Double-conditioning regimen with thiotepa and melphalan for high-risk Neuroblastoma

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Abstract

Appropriate high-dose chemotherapy (HDC) for high-risk neuroblastoma has not yet been established. In Japan, a unique HDC regimen (called double-conditioning regimen) comprising two cycles of total 800 mg/m^2 of thiotepa and total 280 mg/m^2 of melphalan is widely used. To re-evaluate the safety and the efficacy of this regimen for high-risk neuroblastoma, the medical records of 41 patients with high-risk neuroblastoma who underwent the double-conditioning regimen followed by autologous peripheral blood stem cell rescue between 2002 and 2012 were reviewed. *MYCN*-amplified high-risk neuroblastomas were observed in 23 patients. All patients underwent intensive multidrug induction chemotherapy, but none underwent anti-GD2 antibody immunotherapy. The primary tumor was resected at the adequate time point. The median follow-up duration for living patients was 9.2 years (range = 5.5–14.0 years). The 5-year event-free survival (EFS) and overall survival (OS) rates from treatment initiation were 41.5% ± 7.7% and 56.1% ± 7.8%, respectively. The 5-year EFS of *MYCN*-amplified high-risk neuroblastoma patients was 60.9% ± 10.2%, which was significantly superior compared to *MYCN*-non-amplified high-risk neuroblastoma patients (16.7% ± 8.8%; $P < 0.001$). *MYCN* amplification was the most favorable prognostic factor for EFS (hazard ratio = 0.29; 95% confidence interval = 0.12–0.66). Of the 41 patients, 3 died because of regimen-related toxicity (infection, $n = 2$; microangiopathy, $n = 1$). The double-conditioning regimen with thiotepa and melphalan is effective for high-risk neuroblastoma, especially in patients with *MYCN* amplification. However, the double-conditioning regimen is toxic and warrants special attention in clinical practice.

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Abbreviation

HDC	high-dose chemotherapy
EFS	event-free survival
OS	overall survival
SCT	stem cell transplantation
SIOPEN	International Society of Paediatric Oncology European Neuroblastoma
INSS	International Neuroblastoma Staging System
MSI	metastatic site index
INRC	International Neuroblastoma Response Criteria
Ccr	creatinine clearance
SOS	sinusoidal obstruction syndrome
TMA	thrombotic microangiopathy
CR	complete response
VGPR	very good partial response

Abstract: Appropriate high-dose chemotherapy (HDC) for high-risk neuroblastoma has not yet been established. In Japan, a unique HDC regimen (called double-conditioning regimen) comprising two cycles of total 800 mg/m^2 of thiotepa and total 280 mg/m^2 of melphalan is widely used. To re-evaluate the safety and the efficacy of this regimen for high-risk neuroblastoma, the medical records of 41 patients with high-risk neuroblastoma who underwent the double-conditioning regimen followed by autologous peripheral blood stem cell rescue between 2002 and 2012 were reviewed. *MYCN* -amplified high-risk neuroblastomas were observed in 23 patients. All patients underwent intensive multidrug induction chemotherapy, but none underwent anti-GD2 antibody immunotherapy. The primary tumor was resected at the adequate time point. The median follow-up duration for living patients was 9.2 years (range = 5.5–14.0 years). The 5-year event-free survival (EFS) and overall survival (OS) rates from treatment initiation were 41.5% ± 7.7% and 56.1% ± 7.8%, respectively. The 5-year EFS of *MYCN* -amplified high-risk neuroblastoma patients was 60.9% ± 10.2%, which was significantly superior compared to *MYCN* -non-amplified high-risk neuroblastoma patients (16.7% ± 8.8%; $P < 0.001$). *MYCN* amplification was the most favorable prognostic factor for EFS (hazard ratio = 0.29; 95% confidence interval = 0.12–0.66). Of the 41 patients, 3 died because of regimen-related toxicity (infection, $n = 2$; microangiopathy, $n = 1$). The double-conditioning regimen with thiotepa and melphalan is effective for high-risk neuroblastoma, especially in patients with *MYCN* amplification. However, the double-conditioning regimen is toxic and warrants special attention in clinical practice.

INTRODUCTION

Intensive treatment including high-dose chemotherapy (HDC) with autologous stem cell transplantation (SCT) is the standard care for high-risk neuroblastoma.[1] The importance of HDC is well recognized, even after improvement of treatment outcomes by anti-GD2 antibody immunotherapy, so re-evaluation of the efficacy and safety of the HDC regimen is ongoing in several clinical trials.[2] Recently, the International Society of Paediatric Oncology European Neuroblastoma (SIOPEN) conducted a randomized trial and proved the

superiority of busulfan and melphalan over melphalan, etoposide, and carboplatin as a conditioning HDC regimen.[3]

Thiotepa is an alkylating anticancer agent broadly used in the HDC regimen at various dosages and schedules including both single[4] and tandem settings[5–7]. In Japan, a unique HDC regimen called the double-conditioning regimen is widely used in some major pediatric cancer centers, in which thiotepa and melphalan are administered for 2 consecutive weeks. Hara et al. (1998) reported the feasibility and efficacy of the double-conditioning regimen for various solid tumors, including neuroblastoma.[8] Okada et al. (2019) reported toxicity profiles of the double-conditioning regimen in a further cohort.[9] Recently, high efficacy of the double-conditioning regimen for high-risk medulloblastoma was published.[10] In contrast, few studies have investigated the double-conditioning regimen for high-risk neuroblastoma.[11] This study re-evaluated the safety of the double-conditioning regimen and assesses its efficacy for high-risk neuroblastoma in a multi-institutional cohort.

PATIENTS AND METHODS

Patients

We retrospectively reviewed the medical records of 41 newly diagnosed high-risk neuroblastoma patients who underwent HDC with a double-conditioning regimen comprising thiotepa and melphalan with auto-SCT between June 2002 and October 2012 at the National Center for Child Health and Development, Tokyo, Japan; the Osaka City General Hospital, Osaka, Japan; and the Osaka University Hospital, Osaka, Japan. This cohort included 19 patients reported in previous studies.[9,11,12] The diagnosis of high-risk neuroblastoma was made on the basis of histological evaluation of tumor samples showing the presence of elevated urine vanillylmandelic acid and homovanillic acid. High-risk neuroblastoma was defined as follows:*MYCN* -amplified stage 2, 3, 4S, or 4 in patients of any age or*MYCN* -non-amplified stage 4 diagnosed at age >18 months.[13] Staging was performed according to the International Neuroblastoma Staging System (INSS).[14]The metastatic site index (MSI), a score based on the number of metastatic systems/compartments involved, was also calculated.[15]

Treatment

All 41 patients underwent multimodality treatment with induction chemotherapy, surgical resection, and/or radiation for local control according to each institution's policy. The induction chemotherapeutics used varied, although most of them were previously reported as efficacious for neuroblastoma, including platinum, anthracycline, and alkylators.[16–18] The response to induction chemotherapy was assessed according to the International Neuroblastoma Response Criteria (INRC).[14]

After induction chemotherapy, patients underwent the double-conditioning regimen with auto-SCT, which comprised 2 cycles of thiotepa and melphalan at a 1-week interval[8]. Thiotepa (age [?] 2 years, 200 mg/m^2/day; age < 2 years, 8 mg/kg/day) and melphalan (age [?] 2 years, 70 mg/m^2/day; age < 2 years, 1.5 mg/kg/day) were administered on days -12, -11, -5, and -4. If creatinine clearance (Ccr) was <100 mL/min/1.73 m^2 in patients aged [?]2 years, the dosage was principally adjusted before and during HDC using the following formula: given dose (mg/m^2) = (Ccr/100) x 200 mg/m^2/day (thiotepa) or 70 mg/m^2/day (melphalan). The details of this regimen are reported elsewhere.[9]

Some patients received retinoic acid and/or second SCT using cord blood stem cells according to the institution's policy, while no patients underwent GD-2 antibody therapy.

Surgical resection of the primary tumor was conducted at the adequate time point on the basis of the feasibility of resection during induction chemotherapy or post-HDC. In some patients, surgical resection was planned after HDC regardless of tumor resectability during induction chemotherapy because the feasibility of the delayed local control strategy was shown in Japanese nationwide phase 2 study.[19]

Radiation therapy was administered against the residual tumor at the primary site and/or metastatic sites; however, the criteria for determining the target sites for irradiation varied from institution to institution.

Toxicity was assessed from day 1 of the double-conditioning regimen to day 100 after auto-SCT or day 1 of the second conditioning regimen on the basis of the Common Terminology Criteria for Adverse Events version 4.0. Sinusoidal obstruction syndrome (SOS) was diagnosed according to Baltimore criteria.[20] Thrombotic microangiopathy (TMA) was diagnosed according to BMT-CTN criteria.[21] In addition, regimen-related death was defined as death due to any adverse event occurring within the study period.

Statistical analysis

The survival rate was estimated using the Kaplan–Meier method. An event was defined as progression of disease, toxic death, or secondary cancer. In addition, prognostic factors were assessed using univariate and multivariate Cox proportional hazard regression models. Prognostic factors assessed using univariate analysis were age, INSS stage, bone metastasis, bone marrow metastasis, liver metastasis, MSI[15], *MYCN* amplification, INPC pathology, INRC before HDC, radiation, surgery before HDC, retinoic acid, and tandem SCT. Factors showing significant adverse effects on event-free survival (EFS) in a log-rank test were assessed using a multivariable model. All statistical analyses were performed using the R package version 3.3.3.

RESULTS

Patient characteristics

Table 1 summarizes patient characteristics (n = 41). The median age at diagnosis was 35 months (range = 8–75 months).*MYCN* was amplified in 23 patients, 39 patients had stage 4 disease, and the remaining 2 tumors showed *MYCN* amplification. An INPC histologically unfavorable tumor was observed in 35 patients. Bone and bone marrow were involved in 31 patients. The bone metastasis frequency was higher in *MYCN* -amplified high-risk neuroblastoma patients compared to *MYCN* -non-amplified high-risk neuroblastoma patients.

Treatment

All 41 patients underwent intensive multidrug induction chemotherapy in median 5 cycles (range = 4-7 cycles). A sufficient amount of peripheral blood stem cell grafts were collected from 39 patients during chemotherapy, while auto-bone marrow grafts were collected from 2 patients because of insufficient peripheral stem cell collection. Resection of the primary tumor was performed in 11 patients before HDC. Radiation therapy for the primary site was administered in 29 patients. Before HDC, of the 41 patients, 14 (33%) (*MYCN* -amplified high-risk neuroblastoma, 11; *MYCN* -non-amplified high-risk neuroblastoma, 3; P = 0.051) showed complete response or very good partial response (CR/VGPR). Of 26 patients, 7 (27%) who did not undergo resection before HDC showed CR/VGPR. Of 41 patients, 15 (37%) received a reduced dose of thiotepa and melphalan as HDC because of renal function deterioration. In addition, 24 (59%) patients whose guardians requested off-label use underwent retinoic acid therapy, and 8 (20%) patients with poor response after treatment underwent a second SCT using cord blood stem cells, expecting immunological effects. No patients underwent anti-GD2 antibody immunotherapy.

Outcome

The median follow-up duration of all living patients was 9.2 years (range = 5.5–14.0 years). During this period, 20 patients relapsed. Of those 20 patients, 1 developed secondary myelodysplastic syndrome 13 years after the initial treatment for high-risk neuroblastoma. The 5-year EFS and overall survival (OS) rates from treatment initiation were 41.5% +- 7.7% and 56.1% +- 7.8%, respectively (**Figure 1**). The outcomes were not significantly different between the three institutions. Although 15 patients needed dose reduction of thiotepa and melphalan according to their Ccr results, the outcomes were not significantly different (**Supporting Information Figure S1**). The 5-year EFS and OS of patients who showed CR/VGPR/PR before HDC were 50.0% +- 8.6% and 67.6% +- 8.0%, respectively. The 5-year EFS rate of patients with a good remission status (CR/VGPR) before HDC was significantly superior compared to patients with a poor remission status (78.6% +- 11.0% vs. 22.2% +- 8.0%; P = 0.00041) (**Figure 2A**). Similarly, the 5-year OS rate of patients with a good remission status (CR/VGPR) before HDC was significantly superior compared to patients with a poor remission status (92.9% +- 6.9% vs. 37.0% +- 9.3%; P = 0.00019).

Table 1 summarizes prognostic effects according to univariate analysis. Patients with bone metastasis showed a significantly lower 5-year EFS compared to patients without bone metastasis (32.3% +- 8.4% vs. 70.0% +- 14.5%; $P = 0.021$). Eight patients who underwent tandem SCT showed significantly poorer prognosis compared to those who didn't undergo tandem SCT ($P = 0.012$). Interestingly, the 5-year EFS rate of *MYCN* -amplified high-risk neuroblastoma patients was significantly superior compared to *MYCN* -non-amplified high-risk neuroblastoma patients (60.9% +- 10.2% vs. 16.7% +- 8.8%; $P = 0.000065$) (**Figure 2B**). Similarly, the 5-year OS rate of*MYCN* -amplified high-risk neuroblastoma patients was significantly superior compared to *MYCN* -non-amplified high-risk neuroblastoma patients (73.9% +- 9.2% vs. 33.3% +- 11.1%; $P = 0.00018$) (**Figure 2B**). Even when the outcome was analyzed in each group, which showed a good response to induction chemotherapy (CR/VGPR) and poor response, *MYCN* -amplification was associated with good prognoses (P = 0.094 in CR/VGPR patients, P = 0.019 in non-CR/VGPR patients) (**Supporting Figure S2**). Of note,*MYCN* -amplified high-risk neuroblastoma patients who showed good response to induction chemotherapy showed good long-term outcomes (5-year EFS = 81.8%). Among four significant prognostic factors (INRC before HDC, *MYCN* amplification, bone metastasis, and tandem SCT), backward stepwise selection eliminated bone marrow metastasis and tandem SCT in multivariate analysis. Finally, the strongest prognostic factor for EFS was *MYCN* amplification (hazard ratio = 0.29; 95% confidence interval = 0.12–0.66). Similarly, *MYCN* amplification and INRC before HDC were also chosen as significant prognostic factors for OS in multivariable analysis. **Table 2** describes the details of multivariate analysis.

Toxicity

During the assessment period post-HDC, grade 3 or 4 acute mucositis was observed in 33 patients (grade 3, 32 patients; grade 4, 1 patient). Capillary leak syndrome occurred in 8 patients, of which 4 patients required intravenous steroid administration (grade 3). TMA occurred in 1 patient who required dialysis for grade 4 acute kidney injury and finally died of grade 5 pulmonary hemorrhage 1 year post-HDC. In addition, 2 patients died on days 22 and 57 after SCT, respectively because of regimen-related toxicity; they developed grade 5 viral infection (respiratory syncytial virus bronchiolitis in one and cytomegalovirus pneumonia in the other). We also observed acute kidney injury and hypertension in 3 patients (TMA, 1 patient; drug, 2 patients). None of the patients developed sepsis and SOS.

DISCUSSION

In this study, the 5-year EFS rates of all high-risk neuroblastoma patients who underwent the double-conditioning regimen comprising thiotepa and melphalan and of CR/VGPR/PR patients before HDC were 41.5% and 50.0%, respectively. Considering the poor prognosis of patients who did not undergo anti-GD2 antibody immunotherapy, the outcome of our patients who showed CR/VGPR/PR before HDC was comparable to previous studies.[3,22] It is partly because*MYCN* -amplified high-risk neuroblastoma patients show a superior survival rate.

Comprehensive studies have considered *MYCN* amplification as a poor prognostic factor in high-risk neuroblastoma.[23,24] In contrast, in this study,*MYCN* amplification was a favorable prognostic factor, although*MYCN* -amplified high-risk neuroblastoma patients showed a higher response rate after induction chemotherapy compared to*MYCN* -non-amplified high-risk neuroblastoma patients. This result indicated that the double-conditioning regimen might be appropriate for the treatment of *MYCN* -amplified high-risk neuroblastoma.

Some studies have shown favorable outcomes in *MYCN* -amplified high-risk neuroblastoma patients after intensive treatment, including tandem HDC.[5,6] This finding suggests that*MYCN* -amplified high-risk neuroblastoma might be more chemosensitive and more likely to benefit from treatment intensification. The double-conditioning regimen is quite unique in that it consists of two cycles of a drug combination (thiotepa and melphalan) for 2 consecutive weeks to safely administer the maximum dose of these drugs.[8] Therefore, this highly potent regimen could be especially effective in *MYCN* -amplified high-risk neuroblastoma, as shown in some studies using tandem HDC.

In this study, *MYCN* -amplified high-risk neuroblastoma patients who showed CR/VGPR before HDC espe-

cially had good prognosis. Kushner et al. (2014) reported that *MYCN* -amplified high-risk neuroblastoma patients show extreme dichotomy in the clinical course;*MYCN* -amplified and *MYCN* -non-amplified high-risk neuroblastoma patients who showed good response to induction chemotherapy also showed similar good long-term outcomes, while*MYCN* -amplified high-risk neuroblastoma patients who did not show CR/VGPR developed earlier progression with a significantly poor outcome compared to *MYCN* -non-amplified high-risk neuroblastoma patients.[25] The high frequency of chemosensitive*MYCN* -amplified high-risk neuroblastoma in our cohort might have led to our positive results.

Other favorable prognostic factors shown in this study, such as good remission status (CR/VGPR) and bone metastasis negativity, were similar to previous reports.[15,26] High MSI (>1) and older age ([?]5 years), extracted as variables for risk stratification from the analysis of the HR-NBL-1/SIOPEN study, didn't have any prognostic impact.[25]

The toxicity of the double-conditioning regimen is relatively severe. In this study, 2 patients died of regimen-related toxicity, and 1 patient who developed grade 4 TMA died from a pulmonary hemorrhage 1 year post-HDC. Acute mucositis was frequently observed. The dose-finding experience of the double-conditioning regimen for several solid tumors showed severe gastrointestinal toxicity, microangiopathy, renal tubular acidosis, and neurological toxcity[8]. Another study also reported excessive gastrointestinal toxicity and delayed platelet recovery.[27] Okada et al. (2019) reported the successful prevention of renal toxicity by decreasing the doses of thiotepa and melphalan in patients less than 2 years old or in those showing low renal function while gastrointestinal toxicity was still severe.[9] Therefore, the double-conditioning regimen warrants special care when applied to high-risk neuroblastoma patients who undergo intensive induction therapy.

Recently, thiotepa as a double-conditioning regimen therapeutic was approved in Japan along with melphalan with a reduced cumulative dose from 280 to 210 mg/m^2. The feasibility of this modified double-conditioning regimen has been confirmed in early clinical trials.[28] Further studies to confirm the efficacy of this modified double-conditioning regimen are required.

This retrospective study had a few limitations. First, there was selection bias of patients. Especially, the difference in the response rate between *MYCN* -amplified and *MYCN* -non-amplified high-risk neuroblastoma patients warrants careful interpretation. Second, treatments other than the double-conditioning regimen were heterogeneous. However, treatment components did not exhibit any prognostic effects, except for second SCT. Third, we could not cover the entire range of toxic profiles because data collection was based on only medical records. Finally, because of a lack of a common long-term follow-up method, this study did not report long-term toxicity. Therefore, long-term complications in patients who underwent this potent treatment merit considerable care.

CONCLUSIONS

The double-conditioning regimen with thiotepa and melphalan is effective for high-risk neuroblastoma, especially in patients with *MYCN*amplification. However, the double-conditioning regimen is toxic and warrants special attention in clinical practice.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Legends to Figures

FIGURE 1. EFS and OS rates from day 1 of treatment for all patients ($n = 41$). EFS, event-free survival; OS, overall survival.

FIGURE 2. EFS rates from day 1 of treatment for patients (A) with good remission status (CR/VGPR) before HDC ($n = 14$) vs. poor remission status (non-CR/VGPR) before HDC ($n = 29$) and (B) with *MYCN* amplification ($n = 23$) vs. *MYCN* non-amplification ($n = 20$). EFS, event-free survival; OS, overall survival; INRC, International Neuroblastoma Response Criteria; CR, complete response; VGPR, very good partial response; HDC, high-dose chemotherapy.

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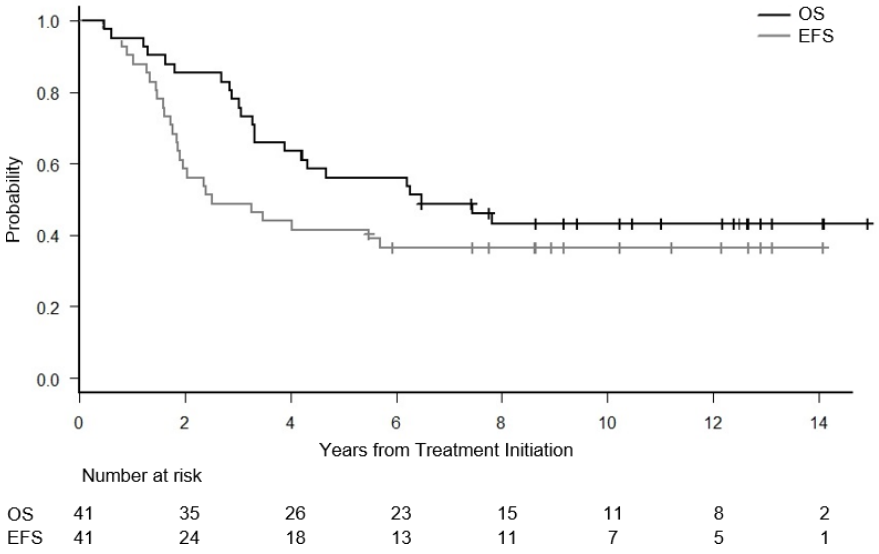
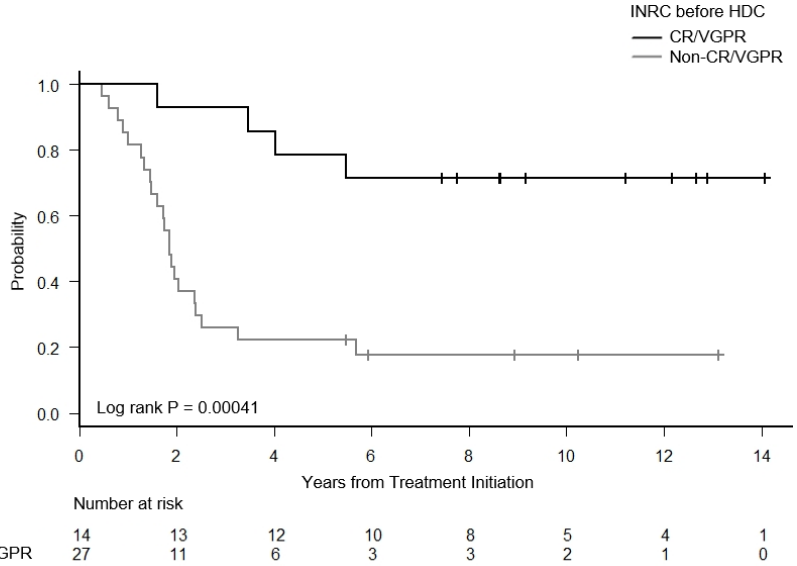


Number at risk

OS	41	35	26	23	15	11	8	2
EFS	41	24	18	13	11	7	5	1

(A)



Number at risk

CR/VGPR	14	13	12	10	8	5	4	1
Non-CR/VGPR	27	11	6	3	3	2	1	0

(B)