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Key Role for Myeloid Cells: Phase II Results of Anti-G_{D2} Antibody 3F8 Plus Granulocyte-Macrophage Colony-Stimulating Factor for Chemoresistant Osteomedullary Neuroblastoma

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Abstract

Anti-G_{D2} murine antibody 3F8 plus subcutaneously-administered (sc) granulocyte-macrophage colony-stimulating factor (GM-CSF) was used against primary refractory neuroblastoma in metastatic osteomedullary sites. Large study size and long follow-up allowed assessment of prognostic factors in a multivariate analysis not reported with other anti-G_{D2} antibodies. In a phase II trial, 79 patients without prior progressive disease were treated for persistent osteomedullary neuroblastoma documented by histology and/or metaiodobenzyl-guanidine (MIBG) scan. In the absence of human anti-mouse antibody, 3F8+scGM-CSF cycles were repeated up to 24 months. Minimal residual disease (MRD) in bone marrow was measured by quantitative reverse transcription-polymerase chain reaction pre-enrollment and post-cycle #2, before initiation of 13-*cis*-retinoic acid. Study endpoints were: 1) progression-free survival (PFS) compared with the predecessor trial of 3F8 plus intravenously-administered (iv) GM-CSF (26 patients), and 2) impact of MRD on PFS. Using all 105 patients from the two consecutive 3F8+GM-CSF trials, prognostic factors were analyzed by multivariate Cox regression model. Complete response rates to 3F8+scGM-CSF were 87% by histology and 38% by MIBG. Five-year PFS was 24%±6% which was significantly superior to 11%±7% with 3F8+ivGM-CSF (p=0.002). In the multivariate analysis, significantly better PFS was associated with R/R or H/R *FCGR2A* polymorphism, sc route of GM-CSF, and early MRD response. *MYCN* amplification was not prognostic. Complement consumption was similar with either route of GM-CSF. Toxicities were manageable, allowing outpatient treatment. 3F8+scGM-CSF is highly active against chemoresistant osteomedullary neuroblastoma. MRD response may be an indicator of tumor

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sensitivity to anti-G_{D2} immunotherapy. Correlative studies highlight the anti-neoplastic potency of myeloid effectors.

Keywords

myeloid effectors; *FCGR2A* polymorphism; immunotherapy

INTRODUCTION

With high-risk neuroblastoma (HR-NB), persistence of osteomedullary metastases, as detected by bone marrow (BM) histology or metaiodobenzylguanidine (MIBG) scan, forebodes a poor outcome.^{1,2} Indeed, scintigraphy after therapeutic doses of ¹³¹I-MIBG reveals more extensive NB than seen in standard diagnostic studies.³ The same holds for imaging with ¹³¹I-labeled monoclonal antibody (MAb) directed against ganglioside G_{D2} which is highly expressed on NB.⁴ Osteomedullary NB is in fact an attractive target for MAb-mediated immunotherapy because the BM compartment contains tumoricidal macrophages and is immersed in blood. These conditions optimize accessibility of NB to MAb and effectors, including granulocytes and natural killer (NK) cells, and bypass the common limitation of immunotherapy, namely, poor trafficking into bulky tumor.

The efficacy of adjuvant anti-G_{D2} MAb ch14.18 plus granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-2 (IL-2) was demonstrated in a randomized trial.⁵ However, histologic or radiologic documentation of anti-NB activity by anti-G_{D2} MAbs is limited, with the largest studies involving patients in remission (adjuvant setting)⁵⁻⁷ and other, smaller studies focusing on toxicity, sometimes without detailing responses.⁸

The murine anti-G_{D2} IgG3 MAb 3F8 mediates antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).⁹ In exploratory studies of 3F8±GM-CSF, activity was noted against BM disease, though not against soft tissue or progressive disease (PD).⁹ When treated in complete/very good partial remission (CR/VGPR), HR-NB patients have an inferior outcome with 3F8 alone compared to 3F8+GM-CSF.⁷ Because Fcγ receptors (FcγR) on effector cells are key mediators of ADCC and they are polymorphic among individuals, the preferential affinity of specific polymorphic allele for MAb can affect treatment outcomes.^{10,11} Similar to other mouse antibodies, 3F8 binds with higher affinity to FcγIIA (CD32) arginine (R) allele over the histidine (H) allele on myeloid effector cells.¹⁰

With 3F8, GM-CSF was initially administered intravenously (iv) over two hours.¹² The aim was to avoid prolonged high serum levels of GM-CSF, given that the neutrophil migration inhibitory and concentration-dependent chemotaxis properties of GM-CSF might impede granulocyte trafficking into tissues.¹³ However, when devising the successor phase II trial, we postulated that those biological effects might not be relevant when targeting NB in BM and, regardless, would be dwarfed by the advantage of greater activation of effector cells with prolonged GM-CSF exposure following subcutaneous (sc) injection.¹⁴

We now report results of the phase II trial that tested 3F8+scGM-CSF in patients with primary refractory NB in BM. These patients had never relapsed or developed PD but had persistence of osteomedullary disease post-induction. Biostatistics were based on results in the preceding phase II trial of 3F8+ivGM-CSF.¹⁰ Large study size and lengthy follow-up of this well-defined group of patients allowed assessment of biological and clinical prognostic factors in a multivariate analysis for progression-free survival (PFS) not reported with other anti-GD2 MAb.^{5,6,8} Prognosticators included FcγR polymorphism,^{10,11} killer immunoglobulin-like receptor (KIR) genotypes of NK cells,^{11,15} minimal residual disease (MRD),¹⁶ *MYCN* status, and overall response.

MATERIAL AND METHODS

Patients

Seventy-nine patients enrolled (12/2003–10/2010) on a prospective phase II study [[ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00072358) NCT00072358] for treatment of primary refractory NB in BM. Eligibility criteria included: HR-NB, i.e., *MYCN*-amplified stage 4 (any age) or *MYCN*-non-amplified stage 4 in patients >18 months old; prior treatment that included standard induction chemotherapy for HR-NB but no immunotherapy; osteomedullary metastases documented by histology and/or ¹²³I-MIBG scan <3 weeks from enrollment; no residual soft tissue NB; and no prior PD. Informed written consents for this trial were obtained following institutional review board rules.

Study design

The primary endpoint of this phase II trial using 3F8+scGM-CSF plus 13-*cis*-retinoic acid (13-*cis*-RA) was PFS. The hypothesis was that outcome would be superior with the sc rather than iv route of GM-CSF. Thus, the study was a one-stage design with type I error 0.05 and 0.85 power. Accrual of 70 patients was planned in order to distinguish two-year PFS of 13% (observed in the predecessor 3F8+ivGM-CSF trial – see below) as “not promising” and 25% as “promising”, where 12 patients had to be progression-free at two years. For comparison, all 26 patients with primary refractory NB in BM in the predecessor 3F8+ivGM-CSF trial [[ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00002560) NCT00002560] were analyzed. This study was identical as regards: 1) eligibility criteria; 2) 3F8+GM-CSF dosage/cycle and schedule; and 3) use of 13-*cis*-RA. Eleven of these 26 patients were in a previous report.¹⁰

The secondary endpoint was applying quantitative reverse transcription-polymerase chain reaction (qRT-PCR) to test the hypothesis that MRD in BM after two cycles of immunotherapy significantly impacts PFS. MRD by qRT-PCR was studied, as described,^{7,16} using BM aspirates pooled from four sites obtained at study entry (Pre-MRD) and post-cycle #2 (Post-MRD).

Treatment

Immunotherapy cycles comprised scGM-CSF alone ×5 days, followed by 3F8+scGM-CSF, with 3F8 (prepared as described⁴) at 100 mg/m²/cycle (Table 1).⁷ Cycles were separated by 3 weeks through 4 cycles after achievement of complete response (CR) in BM. Subsequent cycles were separated by 8 weeks through 24 months from enrollment. Treatments were

deferred if human anti-mouse antibody (HAMA) (measured as described¹⁷) developed; after 7/2008, rituximab (375 mg/m²/dose ×2) ± cyclophosphamide (750 mg/m² single dose) could be used to ablate HAMA. Focal skeletal radiotherapy was allowed. After marrow CR was achieved, 13-*cis*-RA was used as described,¹⁸ ×6 cycles, between cycles of immunotherapy. Toxicity was scored using Common Terminology Criteria for Adverse Events Version 2.0.

Definition of response

Comprehensive extent-of-disease evaluations were performed every 2–3 months ×3 years from enrollment. Disease status was defined by International Neuroblastoma Response Criteria (INRC),¹⁹ using BM histology, computed tomography of the primary site, and urine catecholamine levels, plus MIBG findings. CR: no NB in BM (histology) or elsewhere, and normal urine catecholamine levels. No response: Persistence of BM involvement (histology), and/or no change in number of radiologic lesions. PD: new lesion(s) radiologically, and/or increased number of histologically-positive BM sites. For this analysis, CR(BM) indicates CR in BM by histology and CR(MIBG) indicates CR by scintigraphy. BM histology required standard histochemical studies²⁰ of aspirates/smears and biopsies from bilateral posterior and anterior iliac crests (i.e., four sites, which contrast with the two sites in international guidelines¹⁹) obtained after each cycle of immunotherapy until CR(BM) was documented in successive examinations.

Correlative studies

FcγR polymorphisms and KIR genotypes were determined as described.⁷ Change in total hemolytic complement activity (CH50) was measured in cycle #1.

Statistical analysis

PFS was scored from the first dose of 3F8. Three variables – development of HAMA, number of cycles (≥4 vs. <4), and overall CR – were analyzed as a time-dependent covariate using the hazard model $\lambda(t|Z(t)) = \lambda_0(t) \exp(\beta Z(t))$, where $Z(t)=1$ for any time t after patient developed HAMA (or received immunotherapy cycle #4, or was scored as CR), and $Z(t)=0$ otherwise. $\lambda_0(t)$ was the unknown baseline hazard and $\exp(\beta)$ the hazard ratio corresponding to the variable effect. Kaplan-Meier method was used for survival curves. Log-rank test was used to determine the univariate association between clinical variables and PFS. Multivariate Cox regression model was fitted with variables that had a univariate p -value <0.05. Changes in CH50 were analyzed using paired t -test. The difference between sc and iv groups was tested using exact Wilcoxon rank sum test. Associations between binary variables and treatment groups were analyzed using Fisher exact test. No multiple comparisons adjustment was performed.

RESULTS

Patient characteristics

Prior treatment included dose-intensive induction in all 79 patients enrolled on this 3F8+scGM-CSF study, second-line chemotherapy (because of incomplete response to induction) in 44/79 (56%) patients, and stem-cell transplantation (in an unsuccessful attempt to eradicate persistent osteomedullary NB) in 31/79 (39%) patients. Characteristics of these

79 patients and the 26 patients in the predecessor 3F8+ivGM-CSF study were similar (Table 2). The median time of follow-up from the first dose of 3F8 was 4.6 years (0.9–8.5 years) for scGM-CSF patients and 7.5 years (3.0–17 years) for ivGM-CSF patients.

Primary endpoint: Overall outcome

Based on the trial design's pre-specified decision rule, the 3F8+scGM-CSF treatment met the aim, with the two-year PFS rate of 48% (95% CI 38%, 61%) exceeding the 25% threshold for "promising." In addition, at five years, PFS was $24\% \pm 6\%$, and OS was $65\% \pm 6\%$ (Figure 1). There was no significant association between focal radiotherapy and PFS among MIBG(+) patients ($p=0.32$). Anti-HAMA treatment with rituximab (received by 16 patients) was not associated with PFS ($p=0.65$). Outcome was significantly superior to the 3F8+ivGM-CSF trial, where the five-year PFS rate was $11\% \pm 7\%$ ($p=0.002$).

Secondary endpoint: Impact of MRD

With 3F8+scGM-CSF, Pre-MRD and Post-MRD findings correlated significantly with PFS ($p=0.007$ and $p<0.001$, respectively). Among the 40 patients who were MRD(+) at enrollment, 21 (53%) turned MRD(−) post-cycle #2 of immunotherapy. MRD response was comparable ($p=0.76$) in the 3F8+ivGM-CSF study, where 15 patients were Pre-MRD(+), 9 (60%) of whom became Post-MRD(−). In the combined cohort of 105 patients, 55 patients were MRD(+) prior to 3F8, with 30 (55%) becoming MRD(−) after 2 cycles of immunotherapy. The responders had a 2-year PFS rate of $48\% \pm 9.3\%$ which was significantly better than the 2-year PFS rate of $30\% \pm 9.4\%$ ($p=0.004$) for the non-responders. Moreover, Post-MRD status (positive versus negative) was significantly prognostic of PFS (Figure 2, $p<0.001$).

Osteomedullary response to 3F8+scGM-CSF

Among the 79 patients treated with 3F8+scGM-CSF, evidence of anti-NB activity included CR(BM) in 40/46 (87%) patients (Table 3). CR(MIBG) was noted in 26/50 (52%) patients, including 10/26 (38%) patients who did not receive focal radiotherapy and 16/24 (67%) who received focal radiotherapy (and therefore were not evaluable for response).

BM(+)/MIBG(−) group

Of 29 patients with persistent BM involvement (histology) but normal MIBG findings, 28 (97%) achieved CR(BM) after cycle #1 ($n=18$), #2 ($n=7$), or #3 ($n=3$). In the subset of 16 post-transplant patients, 15 (94%) achieved CR(BM).

BM(+)/MIBG(+) group

Of 17 patients with persistent NB evidenced by histology and MIBG scan, 12 (71%) achieved CR(BM) after cycle #1 ($n=6$), #2 ($n=1$), #3 ($n=2$), or #4 ($n=3$). Six patients (35%) achieved CR(MIBG), including three who received radiotherapy to the abnormal site (hence, not evaluable for response to 3F8+scGM-CSF).

BM(-)/MIBG(+) group

Thirty-three patients had persistence of osteomedullary disease radiologically, but no NB in BM (histology). In this group, 16 patients had 2 sites of abnormal MIBG uptake: 11 achieved CR(MIBG); five also received radiotherapy to abnormal sites. Among the 17 patients who had one MIBG-positive site, nine achieved CR(MIBG), but eight of them also received focal radiotherapy.

Route of GM-CSF and response

The overall CR rate with 3F8+scGM-CSF was 68%, similar to 65% for the 3F8+ivGM-CSF patients (Table 3). CR(BM) rates were 87% with 3F8+scGM-CSF and 78% with 3F8+ivGM-CSF ($p=0.49$), while the respective rates of CR(MIBG) were 52% and 62% ($p=0.76$) (38% and 58% for patients who did not receive focal radiotherapy). There was no significant difference in PFS ($p=0.34$) for BM(+)/MIBG(-), BM(+)/MIBG(+), and BM(-)/MIBG(+) groups.

Impact of FCGR2A polymorphism on outcome

The 5-year PFS rate for patients with the R/R ($n=24$) or H/R ($n=55$) *FCGR2A* genotypes was $24\% \pm 5.8\%$ versus $15\% \pm 7.1\%$ for patients with the H/H ($n=26$) genotype ($p=0.001$). The preferential affinity of 3F8 for the Fc γ RIIA (CD32A) arginine (R) allele over the histidine (H) allele may help explain the survival differences.

Complement activity

CH50 during cycle #1 of immunotherapy decreased significantly with sc ($p<0.001$) and iv ($p<0.001$) routes of GM-CSF, consistent with complement activation. The change in CH50 was similar with 3F8+scGM-CSF and 3F8+ivGM-CSF ($p=0.58$).

Multivariate analysis of prognostic factors

A wide range of key prognostic factors for high-risk NB and immunotherapy were studied in univariate analyses, including clinical and biological characteristics (Table 2); response by BM histology, MIBG, and overall (Table 3); *FCGR2A* and *FCGR3A* polymorphisms; and missing KIR ligand. The sc and iv treatment regimens were then compared in a multivariate model adjusting for potential confounders. Variables that were significant with $p<0.05$ in the combined cohort ($n=105$) were used in the multivariate model. Variables that were no longer significant in the presence of other covariates were excluded from the final model. The multivariate analysis identified Post-MRD-negativity, R/R or H/R *FCGR2A* genotypes, and sc route of GM-CSF as significantly favorable independent prognostic factors for PFS (Table 4).

Toxicity

Immunotherapy was outpatient, with grade 1–2 pain and urticaria.⁷ Late events were attributable to prior therapy: lethal idiopathic pulmonary failure 8 months from study entry (4.5 months post-cycle #4 of 3F8+scGM-CSF) and secondary leukemia 28 months from study entry (successfully treated).

DISCUSSION

The large phase II experience with 3F8+GM-CSF shows the possibility of overcoming histologically- and/or radiologically-visible chemoresistant osteomedullary NB using an anti-G_{D2} MAb regimen administered outpatient. Correlative studies highlight the anti-neoplastic potency of myeloid effectors, rather than lymphocytes, which are usually the focus in the immunotherapy of solid tumors.²¹

When the 3F8+scGM-CSF trial was designed, the study population was defined using results in prior 3F8 phase I/II studies.⁹ These had shown that 3F8 was promising for treating a small tumor burden, but was ineffective against measurable soft tissue disease or PD, so patients with either of those findings were excluded. Biostatistics were based on the PFS of patients with primary refractory osteomedullary NB in the 3F8+ivGM-CSF trial – hence, comparisons between the sc and iv routes were not in a randomized setting. Despite this drawback, given our strict inclusion criteria for analysis of only stage 4 NB with *MYCN* amplification and/or age >18 months at diagnosis in both of the successive trials, we consider the data to be robust enough to at least conclude that there is no harm in using scGM-CSF. Additional factors favoring the sc route over the 2-hour iv schedule include a shorter daily treatment in the outpatient clinic and greater augmentation of myeloid ADCC (see below).²²

BM histologic response was usually rapid, occurring after 1–2 cycles. MRD-negativity post-cycle #2 was an independent predictor of PFS, possibly reflecting NB sensitivity to this immunotherapy. Indeed, the significantly prognostic MRD findings after two cycles may allow early recognition of treatment efficacy and provide guidance about whether to continue this immunotherapy in a given patient.

In addition to early MRD response, the route of GM-CSF administration and *FCGR2A* polymorphism were each significantly prognostic for PFS in the multivariate analysis (Table 4). A recent study of peripheral-blood granulocytes implicated adhesion-molecule CD11b and its activation epitope CBRM1/5 in myeloid-mediated anti-NB effect.²² This finding suggests that the sc route of GM-CSF, as compared to the iv route, improves clinical outcome through greater activation of CBRM1/5. The favorable impact on prognosis of high-affinity *FCGR2A* polymorphic allele (R/R or R/H present on myeloid but not lymphoid cells) further supports the anti-neoplastic potential of myeloid effectors.

Correlative studies point to anti-NB activity not only via ADCC but also CDC. The significant decrease in CH50 likely reflects complement activation, which is well-documented with anti-G_{D2} MAbs *in vitro*.⁹ Changes in CH50 were similar when 3F8 was combined with scGM-CSF or ivGM-CSF.

Clinical and biological factors that were not prognostic of outcome in the multivariate analysis included *MYCN* amplification, missing KIR ligand, prior second-line therapy, prior stem-cell transplantation, and development of HAMA. Of note, two additional treatments – rituximab-cyclophosphamide and focal radiotherapy – were allowed since they were not expected to affect PFS, given the single dose of cyclophosphamide at only 750 mg/m² and

the systemic nature of HR-NB;³ indeed, neither treatment was prognostic in univariate or multivariate analyses.

There is little published histologic/radiologic evidence of anti-neoplastic activity by myeloid cells in the immunotherapy of solid tumors in general²¹ and of NB in particular. Prior reports of phase II trials of other anti-G_{D2} MAbs involve clinically-diverse patients and are limited to one abstract and one formal report: Results with ch14.18+GM-CSF (27 patients evaluable for response) favored use against MRD,²³ and hu14.18-IL2 (IL-2 fused to humanized 14.18) achieved responses in BM and/or MIBG(+) skeletal sites (5/23 patients) but not in soft tissue (0/13 patients).²⁴ When used without cytokines, anti-G_{D2} MAbs achieved responses in small studies (5-to-16 NB patients).⁸ Response data are awaited for hu14.18K322A which is hu14.18 modified to have low CDC to reduce pain.²⁵ The adjuvant setting predominates among large studies of anti-G_{D2} MAbs, precluding documentation of clinical response. Thus, with all study patients in first remission, 3F8 alone (34 patients)⁹ and 3F8+GM-CSF (136 patients)⁷ yielded encouraging results; ch14.18 alone did not improve PFS (166 patients);⁶ and ch14.18+GM-CSF+IL-2 conferred a significant benefit in a randomized study (226 patients).⁵

3F8+scGM-CSF achieves a high response rate in patients with primary refractory NB in BM. The two-year PFS rate of 48% with this immunotherapy plus 13-*cis*-RA was consistent with the favorable results in the adjuvant setting of the randomized trial using ch14.18+GM-CSF+IL-2 plus 13-*cis*-RA (two-year PFS rate of 66%).⁵ Greater anti-NB activity is a possibility with humanized anti-G_{D2} MAbs.^{8,24,25}

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ABBREVIATIONS/ACRONYMS

ADCC	antibody dependent cellular cytotoxicity
BM	bone marrow
CDC	complement-dependent cytotoxicity
CH50	total hemolytic complement activity
CR	complete remission
FcγR	Fcγ receptors
GM-CSF	granulocyte-macrophage colony-stimulating factor
HAMA	human anti-mouse antibody
HR-NB	high-risk neuroblastoma
IL-2	interleukin-2
INRC	International Neuroblastoma Response Criteria

IV	intravenous
MAb	monoclonal antibody
MIBG	metaiodobenzylguanidine
MRD	minimal residual disease
NK	natural killer
PD	progressive disease
PFS	progression-free survival
qRT-PCR	quantitative reverse transcription-polymerase chain reaction
RA	retinoic acid
SC	subcutaneous
VGPR	very good partial remission

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What's new?

The phase II 3F8+GM-CSF experience documents activity against histologically- and/or radiologically-visible chemoresistant neuroblastoma. Large study size and lengthy follow-up allow use of prognostic variables (minimal residual disease, *MYCN*, Fc γ receptors, KIR genotypes of natural killer cells) in a multivariate analysis not described with other anti-GD₂ antibodies. Correlative studies highlight the anti-neoplastic potency of myeloid effectors, rather than lymphocytes, which are usually the focus in the immunotherapy of solid tumors.

Figure 1.

Progression-free survival (PFS) and overall survival (OS) of 79 stage 4 patients treated with 3F8+scGM-CSF for primary refractory neuroblastoma.

Figure 2.

Progression-free survival of 105 patients (79 treated with 3F8+scGM-CSF and 26 treated with 3F8+ivGM-CSF) correlated significantly with MRD negativity after cycle #2 of immunotherapy, $p < 0.001$.

Table 1**3F8+GM-CSF Treatment**

scGM-CSF regimen	Priming doses of scGM-CSF 5 days (Wed-Sun) →	scGM-CSF + 3F8 (20 mg/m ² /d) 5 days (Mon-Fri)		
ivGM-CSF regimen	Priming doses of scGM-CSF 5 days (Wed-Sun) →	ivGM-CSF + 3F8 (10 mg/m ² /d) 5 days (Mon-Fri) →	scGM-CSF 2 days (Sat, Sun) →	ivGM-CSF + 3F8 (10 mg/m ² /d) 5 days (Mon-Fri)

sc, subcutaneous; iv, intravenous

GM-CSF: 250 µg/m²/day for the priming doses and for the first 2 days of 3F8, then 500 µg/m²/day

3F8: 100 mg/m²/cycle, either 10 mg/m²/day ×10 days or 20 mg/m²/day ×5 days, iv over 30 minutes

Rest periods: 3 weeks from end of one cycle to start of 3F8 in next cycle, through 4 cycles after complete response; then 8 week rest periods, through 24 months from the first dose of 3F8

Table 2

Patient and tumor characteristics

Variable	3F8+scGM-CSF (n=79)	3F8+ivGM-CSF (n=26)	Combined cohort (n=105)
Male:female ratio	1.5	1.2	1.4
Age (years) at diagnosis			
Median	4.2	4.4	4.2
Range	1.4–26.8	2.1–19.4	1.4–26.8
MYCN-amplified	17 (22%)	3 (12%)	20 (19%)
LDH 1500 U/L	19/63 (30%)	7/22 (32%)	26/85 (31%)
Cortical bone at diagnosis	69 (87%)	23 (88%)	92 (88%)
Post-induction therapy			
2 nd -line chemotherapy	38 (48%)	12 (46%)	50 (48%)
SCT	13 (16%)	5 (19%)	18 (17%)
SCT + 2 nd -line therapy	18 (23%)	6 (23%)	24 (23%)

LDH, serum lactate dehydrogenase (normal 246 U/L); SCT, stem-cell transplantation after myeloablative chemotherapy

Table 3

Responses to 3F8/GM-CSF*

	scGM-CSF (n = 79)					Response Rate	ivGM-CSF (n = 26)					Response Rate
	BM(+)/ MIBG(-)	BM(+)/MIBG(+)		BM(-)/MIBG(+)			BM(+)/ MIBG(-)	BM(+)/MIBG(+)		BM(-)/MIBG(+)		
		2 MIBG+ sites	1 MIBG+ site	2 MIBG+ sites	1 MIBG+ site			2 MIBG+ sites	1 MIBG+ site			
# of Patients	29 (29)	13 (13)	4 (1)	16 (9)	17 (3)	13 (13)	8 (8)	2 (1)	2 (2)	1 (1)		
Histologic CR in BM	28 (28)	9 (9)	3 (0)	-	-	10 (10)	6 (6)	2 (1)	-	-	78%	
MIBG CR	-	3 (3)	3 (0)	11 (6)	9 (1)	-	4 (4)	2 (1)	1 (1)	1 (1)	62% (58%)	
Overall CR	28 (28)	3 (3)	3 (0)	11 (6)	9 (1)	10 (10)	3 (3)	2 (1)	1 (1)	1 (1)	65% (64%)	
Probability of 2 Year PFS (Std Error)	0.48 (0.09)	0.40 (0.12)		0.53 (0.09)		0.15 (0.1)	0.2 (0.13)		0.5 (0.35)			

BM, bone marrow; CR, complete response; NR, no response; PD, progressive disease; PFS, progression-free survival

- indicates not applicable

* in brackets () are patients without focal radiotherapy

Table 4

Multivariate Analysis of Prognostic Factors in 105 patients

Prognostic Variable	Progression-Free Survival			
	Hazard ratio	lower bound	upper bound	p-value
<i>FCGR2A</i> Polymorphism * R/R or H/R vs H/H	0.41	0.24	0.71	0.001
Post-MRD (Pos vs Neg) **	3.29	2.04	5.32	<0.001
Route of GM-CSF (SC vs IV)	0.52	0.31	0.88	0.02

* R/R, n=24; H/R, n=55; H/H, n=26

** Positive Post-MRD in 38/105 (36%) patients