INTRODUCTION

The NB97 protocol, designed by the French Society of Pediatric Oncology (SFOP) Neuroblastoma Study Group, aimed to evaluate the efficacy of an intensified treatment strategy in children with stage 4 neuroblastoma [1]. This protocol combined the induction chemotherapy regimen of the N7 protocol followed by surgery of the primary tumor and high-dose chemotherapy (HDC) containing Busulfan–Melphalan (Bu-Mel) followed by autologous stem cell transplantation (ASCT). Procedures. From 1998 to 1999, 47 children were included in the NB97 trial and treated with induction chemotherapy according to the N7 protocol, followed by surgery of the primary tumor. HDC (Busulfan, 600 mg/m²; Melphalan, 140 mg/m²) was administered in patients with partial response of metastases with no more than 3 mIBG spots. Radiotherapy was delivered to the primary tumor site when tumors displayed MYCN amplification. Results. Thirty-nine patients received Bu-Mel (83%); 23 who had achieved complete response (CR) of metastases, 20 after induction treatment and 3 after second-line chemotherapy, and 16 in partial response (PR). The toxicity of the whole treatment was manageable. The main HDC related-toxicity was hepatic veno-occlusive disease grade > 2 occurring in 15% of the patients. The 8-year EFS of the whole cohort was 34% (95% CI, 22–48%). The 8-year EFS of the 39 patients who received Bu-Mel and ASCT was 41% (95% CI, 27–57%). Patients who achieved a CR of metastases at the end of induction chemotherapy had a significantly better outcome than the others (8-year EFS, 52% vs. 20%; P = 0.02).


Key words: Busulfan–Melphalan; high-dose chemotherapy; neuroblastoma

METHODS

Patients

From March 1998 to April 1999, 20 SFOP institutions participated into this study. Forty-seven unselected newly diagnosed patients with stage 4 neuroblastoma over 1 year of age were included in this study. Local ethics committee approved this protocol. All parents signed an informed consent form before the initiation of treatment. Patient characteristics are described in Table I.

Evaluation of Disease Extent at Diagnosis

The primary tumor was evaluated by computed tomography (CT) or magnetic resonance imaging (MRI); the size of the lesion was measured in three dimensions. Disease extension was assessed by iodine-123-mIBG scintigraphy. A technetium-99m bone-scan was performed if MIBG uptake in the primary tumor was negative. Biological markers (VMA, HVA, dopamine) and MYCN status were determined in all patients. Two biopsy specimens and at least four aspirates from iliac bones were obtained for morphological evaluation of bone marrow. The diagnosis and staging were

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performed according to the revised International Neuroblastoma Diagnosis and Staging Criteria [9].

**Treatment**

The NB97 protocol induction chemotherapy regimen comprised seven courses according to the N7 protocol. Courses 1, 2, 4, 6 consisted of cyclophosphamide (70 mg/kg/day) on days 1 and 2 (140 mg/kg per course), doxorubicin (75 mg/m²) and vincristine (0.066 mg/kg with a maximal 2 mg dose per course) which were administered in a continuous intravenous infusion for 72 hours, as of day 1 (CAV). Courses 3, 5, and 7 consisted of cisplatin/etoposide association with etoposide (200 mg/m²/day) on days 1–3 (600 mg/m²/course) and cisplatin (50 mg/m²/day) on days 1–4 (200 mg/m²/course).

Surgical resection of the primary tumor was to be performed after the 7th course of induction chemotherapy if metastatic disease responded. Hematopoietic stem cells (HSC) collection was performed at the end of the induction chemotherapy.

Bu-Mel followed by peripheral ASCT was administered to patients who achieved a CR of metastases or with a partial remission of MIBG spots with no more than three persisting spots. Bone marrow evaluation had to be negative. Busulfan was administered orally every 6 hours from day –7 to –3 for a total of 16 doses (600 mg/m²). Melphalan (140 mg/m²) was given as a 15-minute intravenous infusion on day –2. ASCT was performed on day 0 [8].

Radiation therapy was delivered to the primary tumor site at least 70 days after SCT when MYCN amplification had been identified in the tumor cells. Doses of 34 and 24 Gy were delivered in patients ≥2 years and ≤2 years, respectively. The irradiation field corresponded to the size of the primary tumor before surgery.

**Supportive Care**

Supportive care after induction chemotherapy has been described elsewhere [1]. G-CSF (Filgrastim, Amgen, France) at a dose of 5 μg/kg/day was administered intravenously from day 5 post-ASCT until the neutrophil count exceeded 500/μl for 2 consecutive days [10]. Platelets were transfused to maintain the platelet count above 20,000/μl, or to control bleeding. Red blood cells (RBCs) were transfused when the hemoglobin level was below 7 g/dl. All blood products were irradiated.

Clonazepam was administered from day –8 to day 0 to prevent busulfan-related seizures. Ursodiol was administered from the beginning of HDC until day 80 post-SCT to prevent hepatic veno-occlusive disease [11]. All patients received trimethoprim-sulfamethoxazole as prophylaxis against *Pneumocystis jirovecii*.

**Evaluation of Tumor Response**

Response of the primary tumor and metastases was evaluated after the third and seventh courses of induction therapy and before and after ASCT with the same tools used at diagnosis. A CR of metastases was defined as the disappearance of all metastatic spots on MIBG scans associated with the disappearance of cytological and histological bone marrow involvement. Technetium bone scans were obtained in all patients who had negative MIBG scans.

**Evaluation of Toxicity**

The evaluation of toxicity included a physical examination, complete blood cell counts, and serum creatinine, liver enzymes and bilirubin determinations. Complete clinical and biological analyses were required before each course. Echocardiography and glomerular filtration rate (GFR) determination were performed every two courses. Audiometry was used to evaluate hearing loss according to Brock’s grading system [10]. Toxicity of conventional chemotherapy was graded according to World Health Organization (WHO) criteria [12]. Toxicity of HDC was evaluated according to Bearman’s grading system [13].

**Statistics**

The NB97 study included a phase II trial using a three-stage Fleming design evaluating the metastatic response rate to the induction chemotherapy. All the patients recruited in the study are described in the present report (N = 47) whereas only patients with an evaluable MIBG, confirmed by central review (N = 40), were included in the phase II trial analysis previously reported [1].

When the entire population was taken into account, event-free survival (EFS) was defined as the time from the first day of chemotherapy to the date of progression, relapse or death for patients who failed, and to the date of the last follow-up visit for patients in continuous complete remission. OS was defined as the time from the first day of chemotherapy to the date of death for deceased patients or the date of the last follow-up visit for survivors. In the analysis focused on patients who received HDC, similar criteria were used from the date of ASCT. Survival curves were estimated using the Kaplan–Meier method, with 95% confidence intervals calculated with the Rothman method. EFS curves were compared between subgroups with two-sided log-rank tests. Hazard ratios of failure (i.e., progression, relapse or death) were estimated using Cox models. Median follow-up was computed using Schenker’s method. The cut-off date for the present analysis was November 5, 2011.
RESULTS

Treatment Administration and Toxicity

Among the 47 patients who were treated according to this protocol, 42 completed the 7 courses of induction chemotherapy. Three patients stopped N7 induction treatment after the 5th course (1 because of disease progression and 2 because of toxicity). Cisplatin was replaced by carboplatin for the 7th course in two additional patients because of hearing loss. The primary tumor was resected in 41 patients. Surgery was performed at diagnosis in 2 patients, after induction chemotherapy in 35 patients, including 4 who never received HDC, and after HDC in 4. A macroscopic complete resection was performed in 29 patients. HDC with Bu-Mel was administered to 39 patients, at the end of induction chemotherapy in 36 patients and after second-line chemotherapy because of insufficient response of metastases in 3 patients. Radiotherapy was delivered in nine patients with a metastatic status at time of HDC was intrinsically correlated to the metastatic status at the end of induction chemotherapy. The three patients who achieved a complete remission of metastases at the end of induction chemotherapy and then received HDC were alive at 8 years, versus only 5/19 (26%) patients who received HDC but were not in CR after N7 induction chemotherapy (Fig. 3). The metastatic status at time of HDC was intrinsically related to the metastatic status at the end of induction chemotherapy. The three patients, who achieved a CR only after second-line chemotherapy before HDC, relapsed and died. The hazard ratio of failure of chemotherapy. Among these 40 patients, one patient in metastatic CR after induction chemotherapy died of surgical complication before receiving Bu-Mel and 39 received Bu-Mel (23 in CR and 16 in PR). One patient progressed during induction chemotherapy and the six remaining patients had stable or progressive disease at the end of induction chemotherapy. They never achieved the status required to receive HDC despite further lines of chemotherapy. They all died of progressive disease.

Survival

The median follow-up of the study population was 12.1 years. Overall, twenty-nine patients progressed or relapsed, 5 months to 4.1 years after start of treatment. All of them died, 7 months to 5.9 years after start of treatment. EFS of the whole cohort was 36% (95% CI, 24–50%) at 4 years and 34% (22–48%) at 8 years. OS was 51% (37–65%) at 4 years and 34% (22–48%) at 8 years. (Fig. 1). At last follow-up, 16/39 patients who received the Bu-Mel consolidation regimen were alive and free of disease with follow-up exceeding 7.7 years (8-year EFS = 41%, 95% CI, 27–57%), while the eight patients who were unable to receive HDC died (1 toxic death and 7 deaths related to disease progression). Considering the 46 patients who did not progress during induction chemotherapy, and regardless of subsequent treatments, the 21 patients who achieved a complete remission of metastases at the end of induction chemotherapy had a significantly better outcome (8-year EFS = 52%, 95% CI, 32–72%), than the 25 patients who were not in CR of metastases (8-year EFS = 20%, 95% CI, 9–39%, Fig. 2), with a hazard ratio of failure equal to 0.42 (95% CI, 0.20–0.91%, P = 0.02). The impact of the metastasis status at the end of induction chemotherapy on the risk of failure was similar for patients who underwent HDC, albeit not significant (HR = 0.45, 95% CI, 0.20–1.07, P = 0.07); 11/20 (55%) patients who were in CR after induction chemotherapy and then received HDC were alive at 8 years, versus only 5/19 (26%) patients who received HDC but were not in CR after N7 induction chemotherapy (Fig. 3). The metastatic status at time of HDC was intrinsically correlated to the metastatic status at the end of induction chemotherapy. The three patients, who achieved a CR only after second-line chemotherapy before HDC, relapsed, and died. The hazard ratio of failure of chemotherapy. Among these 40 patients, one patient in metastatic CR after induction chemotherapy died of surgical complication before receiving Bu-Mel and 39 received Bu-Mel (23 in CR and 16 in PR). One patient progressed during induction chemotherapy and the six remaining patients had stable or progressive disease at the end of induction chemotherapy. They never achieved the status required to receive HDC despite further lines of chemotherapy. They all died of progressive disease.

Tumor Response and Tumor Status at the Time of HDC

At the end of induction chemotherapy, a CR of metastatic targets was obtained in 21/47 patients (45%, 95% CI, 30–60%). A partial response of metastases (PR), with no more than 3 residual metastatic spots, was obtained after induction chemotherapy in the 16 other patients. Three patients in stable disease after induction chemotherapy finally achieved a CR of metastases after second-line

Overall Survial  
Event-Free Survival

![Fig. 1. Event free survival (EFS) and overall survival (OS) of the whole cohort (N = 47).](image-url)
Event free survival (EFS) of the 39 patients who underwent high dose chemotherapy according to their metastatic status at the end of the induction chemotherapy. Patients who were in CR at time of HDC compared to those who were not in CR was 0.62 (95% CI, 0.27–1.42%, \( P = 0.26\)).

**DISCUSSION**

The treatment of patients with high-risk neuroblastoma has dramatically changed during the last two decades. The positive impact of myeloablative treatment with ASCT has been demonstrated in three randomized studies compared to conventional maintenance chemotherapy [4,5] or no further treatment [6]. In these three studies, HDC was allocated by randomization in patients who had not progressed before, and the tumor status at time of randomization was not taken into account. As the tumor status at time of myeloablative treatment has been demonstrated to have an impact on survival [14], the strategy of the present study was to obtain the best response of metastases with induction chemotherapy and to administer HDC and ASCT only to patients with a sufficient tumor response.

The choice of Bu-Mel combination as HDC was based on the analysis of a cohort of 218 patients treated at the Institut Gustave Roussy. Patients who had received Bu-Mel had a significantly improved EFS in comparison to patients treated with other HDC regimens (\( P = 0.001\) [7]).

Our data suggest an improvement of outcome related to the administration of Bu-Mel. However, due to the design of the study, the current data do not enable to differentiate the Bu-Mel effect from the impact of patient selection to receive HDC according to metastatic response to induction treatment. The benefit related to the administration of Bu-Mel has been recently confirmed in the randomized European study HR-NBL-1/ESIOP comparing BuMel to CEM with a 3-year EFS of 49% (±3%) and 33% (±3%), respectively (\( P < 0.001\)) [15].

Although we did not reproduce the metastasis CR rate published by Kushner et al., a CR of metastases was obtained in 21 patients (45%) and 39 patients (83%) could receive HDC at the end of the first line induction therapy. The 8-year EFS of the whole cohort, including all patients from diagnosis, is 34%. Interestingly we observed a plateau in the EFS curve after 3.5 years from transplantation; the 8-year EFS of patients who received Bu-Mel was 41% (95% CI, 27–57%). It compares favorably with the EFS of the 549 patients of the EBMT registry who received various HDC regimens (5-year EFS = 26%) [14] and with that of patients randomly assigned to autologous bone marrow transplant ABMT within the CCG study (5-year EFS = 30 ± 4%) [16]. These latter patients were subsequently randomized to receive or not retinoic acid, which had a significant impact on their outcome. In the CCG cohort, late events occurred after 5 years from ABMT. Again, the 5-year EFS of German NB97 protocol was 35% (95% CI, 29–41%) for the 295/335 randomized patients; patients with progressive disease before randomization were excluded from the analysis. This study population included stage 1, 2, 3, and 4s tumors with MYCN amplification which could have improved overall results and some patients received maintenance therapy with retinoic acid [4].

The 8-year EFS of the whole cohort is 34% showed a better result as compared to published data concerning patients with stage 4 neuroblastoma, especially as they did not receive any retinoic acid maintenance therapy which has been shown to improve survival after HDC [5]. After administering this intensive induction chemotherapy, the metastasis CR rate remained a significant prognostic factor since patients in CR of metastases had a 8-year EFS of 52% whereas it was 19% for patients who were not in CR. Of note, it is not possible to differentiate the prognostic value of metastatic status at the end of induction from the possible impact of Bu-Mel HDC as both factors are strongly correlated. The intensive induction chemotherapy regimen may have selected patients with sensitive disease who had a better tumor status at time of HDC, that is, minimal residual disease; they obtained the maximum benefits of consolidation with HDC. Our data are not informative to evaluate the impact of second line chemotherapy administered to achieve a CR before HDC. The design of the study and the very small number of patients in this situation (only three patients who all progressed after HDC) do not allow us to draw any conclusion regarding this issue. In a cohort of 99 patients treated with LMCES [17], patients who achieved a CR or VGPR after the four courses of the NB87 SFOP protocol [18] had a significantly improved PFS compared to those who did not (50% vs. 23%, \( P = 0.02\)).

These results demonstrate that an intensive but manageable chemotherapy allowed more than 83% of the patients to receive Bu-Mel HDC with a good tolerance of the HDC regimen, as no toxic death was observed. Although possibly confounded by a positive
selection of patients, the current data suggest a favorable impact of Bu-Mel HDC with a 41% 8-year EFS after transplantation. The metastasis CR rate at the end of the induction therapy was a significant prognostic factor. The analysis of tumor response after induction chemotherapy with adequate tools as defined in the new international classification, therefore appears to be of crucial importance for the conduct of future trials. It is of major importance to design new therapeutic strategies, to evaluate whether second-line chemotherapy improves the outcome of poor responders to induction chemotherapy.

Administration of immunotherapy in addition to retinoic acid has recently been demonstrated to improve at least the 2-year EFS [19]. This should be considered for testing as a backbone in future clinical trials where questions on the benefits of second line of conventional chemotherapy or/and design of the maintenance therapy could be evaluated in randomized trials.

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