FC gamma receptor polymorphism in relapsed/refractory high-risk neuroblastoma patients correlates with outcomes in the SIOPEN dinutuximab beta long-term infusion trial

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Conflict of interest

The academic data supported Apeiron to obtain the dinutuximab beta product licensure in May 2017 in the European Union (EMA). SIOPEN and CCRI had an agreement in place with Apeiron regarding the provision of academic data. Ruth Ladenstein and Holger Lode acted as consultants for Apeiron and EUSA Pharma on behalf of SIOPEN for the development of dinutuximab beta. The other authors declared no potential conflicts of interest.

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Translational relevance

Immunotherapy with dinuximab beta for patients with high-risk neuroblastoma (HRNBL) is clinically effective but associated with neuropathic pain, especially when administered using a short-term infusion schedule. Long-term infusion (LTI) of dinutuximab beta with subcutaneous interleukin-2 (scIL-2) and isotretinoin has previously been shown to result in reduced pain. Our Phase I/II trial evaluated the clinical outcomes of dinutuximab beta LTI plus scIL-2 in patients with relapsed/refractory HRNBL. Overall, the regimen was generally well tolerated, with ≥80% of patients free of intravenous morphine by cycle 1. Dinutuximab beta LTI was also clinically active, resulting in an objective response rate of 45% at the end of treatment and a two-year overall survival rate of 73%. In addition, we identified low-affinity Fc-gamma receptor polymorphisms as adverse risk factor, which has not been reported before, and suggests that alternative treatment approaches may be warranted in patients with this characteristic.

Abstract

Purpose: To identify a tolerable dinutuximab beta long-term infusion (LTI) schedule with immunomodulatory activity for relapsed/refractory high-risk neuroblastoma. **Patients and Methods:** In this Phase I/II trial, dinutuximab beta LTI (five 35-day cycles) with subcutaneous interleukin-2 was evaluated in high-risk neuroblastoma cohorts (1x exploratory, 2x confirmatory). The composite primary endpoint was >80% patients free of intravenous morphine by day 5/cycle 1 plus ≥100 natural killer cells/μL and ≥1 μg/mL dinutuximab beta concentration by day 15/cycle 1. Secondary endpoints included objective response rate, event-free survival, overall survival, Fc-gamma receptor polymorphisms, and natural killer cells. **Results:** Overall, 122 patients were treated. At 10 mg/m^2/day dinutuximab beta LTI, 95% patients (22/24 exploratory cohort; 20/20 confirmatory cohort 1) achieved the composite primary endpoint, with ≥80% patients intravenous morphine-free by day 5/cycle 1. End-of-treatment objective response rate was 45% in 78 evaluable patients. Two-year event-free survival and overall survival were 56% (±4%) and 73% (±4%) overall; and 45% (±5%) and 65% (±5%) in relapsed/refractory disease, respectively. Two-year survival rates were greater in patients with high-affinity Fc-gamma receptor polymorphisms and high-level natural killer cells versus patients with low-affinity Fc-gamma receptor polymorphisms and low-level natural killer cells (event-free survival, 79% [±9%] vs 35% [±11%], p=0.009; overall survival, 84% [±8%] vs 70% [±10%]; p=0.083). Multivariate analysis identified age >5 years, low-affinity Fc-gamma receptor polymorphisms, and relapse/refractory disease as independent risk factors. **Conclusion:** Dinutuximab beta LTI was well tolerated and clinically active in patients with relapsed/refractory high-risk neuroblastoma, with Fc-gamma receptor polymorphisms and natural killer cells identified as prognostic biomarkers.

Trial registration: ClinicalTrials.gov NCT01701479; EudraCT 2009-018077-31

Keywords: Dinutuximab beta, Fc-gamma receptor polymorphism, neuroblastoma

Introduction

Neuroblastoma accounts for 15% of childhood cancer deaths.[1] Approximately 50% of patients have high-risk neuroblastoma (HRNBL) with poor overall survival.[2, 3] Multimodal treatment for HRNBL includes intensive induction,[4, 5] high-dose chemotherapy (HDT) and stem cell rescue (SCR) for consolidation,[2, 6] and isotretinoin with immunotherapy in the maintenance phase.[7, 8]

The disialoganglioside GD_2 is expressed in most neuroblastoma cells and is a suitable target for immunotherapy with the monoclonal antibody ch14.18, which was later developed into two different products named dinutuximab and dinutuximab beta, respectively.[7, 9, 10] Treatment of patients with HRNBL using ch14.18 is clinically effective but is also associated with the GD_2-specific on-target, off-tumor effect of neuropathic pain.[7, 9, 10] Therefore, clinical use of ch14.18 requires heavy co-administration of analgesic drugs, including intravenous (IV) morphine, to increase the tolerability of treatment.

Previously, most treatment schedules with ch14.18 involved short-term infusions over 8–20 hours on 4–5 consecutive days.[7, 11-13] We hypothesized that substantial prolongation of antibody infusion time in neuroblastoma patients would reduce pain and improve tolerability without impairing clinical activity and efficacy. A treatment regimen consisting of dinutuximab beta, given as a 10-day continuous long-term infusion (LTI) in combination with subcutaneous interleukin-2 (scIL-2) and isotretinoin was first explored in a single-center compassionate-use cohort.[14] This LTI regimen showed low pain scores with reduced need for IV morphine, and lower frequency of grade ≥3 adverse events (AEs).[14] In addition, anti-tumor activity and efficacy indicated improvement compared with historical controls.[14]

In preclinical models, dinutuximab beta was demonstrated to mediate its anti-neuroblastoma effect by antibody-dependent cell-mediated cytotoxicity (ADCC) primarily mediated by natural killer (NK cells).[15] While the depletion of NK cells resulted in loss of dinutuximab beta's therapeutic efficacy,[15] the co-administration of interleukin-2 (IL-2) increased its efficacy by activating and expanding NK cells.[16] ADCC requires the recognition of the immunoglobulin dinutuximab beta bound to GD_2 on the cell surface of neuroblastoma cells through Fc-gamma receptors (FCGR).[15] FCGRs involved in ADCC include FCGR3A (CD16) expressed primarily on NK cells and FCGR2A (CD32) expressed on monocytes, macrophages and neutrophils.[17] We also showed that neuroblastoma patients with high-affinity FCGR2A and -3A polymorphisms have higher ADCC levels than those with low-affinity FCGR polymorphisms when treated with dinutuximab beta.[18] Thus, FCGR polymorphisms and levels of NK cells may serve as surrogate markers for ADCC and response to treatment with dinutuximab beta.

Consequently, an international multicenter trial was initiated by the International Society of Paediatric Oncology Europe Neuroblastoma group (SIOPEN) to assess tolerability, immunomodulation, and clinical outcomes of dinutuximab beta LTI in patients with relapsed or refractory HRNBL.[19] Here, we report the effects of dinutuximab beta LTI on pain control and dinutuximab beta levels as well as the impact of FCGR polymorphisms and NK cell levels on treatment outcomes.

Patients and methods

Trial design and patient eligibility

This prospective, open-label, Phase I/II trial (ClinicalTrials.gov identifier NCT01701479; EudraCT identifier 2009-018077-31) had a single-arm phase including one exploratory (dose-finding) cohort and two confirmatory cohorts (**Supplementary Fig. S1A-C**). Dinutuximab beta (provided by Apeiron, with recloning and production done by Polymun) LTI was given in combination with scIL-2 ($6x10^6$ IU/m²/day in two 5-day blocks [days 1–5 and 8–12]; $0.2x10^6$ IU/kg/day for patients ≤12 kg) and isotretinoin 160 mg/m^2/day for 14 days, starting on the day after completion of dinutuximab beta. Detailed criteria for dose modifications and discontinuations are provided in the protocol (**Appendix 1**).

The study was conducted in compliance with Good Clinical Practice guidelines and in accordance with the Declaration of Helsinki, and the protocol was approved by all national regulatory authorities and ethics committees of all participating countries. All patients or their parents or guardians provided written informed assent or consent, as appropriate, before study entry.

The SIOPEN-R-NET web-based system (https://www.siopen-r-net.org/) was used to enroll patients (aged 1–21 years) diagnosed with HRNBL (by the International Neuroblastoma Staging System criteria[20]) who had received ≥1 HDT followed by SCR after induction chemotherapy.

Eligible patients included 1) those with primary refractory disease (defined as insufficient end of induction metastatic metaiodobenzylguanidine (mIBG) response with SIOPEN score >3 following ≥2 front-line treatments within the SIOPEN HRNBL-

1 trial)[9]; 2) front-line patients with major treatment deviations (MTD), including those in the SIOPEN HRNBL-1 trial who were ineligible for immunotherapy randomization[9] due to major delays after completing HDT/SCR andthose receiving other standard front-line therapy protocols for HRNBL; 3) patients with relapsed disease who were high-risk at diagnosis; and 4) patients with relapsed disease who were non-high-risk at diagnosis. For groups 3) and 4), the disease had to be stabilized by second-line therapies prior to recruitment. Patients with previous exposure to an anti-GD2 antibody or those requiring corticosteroids or other immunosuppressive drugs were ineligible for the trial. Detailed eligibility criteria are provided in the protocol (**Appendix 1**) and did not change throughout the trial.

Dose-finding phase

The dose-finding phase was informed by pain control and immunomodulatory activity as a composite primary endpoint. Acceptable pain control was defined as >80% of patients IV morphine-free after day 5 of dinutuximab beta LTI in cycle 1, which is a result from a single-center experience using a 10-day dinutuximab beta LTI schedule combined with IL-2.[14] Immunomodulatory activity analyzed on day 15 of cycle 1 (day 8 of antibody infusion) consisted of two parameters: sufficient dinutuximab beta concentration and increase in NK cells, which are both key components to mediate ADCC, the primary mechanism of action for dinutuximab beta. It was shown that 1 µg/mL dinutuximab beta is highly active to mediate ADCC in neuroblastoma models.[15] Therefore, a dinutuximab beta concentration of ≥1 µg/mL was selected as an endpoint. The expected increase of the number of NK cells following scIL-2 was informed by a previous study where $6x10^6$ IU/m²/day given in six 5-day cycles every 2 weeks achieved a median increase to 118 NK cells/µL and a median relative

increase over baseline of 711%.[21] Therefore, we defined an increase of 500% over baseline or ≥100 NK cells/µL as an endpoint.

Three daily dose schedules of dinutuximab beta were planned (7 mg/m^2, 10 mg/m^2, 15 mg/m^2), corresponding to total doses of 100 mg/m^2, 150 mg/m^2 and 210 mg/m^2 per cycle, respectively. Acceptable dinutuximab beta LTI durations ranged from 10 to 21 days to evaluate 7 dinutuximab beta LTI schedules. Only the first cycle was used for the dinutuximab beta LTI dose-finding algorithm (**Appendix 1**).

The dose-finding algorithm of the exploratory cohort aimed to identify a schedule at which ≥80% of patients could complete cycle 1 with good pain control (defined as >80% of patients IV morphine-free on day 5 of dinutuximab beta LTI in cycle 1) and fulfilling the prespecified efficacy criteria including a dinutuximab beta concentration of ≥1 µg/mL by day 15 of cycle 1 [15] and an increase of 500% or ≥100 NK cells/µl.[21]

Concomitant medication

Prophylactic pain treatment consisted of oral gabapentin and bolus IV morphine 0.02–0.05 mg/kg/hour given before the start of dinutuximab beta. Thereafter, continuous infusion of morphine (0.03 mg/kg/hour) was given on the first day. Morphine infusion was weaned off on a daily basis if the patient was without pain over the first 5 days (to 0.02 mg/kg/h to 0.01 mg/kg/h to 0.005 mg/kg/h). Further details on the morphine administration are provided in the protocol **(Appendix 1**).Oral and transdermal opioids were allowed for breakthrough pain as detailed in the protocol. Prophylactic treatment for fever included metamizole, paracetamol, ibuprofen, or indomethacin according to institutional standards.

Other cancer therapies were not permitted during the trial. Glucocorticoids or other drugs with known immunosuppressive activity were not permitted for 2 weeks before entry or during the trial.

Assessments

Patients were scheduled for disease evaluation prior to treatment start and after cycles 2 and 5. It consisted of whole-body iodine-123 mIBG scintigraphy, computed tomography or magnetic resonance imaging of the primary tumor and other evaluable sites of disease, bone-marrow examination with aspirates and trephines obtained from two sites, and measurement of urinary catecholamine metabolites according to the International Neuroblastoma Response Criteria and as detailed in the protocol (**Appendix 1**).[20]

Dinutuximab beta concentration and NK cell levels were determined by enzyme-linked immunosorbent assay (ELISA)[22] and flow cytometry,[23] respectively. Samples for FCGR polymorphism and human anti-chimeric antibodies (HACA) response were analyzed before dinutuximab beta treatment, and for HACA also during each cycle, by reverse transcription polymerase chain reaction[18] and ELISA.[24] AEs and toxicities were graded using the Common Terminology Criteria for AEs (version 4.0). Pain assessment was done three times per day using self-reporting pain scales as detailed in the protocol (**Appendix 1**).

Statistical analysis

We estimated that 20–40 patients would be required for the exploratory cohort to define the treatment schedule for ongoing evaluation. For the composite primary endpoint, only the first course was taken into account for the dose schedule-finding

algorithm. Since interdependent endpoints were chosen, the exploratory cohort design was based on a cohort of 10 patients.

The composite primary endpoint for the whole trial (including confirmatory cohorts 1 and 2) was IV morphine-free delivery of dinutuximab beta LTI after day 5 of cycle 1 in >80% of patients as well as a 500% increase or an increase of ≥100 NK cells/µL, and a dinutuximab beta level of ≥1 µg/mL by day 15 of cycle 1.

Secondary endpoints included treatment response, event-free survival (EFS) and overall survival (OS), FCGR polymorphisms,[18] HACA response,[24] NK cell count[23] and their impact on EFS and OS. The Kaplan–Meier method was used to evaluate EFS and OS. For EFS, estimates of the date of the first event (relapse or death of any cause) or the last examination date were taken as endpoint of the time interval. Patients were censored at the date of last contact if no event was reported. The effect of the NK cell count on survival was assessed by dividing patients in two groups respectively (high and low) according to the median NK cell frequency. To study the effect of exposure to dinutuximab beta through the area under the curve (AUC) value in cycle 1, patients were also divided in two groups (high and low exposure).

For multivariate analysis, the Cox proportional hazard model for time-dependent variables was planned to identify factors potentially associated with outcomes including age, measurable disease at trial entry, prior relapse, and FCGR polymorphisms. In addition, the impact of HACA response on survival was assessed separately by means of a Cox model for time-dependent variables after adjustment for the variables mentioned above, including HACA response as a time-dependent covariate. Only patients who completed the 5 treatment cycles as planned were

included in this HACA response survival analysis. For non-time-to-event variables the Chi-Square test or, where appropriate, the Fisher exact test were used to compare groups for categorical variables, and the Wilcoxon rank-sum test was used for continuous variables. All p-values <0.05 (two-sided) were considered significant. The statistical analyses were performed with SAS version 9.4 (SAS Institute, Cary, NC, RRID:SCR_008567).

Data availability

The data generated in this study are available upon request from the corresponding author.

Results

Patient characteristics

Between January 2012 and June 2014, 124 patients were screened in 17 hospitals in 8 countries, with 123 patients meeting the eligibility criteria (**Supplementary Fig. S1A, Supplementary Table S1 and S2**). One patient died between enrollment and treatment start with rapid disease progression, leaving 122 treated patients. Prespecified patient numbers recruited to cohorts were 24 (exploratory cohort), 20 (confirmatory cohort 1) and 78 (confirmatory cohort 2) (**Supplementary Fig. S1A-C**). Patient characteristics at baseline are provided in **Table 1**.

Dose finding

In the exploratory cohort, the regimen of 10 mg/m² over 10 days in cycle 1 met the composite primary endpoint criteria of IV morphine-free treatment with dinutuximab beta in 81% of patients after 120 hours (5 days) in cycle 1, with total number of NK cells on days 8 and 15 increased from baseline by factors ranging from 3.2 to 5.7.

Median absolute NK cell levels on days 1, 8, and 15 were 50 cells/µl (range 0.03–2000), 268 cells/µl (range 1–1373), and 242 cells/µl (range 1–1195), respectively. In addition, the mean dinutuximab beta concentration on day 15 was 10.5 µg/mL. As prespecified parameters of the composite primary endpoint were met in the exploratory cohort, this regimen was used for confirmatory cohort 1 and 2.

Treatment tolerability

In cycle 1, 36/44 patients (82%) of the exploratory cohort and confirmatory cohort 1 and 53/78 (68%) patients of the confirmatory cohort 2 received dinutuximab beta without requiring IV morphine by day 5 of cycle 1. Thus, 89/122 (73%) patients overall were IV morphine-free by day 5/cycle 1. Reasons for prolonged IV morphine in the 33 patients who required it after day 5/cycle 1 were: pain/discomfort (n=23, 19%) and other reasons (n=8, 7%), including investigator decision and hospital logistics. Median total dose of morphine steadily decreased from 662 µg/kg/day on day 1 to 47 µg/kg/day on day 5 in cycle 1. Initial doses of IV morphine were lower in each subsequent cycle, with a similar rate of decline over the first 5 days of infusion as observed in cycle 1 (**Fig. 1**).

Overall, 99% of patients experienced ≥1 grade 3–4 toxicity (**Table 2**); the most common events were fever (57%), infections (40%), pain (25%), capillary leak syndrome (16%), and allergic reactions (12%). Hematologic grade 3–4 toxicities occurred in 75% of patients. The most common non-hematologic grade 3–4 toxicities were liver (41%), gastrointestinal (19%, particularly nausea/vomiting, 9%), cardiac (15%, particularly hypotension, 11%), and pulmonary (12%). HACA response did not increase the frequency or the intensity of AEs (**Supplementary Table S3**).

Overall, 35 (29%) patients permanently discontinued dinutuximab beta (**Supplementary Fig. S1A**), which was due to disease progression (n=20) or toxicity (n=15).

Immunomodulatory endpoints

In patients with available data, 80/81 patients had dinutuximab beta concentration >1 µg/mL. One patient who underwent dose reduction followed by premature discontinuation of dinutuximab beta and scIL-2 had a dinutuximab beta concentration of 0.82 µg/mL. Thus, 100% of evaluable patients dosed per protocol met this efficacy endpoint.

On day 15 of cycle 1, 75/80 (94%) patients had a ≥500% increase (≥100 cells/µL) in NK cells. Median NK cell count was 242 cells/µl (range 1–1195) and was used as a cut-off level to distinguish a low- versus high-NK group for further analysis. A similar proportion of patients demonstrated an increase in NK cells on day 15 in cycles 2, 3, 4, and 5 (87%, 93%, 87%, and 88%, respectively). Median (range) NK cell counts in these cycles were 246 (24–1755) cells/µL, 278 (50–974) cell/µL, 285 (0–1326) cells/µL, and 277 (0–1324) cells/µL, respectively.

Determination of HACA in evaluable patients indicated that 26/122 (21%) patients developed a positive response during treatment. A total of 87/122 (71%) patients completed all 5 cycles, and 23 (26%) of those were HACA positive. Development of HACA is time-dependent, occurring over 5 cycles of immunotherapy; thus, only the 87 patients who completed 5 cycles were included in the correlative outcome analysis.

Efficacy endpoints

Response assessments

Overall, 78 of 123 enrolled patients were evaluable for response assessments because 45 patients had no evidence of disease at baseline. At mid- and end-treatment evaluation, 35/78 (45%) patients responded (complete response [CR], n=12; partial response [PR], n=23). Best response of PR (n=27) or CR (n=17) was observed in 44/78 (56%) patients (**Supplementary Table S4**).

Univariate analysis

Two-year EFS and OS rates (±standard errors [SE]) in the overall cohort were 56% (±4%) and 73% (±4%), respectively (**Fig. 2A**). Patients with front-line primary refractory disease and relapsed patients had significantly inferior outcomes compared with other eligible front-line patients with MTD such as those with delayed recovery after HDT/SCR in SIOPEN HRNBL1 trial or other HRNBL standard approaches. Outcomes in the four subgroups (i.e., relapsed – high-risk at diagnosis, relapsed – non-high-risk at diagnosis, front-line primary refractory, and front-line with MTD; **Supplementary Fig. S2A and S2B**) suggested re-grouping patients into two prognostic cohorts: front-line primary refractory and relapsed patients combined (2-year EFS: 45% [±5%]; 2-year OS: 65% [±5%]) and front-line patients with MTD (2-year EFS: 90% [±5%]; 2-year OS: 97% [±3%], p<0.001; **Fig. 2B and 2C**).

Age >5 years at trial entry was associated with inferior survival outcomes in all groups; however, stage at diagnosis and *MYCN* amplification had no impact (**Table 1, Supplementary Fig. S2C and S2D, Supplementary Fig. S3A and S3B**).

Patients with high-affinity FCGR polymorphisms had significantly better survival outcomes than those with low-affinity FCGR polymorphisms (**Table 1**) and this effect was predominantly seen in relapsed or front-line primary refractory patients (**Fig. 3A and 3B**).

The increase of NK cells above or below the median did not significantly impact outcomes (**Supplementary Fig. S3C and S3D**). However, when combining NK cells with FCGR polymorphisms, we observed that low-affinity FCGR polymorphisms and low-NK activation identified a patient group with particularly poor outcomes, whilst high-affinity FCGR polymorphisms and high-NK activation identified a favorable outcome group, with an interim group of one high and one low for each parameter (**Fig. 3C and 3D**). Response rates in these subgroups are in line with survival, but did not reach statistical significance (**Supplementary Table S4**). When analyzing survival outcomes by type of FCGR polymorhism (i.e. FCGR2A and FCGR3A), a survival benefit was specifically observed in patients with high-affinity polymorphisms for FCGR3A but not FCGR2A (**Supplementary Fig. S4A-D**).

Of the 87 patients who completed all 5 cycles of treatment as planned, 23 became HACA positive; including 15 patients with neutralizing antibodies. Interestingly, HACA positivity corresponded to a significant survival benefit in both groups (relapsed/refractory vs front-line other), particularly at longer observation times (**Table 1, Supplementary Fig. S5A and S5B**).

The level of dinutuximab beta concentration (low vs high) had no influence on outcomes in the LTI setting (**Supplementary Fig. S5C and S5D**).

Multivariate analysis

Importantly, significant independent adverse risk factors for EFS and OS were low-affinity FCGR, the group of relapsed and primary refractory disease patients and age >5 years at trial entry (**Supplementary Table S5**).

Discussion

With the aim to improve tolerability, we evaluated an LTI schedule of dinutuximab beta in combination with scIL-2 in refractory or relapsed HRNBL patients to establish an acceptable pain-toxicity profile that also fulfilled immunomodulatory efficacy criteria. Although randomized clinical trials of dinutuximab beta with and without IL-2 showed no benefit of including IL-2 in the maintenance phase of newly-diagnosed patients with HRNBL,[9] its role in combination with dinutuximab beta in relapsed/refractory HRNBL requires systematic evaluation.

Delivery of dinutuximab beta LTI with 10 mg/m^2/day continuously over 10 days (total dose 100 mg/m^2) alongside scIL-2 was found to be generally well tolerated with a relatively low proportion of patients (25%) experiencing grade 3/4 pain. The composite primary endpoint of pain control and immunomodulation was met for the exploratory cohort and confirmatory cohort 1, demonstrating an improved treatment tolerance of dinutuximab beta with the LTI schedule. The freedom from IV morphine with dinutuximab beta treatment avoids opioid-associated side effects. The percentage of patients free of IV morphine within 5 days in cycle 1 was lower (<80%) in the confirmatory cohort 2 for reasons other than pain, such as discomfort, physician choice, or local logistics.

Dinutuximab beta LTI with scIL-2 was also clinically active, as shown by the best objective clinical response rate of 56%, the end-of-treatment response rate of 45%, and the 2-year EFS and OS rates of 56% and 73%, respectively. In a separate study using single-agent dinutuximab beta LTI (without IL-2) in relapsed/refractory patients, a response rate of only 37% was reported, with a 3-year OS and progression-free survival rate of 66% and 31%, respectively,[19] suggesting an additional benefit with IL-

2 which is being evaluated in a randomized trial of dinutuximab beta in relapsed/refractory HRNBL.[25]

Analysis of survival by disease status showed that relapsed patients had significantly worse outcomes compared with refractory patients and other eligible front-line patients, which suggests that relapsed and refractory patients should be considered separately – rather than as one group – in future clinical trials.

Patients receiving per-protocol dinutuximab beta LTI achieved a drug concentration of >1 µg/mL, which is a highly active concentration to mediate ADCC;[15] however, analysis of exposure above or below the median AUC in cycle 1 found that exposure did not impact survival or response rate. Our findings contrast with previous reports for dinutuximab.[26] The variations in dinutuximab beta levels observed in this study may not have been sufficiently large to lead to detectable differences in outcome and/or may be confounded by the complex interplay of patient characteristics, as previously reported for other immuno-oncology clinical studies.[27, 28]

The effect of high-affinity compared with low-affinity FCGR polymorphisms was particularly pronounced in patients with primary refractory disease and relapsed disease but the number of other front-line patients in this study was too low to detect a difference. Interestingly, patients with high-affinity FCGR3A, but not FCGR2A, polymorphisms demonstrated a survival advantage. As FCGR3A is primarily expressed by NK cells, and FCGR2A by myeloid cells including monocytes and macrophages,[17] NK cells seem the primary effector cell population with a subordinate role for myeloid cells (**Supplementary Fig. S4**). Consequently, when combining patients with a high-affinity FCGR polymorphism and high numbers of NK cells, survival rates appeared superior compared with patients with low-affinity FCGR

polymorphism and low NK cell numbers, which may be due to optimal conditions for mediating ADCC. Our results are consistent with those from an earlier study, showing a strong correlation between high-affinity FCGR2A/3A polymorphisms and prolonged survival following dinutuximab beta therapy, which correlated with the increased ability of patients' effector cells to mediate neuroblastoma cell lysis.[18]

In the Children's Oncology Group ANBL1221 Phase 2 trial for patients with relapsed/refractory HRNBL treated with irinotecan plus temozolomide (I/T) combined with the anti-GD_2 antibody dinutuximab and granulocyte macrophage-colony stimulating factory (GM-CSF), a correlative analysis identified that Killer-cell immunoglobulin-like receptor (KIR)/KIR-ligand genotypes and NK cells – but not FCGR2A/3A – are associated with clinical outcomes following chemoimmunotherapy with I/T/dinutuximab/GM-CSF.[29] However, the ANBL1221 trial combined chemotherapy with immunotherapy, unlike the trial reported here, which may suggest that FCGR may be more predictive in patients treated with anti-GD_2 immunotherapy alone in the maintenance phase of relapsed/refractory HRNBL. Another difference may be the assignment of patients to low- and high-affinity FCGR polymorphism status utilizing combined information about FCGR2A and 3A, as done in this trial.[18] Patients with any low-affinity genotype (either FCGR2A [F/F] or FCGR3A [R/R]) were assigned low-affinity FCGR polymorphism status. Patients with all other polymorphisms (FCGR2A [V/V] or [V/F] and FCGR3A [H/H] or [H/R]) were assigned high-affinity FCGR polymorphism status, and we demonstrated that status assignment correlates with the level of ADCC response in patients treated with dinutuximab beta.[18] Finally, the molecular structures of dinutuximab beta and dinutuximab are different; particularly the glycosylation pattern between both

457 antibodies, leading to a higher potency of dinutuximab beta over dinutuximab to
458 mediate ADCC.[15]

459 The poor outcomes for patients with front-line refractory disease and/or relapsed
460 HRNBL and low-affinity FCGR polymorphism, as shown here, may suggest an
461 alternative approach for patients receiving FCGR-independent immunotherapies; for
462 example, chimeric antigen receptor T (CAR-T) cells.[30] Our data support FCGR
463 polymorphism and NK cells as prognostic biomarkers for dinutuximab beta treatment.

464 We observed 21% HACA frequency in patients treated with dinutuximab beta LTI in
465 this study, which is similar to previous reports of 22%[19] and 19%[31] in other studies in
466 which analysis was performed with the same validated assay.[24] We have previously
467 reported the role of HACA development on immunomodulation following dinutuximab
468 beta LTI in combination with IL-2, and showed a strong reduction of dinutuximab beta
469 levels, abrogated complement-dependent cytotoxicity and ADCC in HACA-positive
470 patients.[32] However, the HACA-mediated abrogated effector function of dinutuximab
471 beta had no adverse effects on outcomes in the current study; indeed, the
472 development of HACA appeared beneficial and suggests a role for the induction of
473 an anti-idiotypic immune response, as shown for other anti-GD2 antibodies.[33]

474 The HACA response rate was also evaluated in the ANBL0032 trial, which was the
475 first study to demonstrate a survival benefit for HRNBL patients receiving anti-GD_2
476 immunotherapy with dinutuximab, GM-CSF and IL-2.[34] Only 13/122 (11%) patients
477 receiving dinutuximab developed HACA,[35] although the analysis was performed
478 using a different method and in patients receiving a different anti-GD_2 antibody
479 combined with IL-2 and GM-CSF, which confounds comparison with our trial.
480 Survival by HACA response in the ANBL0032 trial did not show a difference;[35]

however, there were only 13 HACA-positive patients. For patients treated with the anti-GD_2 antibody 3F8, a superior survival was reported for patients developing a transient humoral response against the anti-GD_2 antibody, leading to the hypothesis that there might be induction of an adaptive immune response against neuroblastoma through the anti-idiotypic network.[33]

Importantly, there was no increase in frequency and intensity of AEs observed in HACA-positive patients; in particular there was no increase in allergic or anaphylactic reactions. Hence, there is no reason to stop dinutuximab beta treatment due to HACA positivity.

Age is an established outcome predictor in neuroblastoma.[36] In this study, we confirmed that patients over 5 years of age at baseline are at particular risk of poorer outcomes. In addition, multivariate analysis identified low-affinity FCGR, relapsed and front-line refractory patients, and patients aged >5 years at trial entry as independent risk factors for poorer EFS and OS.

In conclusion, dinutuximab beta LTI over 10 days was well tolerated, clinically active and effective in patients with relapsed/refractory HRNBL. The role of low-affinity FCGR polymorphisms in patients with prior relapse or front-line refractory disease as independent unfavourable predictor has not been reported before and suggests that alternative treatment approaches may be warranted in these patient subgroups.

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Tables and Figures

Table 1. Baseline characteristics and survival outcomes.

	Enrolled patients	Survival outcome					
	(N=123)	2-y EFS	5-y EFS	p-value	2-y OS	5-y OS	p-value
Sex, n (%)							
Male	70 (57)	0.57±0.06	0.47±0.06	0.647	0.73±0.05	0.57±0.06	0.884
Female	53 (43)	0.55±0.07	0.47±0.07		0.74±0.06	0.58±0.07	
Age at diagnosis, n (%)							
≤1.5 years	20 (16)	0.80±0.09	0.75±0.10	0.086	0.85±0.08	0.85±0.08	0.014
1.5-5 years	71 (58)	0.49±0.06	0.38±0.06		0.65±0.06	0.46±0.06	
>5 years	32 (26)	0.56±0.09	0.50±0.09		0.84±0.06	0.65±0.08	
Median (range)	3.69 (0.12–13.17)						
Age at study entry, n (%)							
≤5 years	52 (42)	0.71±0.06	0.65±0.07	<0.001	0.81±0.05	0.73±0.06	0.001
>5 years	71 (58)	0.45±0.06	0.33±0.06		0.68±0.06	0.46±0.06	
Median (range)	5.69 (1.26–27.05)						
Stage at diagnosis	***MYCN* amplification (MNA), n (%)**						
localized	yes	3 (2)	1.00±0.00	1.00±0.00	0.093	1.00±0.00	1.00±0.00	0.349
	no	9 (7)	0.44±0.17	0.44±0.17		0.89±0.10	0.56±0.17	
stage 4	yes	23 (19)	0.74±0.09	0.65±0.10		0.78±0.09	0.70±0.10	
	no	84 (68)	0.51±0.05	0.40±0.05		0.69±0.05	0.52±0.05	
	not available	1 (1)						
stage 4s	yes	1 (1)	1.00±0.00	1.00±0.00		1.00±0.00	1.00±0.00	
	no	2 (2)	0.50±0.35	0.50±0.35		0.50±0.35	0.50±0.35	
Inclusion criteria for LTI entry, n (%)							
Front-line HRNBL, MTD	31 (25)	0.90±0.05	0.77±0.08	<0.001	0.97±0.03	0.90±0.05	<0.001
Front-line HRNBL PRD	39 (32)	0.54±0.08	0.46±0.08		0.72±0.07	0.48±0.08	
Relapsed disease, non-HR at diagnosis	16 (13)	0.50±0.13	0.44±0.12		0.81±0.10	0.63±0.12	
Relapsed disease, HR at diagnosis	37 (30)	0.32±0.08	0.24±0.07		0.51±0.08	0.38±0.08	
Disease status at LTI entry, n (%)							
No evidence of disease	45 (37)	0.64±0.07	0.60±0.07	0.146	0.78±0.06	0.67±0.07	0.226
Measurable disease	78 (63)	0.51±0.06	0.40±0.06		0.71±0.05	0.52±0.06	
Prognostics analysis groups, n (%)							
Front-line HRNBL PRD & relapsed	92 (75)	0.45±0.05	0.37±0.05	<0.001	0.65±0.05	0.46±0.05	<0.001
Front-line HRNBL, MTD	31 (25)	0.90±0.05	0.77±0.08		0.97±0.03	0.90±0.05	
FCGR polymorphisms, n (%)							
High affinity	59 (48)	0.68±0.06	0.61±0.06	0.012	0.83±0.05	0.67±0.06	0.031
Low affinity	63 (52)	0.46±0.06	0.35±0.06		0.65±0.06	0.49±0.06	
Not available	1						
HACA response in 87 patients completing cycle 5, n (%)							
HACA positive front-line PRD & relapsed	9 (10)	0.56±0.17	0.56±0.17	0.021	0.78±0.14	0.67±0.16	0.019
HACA negative front-line PRD & relapsed	50 (57)	0.58±0.07	0.44±0.07		0.80±0.06	0.60±0.07	
HACA positive front-line HRNBL, MTD	14 (16)	1.00±0.00	0.86±0.09	-	1.00±0.00	1.00±0.00	
HACA negative front-line HRNBL, MTD	14 (16)	0.86±0.09	0.70±0.13		0.93±0.07	0.86±0.09	

EFS, event-free survival; HACA, human anti-chimeric antibodies; HR, high-risk; HRNBL, high-risk

669 neuroblastoma; LTI, long-term infusion; MNA, *MYCN* [V-Myc myelocytomatosis viral related
670 oncogene, neuroblastoma derived (avian)] amplification; MTD, major treatment deviations; OS, overall
671 0.90±0.05 survival; PRD, primary refractory disease.
672
673

Table 2. Adverse events (N=123).

		Grade 1/2 n (%)	Grade 3/4 n (%)
OVERALL	ANY OVERALL TOXICITY	1 (1)	121 (98)
KEY SYMPTOMS	ANY KEY SYMPTOM TOXICITY	15 (12)	107 (87)
	Allergic Reaction	82 (67)	15 (12)
	Anaphylaxis	1 (1)	3 (2)
	Capillary leak syndrome	63 (51)	20 (16)
	Cytokine release syndrome	13 (11)	2 (2)
	Eye disorders, other	35 (29)	5 (4)
	Fatigue	54 (44)	2 (2)
	Fever	50 (41)	70 (57)
	Flu-like symptoms	42 (34)	6 (5)
	Infections	33 (27)	49 (40)
	Mood changes	18 (15)	3 (2)
	Pain	69 (56)	30 (24)
	Serum sickness	1 (1)	0 (0)
CARDIAC	ANY CARDIAC TOXICITY	57 (46)	18 (15)
	Cardiovascular/General-other	8 (7)	2 (2)
	Hypertension	4 (3)	0 (0)
	Hypotension	47 (38)	14 (11)
	Myocarditis	0 (0)	1 (1)
	Edema	3 (2)	2 (2)
	Tachycardia	58 (47)	2 (2)
GUT	ANY GUT TOXICITY	81 (66)	23 (19)
	Constipation	56 (46)	2 (2)
	Diarrhea	65 (53)	9 (7)
	Nausea/Vomiting	76 (62)	11 (9)
	Stomatitis	15 (12)	2 (2)
	Other	3 (2)	6 (5)
	Other anorexia/weight loss	20 (16)	8 (7)
HEMATOLOGIC	ANY HEMATOLOGIC TOXICITY	18 (15)	91 (74)
	Blood chemistry changes	28 (23)	42 (34)
	Coagulation perturbances	8 (7)	3 (2)
	Electrolytes disturbances	37 (30)	13 (11)
	Granulocytes	12 (10)	34 (28)
	Hemoglobin	36 (29)	60 (49)
	Inflammatory laboratory signs	3 (2)	8 (7)
	Platelets	33 (27)	54 (44)
	WBC	26 (21)	40 (33)
	Other	9 (7)	5 (4)
LIVER TOXICITY	ANY LIVER TOXICITY	39 (32)	50 (41)
	Bilirubin	7 (6)	2 (2)
	GGT	26 (21)	38 (31)
	SGOT/SGPT	21 (17)	16 (13)
	Other	2 (2)	0 (0)
NEUROLOGICAL	ANY NEUROLOGICAL TOXICITY	32 (26)	5 (4)
	Central neurotoxicity	21 (17)	2 (2)
	Peripheral neurotoxicity	11 (9)	2 (2)
	other – headache	5 (4)	1 (1)
PULMONARY	ANY PULMONARY TOXICITY	26 (21)	15 (12)
RENAL OR UROGENITAL	ANY RENAL OR UROGENITAL TOXICITY	46 (37)	9 (7)
	Creatinine	4 (3)	1 (1)
	Hematuria	5 (4)	0 (0)
	Other urogenital toxicity	8 (7)	4 (3)
	Urinary retention	38 (31)	5 (4)
SKIN	ANY SKIN TOXUCUTY	76 (62)	3 (2)
UNCLASSIFIED	ANY UNCLASSIFIED TOXICITY	46 (37)	7 (6)
	Hearing impaired	2 (2)	0 (0)
	Weight gain	21 (17)	0 (0)
	Other	30 (24)	7 (6)

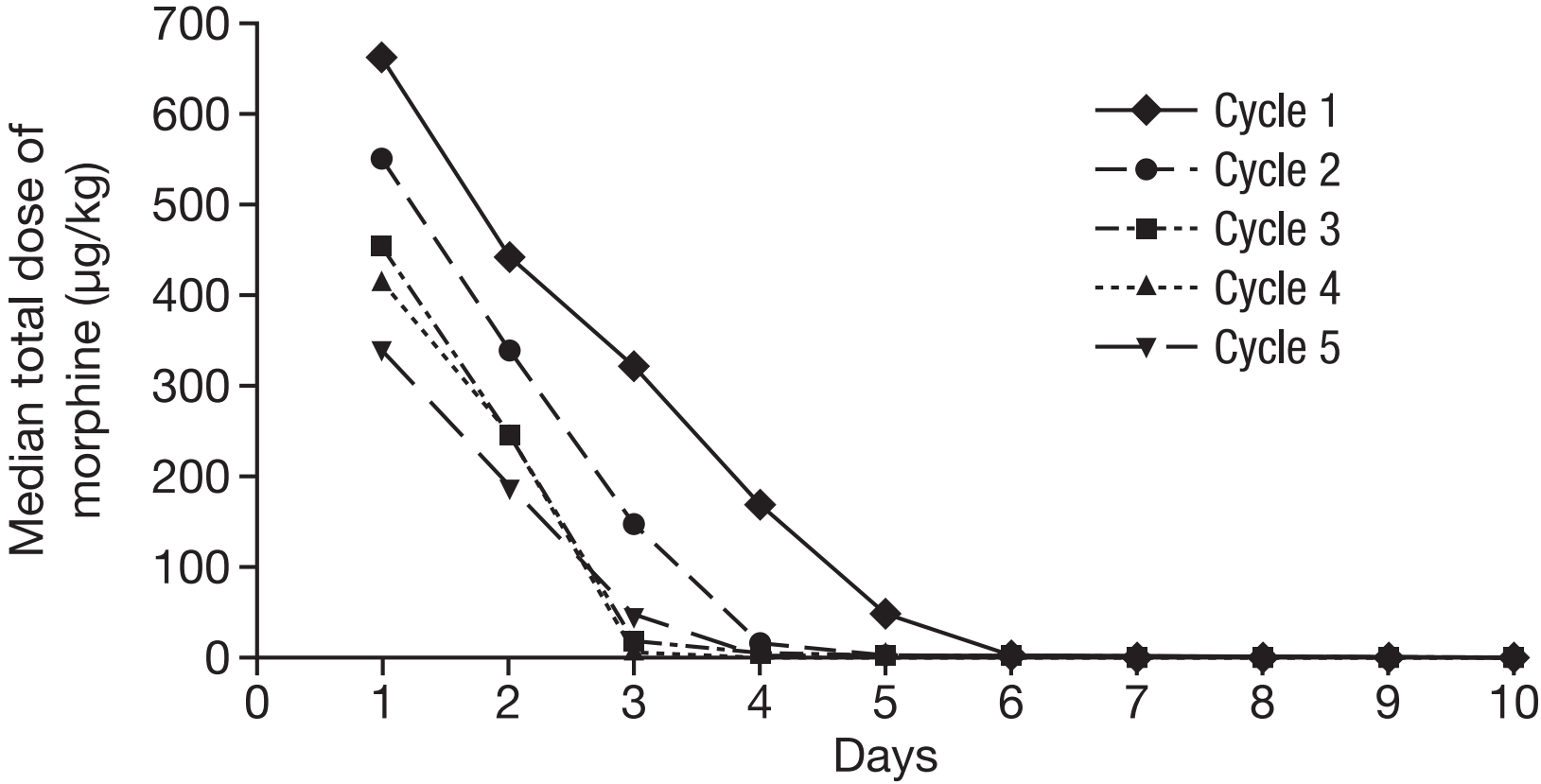
GGT, gamma-glutamyltransferase; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; WBC, white blood cell.

Figure 1. Intravenous morphine use in each dinutuximab beta LTI cycle. LTI, long-term infusion.

Figure 2. Event-free and overall survival in the entire cohort (**A**) and in front-line PRD patients and all relapsed patients versus front-line patients with MTD (**B** and **C**). EFS, event-free survival; HRNBL, high-risk neuroblastoma; MTD, major treatment deviations; NBL, neuroblastoma, OS, overall survival; PRD, primary refractory disease; SE, standard error.

Figure 3. Event-free survival and overall survival by high-affinity versus low-affinity FCGR polymorphisms in front-line PRD patients and relapsed patients versus front-line patients with MTD (**A** and **B**) and by combination of FCGR (high/low) with NK cells (high/low) in the overall population (**C** and **D**). EFS, event-free survival; FCGR, Fc-gamma receptor polymorphisms; MTD, major treatment deviations; NBL, neuroblastoma; NK, natural killer; OS, overall survival; PRD, primary refractory disease; SE, standard error.

Figure 1



Number of patients that received dinutuximab beta	Cycles	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
122	Cycle 1	662	443	320	168	47	0	0	0	0	0
113	Cycle 2	550	336	142	12	0	0	0	0	0	0
95	Cycle 3	453	243	16	0	0	0	0	0	0	0
91	Cycle 4	414	242	0	0	0	0	0	0	0	0
87	Cycle 5	339	186	44	0	0	0	0	0	0	0

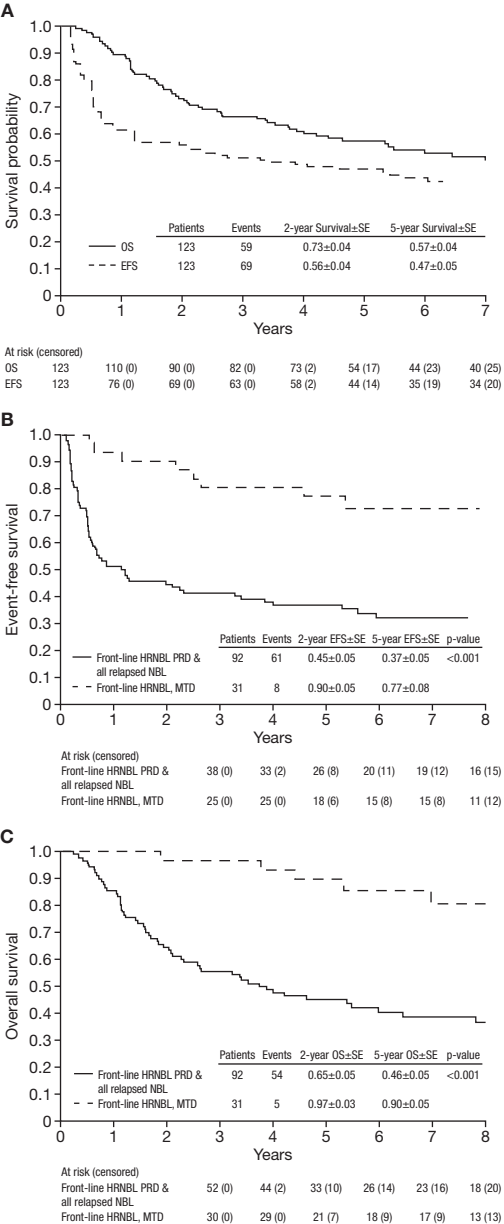
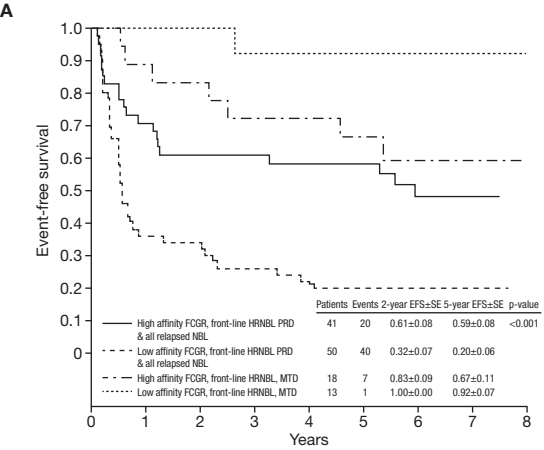
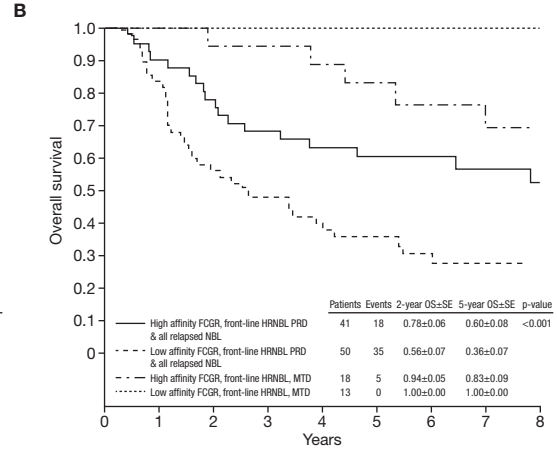

A
Survival probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Patients Events 2-year Survival±SE 5-year Survival±SE
OS 123 59 0.73±0.04 0.57±0.04
EFS 123 69 0.56±0.04 0.47±0.05
0 1 2 3 4 5 6 7
Years
At risk (censored)
OS 123 110 (0) 90 (0) 82 (0) 73 (2) 54 (17) 44 (23) 40 (25)
EFS 123 76 (0) 69 (0) 63 (0) 58 (2) 44 (14) 35 (19) 34 (20)
B
Event-free survival
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Patients Events 2-year EFS±SE 5-year EFS±SE p-value
Front-line HRNBL PRD & all relapsed NBL 92 61 0.45±0.05 0.37±0.05 <0.001
Front-line HRNBL, MTD 31 8 0.90±0.05 0.77±0.08
0 1 2 3 4 5 6 7 8
Years
At risk (censored)
Front-line HRNBL PRD & all relapsed NBL 38 (0) 33 (2) 26 (8) 20 (11) 19 (12) 16 (15)
Front-line HRNBL, MTD 25 (0) 25 (0) 18 (6) 15 (8) 15 (8) 11 (12)
C
Overall survival
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Patients Events 2-year OS±SE 5-year OS±SE p-value
Front-line HRNBL PRD & all relapsed NBL 92 54 0.65±0.05 0.46±0.05 <0.001
Front-line HRNBL, MTD 31 5 0.97±0.03 0.90±0.05
0 1 2 3 4 5 6 7 8
Years
At risk (censored)
Front-line HRNBL PRD & all relapsed NBL 52 (0) 44 (2) 33 (10) 26 (14) 23 (16) 18 (20)
Front-line HRNBL, MTD 30 (0) 29 (0) 21 (7) 18 (9) 17 (9) 13 (13)

Figure 3



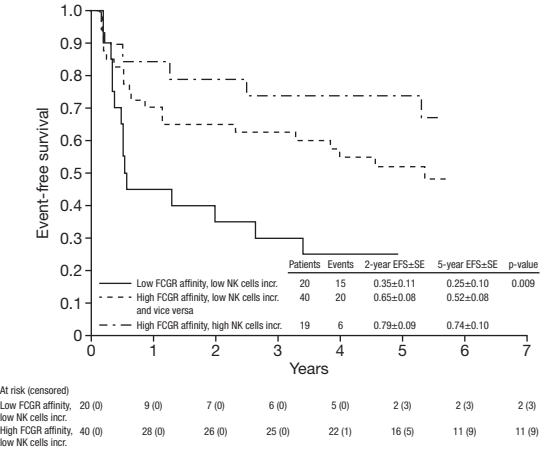
At risk (censored)

High affinity FCGR, front-line HRNBL PRD & all relapsed NBL	22 (2)	19 (5)	13 (8)	13 (8)	12 (9)
Low affinity FCGR, front-line HRNBL PRD & all relapsed NBL	11 (0)	7 (3)	7 (3)	6 (4)	4 (6)
High affinity FCGR, front-line HRNBL, MTD	13 (0)	9 (3)	8 (3)	8 (3)	6 (5)
Low affinity FCGR, front-line HRNBL, MTD	12 (0)	9 (3)	7 (5)	7 (5)	5 (7)



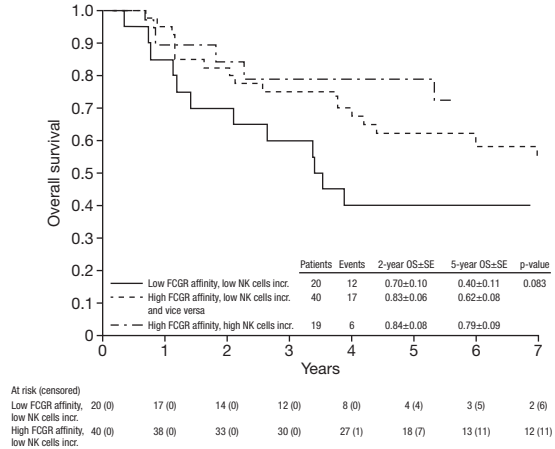
At risk (censored)

High affinity FCGR, front-line HRNBL PRD & all relapsed NBL	24 (2)	19 (6)	16 (9)	15 (9)	12 (11)
Low affinity FCGR, front-line HRNBL PRD & all relapsed NBL	20 (0)	14 (4)	10 (5)	8 (7)	6 (9)
High affinity FCGR, front-line HRNBL, MTD	16 (0)	12 (3)	11 (3)	10 (3)	8 (5)
Low affinity FCGR, front-line HRNBL, MTD	13 (0)	9 (4)	7 (6)	7 (6)	5 (8)



At risk (censored)

Low FCGR affinity, low NK cells incr.	20 (0)	9 (0)	7 (0)	6 (0)	5 (0)	2 (3)	2 (3)	2 (3)
High FCGR affinity, low NK cells incr. and vice versa	40 (0)	28 (0)	26 (0)	25 (0)	22 (1)	16 (5)	11 (9)	11 (9)
High FCGR affinity, high NK cells incr.	19 (0)	16 (0)	15 (0)	14 (0)	13 (1)	11 (3)	9 (4)	9 (4)



At risk (censored)

Low FCGR affinity, low NK cells incr.	20 (0)	17 (0)	14 (0)	12 (0)	8 (0)	4 (4)	3 (5)	2 (6)
High FCGR affinity, low NK cells incr. and vice versa	40 (0)	38 (0)	33 (0)	30 (0)	27 (1)	18 (7)	13 (11)	12 (11)
High FCGR affinity, high NK cells incr.	19 (0)	17 (0)	16 (0)	15 (0)	14 (1)	14 (1)	10 (4)	10 (4)