AD-A204 700

REPORT DOCUMENTATION PAGE

1b. RESTRICTIVE MARKINGS

DTIC FILE COPY

3. DISTRIBUTION/AVAILABILITY OF REPORT
Approved for public release; distribution is unlimited

4. PERFORMING ORGANIZATION REPORT NUMBER(S)
NMRI 87-100

5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION
Naval Medical Research

6b. OFFICE SYMBOL (If applicable)

7a. NAME OF MONITORING ORGANIZATION
Naval Medical Command

6c. ADDRESS (City, State, and ZIP Code)
Bethesda, Maryland 20814-5055

7b. ADDRESS (City, State, and ZIP Code)
Department of the Navy
Washington, D.C. 20372-5120

8a. NAME OF FUNDING/SPONSORING ORGANIZATION Naval Medical Research and Development Command

8b. OFFICE SYMBOL (If applicable)

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER

8c. ADDRESS (City, State, and ZIP Code)
Bethesda, Maryland 20814-5055

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
62233N	MM33C30	06-1003	DN377031

11. TITLE (Include Security Classification)
Autologous bone marrow transplantation for poor-prognosis neuroblastoma

12. PERSONAL AUTHOR(S) Seeger RC, Reynolds CP, Moss TJ, Lenarsky C, Feig SA, Selch M

13a. TYPE OF REPORT
Book chapter

13b. TIME COVERED
FROM ________ TO ________

14. DATE OF REPORT (Year, Month, Day)
1987

15. PAGE COUNT
8

16. SUPPLEMENTARY NOTATION In: Autologous Marrow Transplantation: Proceedings of Third International Symposium, K.Dicke, G. Spitzer, S. Jagannath, (editors), Houston: Univ. of Texas MD Anderson Hospital and Tumor Institute, pp. 375-382, 1987

17. COSATI CODES

FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Neuroblastoma
Autologous bone marrow transplantation

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

DTIC ELECTE 15 FEB 1989 S D E

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION
Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL
Phyllis Blum, Information Services Division

22b. TELEPHONE (Include Area Code)
202-295-2188

22c. OFFICE SYMBOL
ISD/ADMIN/NMRI

DD FORM 1473, 84 MAR

83 APR edition may be used until exhausted.
All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

89 2 14 027

In: *Autologous Marrow Transplantation: Proceedings of Third International Symposium*, K. Dicke, G Spitzer, S Jagannath, (editors), Houston: Univ of Texas MD Anderson Hospital and Tumor Institute, pp 375-382, 1987.

Autologous Bone Marrow Transplantation for Poor-Prognosis Neuroblastoma

Robert C. Seeger, C. Patrick Reynolds, Thomas J. Moss, Carl Lenarsky, Stephen A. Feig, Michael Selch, John Ugelstad, and John Wells

Neuroblastoma, a neoplasm of the sympathetic nervous system, is the most common extracranial tumor of childhood ~~(1)~~. Approximately 60% of all patients have only a 10% probability of long-term, disease-free survival if given conventional therapy that includes chemotherapy, local irradiation, and surgery ~~(2-5)~~. Recent pilot studies of intensive chemotherapy and total body irradiation (TBI) followed by allogeneic bone marrow transplantation (BMT) or autologous bone marrow transplantation (ABMT) have produced encouraging results ~~(6,7)~~. In this report, we update our original study in which 20 patients with advanced neuroblastoma underwent intensive four-drug chemotherapy, TBI, and ABMT or allogeneic BMT; in addition, we provide data from our current study regarding the clinical use of sedimentation, filtration, and magnetic immunobeads for the ex vivo removal of neuroblastoma cells from autologous marrow. Reprints. (AW

TOXICITY AND EFFICACY OF INTENSIVE CHEMORADIOTHERAPY AND BMT

In our initial study (patient entry from January 1983 to October 1985), we investigated toxicity and efficacy of intensive chemoradiotherapy (teniposide [VM-26], doxorubicin, L-phenylalanine mustard, cisplatin, and TBI; VAMP-TBI) followed by allogeneic or ABMT (Table 1). Twenty patients, all of whom were diagnosed after 1 year of age and who had stage IV (n = 18) or stage III (n = 2) disease, received VAMP-TBI and bone marrow. Thirteen patients were given bone marrow transplants before they developed progressive disease (3 received allogeneic marrow from a human leukocyte antigen [HLA]-compatible sibling and 10 received autologous marrow); 7 received transplantations after they developed progressive disease (5 allogeneic BMT and 2 ABMT).

Eight patients received bone marrow from HLA-mixed leukocyte culture (MLC) matched, nonidentical siblings (median of 3×10^8 and range of $2\text{-}4 \times 10^8$ nucleated marrow cells/kg). These patients were given methotrexate after BMT for prophylaxis of graft-versus-host disease (10 mg/m² on days 1, 3, 6, 11, and then weekly to 100 days).

Twelve patients received cryopreserved autologous marrow. Autologous marrow was used to restore hematopoiesis only if tumor cells were not detectable in an aliquot of the cryopreserved specimen by immunoperoxidase staining with anti-cell-surface monoclonal antibodies (mixture of antibodies 390, 459, HSAN 1.2, and 126-4) and anti-neuron-specific enolase serum; analysis of 3×10^5 bone marrow mononuclear cells gives a 95% probability of detecting one neuroblastoma cell among 10^5 normal cells (8,9). Marrow was prepared for cryopreservation by equilibrium density centrifugation over Ficoll-Hypaque (patients 1-7) (10); by sedimentation and filtration (patient 8); or by

Table 1. Pretransplant Intensive Chemoradiotherapy Regimen (VAMP-TBI)*

Day	Treatment
-9	Cisplatin, 90 mg/m² i.v.
-8	No therapy
-7	VM-26, 150 mg/m² i.v.; doxorubicin, 45 mg/m² i.v.
-6	Melphalan, 140 mg/m² i.v.
-5	Melphalan, 70 mg/m² i.v.
-4	VM-26, 150 mg/m² i.v.
-3	TBI, 3.33 Gy, 0.08-0.1 Gy/min
-2	TBI, 3.33 Gy, 0.08-0.1 Gy/min
-1	TBI, 3.33 Gy, 0.08-0.1 Gy/min

* *For patients <2 yrs old or weighing <12 kg, the doses of cisplatin, VM-26, melphalan, and doxorubicin are calculated according to weight, assuming 1 m² = 26 kg (e.g., cisplatin, 3.5 mg/kg; doxorubicin, 1.7 mg/kg; melphalan, 5.4 and 2.7 mg/kg; VM-26, 5.8 mg/kg). Fractionated TBI is administered with a 6-MeV linear accelerator.*

Abbreviations: VM-26, teniposide; TBI, total body irradiation.

sedimentation, filtration, incubation with monoclonal antibodies, and then goat antimouse immunoglobulin-coated magnetic beads (marrows from patients 9-11 were treated with antibodies 390, 459, HSAN 1.2, BA-1, and RB21-7; marrow from patient 12 was treated with antibodies 459, BA-1, and 126-4) (11,12). Recipients of autologous marrow were given a median of 7×10^7 nucleated marrow cells per kilogram (range, $2\text{-}54 \times 10^7$ cells/kg).

Severe oral mucositis and enteritis were observed in all patients after treatment with VAMP-TBI. Total parenteral nutrition via central venous catheter was necessary after BMT for all patients because of mucositis, enteritis, and anorexia; for those surviving the first month after transplantation, the median time until parenteral nutrition was discontinued was 2 months. Skin desquamation was significant in the allogeneic BMT group but not in the ABMT group.

Four deaths occurred during the first month after transplantation among the eight patients undergoing allogeneic BMT. The causes of these early deaths, which all occurred prior to documented engraftment, were renal failure, hepatic veno-occlusive disease, disseminated aspergillosis, disseminated candidiasis. Graft-versus-host disease was not observed in any of the allogeneic marrow recipients.

Among the 12 patients receiving autologous marrow, there were no deaths in the first posttransplantation month. One patient, who failed to recover platelets even though megakaryocytes were present in the marrow, died of a cerebral hemorrhage 3 months after BMT (patient 9); and one whose marrow engrafted died 7 months posttransplantation of bacterial sepsis due to suspected child abuse (patient 8).

Early death as well as morbid toxicity appeared to be greatest in the allogeneic group. Analysis of differences between the two groups suggested that methotrexate given for prophylaxis of graft-versus-host disease was the most probable cause of the added toxicity. Methotrexate toxicity would be increased if renal clearance was impaired secondary to chemotherapy (e.g., cisplatin) and radiation. Thus, it is advisable to assess renal function before administering methotrexate, to monitor clearance and adjust dosage during treatment, and to rescue with Leucovorin as necessary.

Engraftment was defined as follows: 1) absolute neutrophil count greater than 500/mm^3 for 3 consecutive days; 2) platelet count greater than 30,000/mm^3 without transfusion; and 3) hemoglobin count greater than 8 g/dl sustained without transfusions. Engraftment occurred in all 4 patients receiving allogeneic marrow who survived more than 1 month and in 11 of 12 receiving autologous marrow.

Outcome for the 13 patients who received bone marrow before they developed progressive disease was encouraging (Fig 1). Seven are tumor-free survivors for 3+ to 40+ months after BMT; four patients relapsed (all from the ABMT group); one died secondary to toxicity (allogeneic BMT group); and one died of sepsis secondary to suspected child abuse (ABMT group). The estimated disease-free survival rate is 45%, and the actual survival rate is 56% at

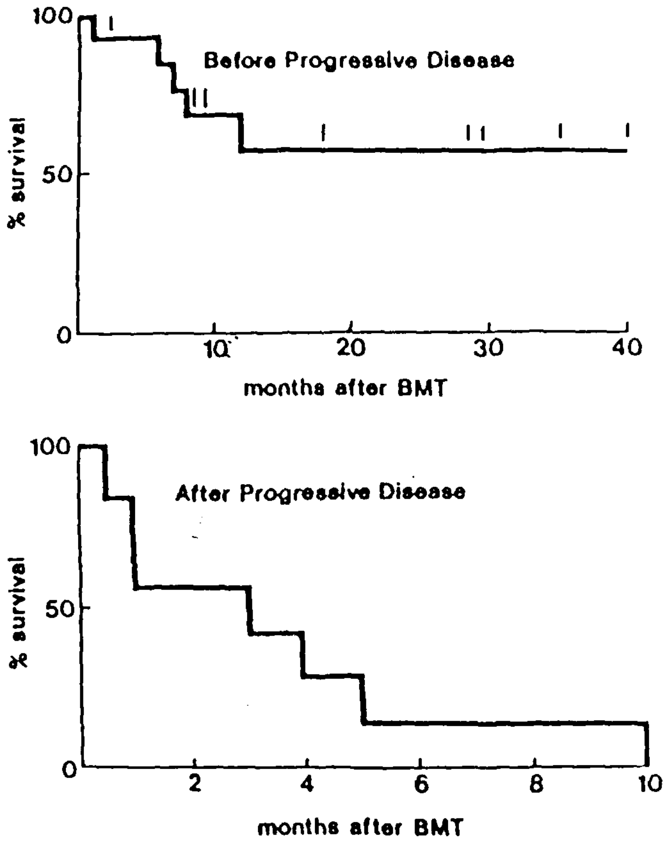

Figure 1. *Survival rates of patients who undergo BMT before or after they develop progressive disease. Initially, all patients received conventional chemotherapy (various regimens); the median times from diagnosis to BMT for groups whose transplantation occurred before and after progressive disease were 6.8 and 11 months, respectively. Tumor status at the time of BMT was as follows: among those who received BMT before progressive disease, five had a complete remission and eight had a partial remission; among those transplanted afterward, one had a complete remission, five had stable disease, and one had progressive disease. Eight patients survive who received BMT before developing progressive disease, and seven are tumor free (two recipients of allogeneic BMT and five of ABMT).*

40+ months. In contrast, all seven patients who received transplants after developing progressive disease had died 10 months after BMT; three deaths were toxicity related, one death occurred from brain hemorrhage, and three deaths followed relapses (Fig 1). These data suggest that treatment with VAMP-TBI followed by BMT improves outcome if carried out before progressive tumor growth occurs.

EX VIVO REMOVAL OF TUMOR CELLS FROM AUTOLOGOUS MARROW WITH SEDIMENTATION, FILTRATION, AND MAGNETIC IMMUNOBEADS

In our current study (CCG-321P3, which opened for patient entry October 1985), we are determining the toxicity and efficacy of aggressive induction chemotherapy followed by surgery and local irradiation (as indicated) and then by VAMP-TBI and BMT. A major objective is to perform BMT by approximately 20 weeks after diagnosis before progressive tumor growth occurs. For those requiring ABMT, we are determining if neuroblastoma cells can be removed ex vivo without impairing the ability of the marrow to restore hematopoiesis. Ex vivo purging will be discussed, since the study is too new to assess long-term efficacy.

Ex vivo purging of autologous marrow employs sedimentation, filtration, and monoclonal antibody-coated magnetic beads (Fig 2). To plan the purging procedure, the numbers of normal and tumor cells in posterior iliac crest marrow are determined 3 days before the large-scale harvest. Sufficient marrow then is obtained from the large-scale harvest to provide approximately 10^8 marrow cells per kg after ex vivo purging. Whole marrow is mixed 1:1 with 3% hetastarch and allowed to sediment, after which supernatant cells are filtered through nylon wool, washed, and mixed with magnetic beads that have been coated with a mixture of monoclonal antibodies via goat antimouse immunoglobulin. Attaching the monoclonal antibodies to the beads, which is a

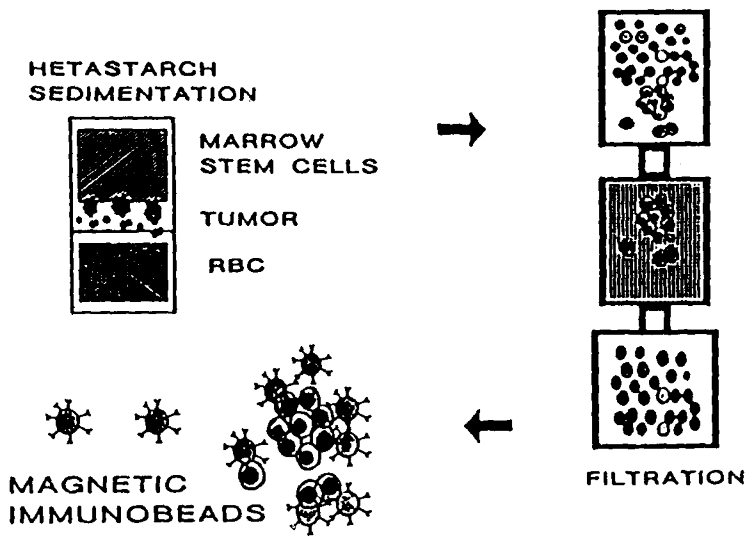

Figure 2. ***Procedure for removing tumor cells from autologous marrow by sequential sedimentation, filtration, and monoclonal antibody-magnetic bead treatment.***

modification of our previous method of first binding them to cells and then to beads (11,12), increases the speed of purging and decreases nonspecific cell loss because cell-washing steps are not required. Following one-half hour of rotation with immunobeads, tumor cells attached to the beads are removed with samarium-cobalt magnets; the immunobead depletion step is repeated if the preharvest marrow contained more than one tumor cell per 10^3 normal cells. Total cell recovery is approximately 66% after the first two steps and 50% after each magnetic immunobead step; thus, 35-40% of the initial cells are recovered after one full sequence. An aliquot of marrow (10^8 cells/kg) that is treated only by sedimentation and filtration is cryopreserved as a backup in case antibody-treated marrow does not engraft.

This procedure has removed immunohistologically detectable tumor from 17 of 17 marrows that have been treated. Approximately 50% had no detectable tumor cells after the filtration step; generally, these were the ones in which the original tumor burden was 0.5-2 tumor cells per 10^5 normal cells. However, magnetic immunobeads were necessary for purging more heavily contaminated marrows. One cycle of treatment with immunobeads was adequate when tumor-cell concentration was less than one per 10^4 normal cells, but two cycles were necessary when it was more than one per 10^3 normal cells. One marrow containing 2% and another with 1% tumor cells were successfully purged with this procedure. Data from the treatment of four representative marrows are shown in Figure 3.

Engraftment of purged marrows is evaluable for 11 patients. Marrows from the first three patients enrolled in this study were purged with a new combination of monoclonal antibodies that was highly effective for removing tumor cells (antibodies 459, BA-1, and 126-4); however, two of these marrows engrafted slowly (82 and 74 days) and one did not engraft. Either antibody 126-4 (anti-G_{D2}) or the particular combination of antibodies was the most likely cause of this complication, because poor engraftment was not a problem previously when antibodies 390, 459, BA-1, HSAN 1.2, and RB21-7 were used. Subsequently, marrows were treated with beads coated with antibodies 390, 459, BA-1, and HSAN 1.2; using a bead to total cell ratio of 1:1 and two cycles of treatment, these immunobeads can clear at least as much as 2% tumor from marrow. Engraftment has occurred in all eight patients given marrow treated with these immunobeads.

SUMMARY

Our initial study suggests that intensive chemoradiotherapy (e.g., VAMP-TBI), if administered relatively soon after diagnosis and before progressive disease develops, may improve the long-term survival rate of patients with advanced neuroblastoma. Our current study of newly diagnosed patients is testing aggressive induction chemotherapy, ex vivo purging of autologous marrow, and VAMP-TBI followed by BMT. Our objectives are to get 90% of newly

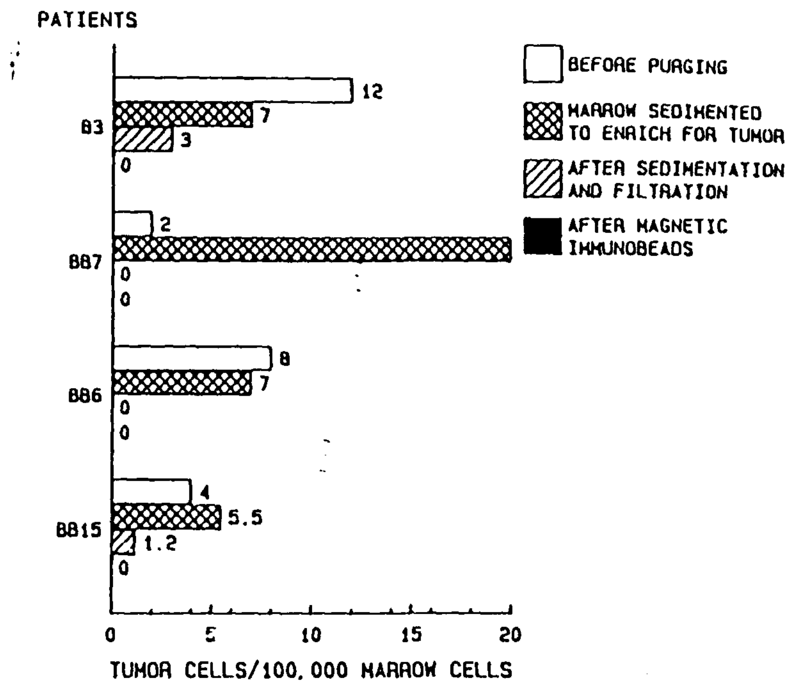

Figure 3. *Removal of neuroblastoma cells from autologous marrow by sequential sedimentation, filtration, and magnetic immunobead treatment. Marrow from four patients (B3, BB7, BB6, and BB15) was treated with one cycle of immunobeads coated with goat antimouse immunoglobulin and monoclonal antibodies 390, 459, HSAN 1.2, and BA-1. Tumor cells were identified in marrow by immunoperoxidase staining with anti-cell-surface monoclonal antibodies (mixture of antibodies 390, 459, HSAN 1.2, and 126-4) and with anti-neuron-specific enolase serum.*

diagnosed patients into the BMT phase by 20 weeks after diagnosis without progressive disease and to emerge from the BMT phase with a 90% survival rate. Because all patients are not expected to remain tumor-free following BMT, efforts are being made to identify prognostic factors for this very aggressive therapeutic approach; additional or different therapy will need to be developed for those who are likely to develop progressive disease after BMT. Collectively, these new strategies may further increase the percentage of patients who survive tumor free.

ACKNOWLEDGMENTS

This study was supported in part by U.S. Public Health Service grants CA12800, CA27678, and CA16042 from the National Cancer Institute, U.S. Department of Health and Human Services; by a grant from Concern II; and by

Naval Medical Research and Development Command Work Unit MF58.527.007.0004. The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Department of the Navy or the naval service at large.

We thank Dr. P. Marangos for providing anti-neuron-specific enolase serum, Dr. R. Reisfeld, Dr. A. Kindler-Rohrborn, and Hybritech Inc. for providing monoclonal antibodies 126-4, RB21-7, and BA-1, respectively, and Sintef for providing magnetic beads. We also thank Ms F. Wiley and nurses of the UCLA Pediatric Bone Marrow Transplantation Unit for providing excellent patient care and Ms S. Rayner, Ms A. Sullivan-Burke, and Mr. F. Hoover for excellent technical assistance.

REFERENCES

1. Young JL Jr, Miller RW. J Pediatr 1975;86:254.
2. Breslow N, McCann B. Cancer Res 1971;31:2098.
3. Hann HL, Evans AE, Siegel SE, Wong KY, Sather H, Dalton A, Hammond D, Seeger RC. Cancer Res 1985;45:2843.
4. Hayes FA, Green AA. Pediatr Ann 1983;12:366.
5. Evans AE. Cancer 1980;45:1799.
6. August CS, Serota FT, Koch PA. J Clin Oncol 1984;2:609.
7. Seeger RC, Wells J, Lenarsky C, Feig SA, Selch M, Moss TJ, Ugelstad J, Reynolds CP. J Cell Biochem 1986;10A:215.
8. Moss TJ, Seeger RC, Kindler-Rohrborn A, Marangos PJ, Rajewsky MF, Reynolds CP. Prog Clin Biol Res 1985;175:367.
9. Bjork R, Marangos P, Moss T, Krailo M, Hammond D, Seeger R. Proc Am Assoc Cancer Res 1986;27:203.
10. Wells JR, Sullivan A, Cline MJ. Cryobiology 1979;16:201.
11. Seeger RC, Reynolds CP, Vo DD, Ugelstad J, Wells J. Prog Clin Biol Res 1985;175:443.
12. Reynolds CP, Seeger RC, Vo DD, Black AT, Wells J, Ugelstad J. Cancer Res 1986;46:5882.