16.

BONE MARROW TRANSPLANTATION (BMT) IN FIRST REMISSION FOR CHILDREN AND ADOLESCENTS WITH ACUTE NON-LYMPHOCYTIC LEUKEMIA (ANLL). N. Ramsay, M. Nesbit, T. Kim, W. Woods, P. McGlave, W. Krivit, D. Hurd, J. Kersey for the University of Minnesota BMT Team, Minneapolis, Minnesota, U.S.A.

A study of BMT for patients (pts) with ANLL in first remission was initiated at the University of Minnesota in 1976. Nineteen pts., < 19 years received a BMT from a sibling matched at the major histocompatibility complex between 1976 and 1982. There were 8 males and 11 females, ages 1-17 years (median 11.5 years). All pts. had completed induction therapy and were in complete remission at the time of BMT. Pts. were transplanted from 2-8 months (median=4 months) following diagnosis. The preparative regimen for transplantation was cyclophosphamide 60 mg/kg/day x 2 days followed by total body irradiation (750 rad at 26 rad/minute). Pts. received from 1.5-8.2 x 10^8 nucleated donor cells/kg (median=3.7 x 10^8). Following BMT, methotrexate (13 pts.) or methotrexate, anti-thymocyte globulin and prednisone (6 pts.) was given for graft-versus-host disease (GVHD) prophylaxis. GVHD occurred in 9 pts. Seven pts. had acute GVHD; of these, two died prior to 100 days after BMT, two pts. had progression to chronic GVHD and two pts. had only chronic GVHD. Three pts. have relapsed at 6, 25 and 27 months. Five pts. have died (relapse-2; infection-2; GVHD-1) and 14 pts. are currently alive from 1 month to 64 months (median 25 months). Kaplan-Meier analysis reveals a predicted disease-free survival of 77% at 1 and 2 years and 61% at 3 years. Recipient age, donor age, recipient sex, donor sex, sex match, presence of GVHD and time from diagnosis to transplant did not significantly correlate with outcome. By life table analysis, the absence of GVHD, however, (n=10) was associated with a better survival (90%), when compared with the survival of pts. (n=9) with GVHD (41%). BMT appears to be an effective treatment for ANLL in first remission.

17.

BONE MARROW TRANSPLANTATION (BMT) FOR CHILDREN WITH STAGE IV NEUROBLASTOMA: A PILOT STUDY AT THE UNIVERSITY OF MINNESOTA. N. Ramsay, W. Woods, W. Krivit, T. Kim, J. Kersey, M. Nesbit for the University of Minnesota BMT Team, Minneapolis, MN, USA.

Long term survival for patients (pts.) with Stage IV neuroblastoma treated with conventional chemotherapy remains very poor. A program of early BMT for these pts. was instituted at the University of Minnesota in 1981 to improve survival. An attempt was made to render the pts. disease-free as soon as possible after initial diagnosis prior to BMT, using chemotherapy, surgery, and radiation therapy. The characteristics of the three pts. transplanted are outlined in the following table:

	Patient #1	Patient #2	Patient #3
Age/Sex	4yr.4mo./male	4yr.9mo./male	4yr.10mo./male
Stage/Extent of Disease	Stage IV Abd mass Bone marrow	Stage IV Retroperitoneal mass, Bone marrow	Stage IV Abd mass, bone Bone marrow
Time from Dx to BMT/Type	7 mos. Autologous	4 mos. Allogeneic	11 mos. Allogeneic

At initial diagnosis, all pts. had received multiagent chemotherapy, followed by delayed surgical removal of the primary. Two pts. received post-operative abdominal radiation. Two pts. were in complete remission and 1 pt. was in partial remission at the time of BMT. All pts. received a preparative regimen consisting of vincristine 1.5 mg/m^2 on day -9, cyclophosphamide 60 mg/kg days -8 and -7, melphalan 180 mg/m^2 on day -2 and 750 rad total body irradiation day -1. All three pts. engrafted and two pts. are surviving disease-free at 4.5 (allogeneic) and 6 (autologous) months after BMT. One pt. died of CMV pneumonitis. These results suggest that BMT, which allows the use of high dose chemotherapy and radiation therapy may be a useful initial therapeutic modality in Stage IV neuroblastoma.

18.

PREVENTION AND TREATMENT OF ACUTE GRAFT-VERSUS-HOST DISEASE - BALTIMORE EXPERIENCE Tutschka, PJ. Marrow Transplant Program, Oncology Center, The Johns Hopkins University School of Medicine, 600 N. Wolfe Street, Baltimore, Maryland 21205 USA.

The strength and kinetics of acute GVHD are thought to be a direct function of the degree of histoincompatibility between donor and recipient. To prevent GVHD, donor and host are commonly matched at the major histocompatibility complex, and are given low doses of cytotoxic agents (Methotrexate or Cyclophosphamide) for prolonged periods after grafting. Despite that, acute GVHD of clinical significance was seen in 57.5% of our patients with a 68% GVHD related mortality. Acute GVHD of clinical significance (grade 2 or above) responded poorly to therapy. If anti-thymocyte globulin (ATG) was given, 54% of patients responded but only 9% survived. If prednisone was given in various dose regimens, (2.5, 5, 10, 20 mg/kg/day) between 33% and 59% responded, but higher doses (10 and 20 mg/kg) were associated with serious toxicities, the best regimen being 2.5 mg/kg/day (59% response, 54% survival). Attempts to improve the prophylaxis of GVHD by incubating donor bone marrow with highly purified ATG have so far not been successful. Of 10 patients treated with marrow incubated with heterologous ATG, five developed clinically significant GVHD. It is hoped that incubation with monoclonal antibodies will improve this result. Cyclosporin A (CsA), a new immunosuppressant that prevents GVHD in animal models by impairing the generation of killer cells yet permitting the generation of suppressor cells, was used as single agent post-grafting to prevent GVHD. In a pilot trial of 22 patients, GVHD occurred in 36% with an overall GVHD associated mortality of only 14%. However, renal failure was encountered frequently, giving CsA a narrow therapeutic range. To improve this range, studies are in progress which combine CsA in lower doses with other immunosuppressive agents.

19.

AUTOLOGOUS BONE MARROW TRANSPLANTATION; AN OVERVIEW WITH EMPHASIS ON THE PROBLEM OF TUMOR CELL CONTAMINATION OF REMISSION MARROW. H.Kaizer[1], R.K.Stuart[1], O.M.Colvin[1], R.Levy[2], and G.W.Santos[1]. The Johns Hopkins Oncology Center[1], Baltimore, Maryland 21205, and Stanford University School of Medicine[2], Palo Alto, California 94305, U.S.A.

Based on currently available information, leukemia and lymphoma are the malignancies most likely to benefit from autologous bone marrow transplantation (ABMT). Successful application of this approach, particularly in acute leukemia, depends on the development of methods to eliminate all clonogenic tumor from the remission marrow to be used for transplantation. Animal model studies have shown that it is feasible to eliminate all clonogenic tumor from marrow-tumor cell mixtures by in vitro pharmacologic or immunologic treatment. We are currently conducting two clinical trials utilizing in vitro treatment of autologous marrow from patients with T-cell leukemia and lymphoma with a monoclonal anti-T-cell antibody and 4-hydroperoxycyclophsphamide (4HC) treatment of remission marrow in patients with non-T-cell ALL and ANLL. Only 1 out of 24 patients treated on these protocols has had a transplant-related fatality. The in vitro treatment of marrow has not significantly affected the pattern or rate of hematologic reconstitution. One surprising result of these studies is the observation that all assayable granulocyte and macrophage colony forming cells may be eliminated with 4HC treatment without detectable effect on hematologic reconstitution. It is too early to assess the therapeutic efficacy in either of these two trials.

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20.

PHARMACOLOGICAL MEAN TO ELIMINATE TUMOR CELLS FROM BONE MARROW WITH A VIEW TO AUTOLOGOUS BONE MARROW GRAFT - *P. Hervé[1], E. Tamaya[1], E. Plauvien[2], A. Nain[2]* - *1. Regional Blood Transfusion Center -2. Pediatrics oncology department* - *Besançon* - *France* -

The danger of regrafting residual leukemic or tumor cells remains the first argument against ABMT.
A new approach is that of ABMT with in vitro elimination of residual malignant cells from the marrow harvested.
We have studied one metabolite of cyclophosphamide (N. BROCK, Asta Lab.) : the 4-Hydroperoxycyclophosphamide (4-HC). We have tested this analogue of Cy with marrow samples taken in 12 acute leukemias patients in remission (table 1).

4-HC (µM)	control	10	20	40	60
CFU-GM	124,5±25	94,5±11	27,3±15	5,4±2	0
recovery	-	75	22	4,3	0

The bone marrow of nine children (6 acute leukemias, 2 NH lymphomas, 1 solid tumor) have been harvested and treated by this pharmacological mean. Just after collection the marrow was concentrated either with Haemonetics M30 or more recently with IBM 2991 blood cell processor. The marrow cells of each of them have been incubated with the dose of 4-HC previously determinated in in vitro assays. According to each

patient the dose of 4-HC has varied from 20 uM to 60 uM. At the end of incubation the marrow cells were washed one time and committed stem cell assays wen done just before freezing step. The isolation of bone marrow mononuclear cells using Ficoll-metrizoate with IBM 2991 appears as the method the best adapted for an in vitro treatment. Two patients have been grafted with b.m. processed in this way (the CFU-GM inhibition was significant = 8 and 20 % of recovery), nevertheless the engraftment time was not significantly delayed.

21.

MONOCLONAL ANTIBODIES ATTACHED TO MICROSPHERES CONTAINING MAGNETIC COMPOUNDS, USED TO REMOVE NEUROBLASTOMA CELLS FROM BONE MARROW TAKEN FOR AUTOLOGOUS TRANSPLANTATION.

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In stage 4 neuroblastoma (Evans' Classification), where cells can metastasise to bone marrow, the use of high dose chemotherapy, with autologous marrow transplantation as a therapeutic regime, carries the risk of reinfusing untreated tumour cells to a patient. Using monoclonal antibodies chosen for their binding to neuroblastoma and not normal bone marrow, we have investigated different approaches to the selective removal of tumour cells from autograft marrow. To date the optimum system involves the use of polystyrene microspheres (2μ diam.) containing 27% wt/wt magnetite and coated with affinity purified goat anti-mouse Ig. Beads coated with anti-mouse Ig will bind to cells incubated with mouse monoclonal antibodies directed against cell surface antigens. When placed in a magnetic field cells binding beads are drawn to the side of the tube leaving unlabelled cells in suspension. To initially model the removal of tumour from bone marrow, the human neuroblastoma cell line CHP 100 was added to different proportions of the leukaemic line Nalm-6 (ratios 1:1 to 1:10). To account for the antigenic heterogeneity observed in neuroblastoma a panel of monoclonal antibodies was added to the mixture. Following washing, cells were incubated with goat anti-mouse Ig coated beads and placed in a magnetic field. 97-99% of neuroblastoma cells could be removed from the suspension without non-specific trapping of Nalm-6. Similar results have been obtained titrating CHP 100 cells into normal bone marrow. Our current experiments suggest the methodology can be scaled up to separate malignant cells from 5 x 10^9 nucleated bone marrow cells.

22.

PURGING NEUROBLASTOMA (NB) CELLS FROM BONE MARROW (BM)

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Various in vitro methods have been used to purge the BM of malignant cells remaining after systemic treatment. We investigated the effect of 6-hydroxydopamine (6-OHDA) on neuroblastoma cells and normal BM. Five human NB cell lines were used. 6-OHDA at 20 ug/ml was found to be most effective and the effect was enhanced with Ascorbic Acid (C) at 100 ug/ml. In vitro incubation of 6-OHDA+C for one hour was 100% cytotoxic at cell concentrations below 7 NB cells/mm^3; at 7-12 NB cells/mm^3 only 0-2% survived. At concentrations of 20 ug/ml 6-OHDA and 100 ug/ml C there were no decreases in CFU-C of various BM's tested (14 samples) while concentrations of 6-OHDA greater than 40 ug/ml were toxic to BM CFU-C. 6-OHDA at 20 ug/ml does inhibit the BFU-e of BM, however, there is no correlation of BFU-e inhibition and subsequent ability for BM engraftment. Two-fold augmentation of specific NB cell kill in vitro by 6-OHDA-C plus 0.12 ug/ml Tropolone, a catechol orthomethyl transferase inhibitor was observed.

Two patients with disseminated NB and residual BM involvement had their BM purged with 6-OHDA+C. The BM's were reinfused after high dose Melphalan, dianhydrogalactitol and total body irradiation. Mild transient hypertension in one patient and diarrhea and mucositis in both patients were noted. Hematopoetic recovery and tumor regression were noted but the follow-up is short at this time and will be discussed. In vitro purging of tumor cells has an important role in the success of autologous stem cell transplants for patients with disseminated neuroblastoma.

23.

HIGH. DOSE CYTOREDUCTIVE REGIMEN FOLLOWED BY AUTOLOGOUS BONE MARROW TRANSPLANTATION (A.B.M.T.) IN CHILDREN WITH ACUTE LEUKEMIA.

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We have treated two children with acute lymphoblastic leukemia (A.L.L.) in relapse or in second remission, and three children with acute myelogenous leukemia (A.M.L.) in complete remission (C.R.) with high dose cytoreductive regimen followed by A.B.M.T. The T.A.C.C. regimen has been used in three children with A.M.L. in remission and in one child with A.L.L. in second relapse. The T.A.C.C. regimen consited of 6- Thioguanine = 400 mg/m2 daily by mouth on day 2 to 5, Cytosine-Arabinoside = 400 mg/m2 daily I.V. on day 2 to 5, C.C.N.U. = 400 mg/m2 by mouth on day 1 and Cyclophosphamide = 50 mg/kg I.V. on day 2 to 5. The A.B.M.T. is transfused two days after the last dose of Cyclophosphamide.

For one child with A.L.L. in relapse, this chemotherapy has failed to obtain a complete remission. Two children with A.M.L. grafted in first C.R., remain in remission for 16 months + and 2 months + without any maintenance treatment. One child grafted in second C.R. of A.M.L. relapsed six months after A.B.M.T., and he went into third C.R. after high dose melphalan (H.D.M. = 200 mg/m2) followed by bone marrow harvested two months before ; actually he is alive and well in C.R. for five months after the second A.B.M.T.

For one child with A.L.L. in second C.R., we used Cyclophosphamide (60 mg/kg/d. x 2 days) and T.B.I. (grays) followed by A.B.M.T. He remains in C.R. for fifteen months + without any maintenance treatment.

24.

THERAPY OF DISSEMINATED NEUROBLASTOMA WITH INTENSIVE THERAPY AND AUTOLOGOUS STEM CELL RESCUE.

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Autologous stem cell transplantation (ASCT) using cryopreserved bone marrow (BM) can be used to circumvent the hematopoietic toxicity of high dose chemotherapy. Two patients with extensive neuroblastoma were managed with 4-6 courses of conventional chemotherapy (N4SE); the patients had residual disease but the BM was not involved. The BM was then cryopreserved and patients were given high dose chemotherapy with melphalan (L-PAM) and dianhydrogalactitol (DAG) at a total dose of 180 mg/M^2 each over 3 days. Patients also received local radiation therapy to bulky disease. The cryopreserved BM was reinfused 48 hrs later. Both patients had good hematopoietic recovery within 17-28 days, and remain disease free 5 months later. Two other patients with disseminated neuroblastoma and BM involvement after conventional chemotherapy had their BM withdrawn and purged with 6-hydroxy-dopamine (6-OHDA) at 20 μg/ml and ascorbic acid (C) at 100 μg/ml for 1 hr. This combination is known to be a selective killer of neuroblastoma cell lines, without causing a decrease in BM CFU-c activity. Both patients then received L-PAM+DAG with total body irradiation (450 rads). Two days later, cryopreserved, purged BM was reinfused, mild transient hypertension (6-OHDA related) was noted. Hematopoietic recovery and tumor response was noted. The follow-up is too short to assess long term benefit. The toxicity of the above treatment includes nadir sepsis, mucositis, and diarrhea. Supportive care includes total parental nutrition, antibiotics and irradiated blood products to prevent graft versus host disease. We feel that early intensive chemotherapy with ASCT rescue has a place in therapy of disseminated neuroblastoma.

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25.

AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN THE THERAPY OF ADVANCED MALIGNANT TUMORS OF CHILDREN AND ADOLESCENTS.

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