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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22 patients (pts) less than 20 years old with advanced malignant tumor (13 abdominal non-Hodgkin's lymphomas: NHL, 3 yolk sac tumors: YST, 3 Ewing's sarcomas: ES, 3 neuroblastomas: NB) received high dose chemotherapy (vincristine 2 mg/m^2, top dose 2 mg iv and adriamycine 60 mg/m^2 iv on day -7 and cyclophosphamide 45 mg/kg iv on days -6 to -3), total body irradiation (600 rad on day -1) and ABMT (day 0). The supportive care included reverse isolation, immunoglobulin 0,4 g/kg iv q 2 weeks, cotrimoxazole and digoxin per os and substitution of erythrocytes and thrombocytes. Granulocytes were given to febrile pts not responding to systemic antibiotic treatment. Hematopoiesis recovered in 20/21 evaluable pts within 3 - 12 weeks. In one pt a severe thrombocytopenia persisted. Disease-free survival after ABMT (number of pts and months): NHL stage III 5/7 (35+ 23+ 20+ 5+ 5+), NHL stage IV 3/6 (7+ 2+ 1+), YST 1/3 (26+) ES 0/3, NB 0/3. One patient has died due to complications of therapy. Tumor was the cause of death in all other pts. In the three surviving pts with NHL stage IV the bone marrow was treated in vitro with a monoclonal antibody (anti Y 29/55) and complement to eliminate NHL-cells before ABMT. We think that ABMT will help to improve the so far poor prognosis of pts with advanced abdominal NHL.

26.

THE EFFECTS OF HIGH DOSE POLYCHEMOTHERAPY WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN 18 CHILDREN WITH RELAPSED LYMPHOMA. O. Hartmann(1), F. Pein(1), T. Philip(2), P. Biron(2), J. Lemerle(1).
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The prognosis of the Non-Hodgkin Malignant Lymphoma in children is particularly poor after the first relapse. In an attempt to improve it, 18 children have been treated with high dose polychemotherapy plus ABMT. 11 were B lymphomas and 7 were T lymphomas. For the majority of the patients (13/18) the bone marrow has been collected and frozen during the first remission. Before using this high dose chemotherapy the relapse was treated by different conventional chemotherapies in order to use this regimen on patients with "residual disease". Among the 18 patients 2 were in second CR, 7 were in second PR, 9 had progressive disease at the time the high dose regimen was administered. This polychemotherapy was the combination of BCNU 200 mg/m2 Days 1,2,3 ; Cyclophosphamide 1600 mg/m2 Days 2, 3,4,5 ; Cytosine-Arabinoside 200 mg/m2 Days 2,3,4,5 ; 6 Thio-Guanine Days 2,3,4,5. The ABMT was performed at day 6. The results of this treatment were : C.R. 13 ; P.R. 3. Progressive Disease 2 ; In terms of survival : 5 pts (4 B Lymphomas, 1 T Lymhoma) are alive and NED et 5+, 11+, 12+, 14+, 16+ months from ABMT ; 1 pt is alive with progressive disease at 4 months from ABMT and 12 pts are dead. 9 of them of the disease.

The toxicity of this regimen was high but tolerable. 2 deaths were observed after hematological reconstitution and related to prolonged immunodepression.

These results are encouraging and the role of this treatment will be discussed depending on the "T" or "B" subtype of the lymphoma.

27.

NON RECOVERY FROM TOXIC APLASIA ASSOCIATED WITH HIGH DOSE METHOTREXATE, AFTER AUTOLOGOUS BONE MARROW GRAFT. T. Philip, P. Biron, L. Dutou, L.Holzapfel, J.B.Cotton, M.Brunat-Mentigny, Oncology Pediatric Unit. Centre Léon Bérard, Lyon, France

We report one case of severe methotrexate (MTX) toxicity who died after 27 days of extreme granulocytopenia and 16 days after autologous bone marrow graft used as a rescue for the toxic aplasia.

Christophe was a 15 years old boy with a Murphy stage IV NHML Burkitt type. Treatment was the French Cooperative LMB 80 in which high dose MTX is used extensively. The drug was well tolerated with no emesis at all. MTX enzymatic dosage at 36 hours was normal and the patient was discharged. The boy came back on day 7 with clinical (Hyperthermia, Toxidermia, continuous diahrrea and abdominal pain) and biological (extreme leucothrombocytopenia, increased BUN and creatinine) signs evocating MTX toxicity. 11 days after the beginning of the extreme granulocytopenia 5 out of the 8 bad prognosis factors of this accident were present. Autologous bone marrow graft was then decided. 1.3 10^8 nucleated cells per kg and 21.10^4 GMCFU c/kg were reinjected. Despite intensive supportive care Aspergillus infection occured and the boy finally died 16 days after ABMG and on day 27 of the aplasia. No sign at all of bone marrow activity was found on WBC, and postmortem bone marrow smears and biopsy.

In conclusion ABMG has not been usefull as a supportive care in this case. One can assumed that an immunological mechanism destroyed first the patient bone marrow cells and secondly the reinjected stem cells. Plasmapheresis just before ABMG could be discussed for future cases.

28.

HAEMOPOIETIC STEM CELLS IN NON CRYOPRESERVED AUTOLOGOUS BONE MARROW TRANSPLANT. J.Ninane, M.J.Latour, G.Cornu, M.Symann, Cliniques Universitaires St Luc & Ludwig Institute for Cancer Research, UCL, 1200 Brussels, Belgium.

Six children aged 18 months to 4 years were given a high dose Melphalan (150-180mg/m^2) as a late intensification for solid tumours in complete or good partial remission. High dose melphalan was followed by an autologous bone marrow transplant (ABMT) kept at 4°C, infused 12 to 24 hours later.

Granulocyte-macrophage progenitor cell (CFUc) and erythroid progenitor cells (BFU-E, CFU-E) were assayed upon bone marrow harvesting and bone marrow reinfusion in order to test haemopoeitic stem cell viability. Furthermore the number of CFUc/kg reinfused was compared to the number of days necessary to obtain an absolute neutrophil count of 0.5.10^9/L. The number of bone marow haemopoietic progenitors recovered was higher than 74 % for CFU-c, higher than 61 % for BFU-E and higher than 83 % for CFU-E. The number of CFUc reinfused per kg varied from 20.10^3 to 22.5 .10^4; there was no direct correlation between the number of CFUc reinfused and the number of days to recover 0.5.10^9 neutrophils/L.

For this small series of patients we conclude that most of cryopreserved bone marrow stem cells are viable for up to 24 hours at 4°C. There seems to be no direct correlation between the number of CFUc reinfused per kg weight and the number of days to recover 0.5.10^9 neutrophils/L.

29.

ROLE OF ^{192}IRIDIUM AFTER-LOADING CURIETHERAPY IN THE TREATMENT OF PEDIATRIC MALIGNANCIES
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- Fifty-three children were treated by Iridium after-loading curietherapy as local treatment,in combination with chemotherapy in forty-seven patients.
- The most common sitesof the primary tumorWERE pelvic-perineal (55 %) and cervico-facial (40 %).
- The pathologic diagnosis was rhabdomyosarcoma in 72 %, yolk sac tumor in 12 %, undifferentiated embryonal sarcoma in 2 cases, malignant mesenchymoma in 2 cases, clear cell carcinoma in 2 cases, fibrosarcoma 1 case, salivary gland epithelioma 1 case.
- Curietherapy was used in 45 previously untreated patients : local control was obtained in 40/45 cases, the two year survival was 82 %.

  For 11 previously treated patients local control was obtained in 9/11 cases, the two year survival was 54 %.

  7 children of 29(at greater than 3 year follow up) HAD sequellae.
- Our results make a strong argument for an important place of ^{192}I afterloading curietherapy in the treatment of pediatric malignancies. However, a rigorous technique and appropriate dosimetry are absolutely necessary in order to obtain maximum local control, with hopefully a lower incidence of sequellae.
