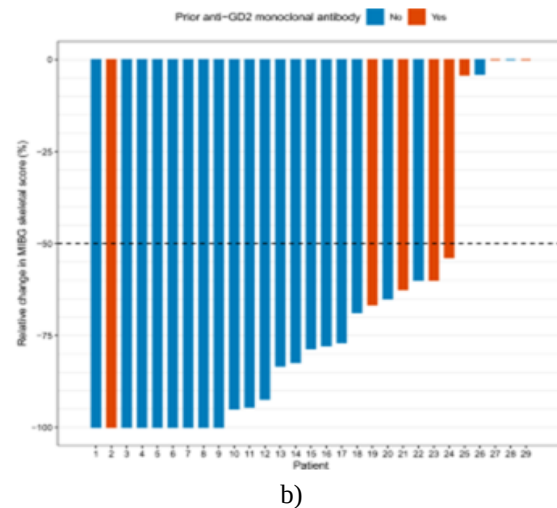
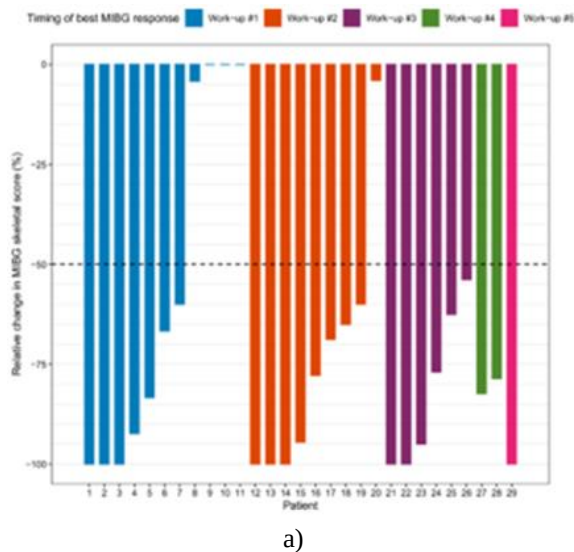


Figure 1. A waterfall plot illustrated the extent of the reduction in the Curie score for each patient. Notably, major responses were observed even among those presenting with a heavy disease burden.

Nine patients had been treated with other anti-GD2 monoclonal antibodies before enrollment. One patient had received both dinutuximab combined with irinotecan-temozolomide and 3F8; this individual attained CR (limited to bone marrow disease) with naxitamab plus GM-CSF, subsequently received the vaccine, and later relapsed at 38 months. Of the three additional patients previously given dinutuximab/irinotecan-temozolomide (with enrollment Curie scores of 13, 13, and 24), two achieved long-term event-free survival (54+ and 63+ months from study entry). Three out of five patients who had previously been treated with 3F8 attained prolonged event-free survival (exceeding 6 years post-enrollment). This group included one patient enrolled with bone marrow disease only and two others enrolled with Curie scores of 8 and 18.

Consolidative radiotherapy to the primary tumor site was delivered after study enrollment in 25 patients, with the following timing: after cycle 1 in 16, after cycle 2 in 4, after cycle 3 in 3, after cycle 4 in 1, and after cycle 6 in

1. Three patients had already received this radiotherapy before joining the trial, while four patients never received it. In addition, radiotherapy was applied to one bony metastatic site in eight patients and to two bony sites in four patients. All 12 of these individuals remained evaluable for response assessment according to INRC criteria [24], as ten had concurrent bone marrow involvement and the remaining two (without BM metastases) presented with Curie scores of 7 and 20. Seven of these patients subsequently experienced disease progression.

Relapse locations varied: isolated single bony site (3 of 6 patients became long-term survivors), central nervous system only (2 of 3 long-term survivors), soft tissue only (0 of 3 long-term survivors), and disseminated disease (2 of 7 long-term survivors). These outcomes followed individualized salvage therapies tailored to each case. For the 19 patients who progressed, the interval from study enrollment to progression ranged from 3.5 to 38 months (median 11 months), while the time from major response (CR or PR) to progression ranged from 2.5 to 37 months (median 10 months).

Survival

Of the 22 patients who completed protocol treatment without progression, 21 received additional therapy afterward. Post-protocol interventions consisted of an anti-neuroblastoma vaccine [30] alone in 17 patients, vaccine followed by DFMO [31] in 2 patients, dinutuximab plus GM-CSF followed by vaccine in the single patient who discontinued after one cycle due to HAHA positivity, and isotretinoin monotherapy in 1 patient. Thirteen of the 32 study participants remained progression-free after enrollment: 12 were alive and progression-free at 4.5+ to 8.6+ years, and one died at 2.1 years from pulmonary toxicity unrelated to the monoclonal antibodies. Eight of these 13 long-term progression-free patients had achieved CR, as evidenced by MIBG-positive disease and negative PET scans.

Figure 2 displays the progression-free survival (PFS) and overall survival (OS) curves. For the entire group, PFS rates were 62% (95% CI: 48%–82%) at 1 year, 53%

(95% CI: 38%–74%) at 2 years, and 38% (95% CI: 24%–59%) at 5 years. OS rates were 100% at 1 year, 90% (95% CI: 80%–100%) at 2 years, and 64% (95% CI: 49%–84%) at 5 years. None of the clinical variables examined showed a statistically significant association with PFS or OS (**Table 3**). Nonlinear relationships were also evaluated for time from diagnosis and baseline Curie score, with no significant associations with PFS or OS identified. Only three patients had MYCN amplification; therefore, its association with survival was not formally analyzed. Of these three, two experienced disease progression and none died.

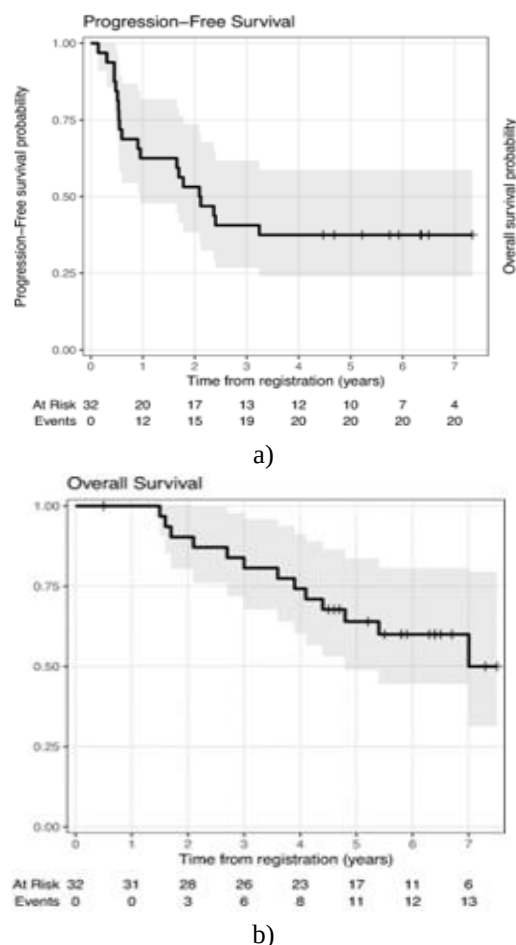


Figure 2. PFS and OS for the whole cohort, with 5-year PFS/OS rates of 38%/64%

Table 3. Prognostic impact of the clinical factors on PFS and OS (Cox regression models; n = 32 for each model)

Characteristic	PFS model				OS model			
	2-year PFS rate (95% CI)	HR	95% CI	P-value	2-year OS rate	HR	95% CI	P-value
Dosage								
RP2D	54% (38%, 77%)	–	–	–	92% (82%, 100%)	–	–	–
Lower than RP2D	50%	0.75	0.22, 2.55	0.64	83%	0.44	0.08, 2.35	0.34

	(22%, 100%)				(58%, 100%)			
Age (years)		1.07	0.88, 1.30	0.51		1.03	0.80, 1.33	0.82
Time from diagnosis (months)		1.03	0.93, 1.14	0.62		1.04	0.94, 1.15	0.40
Baseline Curie score		1.02	0.96, 1.08	0.46		1.03	0.95, 1.11	0.50
2nd-line therapy								
No	33% (13%, 84%)	–	–	–	78% (55%, 100%)	–	–	–
Yes	61% (44%, 84%)	0.57	0.22, 1.44	0.23	95% (87%, 100%)	0.60	0.16, 2.21	0.44
Prior anti-GD2 monoclonal antibody^a								
No	48% (31%, 73%)	–	–	–	91% (80%, 100%)	–	–	–
Yes	67% (42%, 100%)	0.52	0.17, 1.56	0.24	89% (71%, 100%)	0.45	0.14, 1.42	0.17

1. HR = Hazard Ratio, CI = Confidence Interval, RP2D = recommended phase II dosage.

2. ^am3F8 or dinutuximab

Figure 3 presents progression-free survival (PFS) and overall survival (OS) by disease characteristics at study entry. The predominant subgroup comprised patients whose disease was confirmed by both MIBG imaging and bone marrow histology (n = 22). In this cohort, the 2-year PFS rate was 55% (95% CI: 37%–82%), and the 5-year PFS rate was 40% (95% CI: 23%–68%). Corresponding OS rates were 90% (95% CI: 78%–100%) at 2 years and 69% (95% CI: 51%–93%) at 5 years.

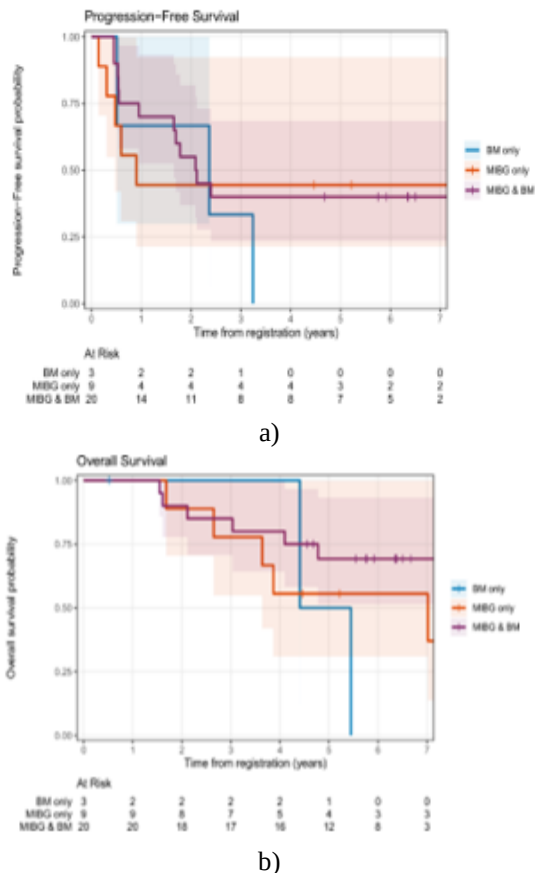


Figure 3. PFS (a) and OS (b) for patient subsets. The 5-year PFS/OS rates were 40%/69% for the largest

group, i.e., patients with disease evident by MIBG scan and BM studies

HAHA and toxicity

Participants underwent 1 to 15 cycles of naxitamab plus GM-CSF (median of 7), for a total of 224 cycles delivered. HAHA that disrupted timely treatment developed in three patients (9%), appearing after cycles 1 or 2. One of these individuals had earlier exposure to 3F8. Two patients became HAHA-negative following two cycles of rituximab combined with irinotecan-temozolomide [20] and were able to restart naxitamab plus GM-CSF. The third patient was withdrawn from the study (as noted earlier) [20]. One additional patient developed HAHA positivity after cycle 9, at which point disease progression was also identified; this patient had previously received 3F8.

Toxicities were generally controllable and compatible with outpatient management. No participant discontinued protocol therapy due to adverse events such as anaphylaxis. As anticipated with naxitamab [15, 19] and other anti-GD2 monoclonal antibodies [6, 17, 22], the most frequent side effects included grade 1–3 pain, fluctuations in blood pressure, hives, fever, and bronchospasm. One patient required hospitalization for hypertension. Grade 1–2 Adie's pupil (characterized by pupil dilation, light sensitivity, and impaired accommodation) occurred in two patients. Two cycles were abbreviated due to breathing issues, and one was shortened due to bradycardia. One patient experienced an allergic reaction to GM-CSF. The step-up dosing schedule for GM-CSF was not associated with any toxicity, whether hematologic or non-hematologic. No cases of capillary leak syndrome were observed.

This trial demonstrated that naxitamab combined with GM-CSF possesses meaningful anti-neuroblastoma activity in individuals with high-risk neuroblastoma (HR-NB) featuring primary refractory disease limited to

osteomedullary locations. Major responses, including both complete and partial remissions, were achieved even in patients presenting with substantial tumor loads (Curie scores 10–25). Acute side effects were manageable and did not compromise delivery in the outpatient setting. The escalated GM-CSF dosing regimen did not result in additional adverse effects. Development of HAHA was uncommon. These observations may help identify which HR-NB patients are most likely to benefit from anti-GD2 monoclonal antibody therapy paired with GM-CSF. Considering that roughly 50% of patients in large cooperative trials show incomplete responses after induction [1-3], the pool of individuals with refractory bone and/or bone marrow disease (without soft-tissue involvement) who could benefit is considerable.

The study cohort was clearly characterized. By comparison, other published experiences have several constraints when attempting to evaluate the effectiveness of anti-GD2 monoclonal antibodies specifically against primary refractory HR-NB: (1) inclusion of patients already in complete remission, which prevents assessment of objective tumor response; (2) enrollment of mixed populations encompassing primary refractory, secondary refractory, and multiply relapsed cases; (3) insufficient information regarding the extent of disease burden before antibody administration; (4) absence of data on anti-drug antibody formation (such as HACA) that can impair therapeutic activity; and (5) concurrent administration with isotretinoin [3, 7, 8, 17, 22].

None of the clinical variables evaluated had a statistically significant effect on response or survival outcomes (**Table 3**). This lack of association could stem from the relatively small sample size, which restricts the statistical power of subgroup analyses. It may also reflect that 23 of 32 patients (72%) had already undergone second-line therapy and 20 of 32 (62.5%) presented with high-burden disease (Curie score ≥ 10), thereby diminishing the discriminatory value of other prognostic factors. The strong responses observed even at doses below the RP2D could be explained by the fact that the lowest dose tested (4.8 mg/kg/cycle, or approximately 144 mg/m²/cycle) still exceeded the conventional dosing range of 70–100 mg/m²/cycle used with other anti-GD2 antibodies [7-9]. Among the nine patients enrolled after prior exposure to 3F8 or dinutuximab, five achieved sustained event-free survival. Although the numbers are limited, these results indicate that naxitamab plus GM-CSF may represent a viable option for individuals whose disease persists

following treatment with alternative anti-GD2 monoclonal antibodies.

Earlier findings with 3F8 plus GM-CSF in primary refractory HR-NB have been described [11]. However, direct comparisons require caution. In addition to receiving isotretinoin, those patients also had substantial differences in baseline disease burden: only 3 of 32 naxitamab-treated patients (9%) had bone marrow positivity with negative MIBG scans, compared to 29 of 72 (40%) in the 3F8 group. Likewise, Curie scores ≥ 10 were present in 20 of 32 (62%) naxitamab patients versus only 5 of 72 (7%) 3F8 patients. Importantly, while all 32 patients in the current naxitamab series were evaluable for response, 23 of the 72 patients in the 3F8 cohort received radiation to their sole MIBG-positive site (without bone marrow involvement), rendering them inevaluable for response assessment. In both the naxitamab and 3F8 experiences, tumor responses typically occurred rapidly, often evident after only two cycles.

Despite the markedly higher disease burden in the naxitamab cohort, the complete response rate of 75% was comparable to the 68% observed with 3F8 plus GM-CSF [11]. It is noteworthy that during the period when 3F8 was primarily used, patients with extensive osteomedullary involvement were generally not considered optimal candidates for anti-GD2 immunotherapy.

With respect to response durability, the 2-year PFS rate of 53% with naxitamab plus GM-CSF was similar to the 48% reported with murine 3F8 plus GM-CSF. The 5-year PFS rate of 38% with naxitamab compared favorably to the 24% achieved with 3F8 [11]. However, the frequent use of an anti-neuroblastoma vaccine [30] following protocol therapy complicates attribution of long-term PFS and OS outcomes solely to naxitamab plus GM-CSF. This vaccine is offered to HR-NB patients who have completed conventional therapy, including anti-GD2 immunotherapy, and are in complete remission. It aims to stimulate antibody production against GD2 and GD3, potentially contributing to the elimination of any remaining neuroblastoma cells.

Five-year overall survival rates were comparable between the present study (64%) and the earlier 3F8 report (65%) [11]. These robust long-term survival figures underscore the potential curability of relapsed HR-NB [10]. The data reinforce the value of immunotherapy in improving survival in patients with residual disease after induction, although the optimal

immunotherapy approach has yet to be defined. Encouraging salvage outcomes were noted in cases of isolated central nervous system relapse and relapse confined to a single bone site.

Radiation to the primary tumor site is a standard component of HR-NB treatment to reduce the risk of local recurrence. Three patients in this trial received such treatment before enrollment, and 25 received it afterward, including 16 patients after just one cycle of naxitamab plus GM-CSF. Twelve additional patients underwent radiation to one or two distant MIBG-avid bone sites. This did not affect response evaluability because disease was present at other locations. Overall, radiotherapy likely contributed to the durability of disease control.

Administering radiotherapy after the start of immunotherapy has been the longstanding institutional approach at our center, typically following the first cycle of anti-GD2 antibody treatment. This differs from the sequence used in the COG [21] and SIOPEX [22] protocols, in which radiation is completed before immunotherapy is initiated. The rationale is to introduce immunotherapy promptly, given the primary concern of controlling metastatic disease. Emerging evidence from preclinical and clinical studies further supports this sequencing, as potential synergistic vaccination-like effects occur when radiation is combined with immunotherapy [32-34].

A range of therapeutic strategies have been employed for HR-NB patients with residual disease at the end of induction [3]. A frequent objective is to achieve sufficient tumor reduction to proceed with consolidation using myeloablative therapy (MAT). In the current study, none of the patients received MAT after naxitamab, even though MAT remains standard practice in many other centers. Several considerations supported this decision. First, the patients had demonstrated chemoresistant disease, yet MAT involves intensive, potentially highly toxic chemotherapy [35, 36]. Second, while older randomized trials suggested a benefit for MAT over non-MAT approaches [37-39], extended follow-up of one trial revealed no long-term survival advantage [40]. Those studies have important limitations that reduce their applicability today, including less effective induction regimens, varying high-dose chemotherapy combinations, infrequent use of radiotherapy, and lack of anti-GD2 immunotherapy. Finally, analyses of non-randomized MSK experience with HR-NB have not shown a survival benefit from MAT [9, 41, 42]. A

prospective randomized trial would be required to determine whether MAT can safely be omitted in this setting.

Chemoimmunotherapy combining dinutuximab, irinotecan, temozolomide, and GM-CSF has become widely adopted for chemoresistant HR-NB based on highly promising outcomes [29]. Similar encouraging results have been reported with other anti-GD2 antibodies paired with irinotecan-temozolomide or alternative chemotherapeutic agents [43-47]. Direct comparisons to determine the preferred approach—monoclonal antibody plus GM-CSF versus chemoimmunotherapy—for purely osteomedullary disease are challenging due to differences in patient populations and disease burden assessments. A randomized study would be needed to resolve this question. In cases involving soft-tissue metastases, we combine naxitamab with chemotherapy, recognizing the limited activity of anti-GD2 antibodies against neuroblastoma in soft-tissue sites [5, 6].

Conclusion

Naxitamab plus GM-CSF offers a compelling treatment choice for patients with extensive chemoresistant osteomedullary high-risk neuroblastoma who have not experienced disease progression, including those with post-induction residual disease. Additional data will help determine its usefulness in individuals who have been previously exposed to other anti-GD2 monoclonal antibodies. The convenience of outpatient administration and minimal immunogenicity represent further advantages of this immunotherapeutic regimen.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Berthold F, Falduum A, Ernst A, Boos J, Dilloo D, Eggert A, et al. Extended induction chemotherapy does not improve the outcome for high-risk neuroblastoma patients: results of the randomized open-label GPOH trial NB2004-HR. *Ann Oncol*. 2020;31(3):422–9.

2. Garaventa A, Poetschger U, Valteau-Couanet D, Luksch R, Castel V, Elliott M, et al. Randomized trial of two induction therapy regimens for high-risk neuroblastoma: HR-NBL1.5 International Society of Pediatric Oncology European Neuroblastoma Group Study. *J Clin Oncol*. 2021;39(23):2552–63.
3. Desai AV, Applebaum MA, Karrison TG, Oppong A, Yuan C, Berg KR, et al. Efficacy of post-induction therapy for high-risk neuroblastoma patients with end-induction residual disease. *Cancer*. 2022;128(15):2967–77.
4. Pinto N, Naranjo A, Hibbitts E, Kreissman SG, Granger MM, Irwin MS, et al. Predictors of differential response to induction therapy in high-risk neuroblastoma: a report from the Children's Oncology Group (COG). *Eur J Cancer*. 2019;112:66–79.
5. Modak S, Cheung NK. Disialoganglioside directed immunotherapy of neuroblastoma. *Cancer Invest*. 2007;25(1):67–77.
6. Shusterman S, London WB, Gillies SD, Hank JA, Voss SD, Seeger RC, et al. Antitumor activity of hu14.18-IL2 in patients with relapsed/refractory neuroblastoma: a Children's Oncology Group (COG) phase II study. *J Clin Oncol*. 2010;28(33):4969–75.
7. Yu AL, Gilman AL, Ozkaynak MF, London WB, Kreissman SG, Chen HX, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. *N Engl J Med*. 2010;363(14):1324–34.
8. Ladenstein R, Potschger U, Valteau-Couanet D, Luksch R, Castel V, Yaniv I, et al. Interleukin 2 with anti-GD2 antibody ch14.18/CHO (dinutuximab beta) in patients with high-risk neuroblastoma (HR-NBL1/SIOPEN): a multicentre, randomised, phase 3 trial. *Lancet Oncol*. 2018;19(12):1617–29.
9. Cheung NK, Cheung IY, Kushner BH, Ostrovnaya I, Chamberlain E, Kramer K, et al. Murine anti-GD2 monoclonal antibody 3F8 combined with granulocyte-macrophage colony-stimulating factor and 13-cis-retinoic acid in high-risk patients with stage 4 neuroblastoma in first remission. *J Clin Oncol*. 2012;30(26):3264–70.
10. Kushner BH, Ostrovnaya I, Cheung IY, Kuk D, Kramer K, Modak S, et al. Prolonged progression-free survival after consolidating second or later remissions of neuroblastoma with anti-G(D2) immunotherapy and isotretinoin: a prospective phase II study. *Oncoimmunology*. 2015;4(7):e1016704.
11. Cheung NK, Cheung IY, Kramer K, Modak S, Kuk D, Pandit-Taskar N, et al. Key role for myeloid cells: phase II results of anti-G(D2) antibody 3F8 plus granulocyte-macrophage colony-stimulating factor for chemoresistant osteomedullary neuroblastoma. *Int J Cancer*. 2014;135(9):2199–205.
12. Cheung NK, Guo H, Hu J, Tassev DV, Cheung IY. Humanizing murine IgG3 anti-GD2 antibody m3F8 substantially improves antibody-dependent cell-mediated cytotoxicity while retaining targeting in vivo. *Oncoimmunology*. 2012;1(4):477–86.
13. Anghelescu DL, Goldberg JL, Faughnan LG, Wu J, Mao S, Furman WL, et al. Comparison of pain outcomes between two anti-GD2 antibodies in patients with neuroblastoma. *Pediatr Blood Cancer*. 2015;62(2):224–8.
14. Kushner BH, Cheung NK. GM-CSF enhances 3F8 monoclonal antibody-dependent cellular cytotoxicity against human melanoma and neuroblastoma. *Blood*. 1989;73(7):1936–41.
15. Kushner BH, Cheung IY, Modak S, Basu EM, Roberts SS, Cheung NK. Humanized 3F8 anti-GD2 monoclonal antibody dosing with granulocyte-macrophage colony-stimulating factor in patients with resistant neuroblastoma: a phase 1 clinical trial. *JAMA Oncol*. 2018;4(12):1729–35.
16. Mora J, Chan GCF, Morgenstern DA, Amoroso L, Nysom K, Faber J, et al. The anti-GD2 monoclonal antibody naxitamab plus GM-CSF for relapsed or refractory high-risk neuroblastoma: a phase 2 clinical trial. *Nat Commun*. 2025;16(1):1636.
17. Desai AV, Fox E, Smith LM, Lim AP, Maris JM, Balis FM. Pharmacokinetics of the chimeric anti-GD2 antibody, ch14.18, in children with high-risk neuroblastoma. *Cancer Chemother Pharmacol*. 2014;74(5):1047–55.
18. Cheung IY, Hsu K, Cheung NK. Activation of peripheral-blood granulocytes is strongly correlated with patient outcome after immunotherapy with anti-GD2 monoclonal antibody and granulocyte-macrophage colony-stimulating factor. *J Clin Oncol*. 2012;30(4):426–32.
19. Mora J, Chan GC, Morgenstern DA, Nysom K, Bear MK, Tornøe K, et al. Outpatient administration of naxitamab in combination with granulocyte-macrophage colony-stimulating factor in patients with refractory and/or relapsed high-risk

- neuroblastoma: management of adverse events. *Cancer Rep (Hoboken)*. 2023;6(1):e1627.
20. Kushner BH, Kramer K, Modak S, Cheung NK. Irinotecan plus temozolomide for relapsed or refractory neuroblastoma. *J Clin Oncol*. 2006;24(33):5271–6.
 21. Park JR, Kreissman SG, London WB, Naranjo A, Cohn SL, Hogarty MD, et al. Effect of tandem autologous stem cell transplant vs single transplant on event-free survival in patients with high-risk neuroblastoma: a randomized clinical trial. *JAMA*. 2019;322(8):746–55.
 22. Ladenstein R, Potschger U, Valteau-Couanet D, Luksch R, Castel V, Ash S, et al. Investigation of the role of dinutuximab beta-based immunotherapy in the SIOPEN High-Risk Neuroblastoma 1 Trial (HR-NBL1). *Cancers (Basel)*. 2020;12(2):309. doi:10.3390/cancers12020309
 23. Kushner BH, Kramer K, Modak S, Cheung NK. Sensitivity of surveillance studies for detecting asymptomatic and unsuspected relapse of high-risk neuroblastoma. *J Clin Oncol*. 2009;27(7):1041–6.
 24. Park JR, Bagatell R, Cohn SL, Pearson AD, Villablanca JG, Berthold F, et al. Revisions to the International Neuroblastoma Response Criteria: a consensus statement from the National Cancer Institute Clinical Trials Planning Meeting. *J Clin Oncol*. 2017;35(22):2580–7.
 25. Usmani N, Deyell RJ, Portwine C, Rafael MS, Moorehead PC, Shammas A, et al. Residual meta-iodobenzyl guanidine (MIBG) positivity following therapy for metastatic neuroblastoma: patient characteristics, imaging, and outcome. *Pediatr Blood Cancer*. 2021;68(12):e29289.
 26. Streby KA, Parisi MT, Shulkin BL, LaBarre B, Bagatell R, Diller L, et al. Impact of diagnostic and end-of-induction Curie scores with tandem high-dose chemotherapy and autologous transplants for metastatic high-risk neuroblastoma: a report from the Children's Oncology Group. *Pediatr Blood Cancer*. 2023;70(8):e30418.
 27. Yanik GA, Parisi MT, Naranjo A, Nadel H, Gelfand MJ, Park JR, et al. Validation of postinduction Curie scores in high-risk neuroblastoma: a Children's Oncology Group and SIOPEN Group report on SIOPEN/HR-NBL1. *J Nucl Med*. 2018;59(3):502–8.
 28. Kushner BH, Kramer K, LaQuaglia MP, Modak S, Yataghene K, Cheung NK. Reduction from seven to five cycles of intensive induction chemotherapy in children with high-risk neuroblastoma. *J Clin Oncol*. 2004;22(24):4888–92.
 29. Lerman BJ, Li Y, Carlowicz C, Granger M, Cash T, Sadanand A, et al. Progression-free survival and patterns of response in patients with relapsed high-risk neuroblastoma treated with irinotecan/temozolomide/dinutuximab/granulocyte-macrophage colony-stimulating factor. *J Clin Oncol*. 2023;41(3):508–16.
 30. Cheung IY, Mauguen A, Modak S, Ragupathi G, Basu EM, Roberts SS, et al. Effect of oral beta-Glucan on antibody response to ganglioside vaccine in patients with high-risk neuroblastoma: a phase 2 randomized clinical trial. *JAMA Oncol*. 2023;9(2):242–50.
 31. Lewis EC, Kravaka JM, Ferguson W, Eslin D, Brown VI, Bergendahl G, et al. A subset analysis of a phase II trial evaluating the use of DFMO as maintenance therapy for high-risk neuroblastoma. *Int J Cancer*. 2020;147(11):3152–9.
 32. Weichselbaum RR, Liang H, Deng L, Fu YX. Radiotherapy and immunotherapy: a beneficial liaison? *Nat Rev Clin Oncol*. 2017;14(6):365–79.
 33. Aiken TJ, Erbe AK, Zebertavage L, Komjathy D, Feils AS, Rodriguez M, et al. Mechanism of effective combination radio-immunotherapy against 9464D-GD2, an immunologically cold murine neuroblastoma. *J Immunother Cancer*. 2022;10(7):e004834. doi:10.1136/jitc-2022-004834
 34. Gill S, Nowak AK, Bowyer S, Endersby R, Ebert MA, Cook A. Clinical evidence for synergy between immunotherapy and radiotherapy (SITAR). *J Med Imaging Radiat Oncol*. 2022;66(6):881–95.
 35. Dandoy CE, Rotz S, Alonso PB, Klunk A, Desmond C, Huber J, et al. A pragmatic multi-institutional approach to understanding transplant-associated thrombotic microangiopathy after stem cell transplant. *Blood Adv*. 2021;5(1):1–11.
 36. Haghiri S, Fayeche C, Mansouri I, Dufour C, Pasqualini C, Bolle S, et al. Long-term follow-up of high-risk neuroblastoma survivors treated with high-dose chemotherapy and stem cell transplantation rescue. *Bone Marrow Transplant*. 2021;56(8):1984–97.
 37. Matthay KK, Villablanca JG, Seeger RC, Stram DO, Harris RE, Ramsay NK, et al. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow

- transplantation, and 13-cis-retinoic acid. *N Engl J Med.* 1999;341(16):1165–73.
38. Pritchard J, Cotterill SJ, Germond SM, Imeson J, de Kraker J, Jones DR. High dose melphalan in the treatment of advanced neuroblastoma: results of a randomised trial (ENSG-1) by the European Neuroblastoma Study Group. *Pediatr Blood Cancer.* 2005;44(4):348–57.
 39. Berthold F, Boos J, Burdach S, Erttmann R, Henze G, Hermann J, et al. Myeloablative megatherapy with autologous stem-cell rescue versus oral maintenance chemotherapy as consolidation treatment in patients with high-risk neuroblastoma: a randomised controlled trial. *Lancet Oncol.* 2005;6(9):649–58.
 40. Matthay KK, Reynolds CP, Seeger RC, Shimada H, Adkins ES, Haas-Kogan D, et al. Long-term results for children with high-risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13-cis-retinoic acid: a children's oncology group study. *J Clin Oncol.* 2009;27(7):1007–13.
 41. Kushner BH, Ostrovnaya I, Cheung IY, Kuk D, Modak S, Kramer K, et al. Lack of survival advantage with autologous stem-cell transplantation in high-risk neuroblastoma consolidated by anti-GD2 immunotherapy and isotretinoin. *Oncotarget.* 2016;7(4):4155–66.
 42. Kushner BH, LaQuaglia MP, Modak S, Wolden SL, Basu EM, Roberts SS, et al. MYCN-amplified stage 2/3 neuroblastoma: excellent survival in the era of anti-G(D2) immunotherapy. *Oncotarget.* 2017;8(56):95293–302.
 43. Federico SM, McCarville MB, Shulkin BL, Sondel PM, Hank JA, Hutson P, et al. A pilot trial of humanized anti-GD2 monoclonal antibody (hu14.18K322A) with chemotherapy and natural killer cells in children with recurrent/refractory neuroblastoma. *Clin Cancer Res.* 2017;23(21):6441–9.
 44. Olgun N, Cecen E, Ince D, Kizmazoglu D, Baysal B, Onal A, et al. Dinutuximab beta plus conventional chemotherapy for relapsed/refractory high-risk neuroblastoma: a single-center experience. *Front Oncol.* 2022;12:1041443. doi:10.3389/fonc.2022.1041443
 45. Wiczorek A, Zaniewska-Tekieli A, Ehlert K, Pawinska-Wasikowska K, Balwierz W, Lode H, et al. Dinutuximab beta combined with chemotherapy in patients with relapsed or refractory neuroblastoma. *Front Oncol.* 2023;13:1082771. doi:10.3389/fonc.2023.1082771
 46. Lode HN, Ladenstein R, Troschke-Meurer S, Struppe L, Siebert N, Zumpe M, et al. Effect and tolerance of N5 and N6 chemotherapy cycles in combination with dinutuximab beta in relapsed high-risk neuroblastoma patients who failed at least one second-line therapy. *Cancers (Basel).* 2023;15(13):3364. doi:10.3390/cancers15133364
 47. Raiser P, Schleiermacher G, Gambart M, Dumont B, Defachelles AS, Thebaud E, et al. Chemo-immunotherapy with dinutuximab beta in patients with relapsed/progressive high-risk neuroblastoma: does chemotherapy backbone matter? *Eur J Cancer.* 2024;202:114001.