

## Double High-dose Chemotherapy with Autologous Stem Cell Transplantation in Patients with High-risk Neuroblastoma: A Pilot Study in a Single Center

Double high-dose chemotherapy (HDCT) was applied to 18 patients with high-risk neuroblastoma including 14 patients who could not achieve complete response (CR) even after the first HDCT. In 12 patients, successive double HDCT was rescued with peripheral blood stem cells collected during a single round of leukaphereses and in 6 patients, second or more rounds of leukaphereses were necessary after the first HDCT to rescue the second HDCT. The median interval between the first and second HDCT (76 days; range, 47-112) in the single harvest group was shorter than that (274.5 days; range, 83-329) in the double harvest group ( $p < 0.01$ ). Hematologic recovery was slow in the second HDCT. Six (33.3%) treatment-related mortalities (TRM) occurred during the second HDCT but were not related to the shorter interval. Disease-free survival rates at 2 years with a median follow-up of 24 months (range, 6-46) in the single and double harvest group were 57.1% and 33.3%, respectively. These results suggest that successive double HDCT using the single harvest approach may improve the survival of high-risk patients, especially who could not achieve CR after the first HDCT despite delayed hematologic recovery and high rate of TRM during the second HDCT.

**Key Words :** Neuroblastoma; Drug Therapy; Transplantation, Autologous

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## INTRODUCTION

Prognosis of high-risk neuroblastoma (NBL) is very poor with conventional treatment. Recently, survival of patients with high-risk NBL has improved with the introduction of high-dose chemotherapy (HDCT) with autologous stem cell transplantation (ASCT), but still not satisfactory (1-3). Complete response (CR) is not achieved in some patients even after HDCT and tumors relapse in many patients after HDCT.

Therefore, new therapeutic approaches such as multiple HDCT are necessary for the patients with high-risk NBL. Multiple HDCT is a novel way to further increase the dose intensity in the treatment of chemo-sensitive high-risk tumors, and this strategy has been evaluated in various tumors including malignant lymphoma, breast cancer, and NBL (4-7).

In this study, double HDCT was applied to improve the survival of patients with high-risk NBL, when CR was not achieved even after the first HDCT or when high probability of relapse was expected with a single HDCT due to the presence of multiple poor prognostic factors at diagnosis. To apply the second HDCT earlier after the first HDCT, we tried to collect as many peripheral blood stem cells (PBSCs) as possible before the first HDCT to rescue successive double HDCT.

## MATERIALS AND METHODS

### Patients

Diagnosis was usually confirmed by pathologic examination of biopsied specimen and the presence of N-myc amplification was also examined. Computed tomography and/or magnetic resonance image and plain radiography for primary or metastatic lesions, <sup>99m</sup>Tc bone scan, and metaiodobenzylguanidine (<sup>131</sup>I-MIBG) scan were performed regularly at 2- to 6-month intervals from diagnosis.

The presence of N-myc amplification, International Neuroblastoma Staging System (INSS) stage 4, and age at diagnosis >1 yr were defined as poor prognostic factors. Patients with two or more poor prognostic factors were assigned as high-risk patients. Otherwise, patients were assigned as low-risk patients.

Among 47 patients who were newly diagnosed as NBL at Samsung Medical Center between June 1997 and February 2001, 30 high-risk patients and 2 low-risk patients who could not achieve CR with conventional treatment received either single or double HDCT. The second HDCT was applied to 18 (17 high-risk and 1 low-risk) of these 32 patients because they could not achieve CR even after the first HDCT (n=14)

**Table 1.** Characteristics of patients and summary of treatment and results

| Pt. No. | Sex/Age (months) | ST         | N-myc Amp. | Risk group | Pre-HDCT1 treatment | Type of ASCT | Pre-HDCT1 status | Regimen of HDCT1 | Post-HDCT1 status | Post-HDCT1 treatment | Interval* (days) | Regimen of HDCT2 | Post-HDCT2 status | Post-HDCT2 treatment | Follow-up† and results |
|---------|------------------|------------|------------|------------|---------------------|--------------|------------------|------------------|-------------------|----------------------|------------------|------------------|-------------------|----------------------|------------------------|
| 1       | M/26             | 3          | +          | H          | CS                  | S/S          | CR               | CEMT             | CR                | c                    |                  |                  |                   |                      | 40 m+ disease free     |
| 2       | F/10             | 4B         | +          | H          | CSR                 | S/S          | VGPR             | CEM              | CR                | lc                   |                  |                  |                   |                      | 37 m+ disease free     |
| 3       | M/91             | 4B,BM,BR   | +          | H          | CSR                 | S/S          | VGPR             | CEM              | VGPR              | -                    |                  |                  |                   |                      | 6 m progression        |
| 4       | F/31             | 3          | +          | H          | CS                  | S/S          | CR               | CEM              | CR                | lc                   |                  |                  |                   |                      | 22 m+ relapse (15 m)   |
| 5       | M/15             | 3          | +          | H          | CS                  | S/S          | CR               | CEM              | CR                | lc                   |                  |                  |                   |                      | 13 m+ disease free     |
| 6       | M/24             | 4B         | -          | H          | CSR                 | S/S          | CR               | CEM              | CR                | lc                   |                  |                  |                   |                      | 10 m+ disease free     |
| 7       | M/33             | 3          | +          | H          | CS                  | S/S          | CR               | CEM              | CR                | lc                   |                  |                  |                   |                      | 9 m+ disease free      |
| 8       | M/63             | 3          | +          | H          | CSR                 | S/S          | VGPR             | CEM              | CR                | lc                   |                  |                  |                   |                      | 8 m+ disease free      |
| 9       | M/20             | 4B         | +          | H          | CS                  | S/hS         | VGPR             | CEMT             | CR                | c                    |                  |                  |                   |                      | 44 m+ disease free     |
| 10      | F/3              | 4LV,LG     | +          | H          | CS                  | S/hS         | PR               | CEM              | CR                | lc                   |                  |                  |                   |                      | 22 m+ disease free     |
| 11      | F/3              | 4B,LV      | -          | L          | CS                  | S/hS         | PR               | CEM              | CR                | lc                   |                  |                  |                   |                      | 19 m+ disease free     |
| 12      | F/4              | 4B,LV      | +          | H          | CS                  | S/hS         | PR               | CEMT             | CR                | lc                   |                  |                  |                   |                      | 19 m+ disease free     |
| 13      | M/56             | 4B,BM,LG   | +          | H          | CSR                 | S/hS         | PR               | CEM              | NE                | -                    |                  |                  |                   |                      | 1 m dead (pn)          |
| 14      | F/57             | 4B         | +          | H          | CS                  | S/hS         | CR               | CEMT             | CR                | lc                   |                  |                  |                   |                      | 14 m+ disease free     |
| 15      | M/17             | 4B,BM,O    | +          | H          | CS                  | D/S          | PR               | CEMT             | PR                | R                    | 69               | CTM              | CR                | c                    | 46 m+ disease free     |
| 16      | F/13             | 4B,BM,O    | +          | H          | CS                  | D/S          | VGPR             | CEMT             | VGPR              | R                    | 60               | CTM              | CR                | lc                   | 43 m+ disease free     |
| 17      | M/49             | 4B         | -          | H          | C                   | D/S          | SD               | CEMT             | PR                | SR                   | 55               | CTM              | CR                | lc                   | 42 m+ disease free     |
| 18      | F/28             | 4B,BM      | +          | H          | CS                  | D/S          | VGPR             | CEMT             | VGPR              | -                    | 47               | CTM              | NE                | -                    | 3 m dead (bleeding)    |
| 19      | F/20             | 4B,BM,O    | +          | H          | CS                  | D/S          | VGPR             | CEMT             | VGPR              | -                    | 80               | CTM              | NE                | -                    | 4 m dead (VOD)         |
| 20      | F/6              | 4B         | -          | L          | CSR                 | D/S          | VGPR             | CEM              | VGPR              | -                    | 72               | CTM              | CR                | Rlc                  | 24 m+ disease free     |
| 21      | F/64             | 4B,BM,LN   | +          | H          | CS                  | D/S          | PR               | CEMT             | PR                | -                    | 58               | CTM              | PR                | lcCM                 | 18 m+ PR               |
| 22      | M/36             | 4B,BM,BR,O | +          | H          | CS                  | D/S          | PR               | CEMT             | CR                | -                    | 88               | CTM              | CR                | -                    | 17 m dead (MOF)        |
| 23      | F/36             | 4B,BM,BR   | +          | H          | CS                  | D/Ss         | VGPR             | CEMT             | CR                | -                    | 81               | CTM              | CR                | lc                   | 13 m+ disease free     |
| 24      | F/52             | 4B,BM      | +          | H          | CS                  | D/S          | VGPR             | CEMT             | VGPR              | -                    | 102              | CTM              | CR                | lc                   | 11 m+ disease free     |
| 25      | F/78             | 4B,BM      | ND         | H          | CS                  | D/S          | VGPR             | CEMT             | VGPR              | -                    | 109              | CTM              | CR                | c                    | 6 m+ disease free      |
| 26      | M/41             | 4BM,LG,LN  | +          | H          | CSR                 | D/S          | VGPR             | CEMT             | VGPR              | -                    | 112              | CTM              | CR                | -                    | 5 m dead (MOF)         |
| 27      | F/125            | 4B,BM      | ND         | H          | CS                  | D/D          | VGPR             | CEMT             | VGPR              | -                    | 83               | CTM              | NE                | -                    | 5 m dead (pn)          |
| 28      | M/54             | 4B         | -          | H          | CS                  | D/D          | VGPR             | CEMT             | VGPR              | CR                   | 270              | CTM              | CR                | lc                   | 38 m+ disease free     |
| 29      | F/60             | 4B,BM      | +          | H          | CS                  | D/D          | PR               | CEM              | PR                | C                    | 329              | CTMT             | CR                | lc                   | 27 m+ relapse (15 m)   |
| 30      | F/34             | 4B,BM,BR   | -          | H          | CSR                 | D/D          | VGPR             | CEMT             | CR                | C                    | 326              | CTM              | CR                | lc                   | 22 m+ disease free     |
| 31      | F/41             | 4B,BM      | -          | H          | CS                  | D/D          | PR               | CEMT             | VGPR              | CR                   | 279              | CTM              | CR                | lc                   | 19 m+ relapse (16 m)   |
| 32      | M/25             | 4B,BM,BR,O | +          | H          | CS                  | D/D          | VGPR             | CEMT             | CR                | C                    | 267              | CTM              | NE                | -                    | 9 m dead (sepsis)      |

ST, stage and metastatic sites; Amp, amplification; B, bone; BM, bone marrow; O, orbit; LV, liver; LG, lung; BR, brain; LN, lymph node; H, high-risk; L, low-risk; C, chemotherapy; S, surgery; R, local radiotherapy; S/S, single HDCT rescued with all of PBSCs collected during a single round of leukaphereses; S/hS, single HDCT rescued with about half of PBSCs collected during a single round of leukaphereses; D/S, successive double HDCT rescued with PBSCs collected during a single round of leukaphereses; D/D, double HDCT rescued with PBSCs collected during double rounds of leukaphereses; CEMT, carboplatin+etoposide+melfalhan+TBI; CEM, carboplatin+etoposide+melfalhan; CTMT, carboplatin+thiotepa+melfalhan + TBI; CTM, carboplatin+thiotepa+melfalhan; NE, not evaluated; HDCT1, first HDCT; HDCT2, second HDCT; I, immunotherapy using IL-2; c, differentiating therapy using 13-*cis*-retinoic acid. \*interval between HDCT1 and HDCT2. †follow-up after HDCT1.

or because they had 3 poor prognostic factors and/or multiple metastases at diagnosis even if they achieved CR after the first HDCT ( $n=4$ ). All of these 18 patients were stage 4 and could not achieve CR before the first HDCT, 15 of them had multiple metastases involving two or more organs, 17 of them were older than 1 yr at diagnosis, and 11 of evaluable 16 patients had N-myc amplification. Characteristics of the patients who received either single or double HDCT are summarized in Table 1. Patients who had been transferred from other hospitals due to relapse or poor response to treatment were excluded to avoid bias.

### Pre-HDCT treatment

Usually, albeit not always, after 5 courses of preoperative conventional chemotherapy, tumor resection was tried and central venous catheter was placed during surgery. Local radiotherapy (1,500-3,000 cGy) was applied when residual tumor was present after surgery. Conventional chemotherapy usually included cisplatin 60 mg/m<sup>2</sup> intravenous (IV) infusion over 8 hr on day 0, etoposide 100 mg/m<sup>2</sup> IV infusion over 2 hr on day 2 and 5, doxorubicin 30 mg/m<sup>2</sup> IV infusion on day 2, and cyclophosphamide 30 mg/kg IV infusion on day 3 and 4. When patients could not tolerate doxorubicin due to car-

diotoxicity, various kinds of conventional chemotherapy regimens were used.

### Collection of PBSCs

PBSCs were collected when tumor cells were not found on bone marrow examination after surgery. When tumor cells were found on bone marrow examination, collection of PBSCs was deferred until they were cleared from bone marrow by additional chemotherapy.

In 16 of 18 patients, 5–10  $\mu\text{g/kg}$  of granulocyte-colony stimulating factor (G-CSF) was infused daily for mobilization of stem cells from the day when absolute neutrophil count (ANC) fell below  $0.5 \times 10^9/\text{L}$  after chemotherapy. Collection of PBSCs was started when white blood cell count exceeded  $1.0 \times 10^9/\text{L}$  after nadir. In the remaining 2 patients, stem cells were mobilized with G-CSF alone without preceding chemotherapy. COBE Spectra (Gambro, U.S.A.) was used for leukapheresis and the numbers of total nucleated cells, mononuclear cells,  $\text{CD}34^+$  cells, and colony-forming cells were counted.

We tried to collect as many PBSCs as possible during the single round of leukaphereses before the first HDCT in order to rescue successive double HDCT, and this strategy was possible in 12 of 18 patients (single harvest group). However, in the remaining 6 patients (double harvest group), second or more rounds of leukaphereses were necessary after the first HDCT to rescue the second HDCT because a sufficient number of PBSCs could not be collected during the first round of leukaphereses or all of PBSCs collected were infused to rescue the first HDCT.

### HDCT

Regimen of the first HDCT included carboplatin 300  $\text{mg/m}^2$  by continuous IV infusion over 24 hr on day-8, -7, -6, and -5, etoposide 200  $\text{mg/m}^2$  by continuous IV infusion over 24 hr on day-8, -7, -6, and -5, melphalan 140  $\text{mg/m}^2$  IV infusion over 30 min on day-7 and 70  $\text{mg/m}^2$  IV infusion over 30 min on day-6, and total body irradiation (TBI) 333 cGy once daily on day-3, -2, and -1. In one patient, TBI was included in the second HDCT.

Regimen of the second HDCT included carboplatin 250  $\text{mg/m}^2$  by continuous IV infusion over 24 hr on day-6, -5, and -4, thiotepa 200  $\text{mg/m}^2/\text{day}$  by once daily IV infusion over 2 hr on day-6, -5, and -4, and melphalan 160  $\text{mg/m}^2$  IV infusion over 30 min on day-3.

G-CSF was used to accelerate neutrophil recovery from day 0 to the day when ANC exceeded  $1.0 \times 10^9/\text{L}$  for 3 consecutive days. Acyclovir, fluconazole, and oral ciprofloxacin were used from day-1 to the day when ANC reached  $1.0 \times 10^9/\text{L}$  and intravenous immune globulin was infused on day 0, 7, and 14 to prevent various infections according to the common strategy of Samsung Medical Center. Total parenteral nutrition was supplied until the patients were able to tolerate an ade-

quate amount of food. Heparin was used from the beginning of HDCT to prevent veno-occlusive disease.

### Post-HDCT treatment

After the first HDCT, local radiotherapy was applied in 5 patients, conventional chemotherapy was applied in 5 patients in the double harvest group, and surgery was performed in 1 patient whose tumor was inoperable before the first HDCT. After the second HDCT, local radiotherapy was applied in 1 patient and conventional chemotherapy and  $^{131}\text{I}$ MIBG therapy was performed in 1 patient (Table 1).

We used interleukin-2 (IL-2) (8, 9) and 13-*cis*-retinoic acid (CRA) (10) after the second HDCT to eradicate possible minimal residual disease. These two drugs were administered simultaneously because their major toxicities do not overlap, and their different mechanisms of action may synergize in the eradication of minimal residual disease and preventing relapse after HDCT. Immunotherapy using IL-2 was started when platelet count reached  $50 \times 10^9/\text{L}$  without transfusion after the second HDCT. After 2 courses of weekly induction (day 0:  $2 \times 10^6$  U/ $\text{m}^2/\text{day}$ , day 1–4:  $4 \times 10^6$  U/ $\text{m}^2/\text{day}$ , continuous infusion), 10–12 courses of maintenance therapy (1st–3rd:  $2 \times 10^6$  U/ $\text{m}^2/\text{day}$ , 4–12th:  $3 \times 10^6$  U/ $\text{m}^2/\text{day}$  subcutaneous injection for 5 days per every 4 weeks) were applied. Six courses (14 days per every 4 weeks) of CRA (125  $\text{mg/m}^2/\text{day}$  or 4.2  $\text{mg/kg/day}$  if body weight <12 kg) were applied for differentiation of minimal residual tumor cells. The first dose of CRA was started on day 0 of the first course of IL-2 maintenance.

### Criteria of response to treatment and toxicities

The international response criteria (11) were modified to evaluate the response to treatment. In summary, CR was defined as no identifiable tumor with normal catecholamine level. Very good partial response (VGPR) was defined as reduction of primary tumor by 90–99% with normal catecholamine level with or without residual  $^{99\text{m}}\text{Tc}$  bone change, PR was defined as reduction of primary tumor and metastatic tumor by more than 50% without a new lesion, and stable disease (SD) was defined as reduction of primary tumor and metastatic tumor by less than 50% without a new lesion. All other cases were considered as progressive disease.

Toxicities were recorded according to the common toxicity criteria of National Cancer Institute of U.S.A.

### Statistics

Continuous variables and categorical variables were represented by median values and frequencies (percentages), respectively. Ranges of continuous variables were also presented. Overall survival rate (OS) and disease-free survival rate (DFS) were estimated by the Kaplan-Meier method. Com-

pletely observed continuous variables between the two different groups were compared by the log-rank test. Statistical significance was declared at  $p < 0.05$ .

## RESULTS

### Collection of PBSCs

The first round of leukaphereses was done median 6 months (range, 4-11) after diagnosis. More PBSCs were collected during the first round of leukaphereses in the single harvest group than in the double harvest group (Table 2). The amount of PBSCs collected during the first round of leukaphereses in the double harvest group was not more than that of PBSCs infused during the first HDCT in the single harvest group which was about half of PBSCs collected during the single round of leukaphereses (Table 3).

The second round of leukaphereses was necessary after the first HDCT to rescue the second HDCT in 6 of 18 patients. In 2 of these 6 patients, 1 or 2 more rounds of leukaphereses were necessary for collection of sufficient cells. The second round of leukaphereses were possible median 6.5 months (range, 2-9) after the first HDCT. The amount of PBSCs collected during the second or more rounds of leukaphereses after the first HDCT in the double harvest group was less

than that of PBSCs infused during the second HDCT in the single harvest group which was about half of PBSCs collected during the single round of leukaphereses (Table 3).

### Interval between the first and second HDCT

The first HDCT was applied median 7.5 months (range, 6-14) after diagnosis and the second HDCT was applied median 85.5 days (range, 47-329) after the first HDCT. In the single harvest group, the second HDCT was applied median 76 days (range, 46-112) after the first HDCT. This interval between the first and second HDCT was shorter than the median interval of 274.5 days (range, 83-329) in the double harvest group ( $p < 0.01$ ).

### Hematologic recovery

During the first HDCT, hematologic recovery was rapid and there was no difference between the single and double harvest group. During the second HDCT, hematologic recovery was delayed compared to the first HDCT, especially the platelet recovery in the double harvest group (Table 3). However, delay of neutrophil recovery during the second HDCT was not clinically significant and there was no difference in the duration of high fever between the first and second HDCT ( $p = 0.37$ ).

### Toxicity

Grade 3-4 toxicities related to double HDCT are listed in Table 4. The most common toxicity was diarrhea. Six (33.3%) treatment-related mortalities (TRM) occurred during the second HDCT. TRM occurred in 4 of 12 patients in the single harvest group and 2 of 6 patients in the double harvest group. The causes of TRM consisted of 1 veno-occlusive disease, 2 multi-organ failures, 1 pulmonary hemorrhage before platelet recovery, and 2 infections. There was no statistical evidence that the shorter interval between the first

**Table 2.** Number of collected PBSCs during the first round of leukaphereses

|   | Single harvest<br>(n=12) | Double harvest<br>(n=6) | p value |
|---|--------------------------|-------------------------|---------|
| Number of leukaphereses                             | 5.5 (3-10)*              | 6.5 (4-7)               | 0.70    |
| TNC ( $\times 10^6/\text{kg}$ )                     | 18.1 (7.1-48.9)          | 15.9 (6.4-31.4)         | 0.48    |
| MNC ( $\times 10^6/\text{kg}$ )                     | 10.4 (3.5-24.8)          | 10.0 (5.5-16.2)         | 0.67    |
| CD34 <sup>+</sup> cells ( $\times 10^6/\text{kg}$ ) | 6.5 (3.7-25.8)           | 3.3 (2.0-5.0)           | <0.01   |
| CFC ( $\times 10^5/\text{kg}$ )                     | 27.3 (9.0-61.9)          | 9.5 (4.9-19.5)          | 0.01    |

TNC, total nucleated cells; MNC, mononuclear cells; CFC, colony-forming cells. \*Values are expressed as median and range.

**Table 3.** Number of infused PBSCs and hematologic recovery in first and second HDCT

|   | First HDCT              |                 |         | Second HDCT    |                 |         |
|---|-------------------------|-----------------|---------|----------------|-----------------|---------|
|   | Single harvest          | Double harvest  | p value | Single harvest | Double harvest  | p value |
| TNC ( $\times 10^6/\text{kg}$ )                     | 9.1 (3.2-29.9)*         | 15.9 (6.4-31.4) | 0.17    | 8.1 (2.4-19.0) | 21.8 (6.2-34.8) | 0.01    |
| MNC ( $\times 10^6/\text{kg}$ )                     | 6.2 (2.0-13.6)          | 10.0 (5.5-16.2) | 0.10    | 4.9 (1.5-11.2) | 15.9 (4.4-22.1) | 0.02    |
| CD34 <sup>+</sup> cells ( $\times 10^6/\text{kg}$ ) | 3.3 (2.0-11.9)          | 3.3 (2.0-5.0)   | 0.78    | 3.3 (1.4-8.4)  | 2.0 (1.4-2.9)   | 0.02    |
| CFC ( $\times 10^5/\text{kg}$ )                     | 13.1 (5.6-36.1)         | 9.5 (4.9-19.5)  | 0.15    | 9.6 (3.4-32.9) | 9.3 (0.7-30.0)  | 0.87    |
| Time (days) to                                      |                         |                 |         |                |                 |         |
| ANC $> 0.5 \times 10^9/\text{L}$                    | 10 (8-14) <sup>†</sup>  | 10.5 (9-14)     | 0.60    | 11 (10-25)     | 13 (10-18)      | 0.55    |
| ANC $> 1 \times 10^9/\text{L}$                      | 11 (9-14) <sup>†</sup>  | 11.5 (9-14)     | 0.50    | 12 (11-27)     | 14 (11-54)      | 0.55    |
| PLT $> 20 \times 10^9/\text{L}$                     | 15 (7-20) <sup>‡</sup>  | 14.5 (12-29)    | 0.60    | 18 (9-49)      | 25 (15-51)      | 0.19    |
| PLT $> 50 \times 10^9/\text{L}$                     | 32 (17-78) <sup>‡</sup> | 29.5 (18-130)   | 0.65    | 55 (31-147)    | 130 (91-156+)   | 0.05    |

ANC, absolute neutrophil count; PLT, platelet count. \*Values are expressed as median and range. <sup>†</sup>The first day when ANC reached  $0.5 \times 10^9/\text{L}$  and  $1 \times 10^9/\text{L}$ , respectively. <sup>‡</sup>The day when PLT reached  $20 \times 10^9/\text{L}$  and  $50 \times 10^9/\text{L}$  for 3 and 7 consecutive days without transfusion, respectively.

**Table 4.** Grade 3-4 toxicities during 18 double HDCT

|                             | First HDCT | Second HDCT |
|-----------------------------|------------|-------------|
| Days of BT>38.0°C           | 8.5 (1-14) | 6 (0-32)    |
| Hemorrhage                  | 6 (33.3%)  | 5 (27.8%)   |
| Stomatitis                  | 14 (77.8%) | 6 (33.3%)   |
| Vomiting                    | 5 (27.8%)  | 6 (33.3%)   |
| Diarrhea                    | 14 (77.8%) | 11 (61.1%)  |
| Hyperbilirubinemia          | 1 (5.6%)   | 6 (33.3%)   |
| Elevation of liver enzyme   | 12 (66.7%) | 6 (33.3%)   |
| Hepatomegaly                | 9 (50.0%)  | 13 (72.2%)  |
| Veno-occlusive disease      | 3 (16.7%)  | 6 (33.3%)   |
| Renal insufficiency         | 0 (0%)     | 6 (33.3%)   |
| Treatment-related mortality | -          | 6 (33.3%)   |

BT, body temperature.

and second HDCT caused a higher rate of TRM ( $p=0.81$ ).

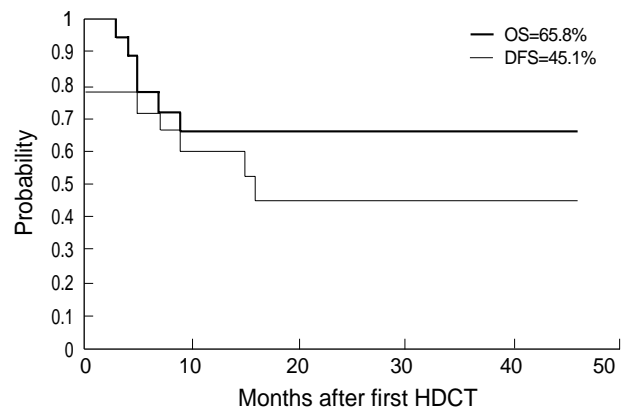
IL-2 and CRA were used safely, and their major toxicities were fever and dermatologic complications, respectively, which were tolerable. There was no life-threatening complication such as capillary leak syndrome.

### Response and survival of patients

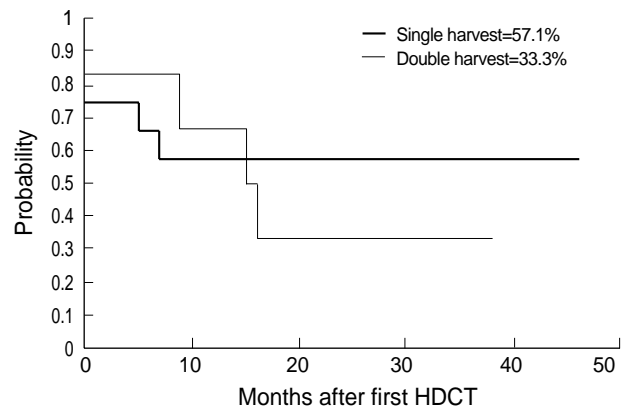
Among 12 patients in the single harvest group, 2 patients achieved CR after the first HDCT but one of them died due to TRM during the second HDCT. Among 10 remaining patients who could not achieve CR after the first HDCT, 7 patients achieved CR after the second HDCT but one of them died due to multi-organ failure after the second HDCT. Overall, 7 of 12 patients in the single harvest group were still free of disease without relapse.

Among 6 patients in the double harvest group, 2 patients achieved CR after the first HDCT but one of them died due to TRM during the second HDCT. Among 4 remaining patients who could not achieve CR after the first HDCT, 3 patients achieved CR after the second HDCT but tumors relapsed in 2 of these 3 patients in whom the intervals between the first and second HDCT were 329 days and 279 days, respectively. Overall, 2 of 6 patients in the double harvest group were still free of disease without relapse.

In summary, among 14 patients who could not achieve CR after the first HDCT, 10 patients achieved CR after the second HDCT, but tumors relapsed in 2 patients in the double harvest group and TRM occurred in 1 patient in the single harvest group. Among 4 patients who achieved CR after the first HDCT, 2 patients were still free of disease without relapse but the remaining 2 patients died during the second HDCT due to TRM. Overall, 9 of 18 patients were still free of disease with a median follow-up of 24 months (range, 6-46) after the first HDCT (Table 1). OS and DFS at 2 yr after the first HDCT in 18 patients who received double HDCT were 65.8% and 45.1%, respectively (Fig. 1). OS and DFS at 2 yr after the first HDCT were 65.6% and 57.1%, respectively in the single harvest group, and were 66.7% and 33.3%, respectively



**Fig. 1.** Probability of overall survival (OS) and disease-free survival (DFS) after the first HDCT in 18 patients who received double HDCT. OS and DFS at 2 yr after the first HDCT were 65.1% and 45.1%, respectively, with a median follow-up of 24 months (range, 6-46).



**Fig. 2.** Probability of disease-free survival (DFS) after the first HDCT. DFS at 2 yr after the first HDCT in the single and double harvest group were 57.1% and 33.3%, respectively, with a median follow-up of 24 months (range, 6-46).

ly in the double harvest group (Fig. 2).

## DISCUSSION

Survival of patients with high-risk NBL has improved with introduction of HDCT, but still not satisfactory (1-3). CR is not achieved in some patients even after HDCT and tumors relapse in many patients after HDCT, and at best fewer than 50% of patients will be cured with the current protocols. PR, the presence of bony lesion before HDCT, and the presence of multiple poor prognostic factors at diagnosis are risk factors for treatment failure after HDCT (12, 13).

Therefore, new therapeutic approaches are necessary. One of these is increasing the dose intensity using multiple HDCT and another is introduction of various kinds of adjuvant therapy after HDCT to eliminate minimal residual disease includ-

ing immunotherapy using IL-2 (8, 9), differentiating therapy using CRA (10), and radioisotope therapy using  $^{131}\text{I}$ MIBG (14).

Multiple HDCT is a novel way to further increase the dose intensity in the treatment of chemo-sensitive tumors and its efficacy has been evaluated in various tumors (4-7). Some investigators used double HDCT in high-risk NBL and reported rapid hematologic recovery and improved survival (6, 7). Although the efficacy was difficult to assess due to the small number of patients, the outcomes were encouraging.

In this study, double HDCT was applied to patients with high-risk NBL, when CR was not achieved after the first HDCT or when high probability of relapse after the first HDCT was expected due to the presence of multiple poor prognostic factors at diagnosis, i.e., presence of 3 poor prognostic factors and/or multiple metastases.

We believe that the second HDCT in double HDCT should be applied as soon as possible after the first HDCT to eradicate possible residual tumor cells and to prevent relapse. Because stem cell collection after the first HDCT is possible only when the bone marrow is fully recovered after the first HDCT, the second harvest after the first HDCT usually tends to be difficult and delayed as in our patients in the double harvest group. Delayed second HDCT may be a cause of relapse. Therefore, in this study, to apply the second HDCT earlier, we tried to collect as many PBSCs as possible during the single round of leukaphereses before the first HDCT to rescue the successive double HDCT, and this approach was possible in 12 of 18 double HDCT.

The advantage of this single harvest approach is to apply the second HDCT as soon as possible after the first HDCT. In this study, the interval between the first and second HDCT in the single harvest group was shorter than that in the double harvest group. In the double harvest group, the second round of leukaphereses was not possible until median 6 months (range, 2-9) after the first HDCT and less PBSCs were collected during the second round of leukaphereses. Two patients in the double harvest group needed another 1-2 more rounds of leukaphereses to collect sufficient number of PBSCs to rescue the second HDCT. In these 2 patients, the intervals between the first and second HDCT were 329 days and 279 days, respectively. They achieved CR after the second HDCT, but tumors relapsed soon thereafter. While tumors relapsed in 2 of 4 patients who achieved CR in the double harvest group, there has been no relapse at present in the single harvest group. When the relationship between longer interval and higher rate of relapse was analyzed in 18 double HDCT cases, there was a borderline correlation ( $p=0.07$ ). However, there was no statistical evidence that the shorter interval between the first and second HDCT was correlated with a higher rate of TRM ( $p=0.81$ ). These suggest that the second HDCT in double HDCT should be applied as soon as possible after the first HDCT if needed. Therefore, we believe that the second round of leukaphereses should be done before the first HDCT when a sufficient number of PBSCs can not be collected during the single round of leukaphereses in a patient who is expected or decided to receive double HDCT.

When PBSCs collected during the single round of leukaphereses were divided to rescue successive double HDCT in the single harvest group, hematologic recovery was not delayed in the first HDCT. Furthermore, although about half of PBSCs collected during the single round of leukaphereses were infused in the first HDCT in the single harvest group, hematologic recovery was as rapid as in other 8 patients (patient No. 1-8) in whom all of PBSCs collected during the single round of leukaphereses were infused to rescue single HDCT (data not shown). However, hematologic recovery was delayed in the second HDCT, especially the platelet recovery in the double harvest group (Table 3). One possible explanation for the delay in hematologic recovery in the second HDCT is a damage to the microenvironment of the bone marrow by the first HDCT, especially including TBI. One patient died before platelet recovery after the second HDCT due to pulmonary hemorrhage. However, delay in the neutrophil recovery after the second HDCT was not clinically significant and there was no difference in the duration of high fever between the first and second HDCT ( $p=0.37$ ).

Six of 18 patients, including 2 of 4 patients who achieved CR after the first HDCT, died due to TRM during the second HDCT. This rate of TRM during the second HDCT was very high compared to 1 TRM among 32 single or first HDCT at our center. Philip *et al.* (6) also reported a high toxic death rate during the second HDCT. In their report, the high rate of TRM after the second HDCT was a critical issue despite encouraging survival. We think that the relatively intense HDCT regimen in this study compared to other reports of double HDCT (4-7) was an important cause of the high rate of TRM in this study. However, we certainly believe that this successive intense regimen not only resulted in the high rate of TRM in the second HDCT, but also resulted in encouraging survival, especially in the single harvest group.

Among 10 of 12 patients in the single harvest group who could not achieve CR after the first HDCT, 7 patients achieved CR after subsequent second HDCT, although TRM occurred in one of them. Overall, 7 of 12 patients in the single harvest group are still free of disease and DFS at 2 yr after the first HDCT with a median follow-up of 24 months (range, 6-46) was 57.1%. At present, there has been no relapse in the single harvest group. We think these results in the single harvest group are encouraging considering the clinical status of patients included in this study, despite the limitation of the study due to the small number of patients and short duration of follow-up.

In summary, the interval between the first and second HDCT in double HDCT could be shortened without further increase in the rate of TRM during the second HDCT by using the single harvest approach. Despite delayed hematologic recovery and high rate of TRM during the second HDCT, successive double HDCT using the single harvest approach may improve the survival of patients with high-risk NBL, especially in whom CR could not be achieved even after the first HDCT.

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