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Feasibility and Safety of Treosulfan, Melphalan, and Thiotepa-Based Megachemotherapy with Autologous or Allogeneic Stem Cell Transplantation in Heavily Pretreated Children with Relapsed or Refractory Neuroblastoma

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The prognosis of resistant or relapsing children with neuroblastoma remains very poor, and the search for new therapies is ongoing. In this analysis, we assessed the toxicity of a treosulfan, melphalan, and thiotepa (TMT) regimen in 17 children with recurrent or refractory neuroblastoma who underwent stem cell transplantation (SCT). For allogeneic SCT, fludarabine and antithymocyte globulin were added. The stem cell source was autologous in 8 patients, haploidentical in 8 patients, and a matched unrelated donor in 1 patient. The reported nonhematologic toxicities included grade 3 mucositis, grade 1 to 3 hypertransaminasemia, and in 3 patients, veno-occlusive disease. No neurologic, cardiac, or dermatologic toxicities were observed. The probability of overall survival (OS) in patients with primary resistance was superior to that in patients with relapsed disease (100% versus 22.6%; $P = .046$). Post-transplantation dinutuximab beta immunotherapy was associated with superior 5-year OS (66.7% versus 11.4%; $P = .0007$). The use of an allogeneic donor, previous autologous SCT with busulfan and melphalan, and pretreatment with high-dose metaiodobenzylguanidine therapy demonstrated no effect on outcomes. In 4 patients, TMT megatherapy alone was enough to achieve complete remission. The TMT conditioning regimen was well tolerated in heavily pretreated patients with neuroblastoma. The manageable toxicity and addition of new anticancer drugs with optional post-SCT immunotherapy or chemotherapy support further trials with the TMT regimen in patients with neuroblastoma.

INTRODUCTION

Neuroblastoma is the most common extracranial tumor in children. Multimodal treatment in the high-risk neuroblastoma subgroup includes standard-dose chemotherapy, tumor resection, megachemotherapy, radiotherapy, and immunotherapy with dinutuximab; however, up to 70% of treated patients die of the disease. The prognosis of resistant or relapsing neuroblastoma remains very poor. Reports on the survival of patients who relapse after high-dose chemotherapy and stem cell rescue are uniformly dismal.

According to data from the Center for International Blood and Marrow Transplant Research, allogeneic stem cell transplantation (SCT) after autologous SCT does not seem to offer an advantage, with only 19% and 6% of patients alive and in remission at 1 year and 5 years after allo-SCT, respectively [1]. Currently, busulfan-based megachemotherapy is the preferred first-line treatment for neuroblastoma, despite the reported 22% incidence of sinusoidal obstruction syndrome (SOS)/veno-occlusive disease (VOD) [2].

Due to poor outcome in resistant and relapsing neuroblastoma the need for optimized high dose chemotherapy to improve survival is warranted, but toxicity and increased risk of non-relapse mortality (NRM) must be considered in the heavily pretreated patients. The popular revival of high-dose thiotepa in recent years raises a question concerning the optimal use of the drug in the transplantation setting. The oldest

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reports on high-dose thiotepa (TT) with auto-SCT in neuroblastoma date back to the early 1990s, but the observed efficacy was low [3–6]. The combination of TT with a second alkylator, such as high-dose carboplatin, was found to produce better outcomes for patients with neuroblastoma, although the follow-up was short [7]. In another study, the combination of TT with etoposide and cyclophosphamide produced inferior 5-year event-free survival (EFS) compared with busulfan-melphalan (21% versus 88%), although the study included only 8 patients in the busulfan-melphalan group, for whom the results were unexpectedly good [8]. Recently, treosulfan has been viewed as a promising option, especially because the data support the in vitro activity of treosulfan on neuroblastoma lines [9]. However, to date only a few reports have investigated megachemotherapy with treosulfan in neuroblastoma, and these reports are limited to single patients [10,11]. Experience with combinations of treosulfan with melphalan and thiotepa (TMT) is virtually nonexistent; only an Israeli pilot study from 2005 reported the use of a TMT protocol in 3 patients with hematologic malignancies [12]. To date, the TMT combination has not been studied in any trials. The purpose of this analysis was to determine the feasibility and tolerability of TMT with SCT and the tumor response in children with recurrent or refractory neuroblastoma.

METHODS

Between 2010 and 2018, 17 children with neuroblastoma underwent megachemotherapy with auto-SCT or allo-SCT at our institution. Patient characteristics and clinical data are presented in Table 1. The cohort for analysis consisted of children who relapsed after auto-SCT (n = 12) or had primary therapy-resistant neuroblastoma (n = 5) and had received prolonged chemotherapy and previous local irradiation, which made them ineligible for busulfan owing to the risk of VOD. The interval from the previous busulfan-melphalan megatherapy and auto-SCT was 19.1 months (range, 8.9 to 48.74 months). MYCN amplification was identified in 6 of the 17 patients. The parents of all 17 children provided written informed consent for the treatment and analysis of clinical data.

Megatherapy

For all patients, the conditioning regimen consisted of treosulfan (10 g/m^2/day for 3 days), melphalan (70 mg/m^2/day for 2 days), and thiotepa (2 × 5 mg/kg body weight [BW] for 1 day). In addition, fludarabine (40 mg/m^2 for 4 days) and antithymocyte globulin (Grafalon; total dose of 30 mg/kg) were added for allo-SCT (Table 2). High-dose metaiodobenzylguanidine (MIBG) therapy with an intended dosage of 12 mCi/kg was administered in 8 MIBG-avid patients at 2 to 3 weeks before the initiation of megachemotherapy.

SCT

The type of SCT performed was based on stem cell availability, previous mobilization, and transplantation history. The intended dose of progenitor cells was 3×10^6 CD34 cells/kg BW, but 3 patients received fewer cells owing to accidental cell loss. In 8 patients, autologous stem cells were used; the patients underwent stem cell apheresis with a Spectra Optia apheresis system (Terumo BCT, Lakewood, CO), and the cells were stored at -196 °C in a liquid nitrogen tank. Patients without autologous backup and those unable to undergo stem cell mobilization were scheduled for allo-SCT. Eight children underwent a haploidentical SCT. All haploidentical donors were mobilized using G-CSF at a dose of 5 μg/kg twice daily for 5 days and underwent stem cell apheresis on day -1.

On the day of transplantation, $\alpha\beta$ T cell depletion was performed using the CliniMacs Plus system (Miltenyi, Bergisch Gladbach, Germany) with CD3 or $\alpha\beta$ depletion reagents, a DTS kit, and the Depletion 3.1 program according to the manufacturer's instructions. The depletion product contained 7.87×10^6 CD34$^+$ cells/kg BW (range, 2.5 to 14.71×10^6) and 6 to 314×10^3 CD3 $\alpha\beta$ lymphocytes/kg BW (median, 43.95×10^3). Post-transplantation immunosuppression after haploidentical SCT consisted of mofetil mycophenolate if the transplanted CD3 $\alpha\beta$ lymphocyte dose exceeded 5×10^4 cells/kg BW. In vivo B cell depletion before haploidentical SCT was performed with administration of rituximab at a dose of 375 mg/m^2 on day -1. A single transplantation from an unrelated donor was performed. Graft-versus-host disease prophylaxis consisted of cyclosporine A from day -1 and methotrexate at a dose of 10 mg/m^{2}on days +1, +3, and +6 post-transplantation for this patient.

Post-Transplantation Therapy

Four patients received radiation therapy to the primary tumor at a total dose of 21 to 36 Gy fractioned into 1.5-Gy single doses. Post-transplantation dinutuximab beta therapy was administered to 6 patients at a dose of 100 mg/m^2 as a continuous infusion over 10 days, repeated for 5 cycles. Patients treated with dinutuximab beta received isotretinoin (13-cis-RA) 160 mg/m^2/day divided into 2 equal doses given orally twice daily for 14 days, followed by a 14-day rest, for a total of 6 cycles. In 3 patients not eligible for dinutuximab beta, temozolomide-irinotecan (TEMIRI) chemotherapy was administered as a 5-day course of irinotecan 50 mg/m^2 (1-hour infusion) and temozolomide 150 mg/m^2 (oral) every 3 to 4 weeks, for up to 6 cycles [13].

Statistical Analysis

The main study endpoint was the evaluation of the organ-specific toxicities. Toxicity data from the patients' medical records were graded in accordance with the Common Toxicity Criteria, version 5 from the National Institutes of Health [14]. SOS/VOD was diagnosed and graded according to the 2018 European Group for Blood and Marrow Transplantation pediatric criteria [15].

The secondary endpoints were overall survival (OS), defined as the time from transplantation to death or the last report from patients with no event, and EFS, defined as the time from transplantation to progression, relapse, second malignancy or death. Survival curves were estimated using the Kaplan-Meier method. Statistical analysis and data presentation were performed with the computer software GraphPad Prism (GraphPad Software, La Jolla, CA) and Statistica 13.0 (StatSoft, Tulsa, OK).

RESULTS

Evaluation of Toxicity

The median duration of hospitalization 31 days (range, 21 to 51 days) and was shorter in the auto-SCT group compared with the allo-SCT group (median, 25 days versus 40 days; range, 21 to 45 days versus 28 to 51 days; P = .014). All 17 patients had grade 4 leukopenia and neutropenia, and 13 (76%) had fever. Two patients were transferred to the intensive care unit for treatment of septic shock, but both recovered and continued hospitalization in the transplantation unit. Reversible grade 3 nonhematologic toxicities included mucositis (n = 15; 88%) with total parenteral nutrition (n = 15; 88%) and diarrhea (n = 5; 29%). One patient exhibited elevated alkaline phosphatase activity, and 6 patients (35%) had elevated aminotransferase activity. Grade 1 hypertransaminasemia was found in 4 patients (23%), and grade 2 or 3 was seen in 2 patients (12%), who developed VOD after previous busulfan-melphalan conditioned auto-SCT, and 1 of these patients experienced recurrence of hepatic VOD after TMT therapy. In total, 3 patients developed hepatic VOD (18%), including 1 patient with a very severe type, but all recovered completely after therapy with defibrotide. In all patients, the maximum creatinine concentration was within the normal range during the procedure and at 30 days post-transplantation, but 8 patients (47%) had elevated urea concentrations. No neurologic, cardiac, or dermatologic toxicities were observed. Neutrophil engraftment was achieved in all patients at median of 9 days (range, 8 to 16 days) after auto-SCT and 10 days (range 9 to 26 days) after allo-SCT. The median duration of follow-up after SCT was 25 months (range, 3.9 to 79 months).

Survival Analysis

The response to TMT chemotherapy was observed in 4 SCT recipients in partial remission (PR) who subsequently achieved complete remission (CR) directly after initiation of the TMT regimen. Two of these 4 patients relapsed during the follow-up period. Five other patients achieved long-lasting CR following immunotherapy (3) or TEMIRI therapy (2). In these patients, the 5-year OS and EFS were 33.6% and 17.8%, respectively (Figure 1A). The probability of relapse or progression at 4 years post-SCT was 55.4% (Figure 1B). NRM was observed in 2 of 8 patients after allo-SCT, due to acute graft-versus-host

Table 1
Patient Characteristics

UPN	Sex	Age at SCT, yr	Diagnosis	Previous Megatherapy and SCT	Status Pre-TMT-SCT	Pre -SCT HD MIBG	SCT Type	Post-SCT Therapies	Best Post-SCT Response	Current Status
709	F	3.1	NBL stage 4/M, metastatic relapse	Bu-Mel, auto-SCT	CR2	Yes	Haplo TCD	None	CR	DOD
737	F	6.2	NBL stage 4/M, MNA, metastatic relapse	Bu-Mel, auto-SCT	CR2	No	Haplo TCD	None	CR	DOD
773	F	5.7	NBL stage 4/M, MNA, metastatic relapse	Bu-Mel, auto-SCT	PR2	Yes	Haplo TCD	Anti-GD2	CR	AWD
823	M	7.3	NBL stage 4/M, MNA, metastatic relapse	Bu-Mel, auto-SCT	PR2	No	Haplo TCD	RTX, anti-GD2	CR	DOD
836	M	5.7	NBL stage 4/M, MNA, metastatic relapse	Bu-Mel, auto-SCT	CR2	No	Autologous	RTX, anti-GD2	CR	A&W
841	F	9.4	NBL stage 4/M, metastatic relapse	Bu-Mel, auto-SCT	PR2	Yes	Haplo TCD	None	CR	DOD
883	M	4.1	NBL stage 3 /L2, relapse	None	CR2	No	Haplo TCD	None	CR	DOD
893	M	5.9	NBL stage 4/M, metastatic relapse	Bu-Mel, auto-SCT	Progression	No	Haplo TCD	TEMIRI	PR	DOD
947	F	7.4	NBL stage 4/M, metastatic relapse	Bu-Mel, auto-SCT	PR2	Yes	Haplo TCD	None	CR	TRM
972	M	4.0	NBL stage 4/M, MNA, nonremission	Bu-Mel, auto-SCT	PR1	No	Autologous	RTX, anti-GD2	CR	A&W
995	F	7.0	NBL stage 4/M, nonremission	Bu-Mel, auto-SCT	PR1	Yes	Autologous	Anti-GD2	SD	AWD
1005	F	8.9	NBL stage 4/M, nonremission	None	PR1	Yes	Autologous	Anti-GD2	CR	A&W
1033	M	3.6	NBL stage 4/M, metastatic relapse	Bu-Mel, auto-SCT	PR2	No	Autologous	None	SD	DOD
1100	F	9.0	NBL stage 4/M, MNA, metastatic relapse	None	CR2	No	Autologous	None	CR	DOD
1107	M	7.0	NBL stage 4/M, nonremission	None	PR1	Yes	MUD	RTX, anti-GD2	SD	AWD
1108	M	6.0	NBL stage 4/M, metastatic relapse	None	SD2	Yes	Autologous	TEMIRI	CR	A&W
1173	M	3.5	NBL stage 4/M, metastatic relapse	Bu-Mel, auto-SCT	CR2	No	Autologous	TEMIRI	CR	A&W

UPN indicates unique patient number; NBL, neuroblastoma; Bu-Mel, busulfan and melphalan; Haplo TCD, haploidentical with T cell depletion; DOD, died of disease; MNA, MYCN-amplified; AWD, alive with disease; RTX, local radiotherapy; A&W, alive and well in remission; TRM, treatment-related mortality; MUD, matched unrelated donor; SD, stable disease.

Table 2
TMT Chemotherapy Backbone

		-8	-7	-6	-5	-4	-3	-2	-1	0
Treosulfan	10 g/m^2	•	•	•						
Thiotepa	5 mg/kg					•	•			
Melphalan	70 mg/m^2							•	•	
Fludarabine*	40 mg/m^2	•*	•*	•*	•*					

* Added only in allo-SCT.

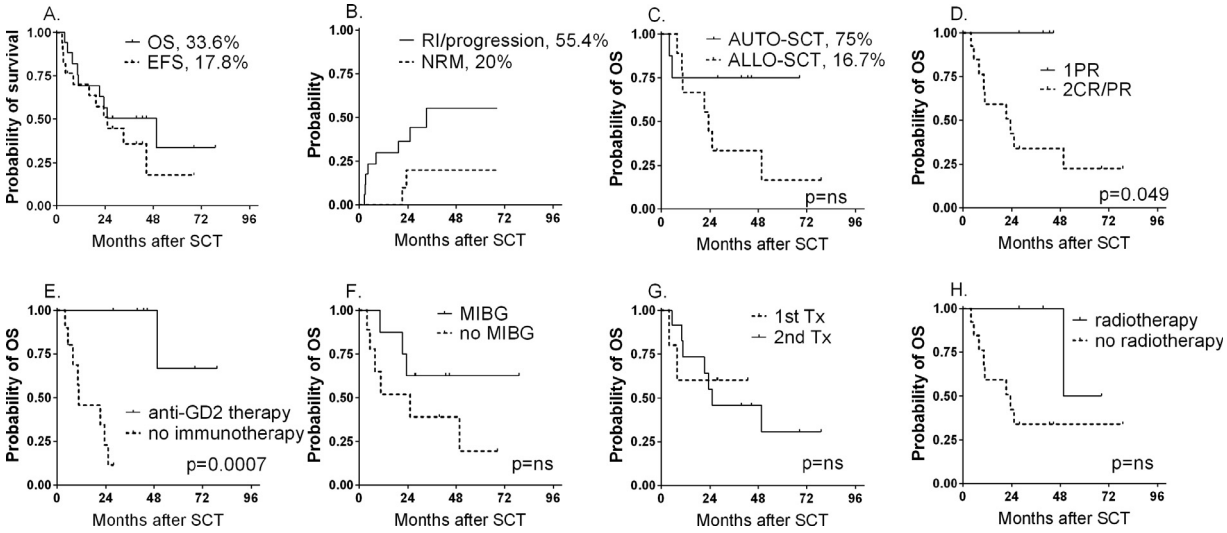

Figure 1. Outcomes after the TMT conditioning regimen: OS and EFS (A); incidence of NRM, relapse, or progression (B); OS after allo-SCT and after auto-SCT (C); impact of primary resistant or relapsing disease before SCT (D); post-transplantation dinutuximab beta immunotherapy (E); preconditioning with MIBG therapy (F); previous busulfan-melphalan therapy (G), and post-transplantation radiotherapy (H).

disease in 1 patient and Epstein-Barr virus (EBV)-induced post-transplantation lymphoproliferative disorder in the other patient. The difference in probability of survival between auto-SCT recipients and allo-SCT recipients was not statistically significant (75% versus 16.7%; *P* not significant) (Figure 1C). All allo-SCT recipients achieved full donor chimerism within 4 weeks post-transplantation. OS at 48 months in patients who underwent transplantation in first PR was superior to that in those who relapsed but achieved second CR or second or greater PR (100% versus 22.6%; *P* = .046) (Figure 1D). Six patients who received dinutuximab beta immunotherapy after SCT had superior 5-year OS (66.7% versus 11.4% in patients who did not receive dinutuximab; *P* = .0007) (Figure 1E). Preconditioning with MIBG in patients with neuroblastoma did not affect OS (62.5% versus 16.4%; *P* not significant) (Figure 1F). No difference in OS was observed between patients who received TMT as the first megatherapy or the second megatherapy following relapse after receiving a busulfan-melphalan regimen (60% versus 30.6%; *P* not significant) (Figure 1G). The use of radiotherapy did not affect OS (50% versus 33.8%; *P*not significant) (Figure 1H).

A second malignancy was diagnosed in 2 patients. The first patient had myelodysplastic syndrome with excess blasts after auto-SCT, and the second patient developed EBV-associated post-transplantation lymphoproliferative disorder after haploidentical SCT. The patient with treatment-induced myelodysplastic syndrome with excess blasts underwent a third SCT from an unrelated donor with treosulfan-fludarabine-thiotepa conditioning, without significant toxicity and with full donor engraftment.

DISCUSSION

Although future therapies may be more targeted and increase efficacy while decreasing toxicity, high-dose chemotherapy is still recommended for management of high-risk neuroblastoma. With the current state of knowledge, chemotherapy still has fundamental advantages for controlling high-risk disease in pediatric malignancies and must not be neglected for the uncertain and noncurative benefits of future therapies.

In this population of heavily pretreated patients, the conditioning TMT regimen was well tolerated and toxicity was generally manageable. The in-hospital stay in both groups was typical for uncomplicated cases after auto-SCT or allo-SCT. Shorter hospitalization and lack of both opportunistic infections and graft-versus-host disease-related issues might speak in favor of auto-SCT.

The regimen did not demonstrate significant hepatic or renal toxicity. The low-degree liver toxicities and the low incidence of VOD support the safety of this conditioning regimen, even for patients with a previous history of busulfan or radiotherapy. The combination of TT-melphalan was reported to be well tolerated in patients with renal impairment, and TMT showed minimal nephrotoxicity [16].

An advantage of TMT chemotherapy is the possibility of inducing remission by a direct cytotoxic effect using a new drug combination and continuation with post-transplantation therapy. Post-transplantation anti-GD2 immunotherapy with dinutuximab beta is an important therapy in neuroblastoma that has shown an advantage even in our selected group, but immunotherapy is more feasible after auto-SCT than after

allo-SCT. Low-intensity chemotherapy with TEMIRI can be initiated without complications within 2 to 3 months after transplantation, which may be a viable option for patients ineligible for immunotherapy.

An alternative approach in neuroblastoma is to investigate tandem transplantations with incorporation of thiotepa into 1 of the procedures, but this requires a higher number of hematopoietic stem cells, the collection of which can be difficult in overtreated patients with a decreased bone marrow reserve [17,18]. The randomized Children's Oncology Group (COG) Phase 3 trial (NCT00567567) compared tandem auto-SCT (thiotepa-cyclophosphamide [TC] followed by modified carboplatin-etoposide-melphalan [CEM]: TC:CEM) with single auto-SCT (CEM) and found significantly higher EFS at 3 years in the patients randomized to TC:CEM compared with those randomized to single auto-SCT (61.8% versus 48.8%; $P = .0082$) [19]. Owing to improved survival probability, the inclusion of thiotepa in upfront neuroblastoma treatment is expected in US protocols.

A drawback of studies investigating tandem transplantations is the high incidence of long-term complications, especially when total body irradiation was administered [20–22]. The efficacy of total body irradiation in neuroblastoma therapy has never been proven, and thus its inclusion should not be encouraged.

A different strategy is offered for patients undergoing allo-SCT. The evolution of conditioning regimens in allo-SCT has led to the widespread use of reduced-intensity/toxicity protocols or even nonmyeloablative regimens and to a greater importance of the graft-versus-malignancy effect.

In our cohort, 9 patients underwent allo-SCT, mostly from a haploidentical parent. Haploidentical SCT can take advantage of both the direct megatherapy effect and post-transplantation graft-versus-tumor activity as a consequence of HLA-related mechanisms mediated by T lymphocyte and natural killer (NK) lymphocyte non-HLA-related mechanisms [23]. Alloreactivity can be observed in neuroblastomas, but there its importance is far less evident than in lymphoid or myeloid malignancies. NK cell activity modulated by KIR ligand mismatches, and the KIR genotype is suggested to play a role in the graft-versus-tumor effect [24]. Indeed, according to Delgado et al [25], the response to anti-GD2 immunotherapy can be improved through KIR-associated mechanisms. Erbe et al [26] reported that in COG study ANBL0032, patients with neuroblastoma with the "all KIR-ligands present" genotype, as well as patients with inhibitory KIR2DL2 with its ligand (HLA-C1) together with inhibitory KIR3DL1 with its ligand (HLA-Bw4), showed improved clinical outcomes when treated with immunotherapy.

Haploidentical SCT with reduced-intensity conditioning in relapsing and refractory patients was considered as a viable strategy after initial reports by Toporski et al [27]. The haploidentical SCT approach was summarized in 2018 by Illhardt [28], showing a 5-year EFS of 19%. The patients in that study had a 75% risk of relapse in the first year after transplantation. This finding could be explained by the lack of immune-mediated cytotoxicity during the early immune reconstitution phase. Although the survival rate was low, COG data from children with recurrent or refractory neuroblastoma show a 1-year PFS of 21% and a 4-year PFS of 6% [29], suggesting some survival benefit accruing from the haploidentical strategies and warranting future studies. Owing to the availability and efficacy of plerixafor in poor mobilizers, a second auto-SCT is possible in overtreated patients, but for patients unable to mobilize autologous stem cells, an allogeneic donor may be a source of rescue [30,31].

Megatherapy with TMT may also expose patients to an increased risk of second malignancy, especially with auto-SCT. Two of the 17 patients had a second malignancy; however, no direct causality with the TMT regimen was recorded. Myelodysplastic syndrome with excess blasts was observed in a patient with a history of intensive chemotherapy with topoisomerase II and alkylating agents. In addition, EBV-associated lymphoproliferative disorder occurred in a child who underwent T cell-depleted haploidentical SCT. Nevertheless, due to pretreatment of neuroblastoma patients and combined chemotherapy and radiotherapy, the follow up must include evaluation for secondary malignancies, hypothyroidism, and drug-specific complications, such as anthracycline-induced cardiomyopathy.

In conclusion, the TMT chemotherapy backbone is a well-tolerated conditioning regimen that can be used in heavily pretreated patients with neuroblastoma, especially in children without previous thiotepa megatherapy. The manageable toxicities, as well as the addition of new anticancer drugs and effective myeloablative effects allowing for engraftment, make the protocol feasible in auto-SCT, and with the addition of fludarabine and antithymocyte globulin, it can be adapted for allo-SCT. We conclude that consolidation with TMT in therapy-resistant children can be a step in post-transplantation immunotherapy or chemotherapy. The results presented by our group warrant prospective study evaluating TMT protocols in patients with neuroblastoma.

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REFERENCES

1. Hale GA, Arora M, Ahn KW, et al. Allogeneic hematopoietic cell transplantation for neuroblastoma: the CIBMTR experience. *Bone Marrow Transplant*. 2013;48:1056–1064.
2. Ladenstein R, Pötschger U, Pearson ADJ, et al. Busulfan and melphalan versus carboplatin, etoposide, and melphalan as high-dose chemotherapy for high-risk neuroblastoma (HR-NBL1/SIOPEN): an international, randomised, multi-arm, open-label, phase 3 trial. *Lancet Oncol*. 2017;18:500–514.
3. Saarinen UM, Hovi L, Mäkipernaa A, Riikonen P. High-dose thiotepa with autologous bone marrow rescue in pediatric solid tumors. *Bone Marrow Transplant*. 1991;8:369–376.
4. Kletzel M, Abella EM, Sandler ES, et al. Thiotepa and cyclophosphamide with stem cell rescue for consolidation therapy for children with high-risk neuroblastoma: a phase I/II study of the Pediatric Blood and Marrow Transplant Consortium. *J Pediatr Hematol Oncol*. 1998;20:49–54.
5. Hara J, Osugi Y, Ohta H, et al. Double-conditioning regimens consisting of thiotepa, melphalan and busulfan with stem cell rescue for the treatment of pediatric solid tumors. *Bone Marrow Transplant*. 1998;22:7–12.
6. Lucidarme N, Valteau-Couanet D, Oberlin O, et al. Phase II study of high-dose thiotepa and hematopoietic stem cell transplantation in children with solid tumors. *Bone Marrow Transplant*. 1998;22:535–540.
7. Kushner BH, Cheung NK, Kramer K, Dunkel IJ, Calleja E, Boulad F. Topotecan combined with myeloablative doses of thiotepa and carboplatin for neuroblastoma, brain tumors, and other poor-risk solid tumors in children and young adults. *Bone Marrow Transplant*. 2001;28:551–556.
8. De Ioris MA, Contoli B, Jenkner A, et al. Comparison of two different conditioning regimens before autologous transplantation for children with high-risk neuroblastoma. *Anticancer Res*. 2012;32:5527–5533.

9. Lanvers-Kaminsky C, Bremer A, Dirksen U, Jürgens H, Boos J. Cytotoxicity of treosulfan and busulfan on pediatric tumor cell lines. *Anticancer Drugs*. 2006;17:657–662.
10. Raciborska A, Ussowicz M, Prost M, Bilska K, Rodriguez-Galindo C, Rychlowska-Pruszynska M. Effective treatment of cytomegalovirus retinitis and neuritis with retrobulbar ganciclovir after treosulfan-based autologous bone marrow transplant. *Klin Oczna*. 2015;117:101–103.
11. Zaucha-Prazmo A, Drabko K, Wójcik B, et al. [High-dose therapy followed by auto HSCT in children with advanced neuroblastoma in four transplant centres in Poland]. *Med Wieku Rozwoj*. 2006;10(3Pt 1):775–784. [in Polish].
12. Bitan M, Shapira MY, Resnick IB, et al. Successful transplantation of haploidentically mismatched peripheral blood stem cells using CD133+-purified stem cells. *Exp Hematol*. 2005;33:713–718.
13. Kushner BH, Kramer K, Modak S, Cheung NK . Irinotecanplus temozolomide for relapsed or refractory neuroblastoma. *J Clin Oncol*. 2006;24: 5271–5276.
14. US Department of Health and Human Services. Common terminology criteria for adverse events (CTCAE), version 5.0. Available at: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf. Accessed July 17, 2018.
15. Corbacioglu S, Carreras E, Ansari M, et al. Diagnosis and severity criteria for sinusoidal obstruction syndrome/veno-occlusive disease in pediatric patients: a new classification from the European Society For Blood And Marrow Transplantation. *Bone Marrow Transplant*. 2018;53:138–145.
16. Termuhlen AM, Grovas A, Klopfenstein K, Rosselet R, Gross TG. Autologous hematopoietic stem cell transplant with melphalan and thiotepa is safe and feasible in pediatric patients with low normalized glomerular filtration rate. *Pediatr Transplant*. 2006;10:830–834.
17. Saarinen-Pihkala UM, Hovi L, Koivusalo A, et al. Thiotepa and melphalan-based single, tandem, and triple high-dose therapy and autologous stem cell transplantation for high-risk neuroblastoma. *Pediatr Blood Cancer*. 2012;59:1190–1197.
18. Pasqualini C, Dufour C, Goma G, Raquin MA, Lapierre V, Valteau-Couanet D. Tandem high-dose chemotherapy with thiotepa and busulfan-melphalan and autologous stem cell transplantation in very high-risk neuroblastoma patients. *Bone Marrow Transplant*. 2016;51:227–231.
19. Park Y, Kreissman SG, London WB, et al. A phase III randomized clinical trial (RCT) of tandem myeloablative autologous stem cell transplant (ASCT) using peripheral blood stem cell (PBSC) as consolidation therapy for high-risk neuroblastoma (HR-NB): a Children's Oncology Group (COG) study. *J Clin Oncol*. 2016;34(18 Suppl). LBA3-LBA3.
20. George RE, Li S, Medeiros-Nancarrow C, et al. High-risk neuroblastoma treated with tandem autologous peripheral blood stem cell-supported transplantation: long-term survival update. *J Clin Oncol*. 2006;24:2891–2896.
21. Sung KW, Son MH, Lee SH, et al. Tandem high-dose chemotherapy and autologous stem cell transplantation in patients with high-risk neuroblastoma: results of SMC NB-2004 study. *Bone Marrow Transplant*. 2013;48:68–73.
22. Lee JW, Lee S, Cho HW, et al. Incorporation of high-dose ^{131}I-metaiodobenzylguanidine treatment into tandem high-dose chemotherapy and autologous stem cell transplantation for high-risk neuroblastoma: results of the SMC NB-2009 study. *J Hematol Oncol*. 2017;10:108.
23. Willems L, Waer M, Billiau AD. The graft-versus-neuroblastoma effect of allogeneic hematopoietic stem cell transplantation, a review of clinical and experimental evidence and a perspective on mechanisms. *Pediatr Blood Cancer*. 2014;61:2151–2157.
24. Pérez-Martínez A, Leung W, Muñoz E, et al. KIR-HLA receptor-ligand mismatch associated with a graft-versus-tumor effect in haploidentical stem cell transplantation for pediatric metastatic solid tumors. *Pediatr Blood Cancer*. 2009;53:120–124.
25. Delgado DC, Hank JA, Kolesar J, et al. Genotypes of NK cell KIR receptors, their ligands, and Fcγ receptors in the response of neuroblastoma patients to Hu14.18-IL2 immunotherapy. *Cancer Res*. 2010;70:9554–9561.
26. Erbe AK, Wang W, Carmichael L, et al. Neuroblastoma patients' KIR and KIR-ligand genotypes influence clinical outcome for dinutuximab-based immunotherapy: a report from the Children's Oncology Group. *Clin Cancer Res*. 2018;24:189–196.
27. Toporski J, Garkavij M, Tennvall J, et al. High-dose iodine-131-metaiodobenzylguanidine with haploidentical stem cell transplantation and post-transplant immunotherapy in children with relapsed/refractory neuroblastoma. *Biol Blood Marrow Transplant*. 2009;15:1077–1085.
28. Illhardt T, Toporski J, Feuchtinger T, et al. Haploidentical stem cell transplantation for refractory/relapsed neuroblastoma. *Biol Blood Marrow Transplant*. 2018;24:1005–1012.
29. London WB, Bagatell R, Weigel BJ, et al. Historical time to disease progression and progression-free survival in patients with recurrent/refractory neuroblastoma treated in the modern era on Children's Oncology Group early-phase trials. *Cancer*. 2017;123:4914–4923.
30. Maschan AA, Balashov DN, Kurnikova EE, et al. Efficacy of plerixafor in children with malignant tumors failing to mobilize a sufficient number of hematopoietic progenitors with G-CSF. *Bone Marrow Transplant*. 2015;50:1089–1091.
31. Teusink A, Pinkard S, Davies S, Mueller M, Jodele S. Plerixafor is safe and efficacious for mobilization of peripheral blood stem cells in pediatric patients. *Transfusion*. 2016;56:1402–1405.