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High-Dose Iodine-131-Metaiodobenzylguanidine with Haploidentical Stem Cell Transplantation and Post-Transplant Immunotherapy in Children with Relapsed/Refractory Neuroblastoma

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Potential conflict of interest

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ABSTRACT

We evaluated the feasibility and efficacy of using high-dose iodine-131-metaiodobenzylguanidine (^{131}I-MIBG) followed by reduced-intensity conditioning (RIC) and transplantation of T-cell-depleted haploidentical peripheral blood stem cells (designated haplo-SCT) to treat relapsing/refractory neuroblastoma (RRNB). Five RRNB patients were enrolled: four with relapse (three after autologous SCT) and one with induction therapy failure. The preparative regimen included high-dose ^{131}I-MIBG on day –20, followed by fludarabine, thiotepa, and melphalan from day –8 to –1. G-CSF-mobilized, T-cell-depleted haploidentical paternal stem cells were infused on day 0 together with cultured donor mesenchymal stem cells. A single dose of rituximab was given on day +1. After cessation of short immunosuppression (mycophenolate, OKT3), four children received donor lymphocyte infusion (DLI). ^{131}I-MIBG infusion and RIC were well tolerated. All patients engrafted. No primary acute graft-versus-host disease (GvHD) was observed. Four children developed acute GvHD after DLI and were successfully treated. Analysis of immunological recovery showed fast reappearance of potentially immunocompetent NK and T-cells, which might have acted as effector cells responsible for the graft-versus-tumor effect. Two children are alive and well with no evidence of disease 40 and 42 months after transplantation. One patient experienced late progression with new bone lesions (sternum) 38 months after haplo-SCT and is being treated with local irradiation and reinstituted DLI. One patient rejected the graft, was rescued with autologous back-up, and died of progressive disease 5 months after transplantation. Another child relapsed 7 months after transplantation and died 5 months later. High-dose ^{131}I-MIBG followed by RIC and haplo-SCT for RRNB is feasible and promising, since two of five children on that regimen achieved long-lasting remission. Further studies are needed to evaluate targeted therapy and immune-mediated tumor control in high-risk neuroblastoma.

INTRODUCTION

The prognosis for relapsing/refractory neuroblastoma (RRNB) remains dismal, with a 5-year disease-free survival (DFS) of less than 20%, and no effective salvage treatment has been identified so far. Palliation is often the sole option for recurrent disease after myeloablative treatment, because salvage therapy may not be feasible or justified due to no realistic chance for cure (1, 2). Treatment of RRNB is still experimental and focused on introducing novel cytotoxic and/or tumor-targeted biological agents.

The norepinephrine analog metaiodobenzylguanidine (MIBG) is selectively accumulated in sympathetic nervous tissue, and several studies have suggested that treatment with iodine-131-labled MIBG (^{131}I-MIBG) induces remission in NB, even in advanced cases. Duration of remission remains the primary concern, and the possibility of incorporating ^{131}I-MIBG into multimodal therapy for RRNB has been proposed (3). An investigation of high-risk patients showed that a combination of high-dose ^{131}I-MIBG, high-dose chemotherapy, and autologous hematopoietic stem cell transplantation (ASCT) was practicable and associated with limited toxicity (4, 5). However, only 36% of the patients responded, and the 3-year event-free survival (EFS) rate was 0.31 ± 0.10.

Studies have not shown any advantage in using HLA-identical donors for allogeneic stem cell transplantation (allo-SCT) to treat high-risk NB (6-8). Also, it has been postulated that absence of graft-versus-host disease (GvHD) in allo-SCT is a major obstacle to an anti-tumor effect. Haploidentical stem cell transplantation (haplo-SCT) for treatment of malignant diseases has become an established procedure (9-11), which has also proven to be feasible and involve acceptable toxicity in children with hematological malignancies (10). Although reports concerning haplo-SCT for pediatric solid tumors are still anecdotal, a potential graft-versus-tumor (GvT) effect in such cases has been discussed (12-14).

Considering the mentioned findings, we studied the influence of high-dose ^{131}I-MIBG treatment followed by reduced intensity conditioning (RIC) with haplo-SCT in a series of consecutive RRNB patients that, to the best of our knowledge, constitute the largest cohort of such patients treated with this combined approach.

PATIENTS AND METHODS

Patient Population

The treatment used was designed for RRNB patients aged 1–18 years who develop resistant disease at any time. This study was approved by the Lund University Ethical Review Board for Research Involving Humans (DNr 385/2005), and informed consent was obtained from all parents/guardians.

Mobilization and Processing of Peripheral Blood Progenitor Cells

At the time the treatment protocol was developed, it was suggested that the mobilization of peripheral blood progenitor cells is more effective in male donors (15, 16). To be able to harvest the optimal number of CD34+ cells, which is particularly important due to additional cell loss caused by further processing of the graft, we chose to transplant all our patients with cells from an HLA haploidentical father. Typing was performed at the two-digit level for HLA class I and at the allelic level for HLA class II. Donor peripheral blood progenitor cells were mobilized with rhG-CSF. To remove T-cells from the graft, a CliniMACS system (Miltenyi Biotech, Bergisch-Gladbach, Germany) was used to subject the harvested cells to immunomagnetic selection of CD34+ cells (the first collection) and to immunomagnetic depletion of CD3+ cells (the second collection), as previously described (17). The first collection secured the optimal number of CD34+ cells with the lowest possible number of contaminating T lymphocytes, and the second collection served primarily as the source of accessory cells, allowing a certain number of CD3+ cells. The target dose was set at $\geq 10 \times 10^6$ CD34+ cells and $\leq 1 \times 10^5$ CD3+ cells per kilogram of recipient body weight.

Culture of Mesenchymal Stem Cells

To facilitate engraftment and reduce the risk of GvHD, mesenchymal stem cells (MSCs) were transplanted directly after hematopoietic stem cell infusion (18). The MSCs were

cultured as described elsewhere (19). Briefly, heparinized bone marrow (45–60 mL) was aspirated from the iliac crest of the haploidentical donor 4 weeks before transplantation. Ficoll-isolated mononuclear cells were seeded in complete mesenchymal stem cell medium, which was changed after 3 days and weekly thereafter. On the day of haplo-SCT, MSCs were harvested, washed, and resuspended in 0.9% saline supplemented with 5% human serum albumin. Flow cytometry showed expression of typical surface marker profiles.

Infusion of ^{131}I-MIBG

On day −21 prior to haplo-SCT, all children received a single 1-hour i.v. infusion of ^{131}I-MIBG under thyroid protection with potassium iodide and i.v. hydration. The patients were subsequently kept in a radiation-protected isolation room until emissions met institutional regulations. Whole-body (WB) activity was measured daily for 5–7 days, and WB/SPECT imaging was performed on day −11.

Conditioning and Post-Transplant Immunosuppression

RIC comprised fludarabine (5 x 25 mg/m² q24 hours, days −8 to −4), thiotepa (2 x 5 mg/kg, q12 hours, day −3), and melphalan (2 x 60 mg/m² q24 hours, days −2 and −1) (20). The T-cell receptor-targeted antibody muromonab-CD3 was administered on days −8 to +15 as rejection prophylaxis. A short course of post-transplant mycophenolate mofetil 600 mg/m^2 bid was given on days −1 to +28 as GvHD prophylaxis. To reduce the risk of EBV-associated post-transplant lymphoproliferative disease, *in vivo* B-cell depletion was performed by a single infusion of 375 mg/m^2 rituximab on day +1.

Donor Lymphocyte Infusion

After terminating immunosuppression, donor lymphocyte infusions (DLIs) were planned to improve immune reconstitution and/or to convert mixed chimerism (MC) to complete donor chimerism (CC). The initial number of infused CD3+ lymphocytes was 2.5–5 x 10^4

cells/kg/dose. The intention was to increase the dose of CD3+ cells if no GvHD occurred. For details, see Table 3. Blood for a DLI was obtained from the donor on the day of the infusion. No GvHD prophylaxis was given after DLI.

Post-Transplant Intrathecal Liposomal Cytarabine

A patient with CNS relapse was given intrathecal liposomal cytarabine every 4–6 weeks for up to 1 year after transplantation.

Engraftment, Immunological Reconstitution, and Chimerism Analysis

Engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) > 500/μL, and platelet recovery as the first of 3 consecutive days with transfusion-independent platelet count > 20,000/μL. Immunological recovery was assessed weekly for the first 3 months and thereafter once over a period of 3–6 months. This was done by immunophenotyping of peripheral blood mononuclear cells with anti-CD3, anti-CD4, anti-CD8, anti-CD19, and anti-CD16/CD56 monoclonal antibodies. Chimerism analysis was performed at the same time points by amplification of variable number of tandem repeats (VNTRs) as polymorphic genetic markers in selected cell populations (T-, B-, and non-T/non-B-cells).

Supportive Care

No G-CSF was administered post-transplant. Prophylactic voriconazole and valacyclovir were given for 6 months after transplantation. As oral prophylaxis against *Pneumocystis carinii*–pneumonia, trimethoprim-sulfamethoxazole was instituted for 6 months following engraftment. Surveillance of CMV, AdV, EBV, and BK viral loads was achieved by PCR performed weekly up to day +100, and preemptive therapy was initiated if increasing loads were detected.

Evaluation of Disease Response

In accordance with the International Neuroblastoma Response Criteria (21), evaluation of disease markers and imaging studies in combination with bilateral bone marrow aspirations and biopsies were performed at regular intervals of 4–6 weeks for the first 6 months and thereafter every 3–6 months.

RESULTS

Patient Characteristics

Between June 2005 and November 2005, five patients with stage IV RRNB were eligible for inclusion in the study. These children (given unique patient numbers NB01–NB05) had been intensively pretreated at the Department of Pediatric Oncology, University Hospital, Lund, Sweden, three of them with high-dose chemotherapy and ASCT. One of the patients had developed secondary AML that had been successfully treated. One had achieved complete remission before haplo-HSCT (CR 2), three were in partial remission (PR), and one had progressive disease (progression). The median interval between initial diagnosis and haplo-HSCT was 3.9 years (Table 1).

Graft Composition

A median of 13 x 10^6 CD34+ cells/kg were transplanted with a median of 0.98 x 10^5 graft-contaminating CD3+ cells (Table 2). A median of 0.75 x 10^6 MSCs/kg were transplanted directly after hematopoietic stem cell infusion (Table 2).

High-Dose ^{131}I-MIBG and RIC

High-dose ^{131}I-MIBG treatment was feasible, and infusion was done at a median dose of 10.9 mCi/kg, corresponding to a median WB absorbed dose of 1.6 Gy (Table 3). The patient whose bone marrow was infiltrated with NB cells at the time of treatment (NB-02) had prolonged post-MIBG marrow aplasia that persisted until the beginning of RIC, whereas the other children recovered their blood counts before start of conditioning. The RIC caused no serious adverse effects.

Hematological Recovery

Neutrophil engraftment was achieved in all five children. The median time to ANC recovery was 13 days (range: 11–16 days), and the median time to platelet recovery, which occurred in four patients, was 12 days (range: 11–37 days) (Table 2). One patient (NB-02) rejected the graft on day 18, and an attempt to rescue the graft by intensified immunosuppression and stem cell boost failed. That child eventually recovered after autologous backup.

Chimerism Analysis of T-Cell Compartment

Although chimerism analysis was performed in different cell populations, DLI interventions were driven by chimerism analysis of the T-cell compartment (CD3+ cells). Patient NB-02, who rejected the graft, exhibited complete donor chimerism (CC) at the time of engraftment. Patient NB-01 showed sustained CC at all time points. In the remaining three patients, analysis of CD3+ cells revealed increasing mixed donor chimerism (MC) after initial CC, which was eventually converted to CC status.

Patient NB-03 displayed a decline to 93% donor chimerism 6 weeks post-transplant and received DLI treatment. Transient conversion to CC was achieved, but, despite that, only recipient T-cells were detectable 9 weeks after transplant. ATG treatment was instituted, and the patient successfully regained CC. In patient NB-04, donor chimerism declined to 40% 6 weeks post-transplant, and that child was given DLI and regained sustained CC by week 10. Patient NB-05 showed a decline to 8% donor chimerism by week 5 post-transplant, despite "preemptive" DLI. Additional DLIs were given, and the peripheral blood CD3+ cells gradually increased to CC by week 9. Chimerism analysis is presented in Figure 1.

Immune Reconstitution

Quantitative analysis was performed on T, B, and NK lymphocytes from four of the patients. The median CD3+ cell counts 2, 3, and 4 months post-transplant were 175 (range: 60–1,050), 265 (range: 90–2,370), and 710 (range: 250–6,160) cells/μL, respectively. NK cell recovery was faster, with a median count of 420 (range: 220–1,050) cells/μL 4 weeks after transplantation and a median 1-year count of 320 (range: 190–1,560) cells/μL. B-cell recovery was delayed until circulating cells reappeared 4 months post-grafting, and the median count 6 months after transplantation reached 180 (range: 50–280) cells/μL. Immune reconstitution is shown in Figure 2. PCR analysis detected multiple viral reactivations with no life-threatening clinical symptoms occurred in three children and were successfully treated preemptively (Figure 1).

GvHD and DLI

None of the patients developed primary acute GvHD. Four children with sustained engraftment received DLIs at an initial dose of 2.5 x 10^4 or 5 x 10^4 T-cells/kg. The first infusion was performed at the earliest on day +25 and at the latest on day +49. Each patient had two to five DLIs. Two of these four patients (NB-03 with imminent rejection and NB-04 with post-transplant detection of NB cells in bone marrow) received high-dose DLIs ≥ 1 x 10^5 of CD3+/kg (Table 3, Figure 1). Secondary to DLIs, all four children experienced acute GvHD, which either responded promptly to reinstitution of short immunosuppression consisting of steroids and CsA (three patients) or subsided spontaneously with no treatment (one patient). Characteristics of the DLIs with regard to time point, T-cell number, and occurrence of secondary acute GvHD are presented in Table 3 and Figure 1. To date, no chronic GvHD has been observed in these patients.

Outcome

The regimen of high-dose MIBG combined with subsequent RIC was well tolerated with no TRM. The most common morbidity was viral reactivation and acute GvHD secondary to DLI, both of which were easy to control by standard treatment. One patient (NB-02) rejected the graft; bone marrow biopsy revealed NB cells, and he was rescued by autologous backup, but died of progressive disease 5 months after haplo-SCT. One child (NB-01) achieved CR but relapsed with bone metastases 7 months after transplantation; additional attempts to control the disease with chemo-/radio-/immunotherapy failed, and the patient died 5 months later. Another patient (NB-04) developed generalized bone pain, and trephine biopsy revealed NB cell infiltration in bone marrow 2 months post-transplant. The clinical symptoms in this child disappeared after intensified DLI with mild GvHD, and his general condition gradually improved; all subsequent biopsies remained negative up to January 2009, when a routine MIBG scintigraphy revealed two new bone lesions in the sternum 38 months after haplo-SCT.

At present (manuscript submitted March 2009), three of the five children included in the study are alive (NB-03, NB-04, NB-05) and show good clinical performance and quality of life, for example attending school regularly. In short, they are doing well, although patient NB-04 is being treated with local irradiation and reinstitution of DLI, now 41 months post transplant. The other two children (NB-03 and NB-05) have neither radiological nor laboratory signs of active disease 42 and 40 months, respectively, after haplo-SCT (Figure 1).

DISCUSSION

Neuroblastoma has an incidence of about 10 cases per million children aged 0–14 years, and hence it is the most common pediatric extracranial solid tumor (22). It accounts for 7.8% of all childhood malignancies, and it is the third leading cause of death due to cancer (23).

The prognosis is particularly poor in children suffering from metastatic disease combined with other risk factors (e.g., age > 1 year, amplification of the *MYCN* oncogene, and unfavorable histology). Even in cases involving complete remission followed by high-dose chemotherapy and ASCT, the 3-year EFS is less than 35% (24). The prognosis is also very dismal for high-risk patients who fail frontline therapy, and it is unlikely that such individuals will benefit from high-dose therapy (25). Furthermore, in studies of therapy-resistant NB patients (7, 26), it was found that survival was not improved by high-dose chemotherapy followed by ASCT and antibody-mediated immunotherapy, retinoids, or immune modulation with IL2. In contrast, allo-SCT offers potential advantages over other treatment modalities. First of all, the graft is always free of contaminating tumor cells, although the contribution of peripheral graft-contaminating neuroblastoma cells to subsequent relapse (post ASCT) and overall survival remains controversial (27-30). Allo-SCT also results in recovery of properly functioning NK cells that can correct any functional defects that exist in their autologous counterparts, which may provide a beneficial GvT effect. The mechanisms of such influence are still unknown, but it has been reported that NB cell immunogenicity is very low, and HLA class I antigens are virtually undetectable (31, 32). Accordingly, NB cells lacking expression of HLA class I molecules may constitute an excellent target for NK cells (12, 33-35). For example, administration or enhanced production of endogenous cytokines or gene transfer might induce increased expression of HLA class I on neuroblastoma cells (12, 36, 37) and thereby expose the tumor to both NK- and T-cell-mediated attacks.

Another option might be to exploit T-cell-mediated cytotoxicity. Traditionally, it has been assumed that NB cells, which apparently lack expression of HLA class I, are not susceptible to the cytotoxic effect elicited by T-cells. However, a post-transplant cytokine storm can cause NB cells to begin expressing HLA class I and thereby make them potential targets for T-cells. Therefore, we speculate that GvHD-induced cytokine release can have a similar

effect. Moreover, it has been suggested that some NB cells may be selected to survive and progress by expressing either higher or lower levels of MHC class I antigens in order to resist NK- or T-cell-mediated anti-tumor responses, respectively (36).

It has also been shown that NB cell lines are susceptible to killing by differentiated CD8+ CTL clones in an MHC class-I-non-restricted manner (38). Thus tumor cells *in vivo* might be recognized and attacked by haploidentical T-cells after transplantation. Perhaps activation of this mechanism can be applied to initiate/enhance death pathways, so that they can subsequently redirect nonspecific T-cells of DLI origin towards NB cells, and in that way be of therapeutic value.

The results of allo-SCT from HLA-identical related donors have been disappointing (6, 8), but the association between GvHD and effectiveness was not assessed in the cited studies. All the patients in those investigations received standard GvHD prophylaxis, and the incidence of GvHD was low among transplanted patients with no scheduled DLI. Accordingly, apart from HLA identity, that therapeutic setting was oriented towards effective immunosuppression to avoid GvHD, which, in turn, might inhibit GvT.

It is generally acknowledged that the GvT effect is less pronounced when allo-SCT is performed in a patient with a large tumor load. Therefore, to minimize the pre-transplant tumor burden, we administered high-dose MIBG to all five of our patients. Our approach with haplo-SCT followed by immunomodulation has potential advantages, one of which is that the pharmacological GvHD prophylaxis is short compared to other transplant modalities. Also, co-transplantation of donor-derived mesenchymal stem cells is done primarily to reduce the risk of GvHD**.** We chose that strategy because our present patients' grafts had relatively large numbers of T-cells, and we also wanted to facilitate and enhance engraftment of HLA-incompatible hematopoietic cells (18). Furthermore, low-dose DLI allows induction of a potentially "controllable" GvHD, which is beneficial, since HLA incompatibility might

enhance the GvT effect. Notably, all our patients with sustained engraftment did in fact develop DLI-induced GvHD, which might have contributed to disappearance of disease and prevention of relapse. The analysis of immunological recovery revealed rapid reappearance of potentially immunocompetent NK and T-cells, which might act as effector cells that are responsible for the GvT effect. However, patient NB-01 received immunosuppression for treatment of GvHD, and that might have counterbalanced potential GvT and finally facilitated tumor regrowth. Patient NB-04 showed substantial clinical improvement after DLI-induced GvT, along with clearance of NB cells from the bone marrow. He had no clinical symptoms of the disease but did have persistent non-progressive bone metastases. The disease was stable up to 38 months after transplantation, at which time two new lesions in the sternum were identified during elective control. This observation demonstrates that long-lasting tumor control was achieved, but the tumor was eventually able to escape immune surveillance. It also suggests that, even quite some time after transplantation, additional measures are necessary to stimulate/maintain/reinduce GvT.

Research results have reported that infection with human cytomegalovirus (CMV) confers resistance to cytotoxic agents and protects against apoptosis in neuroectodermal tumors (39). The cited investigators also postulated that CMV infection before or during tumorigenesis might induce resistance in some NB patients. The available clinical data confirming the relevance of CMV infection in tumor cells concern malignant glioblastomas and prostate and colon cancer (40-43), but CMV is also detectable in the vast majority of tumor cell samples from NB patients (Dr. C. Söderberg-Nauclér, Karolinska Institute, personal communication). If CMV-infected tumors are indeed targeted by CMV-specific cytotoxic T lymphocytes (CTLs), then the transplantation of a new, healthy immune system from a haploidentical donor might induce an immunologic attack and eliminate tumor cells. This may also be a mechanism of effective elimination of residual malignant cells that are primarily infected with

the virus. In our study, all of the patients, except NB-01, and all of the donors were CMV-IgG positive at the time of transplantation. Furthermore, three of the four patients with sustained engraftment had reactivated CMV after transplantation. The "controllable" characteristic of GvHD induced by DLI turned out to be responsive to standard treatment, and no patient developed either life-threatening acute or chronic GvHD.

Our treatment strategy is novel and unique, because it is based on targeting of residual disease by high-dose MIBG and RIC chemotherapy combined with induction of a potentially long-lasting immunomediated and a complex GvT effect achieved using haploidentical donor immunocompetent cells. It appears that this approach led to prolonged DFS in two out of five children with otherwise incurable neuroblastoma, although it is a matter of speculation whether the procedure was actually responsible for disappearance of the tumors. Nevertheless, since it is highly unlikely that a durable response can be obtained after giving only a regimen of RIC or after ^{131}I-MIBG treatment alone, it seems reasonable to assume that the combination of the two procedures, together with implementation of the GvT effect mediated by haploidentical immune systems, contributed to the promising outcomes in two of our five patients. Obviously, this assumption must be confirmed in a larger study.

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Table 1.

Patient characteristics

UPN	Sex Age at Dx/ haplo-SCT (years)	MYC-N	Primary tumor and metastases	1st line treatment	Relapse # 1 (months after Dx) localization	Second line treatment	Relapse # 2 (months after Dx) localization	Third line treatment	Status prior to haplo-SCT (positive sites)	Time from Dx to haplo-SCT (months)	Outcome (months)
NB-01	F 1.4/10.7	yes	abd bone BM	COJEC MEC+ AHCT RT (20Gy) RA	61 paravert., dumbbell BM	RT, CADO BuMel + ASCT RA	110 paravert., bone, BM	RT TVD	PR 3 BM	112	CR Relapse 7 m DoD 12 m
NB-02	M 2.2/7.5	no	adr bone BM CNS	COJEC 131I-MIBG Surgery (tumorrest CNS)	*) 46 Bone BM	CADO TVD RT	60 CNS, BM, abd	131I-MIBG	progressive disease BM + CNS	64	Rejection day +18 Auto-back up Disease progression DoD 5 m
NB-03	M 1.2/5.1	yes	adr bone	COJEC CADO Surgery BuMel+ASCT RA	40 bone	RT TV			CR 2	47	CCR A&W 42 m
NB-04	M 6.8/8.1	no	adr bone BM	COJEC CADO TVD Surgery	Never in remission before haplo-SCT				PR 1 BM + bone	16	Alive 41 m with disease, ongoing DLI,
NB-05	M 5.0/7.0	no	thor bone BM	COJEC BuMel+ASCT RT RA	18 dumbbell CNS	RT TVD x 3			PR 2 CNS + paraspinal	24	CR A&W 42 m

Abbreviations: UPN, unique patient number; Dx, diagnosis; haplo-SCT, haploidentical stem cell transplantation; MYCN, MYCN amplification; F, female; M, male; abd, abdominal; adr, adrenal gland; thor, thoracal; BM, bone marrow; CNS, central nervous system; MEC, melphalan+etopside+carboplatin; BuMel, busulfan+melphalan; 131I-MIBG, iodine-131-metaiodobenzylguanidine; CADO, carboplatin, doxorubicine, vincristine; TVD, topotecan+vincristine+doxorubicine; TV, topotecan, vincristine; ASCT, autologous hematopoietic stem cell transplantation; RT, radiotherapy; Sx, surgery; RA, retinoic acid treatment; CR, complete remission; PR, partial remission; CCR, continuous complete remission; A&W, alive and well; DoD, died of disease.

1

*) Thirty months after diagnosis, the patient developed secondary AML M5 and was successfully treated with the conventional NOPHO-AML-93 protocol.

Table 2.

High-dose ^{131}I-MIBG, graft composition, and engraftment

UPN	Total administered activity (mCi)	Administered activity (mCi/kg)	Whole-body absorbed dose (Gy)	CD34+ (x10^6/kg)	CD3+ (x10^5/kg)	MSC/kg (x10^6/kg)	ANC > 0.5x10^9/L (day)	Platelets > 20 x 10^9/L (day)
NB-01	186	6.97	1.6	12.5	1.43	1.0	+13	+11
NB-02	270	10.5	2.4	11.0	0.45	0.4	+13	na
NB-03	277	13.5	1.5	22.6	0.98	0.3	+11	+11
NB-04	266	11.4	2.1	9.6	0.96	0.9	+16	+37
NB-05	263	10.9	1.5	17.9	0.98	0.75	+11	+13
Median	266	10.9	1.6	12.5	0.98	0.75	+13	+12

Abbreviations: ^{131}I-MIBG, iodine-131-metaiodobenzylguanidine; UPN, unique patient number; MSC, mesenchymal stem cells; ANC, absolute neutrophil count.

Table 3.

Time of post-transplant DLIs, numbers of T cells used, and acute GVHD secondary to DLI

UPN	Time of T-cell add-backs (days after TX)	No. of T-cells (x 10⁴/kg)	Secondary aGvHD	aGvHD treatment/resolution
NB-01	d49; d81; d136; d157	2.5; 5; 2.5; 5	Grade III	yes/yes
NB-02	nd, *)	na	na	na/na
NB-03	d42; d56; d65; d78	5; 5; 25, 50	Grade II	yes/yes
NB-04	d32; d46; d66; d80; d102	2.5; 5; 10; 50; 10	Grade I	no/yes
NB-05	d25; d38	5; 5	Grade II	yes/yes

Abbreviations: DLI, donor lymphocyte infusion; aGvHD, acute graft-versus-host disease; UPN, unique patient number; nd, not done; na, not applicable.

*) Stem cell boost due to rejection on day +18.

CAPTIONS TO FIGURES and abbreviations

Figure 1. Post-transplant chimerism analysis, virus reactivation, time of DLIs, and outcomes

Abbreviations: UPN, unique patient number; CMV, cytomegalovirus reactivation; BKV, BK virus reactivation; AdV, adenovirus reactivation; aGvHD, acute graft-versus-host disease; ATG, anti-thymocyte globulin treatment; DLI, donor lymphocyte infusion; DoD, died of disease; A&W, alive and well; NB, neuroblastoma; BM, bone marrow; m, months after haplo-SCT

Figure 2. Immunological recovery (median values).