BONE MARROW TRANSPLANTATION: CURRENT CONTROVERSIES

Organizers: Robert Peter Gale and Richard Champlin

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Bone Marrow Transplantation: Current Controversies

Transplantation in Nuclear Accidents

K 001 MEDICAL RESPONSE TO NUCLEAR ACCIDENTS, Robert Peter. Gale, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024

Acute exposure to high doses of total boby radiation results in severe damage to somatic tissues. The bone marrow is one important target. At doses < 2 Gy, effects are modest. At doses of 2 -5 Gy there is severe bone marrow depression but spntaneous recovery is likely. Doses > 5 Gy result in severe bone marrow failure and are associated with a substantial risk of intercurrent death from bleeding and/or infection. Several factors influence the likelihood of reversibility of radiation induced bone marrow damage including dose, dose rate, fractionation, uniformity of exposure, type of radiation and energy, and others. Medical interventions can play an important role in increasing the probability of bone marrow recovery and of survival following exposure to total body radiation. At low doses, supportive measures including platelet transfusions, protected environments and prophylactic or therapeutic antimicrobial or antiviral drugs, are important. At higher doses, it is reasonable to consider interventions designed to accelerate the rate of bone marrow recovery. Presently this would involve use of molecularly cloned hematopoietic growth factors such as GM-CSF or Multi-CSF. Experience with these factors in the recent accident in Brazil indicate that this approach can result in rapid increases in granulocytes. Additional data including studies in animals are needed to optimize this approach. At the highest doses of total body radiation it is necessary to consider transplantation of hematopoietic stem cell. This approach is complex and requires careful evaluation. For example, it is possible that transplant candidates may require additional immune suppression to achieve sustained engrasftment. It is also uncertain whether sustained engraftment is required for a beneficial effect. The role of T cell depletion also needs to be defined. Data from the Chernobyl accident illustrate some of the complexities in evaluating the efficacy of therapeutic interventions under accident conditions. Using a combination of these medical interventions it may be possible to rescue a substantial proportion of victims of radiation or nuclear accidents.

K 002 BONE MARROW TRANSPLANTATION FOR RADIATION ACCIDENTS: ANIMAL MODELS, Huibert M. Vriesendorp, The Johns Hopkins Oncology Center, Baltimore, Maryland 21205

The prerequisite of animal models for the analysis of treatment options for human radiation accident victims is that they are predictive of the human response. Three different groups of characteristics are of importance in selecting a model 1. Hemopoietic system. 2. Total body irradiation (TBI). 3. Treatment.

1. The animal hemopoietic system should confirm to the human system in having a low hemopoietic stem cell (HSC) concentration (i.e. $\pm 10^{-5}$) and a high T lymphocyte concentration (i.e. ± 0.25) in bone marrow cells. This can only be achieved in larger animals (> 10 Kg) for HSC and only in primates for T lymphocytes. In non primate models, human bone marrow conditions can be imitated by dilution for HSC and addition of T lymphocytes.
2. TBI dose distribution is going to be inhomogenous in accidently irradiated men. This can be approximated only in large volume animal species. Quick dosimetric methods need to be developed that can be applied to accident situations. New dosimetric possibilities to evaluate dose inhomogeneities include apoptosis in hair follicles and serum diamine oxydase determinations. Part of the radiation received in an accident will be high dose rate, acute; part of the radiation will be low, decreasing dose rate. The later could be modeled with intravenous infusions with radiolabelled immunoglobulins that are also of interest as therapeutic agents in other situations.
3. Treatment needs to be evaluated at different dose levels for the bone marrow syndrome, gastrointestinal syndrome and combined injury (BM + GI + Burns). Human type intensive care should be possible in the selected animal model.

The dog model appears to offer most of the characteristics needed. Allogeneic bone marrow transplantation can offer live saving treatment in this model after LD 100 TBI by providing a temporary (± 3 weeks) graft. This corresponds to the situation observed in surviving human accident victims treated with BMT. Recombinant hemopoietic growth factors (such as CSF-GM and IL-3) appear to cross species barriers to some degree and can be evaluated in the dog model as well. Gastrointestinal decontamination also appears to increase the radiation resistance of the dog.

Bone Marrow Transplantation: Current Controversies

Acute Myelogenous Leukemia

K 003 NEW PREPARATIVE REGIMENS IN ACUTE NONLYMPHOBLASTIC LEUKEMIA (ANL), Frederick R. Appelbaum, Christopher Badger, Irwin Bernstein, C. Dean Buckner, Reginald Clift, Roger Hill, Finn Petersen, Rainer Storb, E. Donnall Thomas, Fred Hutchinson Cancer Research Center and Univesity of Washington School of Medicine, Seattle, WA 98104.

The major problem of marrow transplantation for ANL is the non-existence of a preparative regimen with adequate anti-tumor and immunosuppressive qualities with tolerable toxicity. The most common regimen used (cyclophosphamide [CY] plus 12-15.75 Gy fractionated total body irradiation [TBI]) is not sufficiently tumoricidal as evidenced by relapse rates of 60% for transplantation in resistant relapse, and 30%, 30%, and 25% for transplant in second remission, first untreated relapse, and first remission respectively. Inadequate immunosuppression is evidenced by the 5-15% graft rejection rate in mismatched transplants and 25% rejection rate in matched recipients of T depleted marrow. Further, these regimens are associated with a 10% incidence of life-threatening or fatal toxicities directly related to the preparative regimen. More recently investigators have reported encouraging, albeit preliminary, results using combinations of busulfan plus cyclophosphamide or VP-16 plus TBI. Efforts in Seattle to improve on these results have included Phase I and II studies on the uses of combination chemotherapy with TBI and attempts at optimizing the TBI itself. Busulfan (2.2 mg/kg/day) for 4 days and CY (35 mg/kg/day) for 2 days can be combined with 12 Gy of TBI with relative safety. We found the maximum tolerated dose of TBI combined with CY (60 mg/kg/day x 2) to be 16 Gy if given as 200 cGy BID or 14.4 Gy if given as 110 cGy TID. Newer approaches under study include the use of antibody-isotope conjugates to deliver the radiotherapy more specifically to tumors and to spare normal organs. Another approach is to take advantage of the fact that marrow is in contiguity to bone and to target beta-emitting radioisotopes to bone and thereby also irradiate the marrow relatively specifically.

K 004 AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ACUTE MYELOID LEUKAEMIA, Alan K Burnett, Department of Haematology. Royal Infirmary, Castle Street, Glasgow, G4 OSF, UK.

Based on the considerable evidence that myeloablative chemoradiotherapy with allogeneic marrow transplant resulted in a low chance of relapse if undertaken in first complete remission, much interest, particularly in European Centres has been taken in administering similar chemoradiotherapy protocols or alternatively high dose polychemotherapy, followed by autologous marrow infusion as consolidation treatment for AML. The theoretical disadvantages of such an approach is that the cures achieved by allograft may in part be due to an "allogeneic effect" with or without accompanying GVHD. Syngeneic results, and to a lesser extent the correlation of severity of GVHD to relapse rate and the possibility of increased rates of relapse following T depletion of the allograft, all suggest that ablative chemoradiotherapy alone may only eradicate disease in a proportion of patients. Contamination of the graft with residual leukaemia cells may also increase the risk of relapse. The advantages of a predicted minimal procedural morbidity and mortality and its application to older patients and therefore a higher proportion of those with AML may outweigh the disadvantages.

A number of approaches to myeloablation have been investigated and will be reviewed including multiple drug chemotherapy: high dose Melphalan, single and double autografts as well as conventional cyclophosphamide and TBI. Overall projected survivals are 40-50% with procedural mortality around 5%. In addition, some groups have attempted ex-vivo purging (usually with cyclophosphamide derivatives) without, as yet, demonstrating additional benefit.

Interpretation of these data is complicated by the fact that there was on average 5-6 months pre-autograft delay, which for some patients means a reduced risk of relapse.

Data is presented on 172 patients from European Centres who have had a minimum follow-up of 2 years post-ABMT. Analysis of factors which may determine outcome of autograft will be analysed in these patients, which may assist in the design of prospective controlled trials to determine the value of autograft in the management of AML.

Bone Marrow Transplantation: Current Controversies

K 005 BONE MARROW TRANSPLANTATION (BMT) FOR ACUTE MYELOGENOUS LEUKEMIA, Richard Champlin, University of California, Center for Health Sciences, Los Angeles, CA 90024.

Within the last decade bone marrow transplantation has emerged as an effective treatment for AML. Allogeneic BMT from HLA-identical donors is the preferred treatment for patients less than 45-50 years of age as the initial treatment of leukemia in second or later remission. Selected patients with preleukemia and therapy related AML may benefit from BMT as initial therapy. There continues to be controversy regarding which patients should receive BMT in first remission vs chemotherapy treatment with BMT as treatment for relapse. It has not yet been possible to define patients or disease related prognostic factors to assist in this treatment decision. Most patients have been prepared with cyclophosphamide and total body irradiation (TBI). Alternative preparatory regimens which have reported similar results, including combinations of cytarabine/TBI and busulfan/cytoxan, will be discussed. There is controversy regarding optimal therapy for patients who lack an HLA-identical sibling; possible approaches include HLA-mismatched related transplants, unrelated histocompatible transplants and autologous transplants. Recent results will be reviewed.

K 006 ANIMAL MODELS OF BONE MARROW TRANSPLANTATION FOR ACUTE MYELOCYTIC LEUKEMIA
Anton Hagenbeek, Radiobiological Institute TNO, Rijswijk, The Netherlands.

In general, for leukemias as well as other tumors, the statement holds that a particular animal model should be chosen based upon the specific question to be answered. Apart from investigations in "spontaneously" arising rodent leukemias, the majority of experimental studies are performed in transplantable leukemias. Two important prerequisites for evaluating the efficacy of a given treatment are (a) a reproducible growth pattern upon injection of leukemic cells and (b) a linear relationship between the number of inoculated leukemic cells and the survival time.

Two major groups of transplantable rodent leukemias are recognized, i.e. the fast growing leukemias (e.g. L1210) which are suitable to study the cycle- or phase specificity of (new) drugs and the slowly growing leukemias, which from a kinetic point of view come close to the human counterpart.

From this latter group of models the Brown Norway rat acute myelocytic leukemia (BNML) was chosen as a model to study the biology of leukemia growth and its response to a variety of treatment modalities. The BNML has striking similarities with human AML, i.e. a slow growth rate, a severe suppression of normal hemopoiesis, "AML-like" response to treatment and the absence of specific leukemia-associated antigens. Normal pluripotent hemopoietic stem cells can be discriminated from in vivo clonogenic leukemic cells by modified spleen colony assays. During the past years the BNML has served as a preclinical model to focus on detection and treatment of minimal residual disease (MRD) in acute leukemia.

A number of bone marrow transplantation (BMT) related studies in the BNML will be presented, such as (1) developing effective conditioning regimens prior to BMT (fractionated versus flash total body irradiation; combination with high-dose chemotherapy), (2) methods to detect and eliminate leukemic cells from autologous marrow grafts (biophysical, immunological and pharmacological methods), (3) lodging and regrowth of leukemic cells after infusion with the autologous graft including computer simulations in the area of MRD, (4) late effects of supralethal chemo-radiotherapy followed by BMT and (5) the relationship between conditioning, acute graft-versus-host disease and the occurence of idiopathic interstitial pneumonitis.

Finally the results will be discussed with emphasis to their relevance for bone marrow transplantation in human AML.

K 007 CURRENT STATUS OF CHEMOTHERAPY IN THE MANAGEMENT OF PREVIOUSLY UNTREATED ADULTS WITH DE NOVO ACUTE MYELOGENOUS LEUKEMIA (AML). Robert J. Mayer, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

Complete remission (CR) should now be expected to occur in the majority of previously untreated adults having de novo AML. Patients under age 60 enter CR in 75% of the cases and even individuals older than age 60 experience a complete response approximately 50% of the time. CR can be achieved equally well utilizing two-drug (Ara-C and daunorubicin) and three-drug (daunorubicin, Ara-C, and 6-thioguanine) regimens as well as variations thereof.

Induction chemotherapy alone without postremission therapy is insufficient treatment for patients with AML. The use of some form of post-induction therapy in complete responding patients led to an increase in the duration of CR from 4-8 months to 10-15 months with 17-23% of such individuals projected to remain leukemia-free for longer than four years. The impact of various forms of maintenance and consolidation therapy on such results has been examined in a series of randomized trials. The results of these studies indicate that the median duration of remission in complete responding adults is still 12-15 months while the fraction of such patients remaining disease-free after 24 months may have increased slightly to the range of 25-35%.

Recent investigative efforts in postremission management have focused on methods of intensifying treatment, taking advantage of the steep dose response curve that has been shown for chemotherapeutic agents such as Ara-C in experimental tumors and in human leukemic cells in tissue culture. Several uncontrolled reports have appeared in which several months of intensive treatment have been given to young cohorts of patients. Most of these treatment programs have included high dose Ara-C (i.e., 1-3 gm/m^2) in the therapeutic regimen. After median follow-up times of about two years, most series project a 45-55% likelihood of continued remission after 24 months, similar to that observed following allogeneic bone marrow transplantation. The administration of such therapy often prolonged periods of hospitalization, has been associated with significant toxicity (5-17% mortality), and may not be tolerable in older patients. As such, this promising concept of postremission intensification is presently being objectively evaluated through a comparison with more conventional maintenance programs in prospective randomized trials being conducted by the Cancer and Leukemia Group B and The Eastern Cooperative Oncology Group.

Ref: Mayer RJ. Current chemotherapeutic treatment approaches to the management of previously untreated adults with de novo acute myelogenous leukemia. Sem Oncol 1987; 14:(in press).

Panel Discussion

K 008 MONOCLONAL ANTIBODY AND COMPLEMENT-MEDIATED PURGING OF BONE MARROW FOR AUTOLOGOUS BONE MARROW TRANSPLANTATION IN ACUTE MYELOBLASTIC LEUKEMIA. Edward D. Ball, Dartmouth- Hitchcock Medical Center, Hanover, NH 03756. Autologous bone marrow transplantation for leukemia is potentially limited by the ability to eliminate disease from the bone marrow. We have been exploring the use of complement-fixing myeloid-specific monoclonal antibodies (MoAbs) for marrow purging for patients with acute myeloblastic leukemia (AML) in remission and presently have several useful MoAbs. Two of these, PM-81 (CD 15) and AML-2-23 (CD 14), have been in use for >3 years in a Phase I/II clinical trial of purging in AML in 2nd and 3rd complete remission (CR) and have been used for >1 year in a Phase II trial in AML in 1st CR. Following cyclophosphamide and total body irradiation (1200 cGy in fractionated schedule), 19 patients in 2nd and 3rd CR and 5 patients in 1st CR have been transplanted with MoAb-treated autologous bone marrow to date. Leukocyte engraftment has been satisfactory in all but one patient (CR3), with a mean neutrophil recovery time of 30 days (>500 PMNs/ul). Platelet recovery has generally been satisfactory, but has been delayed in 4 patients and contributed to the demise of 3 patients. Mean time to reach >20K platelets/ul was 45 days. Red cell recovery was documented by a rise in reticulocytes at a mean time of 17 days. In the CR2/3 group, 6 patients have relapsed and 4 early transplant-related deaths have occured. After salvaging one of the relapsed patients and performing a second transplant, 9 patients survive with a median follow-up of 10 months. Four of 5 patients transplanted in CR1 are disease-free at a median follow-up time of 10 months. These early data indicate a favorable effect on relapse-free and overall survival compared to chemotherapy alone in CR2 and 3. Longer follow-up and more patients are needed to assess the impact of this therapy in CR1.

K 009 PROSPECTIVE EVALUATION OF AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN AML, B. Löwenberg, For the Netherlands Leukemia Working Party "HOVON". The Daniel den Hoed Cancer Center, Rotterdam, The Netherlands.

ABMT in patients with AML appears a therapeutic modality that consolidates remissions in 30-50% of cases but its definite value has not been assessed in prospective studies. We present the results of a study that was designed to assess the role of nonpurged ABMT in patients with AML in first complete remission (CR) in comparison to that of alloBMT. Patients of age 15-60 yrs with AML were enrolled at diagnosis. The patients were subjected to one or two cycles of remission induction chemotherapy with daunomycin and cytosine arabinoside, then consolidated with another schedule of the same agents, and subsequently treated with ABMT or alloBMT for intensification unless CR was not reached. Autologous marrow was harvested following the consolidation cycle during CR and cryopreserved. Pretransplant conditioning consisted of two separate schedules with 4 wk intervals: Ara-C (1 g/m^{2}x4) plus AMSA (115 mg/m^2) and subsequently Cyclophosphamide (120 mg/kg) and TBI (8 Gy). AlloBMT was carried out after Cyclosphamide/TBI when an HLA-identical donor was available and the patient was less than 45 yrs age. 145 patients have been entered. CR was attained in 112 patients. Early relapse, hematological support problems or other reasons prohibited BMT in 34 cases while 7 patients remain potential candidates for BMT. A total of 39 ABMTs and 32 alloBMTs have been carried out. Hematological regeneration following ABMT was usually delayed. The median intervals to granulocyte counts of 0.5x10^9/l and platelet values of 50 x10^9/l were 40 and 82 days respectively. 20 ABMT recipients survive free of disease, and three following relapse. Eighteen subjects have died from relapse (n=13), infection (n=2) or other causes (n=3). The complete update of the analysis (survival, relapse, complications) will be presented. At the present time overall survival nor disease free survival between ABMT and alloBMT recipients are different.

Acute Lymphoblastic Leukemia

K 010 CHEMOTHERAPY FOR ALL IN ADULTS
D. Hoelzer for the German ALL/AUL Study Group, Dept. of Hematology, University of Frankfurt, 6000 Frankfurt 70, F.R.G.

The chemotherapy results concern mainly the German multicenter studies for treatment of adult ALL with over 800 patients. In the first prospective trial (01/81) 10/1978 to 6/1983, 368 previously untreated patients aged 15 to 65 years were given an intensive induction and consolidation regimen. 74% achieved complete remission (CR), the median remission duration (MRD) was 25 months and 35% are in continuous CR (CCR) at 7 1/2 years. Low risk patients, defined as having CR within 4 weeks, WBC <30000/ul, subtype c-ALL or T-ALL, age <35 y, had a CCR rate of 62% and high risk patients, defined as having one or more of the factors CR after >4weeks, WBC >30000/ul, subtype null-ALL or age >35 years, had a CCR rate of 28% ($p = 0.0005$).

With this experience, a risk adapted therapy trial (02/84) was activated in July 1983. For high risk patients treatment was further intensified with 4 monthly cycles of Ara-C (75 mg/m^2) and VM-26 (60 mg/m^2) i.v. daily for five days, two after induction and two after reinduction therapy. 507 adult ALL patients entered the study and 442 completed induction therapy (11/1986). The CR rate was 79%, the MRD for the 349 remitters 29 months and the CCR rate at 3 years was 48%. For the 96 low risk patients the 3-year CCR rate of 59% was similar to the 65% in the earlier study. For the 182 high risk patients, despite intensification, the MRD of 17 months and the 3-year CCR rate of 37% showed no overall improvement compared to the former study. However results were better for patients with common-ALL and for the older patients (35 - 65 y), the latter receiving now the high risk protocol. The four prognostic factors proved to have independent influence also in the risk adapted study.

In a newly activated therapy trial (03/87), high risk patients receive, instead of Ara-C/VM-26, one course of high dose cytosine arabinoside, 3 g/m^2 twice daily (days 1-4) for patients aged 15-50 y and 1 g/m^2 for patients aged 50-65 y, in combination with mitoxantrone 10 mg/m^2 (days 2-6). In this study these high risk patients, defined as having one or more of the adverse prognostic factors late CR, WBC >30000/ul, subtype null-ALL or B-ALL, Ph`+ ALL, are also candidates for bone marrow transplantation, allogeneic or autologous, in first remission.

K 011 **AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL),** Norma Ramsay, Tucker LeBien, Daniel Weisdorf, William Woods, Bruce Bostrom, Mark Nesbit, Daniel Vallera, Fatih Uckun, Anne Goldman, Tae Kim, John Kersey, University of Minnesota, Minneapolis, Minnesota 55455.

Autologous bone marrow transplantation for ALL has been investigated at the University of Minnesota since 1982 for patients who lack a compatible donor. Eligible high risk patients were those who had relapsed while receiving primary therapy. Patients were also eligible for transplant in first remission if they were $\geq$ 21 years of age or age 16-20 years with high risk features. Sequential Minnesota protocols utilized cyclophosphamide (Cy) + fractionated 1320 cGy TBI followed by randomization to maintenance therapy with methotrexate + 6-mercaptopurine (n=28) and 850 cGy TBI followed by high dose cytosine arabinoside (AraC) (n=37). In all patients with B-lineage ALL autologous marrow was purged with the monoclonal antibodies BA-1 (CD24), BA-2 (CD9/p24) and BA-3 (CD10). Recently 4-hydroperoxycyclophosphamide has been added to the purging cocktail (n=14). The median age of the patients at transplant was 8.6 years with a range from 2.3-41.7 years. The time from diagnosis to transplant ranged from 0.5-15.8 years with a median of 2.5 years. Two patients were in first remission at transplant, 39 in second, 23 in third and 1 in fourth remission. Twenty patients had prior extramedullary disease.

Years	Preparative Regimen	N	Purging	Relapse	Disease-Free Survival (2 yr)	Median Followup (years)
1982-84	Cy + fTBI	28	BA1,2,3 + C'	78%	21% $\pm$ 14%	4.2
1985-86	TBI + AraC	23	BA1,2,3 + C'	76%	22% $\pm$ 17%	1.7
1986-87	TBI + AraC	14	BA1,2,3 + C' + 4HC	---	---	0.4

The time to relapse post transplant has been early with a median of 3.3 months ranging from 1.3 - 24 months. Peritransplant mortality has been low with only 4 patients dying of non-leukemic causes. Improved anti-leukemic therapy is needed to decrease the incidence of recurrent leukemia post-transplant and improve survival.

K 012 CHEMOTHERAPY FOR ACUTE LYMPHOCYTIC LEUKEMIA IN CHILDREN, Gaston K. Rivera, St. Jude Children's Research Hospital, Memphis, TN 38105.

The lymphoid malignancies of childhood are among the most drug sensitive human tumors. The value of intensified leukemia therapy for improving event free survival rates in childhood lymphoblastic leukemia (ALL) is widely accepted today.[1] Still to be determined, however, is whether chemotherapy protocols have reached a plateau in effectiveness (55 to 75% cure rates). About one-third of patients are expected to fail front-line treatment and require improved therapeutic alternatives. Groups of patients at high risk of early failure include infants below the age of one year, those with Philadelphia chromosome positive leukemia or B-cell ALL. Optimal management for these patients remains controversial. Lately, improved results have been reported for certain subsets of patients with use of either more intensive chemotherapy[2] or marrow transplantation.[3] Additional patients in second (or subsequent) hematologic remission may be cured with more recent regimens of salvage chemotherapy[4] and/or autologous or allogeneic marrow transplants.[5,6] A critical review of these data will be presented.

1. Seminars in Hematology, 24:12, 1987
2. Cancer Treatment Reports, 71:1033, 1987
3. Clinics in Haematology, 15:205, 1986
4. New England Journal of Medicine, 315:273, 1986
5. New England Journal of Medicine, 315:461, 1987
6. New England Journal of Medicine, 317:1618, 1987

Bone Marrow Transplantation: Current Controversies

Chronic Leukemia

K 013 THE ROLE OF CHEMOTHERAPY AND BONE MARROW TRANSPLANTATION IN THE TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA, Robert Peter Gale, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

Chemotherapy and bone marrow transplantation are both important therapeutic modalities in the treatment of acute lymphoblastic leukemia. Optimal utilization of these therapies is complex. This report analyzes data reported in the literature and to the IBMTR regarding results of these modalities and suggests likely therapeutic strategies.Decisions regarding optimum treatment require separate analyses of standard and high risk children and adults. Few if any cildren are candidates for transplants in 1st remission since chemotherapy results in 70 -95% leukemia free survival in high and standard risk patients respectively. Possible exceptions are infants, B-ALL,and individuals with adverse cytogenetic abnormalities such as t(9;22) or t(4;11). Patients who relapse from chemotherapy within about 18 months have a poor survival with retreatment; results of transplantation in this setting seem superior. Chemotherapy relapses after about 18 months should be retreated with chemotherapy and transplanted only after a 2nd failure i.e. 3rd remission. Adults with ALL achieve only 35% leukemia free survival with chemotherapy. Results of transplants in !st remission are similar when corrected for age distribution and censoring related to transplant timing. As a consequence the most reasonable strategy is to delay transplantation to the time of relapse in most instances. Some adults, such as those with extremely high WBC or the t(9;22) may be candidates for a transplant in 1st remission. By combining chemotherapy and transplantation, it should be possible to further increase cure rates in children and adults with ALL.

K 014 BONE MARROW TRANSPLANTATION IN CML: PROBLEM AREAS. John M Goldman, Royal Postgraduate Medical School, London, UK.

Throughout the present decade patients with CML have been treated by BMT at a number of specialist centres worldwide and certain conclusions can now be drawn. There remain however a number of controversial points. (1) Results of BMT using HLA-identical siblings are substantially better if BMT is performed in chronic phase (CP) than in later phases; nevertheless about 15% of patients transplanted in transformation are cured; (2) The actuarial cure rate for BMT in chronic phase is about 90% if donor marrow includes T-cells; the comparable figure for recipients of T-depleted donor marrow is 50%; (3) The major factors assessed pre-transplant that predict for survival after BMT for CML in CP are disease phase and patient age; the major factors that predict for probability of survival and/or relapse after BMT in CP are patient age, incidence and severity of GVHD and use of T-depleted donor marrow; (4) The optimal timing of BMT within the CP is not yet agreed; some evidence suggests that the probability of survival after BMT is correlated inversely with the duration of disease before BMT, while other analyses fail to confirm this contention; (5) The best method of treating the spleen at the time of transplant is not clearly established; enlarged spleens may require removal but there is no evidence that small spleens must receive additional irradiation; (6) The preliminary results of the use of matched unrelated donors (with or without T-cell depletion) for patients in CP who lack HLA-identical siblings look promising but further follow-up is required before this approach can be recommended more widely.

K 015 MARROW TRANSPLANTATION FOR CHRONIC MYELOGENOUS LEUKEMIA (CML), E. Donnall Thomas, Fred Hutchinson Cancer Research Center, Seattle WA 98104.
In Seattle, patients with chronic myelogenous leukemia have been prepared with cyclophosphamide and total body irradiation, given an untreated marrow graft from an HLA-identical sibling followed by methotrexate, cyclosporine or methotrexate-cyclosporine with follow-up times of 1 to 10 years. Survival is as follows: 158 patients in chronic phase plateau at 61%, 19 in second chronic phase at 32%, 73 in accelerated phase at 21%, and 69 in blast phase at 14%. Multivariate analysis of survival for patients transplanted in chronic and in accelerated phase demonstrated that increase of age and of the interval from diagnosis to transplant were significantly associated with increased mortality. Survival by age for patients in chronic phase ranges from 72% for those 21 to 30 years to 45% for those 40 to 52 years. Survival by the duration of the interval from diagnosis to transplant ranges from 78% for those transplanted within 6 months to 38% for those transplanted more than 3 years after diagnosis. The major cause of death was interstitial pneumonia for patients in the chronic phase and recurrence of leukemia for those in blastic or accelerated phase. Acute graft-versus-host disease (GVHD) had an adverse effect on survival. Marrow depleted of T-cells was used in transplanting 7 patients in chronic phase. Failure of engraftment in 3 patients, recurrence of the leukemia in 3 patients and death from chronic GVHD in 1 patient led to the discontinuation of the use of T-depleted marrow. The survival of marrow grafted patients will be discussed in relation to the optimal time for transplantation. Causes of failure and possible future directions will be discussed based on published data of marrow grafting for CML.

K 016 **OVERVIEW OF BONE MARROW TRANSPLANTATION FOR MULTIPLE MYELOMA**, Sante Tura[1], Michele Cavo[1], Gösta Gahrton[2] and Giuseppe Bandini[1], for the Advisory Committee of the International Bone Marrow Treatment Registry. [1]Institute of Hematology "Seràgnoli", University of Bologna, Italy and [2]Department of Medicine, Huddinge University Hospital, Huddinge, Sweden.
Between January 1978 and December 1987 38 pts. with MM who received an allogeneic BMT (35 from matched siblings) were reported to the IBMTR by 18 teams (11/38 pts., or 29%, by Bologna team). The median time from diagnosis to BMT was 26 (range 4-134)mos. PATIENTS: There were 23 males and 15 females, varying in age from 23 to 50 (median 38) yrs. All pts. but one were conditioned with TBI, either in a single dose (16 cases) or in fractionated doses (21 cases), and chemotherapy. Cyclophosphamide (median 120 mg/kg) was given most frequently, either alone (16 cases) or in combination with melphalan (median 4 mg/kg)(8 cases), BCNU (5mg/kg)(1 case) or both (8 cases). Also prophylactic treatments against GVHD varied considerably and consisted mainly of T cell depletion $\pm$ other (15 cases), CSA $\pm$ steroids (10 cases), MTX $\pm$ CSA (9 cases) or MTX $\pm$ other (4 cases). RESULTS: The actuarial probability of survival and disease-free survival at 2 yrs. was 48% and 38%, respectively. Twenty-two pts. (or 58%) died (interstitial pneumonia: 8 cases; relapse: 4 case; GVHD: 2 cases; hemorrhage: 2 cases; GVHD and hemorrhage: 1 case; multi-organ failure: 1 case; heart failure: 1 case; suicide: 1 case) 1 to 38 mos. after BMT, while the other 16 are alive (10 of them 1 or less than 1 yr. after BMT, 3 after 1 to 2 yrs. and 3 after 3 to more than 4 yrs.). Complete remission was achieved in 25 out of 35 (or 71%) evaluable pts. In addition, 3 other pts. with advanced refractory myeloma had a sustained decrease in tumour cell mass, which ranged from 70% to 90%. The two yr.actuarial probability of relapse for pts. in complete remission was 58% and appeared to be higher for the group treated with a single drug than for that receiving two or more drugs as preparation for engraftment (70% vs. 15%). DISCUSSION: These results indicate that: 1) BMT may be an effective treatment for selected pts. with MM, especially if responding to prior conventional chemotherapy, and may eradicate the malignant clone in some of them; 2) intensified preparative regimen (with at least 2 drugs) has not a greater toxicity than the standard conditioning and may reduce the risk of relapse following BMT; 3) resistance to conventional chemotherapy may be overcome by increasing the dose of the drugs, thus suggesting the use of high-dose chemoradiotherapy also in selected pts. with refractory MM.

Bone Marrow Transplantation: Current Controversies

Aplastic Anemia

K 017 IMPACT OF THE CONDITIONING REGIMEN ON THE OUTCOME OF BONE MARROW TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA. E.Gluckman. Bone Marrow Transplantation Unit. Hopital Saint Louis. 1, Av. Claude Vellefaux 75475 Paris Cedex 10; France.

Bone marrow transplantation (BMT) is the therapy of choice for patients with SAA who have an HLA identical donor. During a recent period, results have been markedly improving with a long term survival of 70%. This improvement can be related to a better choice of the indications, a decreased delay between diagnosis and BMT, an improvement of supportive care. Modifications of conditioning and prophylaxis of GVHD are also major contributory factors of success. Four main protocols of conditioning are used :(1) Cycloposphamide alone, (2) Cyclophosphamide + buffy coat from the donor, (3) Cyclophosphamide + TBI and (4) Cyclophosphamide + TLI/TAI. A preliminary analysis from the IBMTR shows that the 5 years actuarial probability of survival is respectively 70 ± 9%, 52 ± 12%, 54 ± 12% and 64 ± 8%. Rejection has been decreased to 11% since Cyclosporine A was used instead of Methotrexate for prophylaxis of GVHD. Results have been improved furthermore when Cyclosporine A is used in association with Methotrexate. The analysis of the chimerism by RFLPs or DNA fingerprinting shows a major difference between conditioning using Cyclophosphamide alone or associated with TAI. Patients receiving 6 Gy TAI had no rejection and a 100% complete chimerism on long term follow up studies while patients receiving Cyclophosphamide followed by Cyclosporine A had 10% graft rejection and 20% mixed chimerism or autologous reconstitution. Acute and chronic GVH was more frequent in patients receiving TAI/TLI or buffy coat cell transfusions than in patients receiving Cyclophosphamide + Cyclosporine A. TBI gave poor results because of the increase of interstitial pneumonitis and GVHD. In FA patients, the decrease of the dose of Cyclophosphamide to 20 mg/kg with 5 Gy TAI led to a 70% long term survival. HLA mismatched transplants give poor results even with an intensification of the conditioning with or without T cell depletion except in the cases with a phenotypically matched related or unrelated donor. ATG or Cyclosporine A give a one year probability of survival of 70% , they are clearly indicated in patients older thant 50 years or without HLA identical sibling donor.

K 018 UNRELATED AND MISMATCHED TRANSPLANTS IN APLASTIC ANEMIA. Jill M Hows, Edward Kaminski, Benjamin A Bradley*, Departments of Hematology and Immunology, Royal Postgraduate Medical School, London, UK, and UK Transplant Services*, Bristol, UK.

The superiority of HLA identical sibling bone marrow transplantation (BMT) over immunosuppressive treatment for severe accquired aplastic anemia (SAA) is most dramatic in very severe aplastic anemia (VSAA) defined by 0.2×10^9/L neutrophils at presentation. Factors which predict survival after BMT for SAA are younger age, short disease duration, and absence of sensitisation from multiple blood transfusions. Preliminary results of partially matched family donor (PMFD) and matched unrelated donor (MUD) BMT are significantly worse than for HLA identical sibling BMT. A preliminary survey from the EBMTG included 31 patients, PMFD (N=24), MUD (N=7). Actuarial survival for the whole group was 25% at 72 months. Survival after BMT for HLA A, B and DR matched donors was better (45%) than after 1 antigen mismatched (25%) and 2-3 antigen mismatched (11%) BMT. At Hammersmith, 15 patients were transplanted for SAA from donors other than HLA identical siblings MUD=9, PMFD=6. 14/15 patients had previously failed to respond to immunosuppressive treatment, all were multiply transfused. Median duration of SAA pre BMT was 8 mmths (2-200). All patients received cyclophosphamide 200 mg/kg iv pre BMT and cyclosporine post BMT. Pre BMT, 5 patients also received antilymphocyte globulin and 4 total nodal irradiation. Donor marrow was lymphocyte depleted ex vivo with Campath-1 in 3/15 cases, 2/3 also received in vivo Campath 1, 2 and 3. 4/15 patients survive (27%) a median of 1754 days post BMT. The most frequent cause of death was graft failure (N=6). Graft failure following sensitisation of recipients by transfusions may be more difficult to overcome than in HLA identical sibling BMT. We have therefore investigated recipient sensitisation by measuring recipient donor specific CTL pre BMT using limiting dilution analysis. In MUD BMT for SAA a serious limiting factor is the time taken for MUD identification. Current developments in immunogenetic techniques analysing restriction fragment length polymorphisms (RFLP) for the assessment of HLA class II gene differences may decrease delays in donor identification. Advantages of RFLP analysis include speed of donor screening, and better definition of class II specifities in pancytopenic patients. We conclude that better donor selection combined with early treatment may improve the results of BMT for SAA from donors other than HLA identical siblings.

K 019 PATHOPHYSIOLOGY OF BONE MARROW GRAFT REJECTION. Rainer Storb, Fred Hutchinson Cancer Research Center and University of Washington School of Medicine, Seattle, WA 98104. Transplants from monozygous twins are successful in half of the patients (pts) without prior immunosuppression. In the other half, grafts have only been successful after cyclophosphamide (CY) suggesting that these involve other mechanisms, perhaps autoimmune causes. HLA-identical sibling marrow grafts after CY are effective treatment. A frequent and highly fatal problem has been rejection of the graft, seen until the mid-seventies with a frequency of 30-60%. Most likely, rejections are due to sensitization of pts to non-HLA antigens induced by blood transfusions, as suggested by studies in experimental animals and by positive in vitro tests of cell-mediated immunity of pt against donor. Transplantation before transfusion obviates rejection, and more than 80% of pts so transplanted survive. An increased marrow cell dose is effective in reducing rejection without increase in acute and chronic GVHD. Many programs using more intensive immunosuppression to avoid rejection have been carried out. All include CY either combined with total body irradiation (TBI), total lymphoid irradiation (TLI), or thoraco-abdominal irradiation (TAI), or CY combined with cyclosporine or viable donor buffy coat cells post-transplant. As a result of these manipulations, rejection has generally decreased. The Bone Marrow Transplant Registries reported rejection rates of 17% and Seattle of 11%. Recent survivals of CY conditioned pts were 55% and 60% for the registries and 73% for Seattle pts. The registries reported approximately 65% survival in pts given CY combined with TLI or TAI and 46% when it was combined with TBI. Most of the programs used to overcome rejection have associated risks. The addition of buffy coat cells increases the incidence of chronic GVHD. Radiation regimens carry the risks of late malignancies, cataracts, central nervous system damage, retardation of growth and development, and sterility. Because of these risks emphasis should be placed on measures to avoid rather than overcome sensitization related to blood transfusions. A combination of CY and antithymocyte globulin, found effective for second grafts in pts rejecting first grafts, might be as effective as CY + TBI, TLI, or TAI for first grafts without associated toxicity. Among HLA-nonidentical pts conditioned with CY, best results and no rejection were seen in those given phenotypically matched grafts, compared to 30% rejection and 29% survival in one-antigen mismatched and 73% rejection and 0% survival in two-antigen mismatched pts. T-cell depletion of the grafted marrow increases the rate of graft rejection. Rejection in unmatched pts is the combined result of natural "resistance" to marrow grafts and of transfusion-induced sensitization. More effective programs including CY + TBI are needed for consistent engraftment to occur. Perhaps monoclonal antibodies to immunologically active cells either alone or combined with short-lived radioactive isotopes will provide less toxic alternative conditioning programs.

K 020 THERAPEUTIC ALTERNATIVES TO BONE MARROW TRANSPLANTATION IN APLASTIC ANEMIA. Neal S. Young, Patricia Griffith, Eileen Leonard, Arthur Nienhuis. Clinical Hematology Branch, NHLBI, Bethesda MD 20892.
Most patients with aplastic anemia (AA), either because of their age or the absence of a histocompatible sibling donor, are not good candidates for BMT. IgG from horses immunized with human thymocytes (ATG) or thoracic duct lymphocytes (ALG) has been shown to be effective therapy for AA. In a large NHLBI-sponsored multicenter trial, ATG was administered to patients with acute severe AA, moderate or chronic AA, and other bone marrow failure syndromes. Hematologic responses were assessed by transfusion independence, blood counts, and review of clinical abstracts by observers blinded to the treatment protocol. In 77 patients with acute severe AA, there were no major differences in responses to 10 versus 28 days of ATG. About 1/3 of patients were transfusion independent at 3 mos, and 47% had had meaningful clinical improvement; neutrophil and platelet counts were higher in surviving patients who had received larger amounts of ATG. Sex was a major prognostic determinant, with men having a higher probability of survival and recovery; older women did more poorly than younger. The baseline neutrophil count ($<200/mm^3$) and nonidiopathic etiology also were predictive of poor survival. Follow-up over 1 yr showed that the failed transfusion-dependent state was unstable: only 10% of patients were receiving transfusions, the remainder being equally divided among deaths and recovery. Of patients with chronic severe or moderate AA randomized to receive ATG, 28% recovered to transfusion independence at 3 mos compared to 0% treated with nandrolone decanoate. Pancytopenia and cellular marrow showed a response rate similar to chronic AA. ATG activity might be due to immunosuppression, stimulation of lymphocytes to produce growth factors, or directly on hematopoietic stem cells. Cyclosporine, which more specifically affects T cell function, has also been effective in aplastic anemia. In our trial, 5 of 15 patients who had failed ATG showed a clinical response, and three were sustained. Improvement required high blood levels and corticosteroids. Some patients with virus-related bone marrow failure have responded to Acylovir (Epstein-Barr virus) and specific immunoglobulin (B19 parvovirus). Current trials of GM-CSF may be helpful in determining the role of growth factors in bone marrow failure as well as in supporting severely neutropenic patients.

Graft vs. Host Disease

K 021 CHEMOPROPHYLAXIS AND THERAPY FOR GRAFT-VERSUS-HOST DISEASE (GVHD)

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Donor derived T lymphocytes play a pivotal role in the development of GVHD. Thus, elimination of the responsible lymphocytes from donor marrow should prevent GVHD. Attempts have included pretreatment of the donor before marrow harvest, purging of the marrow in vitro prior to infusion, and treating the recipient in the peri- and post-transplant period. Cytotoxic and immunomodulatory drugs, monoclonal and polyclonal antibodies, as well as physical and chemical means have been utilized. Recent emphasis has been on the use of monoclonal antibodies directed at T lymphocytes. Although quite successful in regards to reducing the incidence of acute GVHD, this approach has been associated with an unexpectedly high risk of failure of sustained engraftment and recurrence of the underlying disease. Consequently drug based regimens have remained the mainstay of GVHD prophylaxis. Methotrexate (MTX), cyclophosphamide (CY), prednisone (PD), antithymocyte globulin (ATG), cyclosporine (CSP) are being used widely. Regimens combining MTX, ATG and PD, CSP and PD or CSP and MTX have reduced the incidence of acute GVHD in patients given HLA identical marrow grafts to 20-30%. In patients given HLA non-identical grafts these regimens have been less successful, dependent upon the degree of mismatch between donor and patient. New modifications of those regimens are being developed. For example, with the CSP/ MTX regimen, reduction of the MTX dose on day 1 and omission of the day 11 dose has significantly reduced hepatic and renal toxicity without jeopardizing GVHD prophylaxis. Use of any of these regimens in conjunction with patient placement in laminar airflow isolation and intestinal decontamination, may also affect GVHD. Despite all efforts, some patients still develop GVHD and require treatment. PD has been considered standard therapy although only one third of patients will respond. Increased doses of CSP if tolerated, or ATG are useful. Recently monoclonal antibodies either alone or conjugated to immunotoxins have been used successfully. With all prophylactic regimens attempted, however, chronic GVHD has continued to be a problem not only in patients given HLA non-identical but also with HLA matched transplants. Prolonged administration of PD has not been uniformly successful. It has been suggested that the prophylactic administration of immunoglobulin may be beneficial. For therapy, PD alone or alternating with CSP has been found to be effective in two thirds of patients so treated. Resistant patients may respond to azathioprin alone or combined with CSP. Preliminary data indicate that thalidomide may be a useful drug in this setting. Further studies are necessary.

K 022 ROLE OF T CELL SUBSETS IN GVHD, Robert Korngold and Jonathan Sprent, Dept. of Microbiology, Jefferson Medical College, Philadelphia PA and Scripps Clinic & Research Foundation, La Jolla CA.

The transfer of unprimed T cells to irradiated allogeneic mice expressing differences at either multiple minor histocompatibility (H) or major histocompatibility complex (MHC) loci can result in a high incidence of lethal graft-versus-host disease (GVHD). Severity and kinetics of disease depend largely on the dosage of donor cells and the particular immunogenetic barriers involved. In the MHC transplant situation, both H-2 (K/D) class I and class II (Ia) incompatibilities can lead to lethal GVHD, although anti-class II GVHD seems to be more severe. The association of particular T cell subsets and GVHD induction has been recently elucidated in that helper-type (L3T4+) T cells are capable of mediating anti-class II GVHD, whereas cytotoxic-type (Lyt 2+) T cells cause anti-class I GVHD. In addition, Lyt 2+ cells can lead to GVHD in the apparent absence of L3T4+ cells. In the case of multiple minor H GVHD, Lyt 2+ T cells appear to be capable of mediating disease in all strain combinations tested, and also appear to function independent from L3T4+ T cells. L3T4+ T cells, themselves, are also capable of mediating anti-minor H GVHD in certain strain combinations, although this does not correlate with in vitro proliferation responses. Further investigation of the mechanisms of GVHD pathogenesis related to individual T cell subsets is underway. A better understanding of the role of T cell subsets and GVHD may lead to approaches by which we can selectively deplete a T cell subset responsible for GVHD in a particular histoincompatible situation and leave the other T cell subset to provide an immediate level of immunocompetence in the recipients.

K 023 NOVEL APPROACHES TO THE PROBLEM OF GRAFT-VERSUS-HOST DISEASE, PROPHYLAXIS AND TREATMENT, G.W. Santos and G.B. Vogelsang, The Johns Hopkins Oncology Center, Baltimore, Maryland 21205 U.S.A.

Are all the patients at risk for GVHD? Is there an approach to the prevention or treatment for those at risk that is effective but less toxic than present methods? We wish to outline our "Novel" approaches to these questions. Currently our skin explant predictive test for acute graft-versus-host disease (GVHD) has been employed in over 70 allogeneic HLA identical marrow transplants. Of 35 patients with positive tests, 32 developed histologic grade 2 or greater GVHD of the skin. Of 35 patients with negative tests, 30 did not develop GVHD. Test correlation with clinical result was significant ($P < 0.001$). A prospective randomized study is underway where in patients with a negative test will receive cyclosporine or no prophylaxis for GVHD. Thalidomide is very effective in preventing or treating acute GVHD and treating chronic GVHD in a rat model. Preliminary results in treating azathioprine/prednisone resistant and high risk chronic GVHD in patients are encouraging. Two patients on therapy 3 months or more have had a significant and continued response (sclerodermatous GVHD) or a complete response (Lichenoid GVHD). Three patients on therapy for a shorter time also appear to be responding. Two other patients are unevaluable due to leukemic relapse and failure to absorb drug. Drug serum levels (0.5-1 ug/ml) measured by HPLC were found. Currently drug doses are being escalated to obtain levels found most efficacious in the rat model (7-10 ug/ml). Studies of treatment of acute GVHD are hampered by very poor oral absorption of drug in the early post transplant period. Methods designed to develop an active intravenous preparation are being persued. It is hoped that these two approaches will decrease the problems of GVHD and the toxicity engendered by its prophylaxis or treatment.

K 024 CHRONIC GRAFT-VERSUS-HOST DISEASE: RECENT ADVANCES IN DIAGNOSIS AND TREATMENT. Keith Sullivan, Robert Witherspoon, Rainer Storb, Jean Sanders, Thomas Loughran, Jr., Kristine Doney, Frederick Appelbaum, Claudio Anasetti and E. Donnall Thomas. Fred Hutchinson Cancer Research Center and University of Washington School of Medicine, Seattle WA 98104.

Chronic graft-versus-host disease (GVHD) and associated infections are major determinants of morbidity and mortality observed in 25-50% of long-term survivors of allogeneic marrow transplantation. Older patient age, prior acute GVHD, and viable buffy coat or bone marrow reinfusions increase the risk of these complications. Impaired thymic function, failure to generate antigen-specific suppressor T-cells mediating stable graft-host tolerance, and generation of autoreactive helper T-cells and lymphokines have been implicated in the immunopathogenesis of this autoimmune-like disorder. The most important clinical aspect of chronic GVHD is immune suppression and associated infection. Prophylactic trimethoprim sulfamethoxazole (TMP-SMX) reduces the risk of infection. Other antibiotic combinations and trials of prophylactic high-dose immunoglobulin infusions are currently in progress. Diagnostic screening studies and biopsies are routinely obtained at 80 days posttransplant. The spectrum of clinical findings in chronic GVHD has altered as an increasing proportion of patients are diagnosed early in the disease and receive immunosuppressive and antibiotic treatment. Since 1980, the incidence of disabling contractures has decreased from 50% to 5-10%. Obliterative bronchiolitis, however, is now recognized in 10-15% of patients. Without treatment, <20% of patients with multiorgan clinical extensive chronic GVHD survive with Karnofsky scores $\geq$70%. Between 1980 and 1983 we conducted a randomized, double-blind comparison of prednisone (1.0 mg/kg/QOD) and placebo (Group I, N=63) vs azathoprine (1.5 mg/kg/day) and prednisone (Group II, N=63) as early treatment of chronic GVHD. Patients with platelet counts <100,000/μl were placed into therapy with prednisone alone (Group III, N=38). All groups received prophylactic TMP-SMX (1 DS BID). Non-relapse mortality was observed in 21%, 40% and 58% of Groups I, II and III, due to increasing rates of infection. Actuarial 5-year survival was 61%, 47% and 26%, respectively (I vs II, p=0.03; I vs III, p=0.0001). A pilot study was started in late 1982 in high-risk patients who had either failed prior therapy (N=21) or were previously untreated but had persisting thrombocytopenia (N=40). Oral cyclosporine (6 mg/kg/q12°/QOD) was combined in an alternate-day regimen with prednisone (1 mg/kg/QOD). Toxicity appeared acceptable and long-term survival has increased to over 50% in these high-risk patients. Controlled trials of single and multiagent therapies are now in progress in different prognostic groups. Improved understanding of the pathogenesis, diagnosis, treatment and prognostic factors of chronic GVHD has reduced the late morbidity of allogeneic marrow transplantation.

K 025 EFFECTOR CELLS IN THE GRAFT-VS-LEUKEMIA REACTION, Robert L. Truitt and Ann V. LeFever, Midwest Children's Cancer Center, Medical College of Wisconsin at Children's Hospital, Milwaukee, WI 53233

Graft-versus-host (GVH) disease is a complex immunologic reaction initiated by T-lymphocytes present in allogeneic bone marrow. Retrospective statistical studies in man suggest that acute and/or chronic GVHD disease is associated with decreased leukemia recurrence. This has been termed the graft-versus-leukemia (GVL) effect. In some animal models, the GVL effect can be distinguished from GVH reactivity, leading to speculation that GVL and GVH reactive cells might be distinct and, therefore, separable. Alternatively, the same cells may mediate both GVL and GVH reactions, but quantitative differences in sensitivity of leukemic and "normal" cells could account for apparently distinct GVL/GVH reactions (i.e., a threshold effect). We have used limiting dilution analysis and T-cell cloning techniques in vitro together with bioassay systems in vivo to isolate and identify cytolytic effector cells that mediate GVL/GVH reactions. We describe three types of effector cells that lyse leukemic target cells in vitro and mediate a GVL effect in vivo. These effector populations differ from each other in their ability to recognize and react against nonleukemic (normal) target cells in vitro and to cause (or contribute to) GVH disease in vivo. The first type of effector is characterized by MHC-restricted minor histocompatibility antigen specific cytotoxic T-lymphocytes (CTLs) that mediate both GVL and GVH reactivity. Because the GVL and GVH reactions are mediated by the same cell, they cannot be separated; however, the intensity of the GVL/GVH reaction is dose dependent and can be manipulated to obtain a beneficial GVL effect. The second type of effector is represented by CTLs that recognize normal class I MHC molecules with limited tissue distribution in the host (e.g., Qa-1 molecules). These effector cells lyse both leukemic and normal target cells in vitro and mediate a preferential GVL effect without causing GVH disease in vivo. A third type of lytic GVL effector cell is represented by those cells which are truly leukemia (or tumor) specific. That is, they recognize cell surface molecules that are unique to neoplastic cells. This includes both MHC-restricted CTLs and nonrestricted lymphokine activated killer (LAK) cells. Initial studies on LAK cells suggest that they can mediate a GVL specific reaction without reacting against normal tissues in vivo or in vitro. Thus, LAK cells represent a class of GVL effector cells that are distinct and separable from cells that cause GVH disease. In summary, the cells which mediate the GVL reaction are complex. They can be distinct from or identical to cells that contribute to GVH disease and are not limited to leukemia-specific target antigens.

K 026 SOLUBLE SUPRESSOR FACTORS INTERFERE WITH GvHD AND T CELL RESPONSES IN VITRO, D.W. van Bekkum, S. Knaan-Shanzer and L. Nagelkerken, Radiobiological Institute and Institute for Experimental Gerontology TNO, P.O. Box 5815, 2280 HV Rijswijk, Holland.

Culture supernatant of hybridomas of mouse neonatal spleen cells and lymphoma cells contain a soluble mediator (SUF) that suppresses T cell responses both in vivo and in vitro. In vitro incubation of spleen and bone marrow cells with SUF prevents the acute Graft-versus-Host (GvH) reaction (in lethally irradiated mice) without affecting the haemopoietic stem cells. The addition of SUF to stimulated lymphocyte cultures inhibits cell proliferation. This suppressive activity of SUF is exerted across species barriers. Fractionation of the crude supernatant revealed the presence of two suppressive moieties, one of high and one of low molecular weight (respectively >100 and <3 kDa). They exert suppression independently and by distinct mechanisms. The suppressive activity of low molecular weight SUF can be demonstrated only in vitro. It prevents T cell proliferation by interfering with the utilization of IL-2 (1).
The high molecular weight SUF (SUF-4) interferes both with in vivo GvHD and with in vitro T cell proliferation. In vitro studies revealed that this moiety primarily affects an early event in the proliferative response to alloantigen, mitogen or anti CD3 antibodies. SUF-4 inhibits the expression of stimulation induced surface receptors (IL-2 and Transferrin receptors) while the expression of T cell differentiation antigens (CD2, CD4, CD8, TcR) remains unaffected. SUF-4 also suppresses blastogenesis, DNA replication and the synthesis of γ-IFN and IL-3. SUF-4, however, does not interfere with the production of IL-2 as found in cultures of stimulated lymphocytes, stimulated Jurkat cells and in cultures of MLA 144 cells. Neither does SUF-4 interfere with the utilization/binding of the lymphokines IL-1, IL-2, IL-3 and /-INF by cells bearing the proper receptors. Furthermore, SUF-4 suppresses the proliferation of separated CD4 as well as CD8 cells.
These findings suggest that SUF-4 blocks signals essential for IL-2 receptor expression but not for IL-2 synthesis.

1. S. Knaan-Shanzer and D.W. van Bekkum. Soluble factors secreted by naturally occurring suppressor cells that interfere with in vivo graft-vs.host disease and with T cell responsiveness in vitro. Eur. J. Immunol. 1987, 17:827.

T Cell Depletion

K 027 T-CELL DEPLETION AND GRAFT-VERSUS-LEUKEMIA: RESULTS OF CLINICAL TRIALS, Anna Butturini, Leukemia Biology Unit, University of Parma, Parma, Italia 43100.
In animal models, donor T cells impacts on several important transplant outcomes including graft-versus-host disease (GvHD), the likelihood of sustained engraftment, prevention of graft rejection, immune reconstitution, and an immune mediated anti-leukemia effect referred to as GvL. The outcome of transplantation depends on the interaction of these factors. Other recipient and host related variables are also important including the degree of genetic disparity, the intensity of anti-leukemia and immune suppressive conditioning, and the size of the residual recipient T cell compartment. Recently clinical trials of T cell depletion have been reported often with contrdictory results. For example, in some instances GvHD is completely eliminated while in other circumstances it is merely decreased. There is also debate as to whether graft failure is increased and whether there is an increased risk of leukemia relapse. Some of these difference reflect the lack of controlled randomized trials, small patient numbers, or inadaquet follow-up. Other differences reflect statistical varability. Different conditioning regimens, technics of T cell depletion, and diseases or disease states may result in bona fide differences in treatment outcome. In this review I consider results of T cell depleted transplants in > 20 studies involving > 400 recipients of T cell depleted transplants. These data are compared to results in > 2000 transplant recipients without T cell depletion reported in the biomedical literature and to the IBMTR. These data indicate that T cell depletion decreases acute GvHD from about 45% to about 10% in HLA-identical transplants and from about 75% to about 30% in HLA-nonidentical transplants. Graft failure is increased from < 1% to about 10% in HLA-identical transplants and from about 10% to about 30% in HLA-nonidentical transplants. Leukemia relapse was also increased. This increase was most marked in chronic myelogenous leukemia where it increased from about 10% to about 50%. Increases of a lesser magnitude were also detected in acute myelogenous leukemia and acute lymphoblastic leukemia in remission. The increase in leukemia relapse could, in some instances, be distinguished from the prevention of GvHD. Because of the complex effects of T cell depletion on transplant outcome, survival was not convincingly improved. Future approaches to addressing problems engendered by T cell depletion will be discussed.

K 028 GRAFT FAILURE FOLLOWING T CELL-DEPLETED MARROW TRANSPLANTATION, Paul J. Martin, Fred Hutchinson Cancer Research Center, Seattle, WA
The incidence of graft failure has been increased in virtually every study in which T cell depletion has been used to prevent acute GVHD, regardless of the method employed for removing T cells from the donor marrow. It is possible that stem cell damage or loss during in vitro processing has played a contributory role. On the other hand, there are data from animal models clearly demonstrating that donor T cells can facilitate engraftment, suggesting that some of the difficulty in human trials has occurred because of a loss of this beneficial effect. The mechanisms for this effect have not been elucidated. T cells might facilitate engraftment by providing lymphokines and accessory factors that promote proliferation and differentiation of hematopoietic cells. T cells in donor marrow might also suppress host immunity. T cell depletion has been associated with a high incidence of lymphoid and hematopoietic mixed chimerism (i.e. persisting host cells) after transplantation and also with a high relapse rate in patients transplanted for chronic myelogenous leukemia. These findings suggest that donor T cells help to eliminate residual host lymphoid cells and hematopoietic stem cells that survive the preparative regimen. The loss of this anti-host effect may contribute to the increased incidence of graft failure after T cell depletion. Data from a variety of animal models have implicated at least three types of host cells in causing graft failure after marrow transplantation: a non-T cell or NK-like population responsible for rejection of MHC-homozygous marrow in certain strains of mice and for rejection of MHC-incompatible marrow in dogs; a T cell population that can be sensitized by the marrow graft; and T cells sensitized by prior transfusions. Further experiments have shown that donor T cells can overcome rejection mediated by host NK-like populations, but donor cells had little effect on host T cells sensitized by transfusions. The host cells and donor antigens responsible for graft failure after human T cell-depleted marrow transplantation have not been fully identified, but the demonstration of cytotoxic host cells with anti-donor MHC specificity has implicated the involvement of T cells in certain patients. It also remains to be determined whether the donor T cells that facilitate engraftment constitute a subset distinct from those that cause GVHD.

K 029 STEM CELL COMPETITION VS. IMMUNOLOGICAL REJECTION IN MURINE MODELS FOR BONE MARROW ALLOGRAFT REJECTION, Zvi Lapidot, Adelmo Terenzi, Titi Singer, Ophira Salomon and Yair Reisner, Department of Biophysics, Weizmann Institute of Science, P.O.B. 26 Rehovot 76100, Israel.

Graft rejection presents a significant obstacle for transplantation of T cell-depleted allogeneic bone marrow in leukemia patients. A major problem in murine models for bone marrow allograft rejection is the narrow margin in which graft rejection can be analysed. Following doses of >9 Gy TBI rejection is minimal, whereas after administration of 8 Gy TBI, which spares a significant number of clonable T cells, a substantial frequency of host stem cells can also be detected. Thus, in current murine models, unlike in man, bone marrow allograft rejection is generally associated with full autologous hematopoietic reconstitution. In a previous study, we found that in the "classical" unsensitized murine model, chimerism can be changed from 30% to >90% donor type either by treatment with the myeloablative drug dimethyl myleran (DMM) (0.2 mg/mouse, i.v. one day post 8 Gy TBI), or by increasing bone marrow dose from 3-5 x 10^6 to 10-15 x 10^6 cells. These results suggested that in unsensitized mice stem cell competition plays a major role in bone marrow allograft rejection. In the present study we used DMM to abolish the host stem cell compartment and we presensitized the recipient mice (C3H/HeJ) against irradiated donor type (C57BL/6) lymphocytes in order to enhance immunological rejection. In this model, which resembles graft rejection in leukemia patients, DMM does not enhance donor type chimerism as it will not affect significantly the immunological responses which become dominant following presensitization. Alternatively, by adding host-type PNA^- thymocytes to DMM-treated mice, we were able to determine the minimum number of T cells required for graft rejection. Engraftment of allogeneic bone marrow in DMM-treated mice can be enhanced by using large bone marrow inoculum (20 x 10^6 cells) in mice sensitized with 1-5 x 10^6 cells. Graft rejection cannot be overcome by a transplant of 20 x 10^6 cells in mice presensitized with $\geq$15 x 10^6 cells. New approaches, aimed at overcoming immunological rejection of allogeneic bone marrow in leukemia patients, could be evaluated in this stringent model prior to clinical trials.

Interstitial Pneumonia and Immune Deficiency

K 030 PREVENTION AND TREATMENT OF CYTOMEGALOVIRUS INFECTION, Joel D. Meyers, Fred Hutchinson Cancer Research Center and the University of Washington, Seattle, WA 98104.

Primary cytomegalovirus (CMV) infection among seronegative patients receiving marrow from seronegative donors can be effectively prevented by the use of seronegative blood products. A potential alternative is the use of leukocyte-depleted blood products, although further documentation of feasibility and efficacy is needed. Neither of these approaches would be wholly effective among seronegative patients receiving marrow from seropositive marrow donors. Passive immunoprophylaxis has been tested extensively, but with uncertain results. Additional studies of efficacy as well as attention to the cost of available immunoglobulins are needed before this modality can be recommended. Seropositive patients appear to develop active infection from reactivation of latent virus, and use of an effective antiviral agent to suppress virus reactivation should also be effective in preventing CMV disease. Intravenous acyclovir has been shown to significantly reduce the probability of CMV disease in seropositive patients. The newer antiviral agents ganciclovir and foscarnet may provide better results, although the marrow toxicity of ganciclovir may limit its utility. The combination of ganciclovir and intravenous CMV immunoglobulin shows promise for treatment of CMV pneumonia. Ultimately the ability to provide CMV-specific immune reconstitution will be needed for optimal control of CMV infection.

K 031 EFFECT OF RECOMBINANT HUMAN GRANULOCYTE MACROPHAGE COLONY-STIMULATING FACTOR (rHuGM-CSF) ON HEMATOPOIETIC RECONSTITUTION AND GRANULOCYTE FUNCTION FOLLOWING HIGH DOSE CHEMOTHERAPY (HDC) AND AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT), W.P. Peters, S.J. Brandt, S.K. Atwater, M.J. Borowitz, J. Kurtzberg, R.B. Jones, E.J. Shpall, J. Shogan, R.C. Bast, Jr., D.H. Oette, Duke University Medical Center, Durham, NC 27710, and Sandoz Research Institute, East Hanover, NJ 07936.

Prolonged myelosuppression is associatied with increased morbidity and mortality due to infection and other causes. With the cloning and expression of human colony stimulating factors, which in vitro stimulate the proliferation and differentiation of bone marrow progenitors, the opportunity exists for shortening the extended but well defined period of myelosuppression which follows HDC and ABMT. We performed a phase I-II trial of rHuGM-CSF to determine the safety and efficacy of rHuGM-CSF in man. We treated 27 patients with metastatic breast cancer (19 pts) or melanoma (8 pts) with cyclophosphamide (5625 mg/M^2), cisplatin (165 mg/M^2) and carmustine (600 mg/M^2) followed by infusion 3 days later of autologous bone marrow cells. rHuGM-CSF was infused continuously for either 14 (21 pts) or 21 days (6 pts). Groups of 3-11 patients were treated with 2, 4, 8, 16, or 32 ug/kg/day of rHuGM-CSF and compared to a group of 24 age, diagnosis and treatment matched historical controls. WBC at day 15 of infusion was higher in rHuGM-CSF treated patients (2229/mm^3 +/- 1464) compared to historical controls (863/mm^3 +/- 645). Three patients required early discontinuation of rHuGM-CSF because of drug related renal dysfunction, weight gain, hypotension. WBC declined rapidly after rHuGM-CSF was discontinued and hematopoietic recovery proceeded similarly to historical controls. Granulocyte margination using In-111 granulocytes was similar prior to (38.6% +/- 10.2) and during infusion (34.2% +/- 9.9) of rHuGM-CSF. Phagocytosis of Cryptococcus neoformans was enhanced during rHuGM-CSF infusion; granulocyte hydrogen peroxide production was similar before and during rHuGM-CSF infusion. However, migration of granulocytes using a standarized skin chamber assay was markedly reduced during rHuGM-CSF infusion (1.5 +/- 0.4 x 10^6 WBC/cm^2/24 hr) compared to baseline (41.9 +/- 20.3 x 10^6 WBC/cm^2/24 hr) ($p<0.0008$). These findings may be of relevance when extravascular granulocytes are required for host defense.

K 032 USE OF IVIG AND DHPG: DO THEY WORK? Drew J. Winston, Winston G. Ho, and Richard E. Champlin. UCLA Medical Center, Los Angeles CA 90024.

Although the incidence of interstitial pneumonia appears to be decreasing at many marrow transplant centers, established CMV interstitial pneumonia still has a high mortality (80-90%) when it occurs (1,2). Treatment of CMV interstitial pneumonia with available antivirals continues to be unsatisfactory (1). DHPG (ganciclovir) has increased potency against CMV in vitro but only 3 of 19 marrow transplants with CMV pneumonia treated with DHPG at Seattle or UCLA survived (3,4). Combining high doses of corticosteroids with DHPG was also of no benefit (5). Recently, more favorable results and improved survival from CMV pneumonia have been obtained by using a combination of DHPG plus high doses of intravenous immunoglobulin (IVIG) given several times each week (6)

In CMV-seronegative transplant recipients, CMV infection can be prevented or modified by the use of CMV-seronegative blood products and prophylactic IVIG (7,8). The relative efficacy of CMV-seronegative blood products plus IVIG versus CMV-seronegative blood products alone is being evaluated in a controlled clinical trial. For CMV-seropositive transplant recipients, effective prophylaxis for CMV disease and pneumonia has not yet been clearly established. We are currently conducting a placebo-controlled, double-blinded trial of prophylactic DHPG in UCLA allogeneic marrow transplants seropositive for CMV. Fifteen (15) patients have completed this study to date. All patients had marrow engraftment, but the study code has not yet been broken.

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K 033 ENHANCEMENT OF IMMUNOLOGIC RECOVERY AFTER BONE MARROW TRANSPLANTATION: IS THERE A ROLE FOR INTERLEUKINS? Robert P. Witherspoon, Rainer Storb, Keith M. Sullivan, E. D. Thomas. The Fred Hutchinson Cancer Research Center and the University of Washington, Seattle, WA 98104.

During the first 6 months after allogeneic marrow grafting from HLA identical siblings, patients are at risk to develop a variety of opportunistic infections. Beyond 6 months most patients lead normal lives and develop only rare infection; however, 30-50% of patients develop chronic graft versus host disease (GVHD) and are at increased risk to develop serious infection especially from encapsulated bacterial organisms. In vivo and in vitro studies of such patients demonstrate persistent T and B lymphocyte immune deficiency. Cellular immune deficiency was shown by cutaneous anergy to recall antigens, deficient numbers of peripheral blood helper T lymphocytes, poor lymphocyte proliferation and IL-2 production following stimulation with mitogens or alloantigens. Patients with chronic GVHD also had low levels of serum thymic factor. Humoral immune deficiency was shown by poor serum antibody production to pneumococcal polysaccharides, inability of B lymphocytes to produce immunoglobulin when cocultured with normal marrow donor T lymphocytes and pokeweed mitogen, and deficient in vitro proliferation of purified B lymphocytes in response to crude T cell supernatant containing B lymphocyte growth and differentiation factors. The patients with chronic GVHD had persistant B lymphocyte deficiencies contributing to their morbidity and mortality from bacterial infection. Our initial attempts to accelerate immunologic recovery were directed at improving T lymphocyte growth and differentiation by use of thymic grafts, thymosin fraction 5, or thymopentin. These in vivo treatments did not alter immune function and did not appear to alter the incidences of infection, GVHD, or leukemic relapse. Addition of IL-2 to lymphocyte cultures from transplant recipients resulted in T lymphocyte proliferation and generation of cytotoxic T cells directed against alloantigens. The potential for in vivo IL-2 administration to bring out GVHD in allograft recipients is of concern. However, IL-2 may be useful in autologous transplant patients by stimulating natural killer cell or lymphokine activated killer cell function resulting in immunity against malignancy or viral infection. The influence of natural killer cell function on hematopoiesis may or may not be beneficial. Other interleukins such as B cell growth and differentiation factors might be useful to expand highly purified donor B lymphocytes in culture for infusion into marrow graft recipients, or to assist differentiation of B lymphocyte precursors to immunoglobulin production. If successful in vitro, these factors may merit trials in animal and human marrow transplantation.

Autotransplants in Lymphoma and Solid Tumors

K 034 HIGH DOSE CHEMOTHERAPY & BONE MARROW AUTOTRANSPLANTS (BMA) FOR BREAST CANCER Karen Antman, M.D. & Robert Peter Gale, M.D. Ph.D.
Departments of Medicine, Dana Farber Cancer Institute; Harvard Medical School, Boston, Massachusetts 02115 & University of California at Los Angeles School of Medicine, Los Angeles, California 90024

Breast cancer is the most common cancer in women (10% lifetime risk). While half can be cured by surgery, radiation therapy &/or adjuvant chemotherapy, recurrent disease remains virtually uniformly fatal with a median survival of ~2 years after first metastasis. A steep dose-response relationship in retrospective studies exists between chemotherapy & remission rate & duration. Because many women with advanced breast cancer have no apparent tumor involvement of bone marrow & no prior pelvic radiation, high-dose chemotherapy and/or total body irradiation (TBI) & BMA is a possible therapeutic approach.

There are 12 studies of high dose single agent chemotherapy & BMA in 73 patients with advanced breast cancer. All 4 (5%) complete responses (CR) were obtained with melphalan; partial responses (PR) were observed in 19 patients (20%). The response rate (RR) was 40% for alkylating agents versus 20% for non-alkylating agents. Combination chemotherapy ± TBI has been reported in 40 patients with advanced breast cancer. Regimens of two or more alkylating agents produced the highest RRs. The RR for TBI containing regimens (10 /14, 71%) was similar to regimens without TBI (19/26, 73%).

There are three studies of BMA in 27 previously untreated patients with inflammatory or metastatic breast cancer. 13 had CRs and 7 PRs. Finally 48 patients responding to several cycles of "induction" therapy were treated in 3 studies. 38 achieved CR & 5 PR. **Thus a summary of the published data reveals the following:**

	#	%CR	%RR
Single agents in refractory diseases	73	5	31
Combinations in refractory diseases	40	25	73
ABMT only in untreated patients	27	48	74
ABMT after induction	48	79	89

Thus high-dose chemotherapy ± TBI produces remissions of refractory breast cancer. However substantial prolongation of survival in patients with advanced refractory disease is rare. The 2-5 month response durations without maintenance therapy approximates that achieved with second line salvage regimens at standard doses given at repeated intervals. Transplant earlier in the course of the illness, after a good response to standard dose therapy yields a high CR rate. Any treatment related toxic deaths are least acceptable in this group & whether there will be long term survivors is as yet unknown.

K 035 BONE MARROW AUTOTRANSPLANTATION IN LYMPHOMA. James O. Armitage, University of Nebraska Medical Center, Omaha, Nebraska 68105.

Autologous bone marrow transplantation is an increasingly utilized treatment modality in patients with both non-Hodgkin lymphoma and Hodgkin disease. Almost 2,000 patients have been treated worldwide, and the number being treated annually is increasing. While initially used mainly as a salvage therapy for patients with far advanced disease, investigators have recently begun to apply this treatment modality earlier in the course of these illnesses. This would be expected to improve treatment results. Autologous bone marrow transplantation offers only a quantitative--not qualitative--advance in cancer therapy (i.e. more intense use of the same treatment modalities but not a new treatment modality).

Several principles regarding the use of autologous bone marrow transplantation in lymphoma have become apparent over the past few years. It is certain that this approach has curative potential--even in patients resistant to conventional doses of drugs. This was documented in a study of 100 patients with aggressive non-Hodgkin's lymphoma who were treated with autologous bone marrow transplantation. Twenty-two patients had drug resistant (i.e. no response to an immediately preceding chemotherapy regimen) relapsed disease, and 14% of those patients achieved long-term disease-free survival. Several studies have documented that patients with non-Hodgkin lymphoma who have minimal, chemotherapy responsive disease have the best outlook with long-term, disease-free survivals ranging from 35-75%. In Hodgkin disease the primary factors determining chance for cure with autologous bone marrow transplantation seem to be performance status, extent of disease at the time of therapy, and the amount of preceding therapy. In Burkitt lymphoma investigators have found timing of therapy, responsiveness of the tumor to preceding therapy, and extent of disease as the most important determinants of treatment outcome. Thus, the theme running through all these studies is that healthier patients with less disease and less previous therapy are the most likely to benefit.

The foregoing strongly suggest that autologous bone marrow transplantation should be included early in the therapy of poor risk patients with lymphomas if it is to make an important contribution to treatment outcome. The best strategy for accomplishing this, along with identification of the best treatment regimen, improved supportive care techniques, determination of the value of marrow purging, and investigations in the use of this approach in indolent lymphomas, are the major immediate challenges facing investigators studying autologous bone marrow transplantation in lymphomas.

K 036 EUROPEAN EXPERIENCE OF AUTOLOGOUS BONE MARROW TRANSPLANTATION IN LYMPHOMA. Goldstone A.H., Linch D.C. & Gribben J.G. For the EBMT Group. University College and Middlesex Hospital Transplant Team, London WC1, UK.

We report 422 cases of lymphoma autografted in 42 EBMT centres updated to 1987. In addition we will report in greater detail on the first 100 adult lymphomas grafted by our own group. Of the 422 there are 305 NHL, median age 28 years, 18% intermediate grade, 17% Burkitt, 33% lymphoblastic and 32% other high grade. 28% were in first CR, 16% in later CR, 10% in responding relapse and 28% in resistant relapse. After transplant 70% of patients were in CR at 3 months and 12% had a PR. 36% relapsed from CR. 177 patients had a minimal follow up of 2 years. Of these the patients in first CR have a plateau at 61% event-free survival. The second relapse and responding relapse patients have a plateau at 50% and the resistant relapse 15-20%. Multivariant analysis shows no influence of histology, purging, number of courses of previous therapy on overall survival but status at transplant was very significant with $p = 0.00001$. 117 Hodgkin's patients have been grafted, median age 27 years. In this group only 3% were in first CR and 7% in any later CR. 21% were in responding relapse and 47% in resistant relapse. After transplant 57% were in CR and 27% in PR. 33% of patients relapsed from CR. Event-free survival of Hodgkin's patients entering CR was 40% for the group with a minimum of 2 years follow up. We report 100 adult lymphomas grafted in our own centre. There were 56 HD, median age 27 years, and 44 NHL, median age 31 years. Procedure-related death was 13% and CR rate 39% in each group. No purging was used. The vast majority of patients relapsing did so at sites which included those originally involved by disease. Treatment failure remains as yet failure to eradicate disease rather than due to infusion of contaminated marrow. The EBMT experience will be updated to 700 cases for this meeting.

K 037 BONE MARROW PURGING IN SOLID TUMORS, Thierry Philip, Head Bone Marrow Transplant department Centre Léon Bérard 28, rue Laënnec 69373 Lyon Cx 08 France.

The contamination of autologous bone marrow with malignant cells is the major limitation to Autologous Bone Marrow Transplantation even in solid tumors. We will review :
1) the purging technology ie physical separation methods, chemical methods and immunological methods.
2) experimental models,
3) clinical or preclinical assessment of the purging methods,
4) comparison of the purging methods and their possible combinations,
5) past and ongoing clinical studies.

K 038 AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR POOR PROGNOSIS NEUROBLASTOMA. R.C. Seeger, T.J. Moss, S.A. Feig, C. Lenarsky, M. Selch, K.K. Matthay, J. Wells, and C.P. Reynolds. Jonsson Comprehensive Cancer Center and UCLA School of Medicine, Los Angeles, CA 90024; UCSF School of Medicine, San Francisco, CA 94143; Children's Cancer Study Group, Pasadena, CA 91101.

We have conducted pilot studies that suggest intensive chemotherapy and radiotherapy in conjunction with bone marrow transplantation (BMT) may improve survival for poor prognosis neuroblastoma patients if carried out prior to disease progression. Analysis of all 31 patients that we have transplanted with autologous marrow (January, 1982, to November 1987) indicates a projected survival of 56% at 42+ months after BMT. Our current study (November 1985 to the present) employs aggressive induction chemotherapy (CPAV, Proc Am Soc Clin Oncol 5:210, 1986) for 16 wks followed by marrow harvest and ex vivo treatment of marrow; 70% of patients receiving CPAV induction therapy were able to undergo BMT before developing progressive disease. Intensive chemoradiotherapy (VM-26, Adriamycin, melphalan, CDDP and total body irradiation = VAMP-TBI) at approximately 20 wks is followed by autologous BMT; in our current study, we have transplanted 21 patients before they developed progressive disease. Marrow was purged by sedimentation and filtration which both remove tumor clumps, and then by immunomagnetic separation using monoclonal antibodies 390, 459, HSAN 1.2, and BA-1 attached to magnetic beads via goat anti-mouse Ig. Immunocytological detection of tumor after purging using anti-cell surface monoclonal antibodies and anti-neuron specific enolase serum provides a sensitivity of 1/100,000 (Proc Amer Assoc Cancer Res 28:219, 1987). Detectable tumor in marrow varied prior to purging (0 to 1800 tumor cells/10^5), but no tumor was detected in 21 of 21 after purging. All patients engrafted (ANC > 500 in < 28 days). To date 17 of 21 (81%) patients transplanted are disease free survivors (range 6 to 26 mos, median = 15 mos). The four events following BMT have been 1 toxic death from hepatic veno-occlusive disease, 1 death from progressive tumor growth, and 2 tumor recurrence patients still alive. Thus, the combination of aggressive induction therapy (which reduces tumor content of the marrow in vivo), multi-modality purging ex vivo, and sensitive detection of tumor in marrow, minimizes the chance of infusing tumor with the transplant, but does not compromise marrow engraftment. The results of our studies suggest that long-term survival may be achieved in 1/2 of patients with this therapy.

Bone Marrow Transplantation: Current Controversies

Alternative Donors

K 039 **Marrow Transplantation From Relatives Other Than HLA Genotypically Identical Siblings** Patrick G. Beatty, Claudio Anasetti, Rainer Storb, E Donnall Thomas, John A Hansen Fred Hutchinson Cancer Research Center, Seattle Wash.

From March 1975 through March 1986 281 patients have received marrow grafts in Seattle from related donors other than HLA genotypically identical siblings. 137 donors were parents, 119 were siblings, 4 were half-siblings, 12 were children, and 9 were other relations. Minimum followup is 18 months. All patients shared one HLA haplotype with their donor, and differed variably with respect to the non-shared haplotypes: 29 were phenotypically matched for HLA-A,B,,D; 119 differed for one of these three loci; 104 differed for 2 of three loci, and 29 differed for all three loci. Graft rejection was correlated with the degree of incompatibility: phenotypic matches had a rate of 7%, one locus mismatched 9%, two locus 21%, and three locus 5% (p=.028). In a multivariate analysis, donor HLA-incompatibility at HLA-B and -D (p=.0004) and presence of patient-anti-donor lymphocytoxic antibodies (p=.0028) both were predictive of graft rejection. The use of the combination of Cyclosporine plus Methotrexate as compared to Methotrexate only has led to reductions in the rate, incidence, and severity of acute GVHD (p=.0001). Graft versus host disease in patients receiving the combination of CYA plus MTX coorelated with degree of incompability: phenotypic matches had no cases of Grade III-IV acute GVHD, one locus mismatches 32%, and 2 or 3 locus mismatches 62% (p=.0001). Relapse in mismatched patients correlated with presence or absence of acute GVHD in patients transplanted for ALL, but not in those transplanted for ANL or CML. Overall survival in patients transplanted from phenotypic or one locus mismatched donors approximates the survival of patients transplanted from genotypically identical siblings. Patients transplanted from 2 or 3 locus mismatched donors fare more poorly than patients transplanted from matched donors whether transplanted in remission or relapse. We conclude that transplantation from phenotypic or one locus mismatched donors should be considered standard treatment for any patient who is a candidate for marrow grafting. Transplants from 2 or 3 locus mismatched donors should be considered experimental pending advancements in marrow transplant technology.

K 040 ACTIVITIES OF THE NATIONAL BONE MARROW DONOR REGISTRY (NBMDR), Jeffrey McCullough, David Stroncek, Glenn Bartsch, Herbert Perkins, John Hansen, the National Bone Marrow Donor Registry Coordinating Center, American Red Cross, St. Paul, MN 55107.
Approximately 65,000 HLA-A, B typed apheresis blood donors from 50 blood banks in the U.S. are being approached to become potential bone marrow donors. Recruitment of about 30,000 of these has been completed and 13,500 are now on a central registry operated by the NBMDR located in Minnesota. In September, 1987, the NBMDR began accepting donor search requests from the 17 participating marrow transplant centers. As of January 12, 1988, a preliminary search to match for (HLA-A, B) was done for 348 patients with the following diseases: CML 154, AML 35, ALL 47, AA 46, myelodysplastic syndrome 17, other 40. An extended search was completed or is underway for 80 of these patients. Twenty-one of these extended searches are completed. A suitably matched donor has been found for eight. Two of these patients have been transplanted and six more are awaiting transplant, 4 patients died, 3 withdrew, no donor was found for 7. The median time required to locate a donor who was A,B DR compatible was 11 days (range 1-27 days). The time required for completing the MLC testing and identifying a donor for these patients was 29 days and was determined primarily by the transplant center physicians. Two transplants have been carried out between the following donors and patients: ALL patient at Fred Hutchinson Center - donor in Milwaukee; AML patient at UCLA - donor in Minnesota. The HLA types of the patients for whom donors have been located are:

Patient	A	B	DR	
1	2,2	27,27	2,5	
2	1,2	8,44	1,3	* Patients transplanted
3	2,24	8,27	3,3	
4	2,3	7,44	2,7	
5	2,2	13,44	4,7	
6	1,24	8,w60	3,4	
7*	2,29	7,44	2,7	
8*	2,3	7,w62	2,4	

The Registry, although new, has been successful in locating donors for patients for whom bone marrow transplantation is medically indicated.

Genetic, Immune and Metabolic Diseases

K 041 MARROW TRANSPLANTATION IN PATIENTS WITH THALASSEMIA, Baronciani D., Angelucci E., Lucarelli G., Galimberti M., Polchi P., Delfini C., Giardini C., Manenti F.,Durazzi S.M.T.,Giorgi C., Politi P., Divisione Ematologica e Centro Trapianto di Midollo Osseo di Muraglia, Ospedale di Pesaro, Pesaro, Italy.

We report here a consecutive series of 173 patients with beta homozygous thalassemia ages 1-15 years (mean age 7.8 yrs) that received bone marrow transplant (BMT) from 165 HLA identical siblings and 8 identical parents. All 173 patients received unmodified bone marrow after busulphan 14 mg/Kg and cyclophosphamide 200 mg/Kg. Acute GVHD prophilaxis was done with methotrexate in 48 patients, with cyclosporine in 99 and with cyclosporine plus methotrexate in 26. One hundred and forthyseven (84%) are alive 40-1608 days after transplant. One hundred and thirtyfour (77%) are disease free 40-1594 days after transplant and 13 (7%) are alive with return of thalassemia 131-1608 days post-transplant. Eighteen patients died of transplant complications within 100 days and eigth died between 100 and 235 days post-transplant of infectious complications associated with GVHD. In total there was failure of engraftment or rejection in 19 patients (11%). One hundred and twentyfour of the 134 DSF patients have a Karnofsky score of 100, nine with chronic GVHD have a score of 80 and one of 40. Liver biopsies regularly performed before and at six months interval after the transplant show that liver siderosis remains unmodified for the first two years post-transplant and thereafter starts to decrease impressively. In this group of patients the Kaplan-Meier calculation of the probability of survival after the transplant is 85% at day 376 and the probability of disease free survival is 76% at 340 days post-transplant.

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K 042 BONE MARROW TRANSPLANTATION IN GENETIC DISORDERS, Barrett A J, McCarthy D M, Department of Haematology, Westminster Hospital, Charing Cross and Westminster Medical School, London SW1P 2AP, UK.

Genetic disorders susceptible to correction by bone marrow transplantation (BMT) fall into two broad categories: (1) Defects of the lymphohaemopoietic stem cell and its progeny - red cell, leucocyte and platelet disorders, immunodeficiency diseases and osteopetrosis; (2) Defects which do not primarily effect the lymphohaemopoietic stem cell or its progeny - lysosomal storage diseases, and other rare metabolic disorders. A variety of BMT conditioning regimens have been used in genetic disorders. The most successful and widely applied is the combination of busulphan and cyclophosphamide. It has yet to be established, however, whether this chemotherapy only regimen avoids long term complications associated with the use of whole body irradiation. While BMT can be expected to fully correct defects associated with the haematopoietic stem cell, the degree of correction of other metabolic disorders is still not fully defined. 30 BMT for Hurlers disease (mucopolysaccharidosis type I) have been reported. Biochemical correction with blood enzyme levels similar to those of the heterozygous or normal donor are routinely achieved, correction of reticuloendothelial and tissue abnormalities are apparent by six months but correction of bony deformities and central nervous system defects is only slowly and incompletely achieved. Long term follow up beyond 5 years from transplant is now available in 12 patients who show varying degrees of social adaptatation and mental development. The most successful cases are attending normal schools and are developing normally. A crucial question is whether BMT can be of benefit in neuronal storage disorders such as metachromatic leukodystrophy, gangliosidoses and Krabbés disease. Animal models suggest that it is possible to protect the CNS from damage by early bone marrow transplantation in feline models of metachromatic leukodystrophy and in murine models of Krabbés disease. The small amount of BMT data available in man does not provide conclusive evidence that CNS damage can be prevented. Recent attempts at BMT in Gaucher Disease, Pompé disease, adrenoleukodystrophy and Womlman's disease will be reviewed.

These encouraging results clearly establish the therapeutic role of BMT in a number of congenital metabolic disorders and point the way to further attempts at BMT in rarer conditions. Ultimately the BMT manoeuvre could be adapted for the correction of metabolic disorders by genetic manipulation of the patient's own haematopoietic stem cell prior to retransplantation.

K 043 BONE MARROW TRANSPLANTATION IN ANIMAL MODELS FOR LYSOSOMAL ENZYME DEFICIENCY. Peter M. Hoogerbrugge[1,2] and Dirk W. van Bekkum[1]. [1]Radiobiological Institute TNO, Rijswijk and [2]Dept. of Pediatrics, Univ. Hosp., Leiden, the Netherlands.

Evaluation of the effects of bone marrow transplantation (BMT) in the treatment of patients with lysosomal storage diseases is complicated by the large variation in disease pattern in untreated patients. In contrast, the enzymatic defect in inbred strains of animals results in an almost identical disease pattern in all affected animals. This makes animal models suitable for studying various questions related to BMT for lysosomal enzyme deficiency, eg. what is the optimal timepoint for BMT, which organs and cell types will take up the donor enzyme, which lesions are reversible, etc. The mechanisms leading to beneficial effects of BMT can be studied in animal models as well, eg. it can be studied whether transfer of enzyme into deficient cells occurs, or whether replacement of deficient cells by enzymatically competent, donor-derived cells is the only phenomenon leding to beneficial effects after BMT. Also, treatment regimens to prevent neurological deterioration, one of the major clinical problems in patients with lysosomal storage diseases, can be developed. E.g., the role of intrathecal inoculation of enzymatically competent cells in combination with BMT can be studied, and the consequences of the infiltration of donor-derived cells in the CNS of transplanted mice (Hoogerbrugge et al., this meeting) can be evaluated. Finally, animal models can be used for studying the possible role of gene transfer into hemopoietic stem cells as means of therapy for lysosomal enzyme deficiency. The animal models most commonly used in studies to the role of BMT in the treatment of lysosomal storage diseases are murine models for beta-glucuronidase deficiency (C3H mouse), Krabbe's disease (Twitcher mouse) and Niemann-Pick disease. Other animal models used are dog-models with iduronidase and fucosidase deficiency and a feline model for aryl-sulphatase deficiency. The results of BMT in these animal models will be reviewed and the possible implications for clinical BMT will be discussed.

K 044 BONE MARROW TRANSPLANTATION FOR CONGENITAL AND ACQUIRED IMMUNODEFICIENCY SYNDROMES, Robertson Parkman, Carl Lenarsky, Donald Kohn and Kenneth Weinberg, Division of Research Immunology/Bone Marrow Transplantation, Childrens Hospital of Los Angeles and the University of Southern California School of Medicine, Los Angeles, CA 90027.

Allogeneic bone marrow transplantation (BMT) is the treatment of choice for congenital immune deficiency states, which involves either lymphoid and/or hematopoietic stem cells. All forms of severe combined immune deficiency (ADA deficiency, IL 2 deficiency, NP deficiency, etc.) except IL 1 deficiency can be corrected by the replacement of the abnormal lymphoid stem cells with normal histocompatible or haploidentical lymphoid stem cells with the persistence of hematopoiesis of recipient origin. Other defects of T lymphocyte function (Wiskott-Aldrich syndrome, gpL-115 deficiency, Chediak-Higashi syndrome) can be treated by histocompatible BMT although the results with haploidentical BMT are less successful. At present, BMT is not used to treat immune deficiency states due to defects in B lymphocyte differentiation or function. Defects of myeloid differentiation and function (infantile agranulocytosis, chronic granulomatous disease, actin deficiency, etc.) can be corrected by the replacement of the abnormal recipient hematopoietic stem cells by normal hematopoietic stem cells. Ablation of the recipient's normal lymphoid stem cells is necessary to assure stable donor engraftment. BMT for acquired immune deficiency syndrome has been limited. BMT for the acquired immune deficiency syndrome will have its greatest likelihood of success if 1) the number of virally infected cells is reduced prior to transplantation; 2) the patients are transplanted before opportunistic infections occur; and 3) recipient lymphoid and hematopoietic stem cells are ablated to permit maximal donor stem cell engraftment.

Leukemia

K 100 BONE MARROW PURGING WITH VINCRISTINE AND METHYLPREDNISOLONE, M.Andolina, E.Agosti L.Meraviglia, M.G.Drobinz, A.de Manzini, Istituto per l'Infanzia, Trieste. Italy
The incubation with vincristine 1 mcg/ml and methylprednisolone 3 mg/ml for 30' is hyghly effective against leukemic cell lines (Nalm 6 and Molt 4) while partially respecting the normal marrow stem cells (approx.20% CFU GM survive). 1 mcg/ml of vincristine is at least ten times the minimum dose that completely abolishes the growth of the cell lines in long term cultures. At lower doses methylprednisolone increases the effect of vincristine.
We have used such a purging procedure in 13 children affectd by high risk LLA (most in CR 2). The protocols were: Cy TBI, V.C.R. (vincristine in continuous infusion, cytoxan, TBI), A.C.R. (Ara C, cytoxan, TBI), V.C.B. (vincristine, cytoxan, busulphan). One patient died for septic shock, one relapsed after one month (transplanted in ongoing relapse, as estimatd at FACS analysis of the OKT11+TdT+ cells: 5%), the others are alive in complete remission at 24, 20, 16, 12, 11, 9, 7, 5, 4, 1 months.
Comment: no patient transplanted in remission relapsed. 4 patients (CR 2 and CR 3) are theoretically cured. Our results suggest that this method of purging is at least as effective as the others. As compared to the immunological methods it is fairly cheaper and suitable in patients referred from other hospitals without informations about the phenotype. As compared to cyclophosphamide derivatives vincristine is surely less toxic for both normal marrow cells and for lymphocytes.

K 101 ANALYSIS OF BCR SEQUENCES AND STANDARD CYTOGENETICS AFTER BONE MARROW TRANSPLANTATION OF PH POSITIVE CHRONIC MYELOID LEUKEMIA (CML), R. Arnold, B. Heinze, C.R. Bartram, W. Heit, T. Schmeiser, M. Wiesneth, B. Hertenstein, H. Heimpel and B. Kubanek, University of Ulm, D.7900 Ulm, FRG.
26 patients with Ph positive CML (chronic phase n = 21, accelerated phase n = 5) were treated with an allogeneic bone marrow transplant. 18/26 patients received a T-cell-depleted bone marrow transplant. In all patients cytogenetic follow up was done in regular intervals after bmt. 16/26 patients are alive with a median of 19 months (2 - 63) after bmt. Bcr sequences were studied in 16/26 patients. 7/16 patients are Ph negative and bcr/c-abl negative (investigated 2 - 58 months after bmt). 5/16 patients are Ph negative, bcr/c-abl analyses are pending. 4/16 patients have relapsed after bmt (hematological relapse n = 1, cytogenetic relapse n = 3). 2/4 patients are Ph positive, bcr/c-abl positive. 1/4 patients is Ph positive, bcr/c-abl negative. 1/4 patients is Ph negative, bcr/c-abl positive. Conclusions: 1. Cytogenetics and molecular analysis of the bcr region matched in 9/11 patients. 2. In 2/11 patients results differed. In the Ph negative, bcr/c-abl positive patient this is probably due to the higher sensitivity of the molecular analysis (1 - 5 % detection limit). In the other patient (Ph positive, bcr/c-abl negative) bcr analysis of the marrow before bmt is pending. 3. The molecular analysis of the bcr region is a suitable method for the clonal detection of residual leukemia after bmt in CML patients with a higher sensitivity and less time consuming than cytogenetic analysis.

K 102 RESULTS OF A COMPARATIVE TRIAL OF 4-DEMETHOXYDAUNORUBICIN (IDARUBICIN,IDR) AND ARA-C WITH DAUNORUBICIN (DNR) AND ARA-C IN PATIENTS WITH UNTREATED ANLL. E. Berman, W. Miller, G. Raymond, TS Gee, S. Kempin, S. Gulati, M. Andreeff, J. Gabrilove, J. Kolitz, L. Reich, K. Mayer, CW Young, BD Clarkson. Memorial Sloan-Kettering Cancer Center, New York, NY USA.
IDR, a new analog of DNR, differs from its parent compound by the absence of a methoxy group at position 4. IDR is more potent on a molar basis than DNR and is extensively converted to its active metabolite 13-OH-idarubicinol which has prolonged plasma half life. As a single agent, IDR demonstrated activity in heavily pretreated pts with ANLL. Since 1983, 2 successive protocols (L-19,L-22) have randomized a total of 72 pts with newly diagnosed ANLL between the ages of 16 and 60 to either IDR (12mg/m2 iv qd x 3d)/Ara-C (25mg/m2 iv bolus followed by 200mgs/m2 continuous infusion q d x 5d) or DNR (50mg/m2 iv qd x 3d)/Ara-C (as above) for induction therapy. Thirty-six pts are evaluable on the IDR/Ara-C arm and 33 pts are evaluable on the DNR/Ara-C arm. The median ages of the IDR/Ara-C and DNR/Ara-C arms are 34 and 40 respectively. The distribution of pts within each FAB category is approximately equal. 30/36 pts (83%) on the IDR/Ara-C arm and 20/33 pts on the DNR/Ara-C arm (61%, p=.04) achieved CR. More pts achieved CR with 1 course of IDR/Ara-C than with 1 course of DNR/Ara-C (24/30 pts vs 11/20 pts, p=.06). In summary, IDR/Ara-C is at least as effective and may prove superior to DNR/Ara-C for remission induction therapy for pts with untreated ANLL. Because post induction therapy differed on each protocol, remission duration and long term survival data cannot be combined for analysis.

K 103 ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN 1st COMPLETE REMISSION (CR) : OUTCOME AFTER ALLOGENEIC (n=25) OR AUTOLOGOUS (n=22) BONE MARROW TRANSPLANTATION (BMT).
D. Blaise, D. Maraninchi, M.H. Gaspard, G. Michel, A.M. Stoppa, and Y. Carcassonne. BMT Unit, I. Paoli-Calmettes, 13273 Marseille cedex 9, France.
Between 12/81 and 6/87, 47 patients (pts) with high risk ALL received BMT, allogeneic (allo) when a HLA matched donor was suitable, or autologous (auto) when no donor was identified. Median age was 20 years (range 7-47), including 7 children, with a sex ratio of 28/19. Pts of both groups were comparable in terms of age, initial presentation of ALL, induction chemotherapy : allo. pts were transplanted earlier (me. 2.5 mths after CR) than auto. pts (med. 5. 5 mths after CR) who received more consolidation chemo before BMT. All pts received TBI 2.2 Gy/dx5 days after Cycloph. 60 mg/kgx2 (18 allo - 5 auto) or Melphalan 140 mg/m2 (7 allo-17 auto). Prevention of GVH was done by conventional immunosuppression in 18 allo BMT and T depletion in 7. 7 pts (28%) developped moderate to severe AGVHD. Autologous BMT were treated in vitro by relevant m Ab (B lineage : 14 - T lineage : 4) or 4HC (n=4). Seven pts died in CR from BMT complications (5 allo and 2 auto). With a median follow-up of 26 mths for allo and 17 mths for auto BMT, projected prob. of relapse is respectively of 9% and 52% ($p < 0.01$), leading to disease free survival of 72% (allo) and 44% (auto) (p = N.S.). These retrospective results confirm that early BMT is an efficient form of consolidation of high risk pts with ALL in 1st CR and confirm, in this setting, that the allogeneic effect is an important part of the efficacity of the method.

K 104 TREATMENT OF SERIOUS CYTOMEGALOVIRUS (CMV) DISEASE WITH 9(1,3-DIHYDROXY-2-PROPOXYMETHYL) GUANINE (GANCICLOVIR, DHPG) AND INTRAVENOUS IMMUNOGLOBULIN (IVIG) IN THIRTY-SEVEN ALLOGENEIC BONE MARROW TRANSPLANT (BMT) PATIENTS (PTS), N.C. Bratanow, R.C. Ash, P.A. Turner, R. Smith, G. Haasler, C. Chitambar, R. Hansen, and J. Casper, Medical College of Wisconsin, Milwaukee, WI 53226. We have treated a total of 37 allogeneic BMT pts with CMV disease using a regimen of DHPG and concomitantly administered IVIG. 15 pts had tissue-proven CMV pneumonia. The 1st 4 received lower dose DHPG (6-7.5 mg/kg/day for a mean of 10 days); 2 of 4 had no response (NR), and 2 partial response (PR-improvement in infection or negative cultures, but death due to disease progression or non-CMV events). The subsequent 11 pts with pneumonia received DHPG at 10mg/kg/day for a mean of 10 days, then 6 mg/kg/day, extended maintenance for a mean of 48 days. 8 of 11 (73%) had complete response (CR-clearing of CMV infection and return to normal outpt activities), and 1 had PR (82% total favorable response). Of the 22 pts with CMV disease of other sites (10 viremia with other manifestations, 4 colitis, 3 hepatitis, 1 nephritis, 1 cystourethritis, 1 cystitis, 1 retinitis, and 1 parotitis), 18 had CR, and 2 PR (91% total favorable response). Early intervention with DHPG at evidence of CMV viremia has reduced the number of pts progressing to fulminate pneumonia. These results demonstrate that with early diagnosis, adequate therapy with DHPG and use of IVIG, serious CMV infections including pneumonia, can now be successfully treated in many BMT pts.

K 105 DEVELOPMENT OF A NATIONAL REGISTRY OF UNRELATED BONE MARROW DONORS IN CANADA.
Noel Buskard, Gail Rock, for Canadian Red Cross Society Blood Transfusion Service, Vancouver, British Columbia, Canada.
Seventeen Red Cross Centres are responsible for collecting, testing and distributing all blood products in Canada. Approximately 30,000 tissue typed apheresis donors exist in Canada. Most are Red Cross donors who have been tissue typed to provide HLA-matched single-donor platelets for patients with acute leukemia and those undergoing bone marrow transplantation. Both apheresis and regular whole blood donors have been approached to become unrelated bone marrow donors. Initial experience in developing a national registry indicates that apheresis donors are more likely to become bone marrow donors. As they are already tissue typed it is more cost effective to approach them rather than regular blood donors. Over 75% of apheresis donors responded to a questionnaire about the program. Most of those who did attended information sessions about the program. Over 95% of apheresis donors who attended them signed consent forms. In contrast, less than 5% of whole blood donors who were approached about the program showed interest. We believe that the ethical issues related to approaching tissue typed apheresis donors are overcome by requesting a donation before identification of a patient. With our experience to date, it is anticipated that over 20,000 apheresis donors in Canada will join the registry at little additional cost. The second phase of recruiting and tissue typing regular blood donors will be expensive. A national coordinating centre for all donor requests is being established. A review of the national program will be presented.

K 106 HIGH DOSE VINCRISTINE BY CONTINUOUS INFUSION AS ADDITIONAL DRUG IN CONDITIONING REGIMEN FOR ABMT IN CHILDHOOD ALL, Paolo Colleselli, Marino Andolina, Fulvio Porta, Guido Sotti, Chiara Messina, Maurizio Belloni, Ediberto Agosti, Giorgio Dini, Federico Bonetti and Luigi Zanesco, for Italian ALL ABMT Pediatric Group, Department of Pediatrics, University of Padova, Padova, Italy.
Autologous bone marrow transplantation allows a prolonged disease free survival in about 20% of patients, but the relapse rate after transplant remains the major problem. In an attempt to reduce this high relapse rate we have added high dose Vincristine by continuous infusion to classic Cyclophosphamide-TBI conditioning regimen. The low toxicity of high dose Vincristine results from experience in neuroblastoma transplant. The specific efficacy of Vincristine for ALL is also well known. 11 children affected by ALL entered this study. The ages ranged from 4 to 1 years. ABMT was performed in 1st (3), 2nd (5) or 3rd (3) complete remission. Blood marrows were purged by incubation with Asta-Z or Vincristine-Prednisone. Conditioning regimen was done as follows: Vincristine 1,5 mg/sqm push day 1st, continuous infusion Vincristine 0,5 mg/sqm/day from 1st to 5th day, TBI 1200 cGy: 6 fractions from 2nd to 4th days, Cyclophosphamide 60 mg/kg days 6th and 7th, ABMT day 9. All patients achieved hematologic recovery. No severe toxicities were observed. 7 patients are in complete remission until now from 4 to 18 months after ABMT. 4 patients relapsed 6,5,5,1 months after ABMT. High dose Vincristine doesn't seem to add any additional toxicity to standard conditioning regimen. Because of too early results, the advantages of high dose Vincristine in ABMT are still under investigation.

K 107 BINDING SITES FOR TISSUE SPECIFIC AND DEVELOPMENTAL STAGE SPECIFIC NUCLEAR PROTEINS PRESENT IN LEUKEMIA CELLS LOCALIZED 5' TO ZETA GLOBIN GENE, C.Kane, Z.Howard, K.Purohit, and A. Deisseroth, M.D. Anderson Hospital, Houston, Texas, 77030.
The globin gene family exhibits differential expression of its members during the embryonic, fetal and adult stages of development. Gene transfer experiments in our own and other laboratories have suggested that nuclear transcriptional regulatory proteins specific for each stage of develpment govern the expression of these globin genes during development in man. In order to isolate and characterize such regulatory proteins, we first used nuclease hypersensitivity assays to identify potential areas of interest. We mapped two nuclease hypersensitivity sites specific for cells in which embryonic gene expression occurred 266 base pairs 5' to the start and 80 base pairs 3' to the start of exon I of the zeta globin gene. Three nuclear proteins bound to a 32 nucleotide oligomer in this region: one specific for cells in which embryonic globin genes were expressed, one present in all erythroid cells tested, and one present in non-erythroid but not erythriod cells. We are currently attempting the isolation of these proteins by oligonucleotide affinity chromatography.

K 108 EVALUATION OF MONONUCLEAR CELL LEUKEMIA USING A CELLULAR TRANSPLANT MODEL IN RATS: STUDIES OF PYRIDINE,TRICHLOROPHENOL,ETHOXYETHANOL,AND HEXYLRESORCINOL. M.P. DIETER, J.E. FRENCH, C.W. JAMESON, S.A. STEFANSKI, AND R.S. CHHABRA. NIEHS/NTP, RESEARCH TRIANGLE PARK, NC 27709. An in vivo leukemia transplant model was developed in F344 rats to facilitate the study of the disease. The prognostic ability of this short-term test was evaluated with chemicals that caused positive or negative trends for leukemia in 2-yr carcinogenesis studies. Eight week old male rats were injected s.c. with 2 x 10^7 leukemic spleen cells from syngeneic donors, and chemical dosage was initiated simultaneously or prior to transplant. At 60-90 days after transplantation, organ/body weight, hematology, histology, and mononuclear cell enzyme activity was compared in dosed or undosed rats. Four chemicals of dissimilar structure were evaluated. Data from the transplant model agreed exactly with the conclusions reached in 2-yr carcinogenesis studies. The positive chemicals pyridine and trichlorophenol exacerbated WBC counts and splenomegaly, while the negative chemicals ethoxyethanol and hexylresorcinol ameliorated these responses. Tumor marker enzyme assays in blood mononuclear cells confirmed these results, as did histological examination of spleen and liver. Dosage prior to leukemia transplant enhanced the sensitivity of the model to detect negative, but not positive chemicals, and the detection of both positive and negative chemicals was dependent on the duration of the post-transplant interval, as advanced tumor development masked the differences caused by chemical intervention. Maximum sensitivity in the leukemia transplant model was achieved when neoplasia could first be detected. This event was determined either by the degree of splenomegaly in rats killed at different post-transplant intervals, or non-invasively by the serial assay of tumor marker enyzmes in blood mononuclear cells.

K 109 IMMUNOLOGIC RECONSTITUTION OF PATIENTS WITH REFRACTORY LEUKEMIAS TREATED WITH BONE MARROW TRANSPLANTATION USING CT-2 DEPLETED MARROW FROM MISMATCHED RELATIVES. Patricia Dinndorf, Bridget Flynn, Michael Trigg, Paul Sondel, Jonathan Finlay, Richard Hong, Department of Pediatrics, University of Wisconsin, Madison, Wisconsin, 53792.

We studied the kinetics of reconstitution of 8 children who received transplants for refractory leukemia (3CML, 3AML, 2ALL) using bone marrow from mismatched relatives that was depleted of T-cells using the monoclonal antibody CT-2 and complement. Short term (3-4 mos) and long term (>9 mos) measurements of B cell and T cell function were evaluated.

Short term measurements were obtained on seven patients and categorized as severely impaired, moderate, and normal function:

	B cell:	IgM	IgA	T cell:	PHA	MLC	CML	proliferation to candida
severe		0/5	0/5		2/6	0/5	1/4	2/6
moderate		2/5	0/5		4/6	2/5	0/4	2/6
normal		3/5	5/5		0/6	3/5	3/4	2/6

Long term data was obtained on 5 patients (one at 9 mos, 4 at 1 yr). The patient with severely compromised studies had severe active GVHD:

	B cell:	IgM	IgA	IgG	T cell:	PHA	MLC	CML	proliferation to candida
severe		0/4	2/5	0/5		2/5	0/4	1/5	0/3
moderate		1/4	0/5	1/5		1/5	0/4	0/5	0/3
normal		3/4	3/5	4/5		2/5	4/4	4/5	3/3

The reconstitution seen is similar in quality and quantity to that seen in HLA-matched transplants. We feel acquisition of proliferation to PHA, and candida, and cell mediated lytic activity is significant as these assays may best predict clinical immunity.

K 110 KINETICS OF HEMOPOIETIC RECOVERY CORRELATE WITH LOGARITHMUS OF CFU-GM TRANSFUSED IN AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT), Waltraud Emminger-Schmidmeier, P. Kier, W. Emminger, P. Höcker, W. Hinterberger and H. Gadner, St. Anna Childrens'Hospital, Vienna, Austria

24 children and 48 adults with a bone marrow donor were compared to 21 children and 7 adult patients (pts), who received autologous bone marrow with or without peripheral stem cells. 14 ABMT were carried out without an in vitro manipulation of the marrow. In 18 cases (2 double, one triple ABMT) purging procedures were applied: Asta-Z 15x, monoclonal antibodies (MoAb) 2x, Asta-Z +MoAb 1x). In allogeneic BMT no correlation could be found between cell number and kinetics of engraftment. In 32 ABMT, the number of nucleated cells (log NC/kg) correlated only weakly ($p > 0.05$). The logarithmus of CFU-GM/kg however, correlated with the appearance of reticulocytes in the peripheral blood with a $p < 0.01$ and even more convincingly with the time to recover 0.5×10^9/l and 1.0×10^9/l leucocytes as well as 0.5×10^9/l granulocytes with a $p < 0.001$. Pts. with an allogeneic BMT received median 13.44×10^4/kg CFU-GM (2.94-41.88) compared to the rather low number of median 0.57×10^4/kg CFU-GM (0-22.96) in ABMT. It seems conceivable, that correlations can only be found when suboptimal numbers of stemcells are transfused, above a certain optimal limit the time to engraftment may not be shortened by increasing stem cell numbers.

K 111 PHARMACOKINETICS AND ACTIVATION OF CYCLOPHOSPHAMIDE AFTER IRRADIATION, G. Ehninger, H.P. Waidelich, H.J. Kolb, T. Wagner, and U. Schuler, Medizinische Universitätsklink, Tübingen, FRG.

Radiation induced inhibition of microsomal enzymes has been reported. Cyclophosphamide (CP) is an inactive prodrug metabolized by hepatic microsomal enzymes forming the active alkylating agent 4-hydroxy-CP (4-HOCP). Therefore a reduced exposition to cytotoxic compounds might occur after prior irradiation. Aim of our study was to compare the elimination and activation of CP in 7 patients who had TBI prior chemotherapy (60 mg/kg/day x 2 or 50 mg/kg/day x 4) with our previously reported data of patients who had chemotherapy first. CP levels were measured by N/P-flame ionization gas chromatography, 4-HOCP by liberation of acrolein and its fluorometric determination.

		Before	After TBI
CP half-lives	1st day	7.13 (1.69) N=11	4.31 (1.19) N=5
in hours (STD)	2nd day	5.53 (2.33) N=11	2.35 (0.66) N=5
	4th day	4.33 (0.78) N= 4	2.18 (0.23) N=4
4-HOCP	1st day	10.53 (5.78) N=10	11.37 (5.35) N=7
exposition	2nd day	18.23 (7.70) N=10	23.28 (8.49) N=7
in nmol*h/ml (STD)	4th day	26.05 (6.26) N= 3	16.72 (2.38) N=4

Metabolism and activation of CP is not significantly altered by prior TBI. An increase in the exposition towards 4-HOCP was observed in both groups on the second and fourth day of treatment ($p < 0.05$).

K 112 MARKED REDUCTION IN SYMPTOMATIC CMV-INFECTIONS AFTER BMT BY INTRAVENOUS ADMINISTRATION OF A CMV HYPERIMMUNE GLOBULIN WITH A HIGH TITER OF NEUTRALIZING ANTIBODIES, Hermann Einsele, Angelika Vallbracht, Helmuth Schmidt, Michael Haen, Käthe Schüch, Roland Dopfer, Dietrich Niethammer, Hans-Dierk Waller, Gerhard Ehninger, Medizinsche Klinik, Abt. II, Eberhard-Karls Universität, 7400 Tübingen, FRG
83 patients undergoing bone marrow transplantation for different underlying diseases received intravenous hyperimmune globulin ("Cytotect®", 100 mg/kg body weight) twice before and every third week following BMT until day +100 to prevent CMV infections. Blood products for cell substition were obtained from donors unscreened for CMV serostatus, ten of the patients received therapeutic granulocyte transfusions. Inspite of this only 8 patients, 4 of them during the time they received hyperimmune globulin prophylaxis were found to develop primary or reactivation of a latent CMV infection by serological and cultivation techniques performed routinely once a week or at the onset of symptoms. Only two patients, one when receiving passive immunization, developed symptomatic CMV infection. Both patients suffered from gastroenteritis histologically and by cultivation techniques found to be caused by cytomegalovirus. None of the 83 patients developed CMV pneumonitis or died due to complications of CMV infections.

K 113 AUTOLOGOUS PERIPHERAL STEM CELL TRANSPLANTATION (ASCT) IN A CHILD WITH GENERALIZED NON HDGKIN'S LYMPHOMA, Wolfgang Emminger, P. Höcker, W. Hinterberger, Th. Radaszkiewicz, P. Ambros, W.Emminger-Schmidmeier and H. Gadner, St. Anna Childrens' Hospital, Vienna, Austria
A five year old boy underwent ASCT during partial remission of generalized non Hodgkin's Lymphoma five months after initial diagnosis. The preparative regimen consisted of 12 GY fractionated total body irradiation and 60mg/kg VP-16. According to our results from a previous prospective study in patients (pts) with acute lymphoblastic leucemia and osteogenic sarcoma we determined the optimal time for stem cell pheresis. By two cytaphereses only, we collected 22.96×10^4/kg CFU-GM. On day 9 already, erythropoetic precursors circulated, 1×10^9/l leucocytes were reached on day 11 and 0.5×10^9/l granulocytes on day 13. The pt. required 3 platelet transfusions, reached 100×10^9/l platelets on day 17 and was discharged on day 18. The clinical course was uneventful except for a mild mucositis. The pt. is in complete remission 7 months after ASCT, although he had still tumor cells in his bone marrow at the time of stemm cell collection and also when his conditioning regimen was started.
Autologous stem cell transplantation resulted in rapid engraftment of all three cell lines, a complete remission could be induced in a therapyresistent malignancy, which lasts 7 months now. It may offer an alternative to bone marrow transplantation in selected cases.

K 114 EFFECT OF CYCLOSPORINE (CSA) ON OUTCOME OF PATIENTS (PTS) WITH ACUTE MYELOCYTIC LEUKEMIA (AML) TREATED WITH ALLOGENEIC TRANSPLANTATION, Robert B.Geller, Georgia B.Vogelsang, John R. Wingard, William H.Burns, Rein Saral, and George W.Santos, Johns Hopkins Oncology Center, Baltimore, MD 21205.
Ninety-nine pts (med 26,range 1-48 yrs) with AML received bone marrow transplants from HLA-identical sibling donors after preparation with high doses of busulfan (1mg/kg q6h, 16 doses) and cyclosphosphamide (Cy) (50mg/kg/d,4d). Fifty-three received Cy with or without steroids for graft versus host disease (GVHD) prophylaxis; the remaining 46 received intravenous (iv) CSA. Disease-free-survival (DFS) at three yrs for patients transplanted in first complete remission (CR) was 68% for 22 CSA-treated pts and 30% for 27 pts receiving other GVHD regimens (p=.009). There was a significantly lower incidence of both acute and chronic GVHD (37% vs 78%,p=.008) and death related to GVHD, interstitial pneumonitis (IP),and other viral infections (9% vs 67%,p<.001) in CSA-treated pts. The administration of cytomegalovirus-negative blood products and high dose acyclovir was used primarily in pts who received iv CSA (most recently transplanted). In constrast, for pts transplanted in second and third CR and early relapse, DFS at three yrs was 29% for 24 CSA-treated pts and 27% for 26 pts receiving other regimens (p=.76). GVHD was less frequent in the CSA-treated group (25% vs 62%,p=.015) as was death related to GVHD, IP, and other viral infections (25% vs 54%,p=.073), however the rate of relapse was higher (25% vs 8%,p=.20). We conclude that transplantation for pts in first CR who receive CSA for GVHD is highly effective treatment; pts with more refractory disease treated with CSA have less GVHD and IP, but survival did not improve.

K 115 ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) FOR RELAPSED CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): FAVORABLE PRE-BMT PROGNOSTIC FACTORS PREDICT AN EXCELLENT OUTCOME. Richard E. Harris, Stephen A. Feig, Peter F. Coccia, Phyllis I. Warkentin, Beatrice C. Lampkin, Charles S. August, John H. Kersey, Harland N. Sather, and G. Denman Hammond. The Children's Cancer Study Group, Pasadena, CA 91101.
In a limited institution CCSG trial (CCG-181P) comparing intensive maintenance chemotherapy (CT) to HLA-matched allogeneic marrow transplantation after first relapse in childhood ALL, the relapse-free survival (RFS) for the CT group (N=52) at 4 yrs was 16% vs 38% for the CTX/TBI transplant arm (N=21) vs 52% for the HDAraC/TBI transplant arm (N=19), p=0.02. The following prognostic factors known to impact on the length of first remission were examined for their effect on survival, RFS, and relapse rates for all patients and separately for the CT vs the BMT groups: sex, age, WBC, HGB, platelet count, FAB morphology, blast cell phenotyping, mediastinal mass, lymphoma syndrome, extramedullary disease at or before relapse, rapidity of achieving initial remission, intensity of front-line therapy, and length of first remission. No factor significantly impacted on survival, RFS, or relapse rates in the CT arm. However, in the BMT arms, an age >9 yrs and a WBC >50k/ul at diagnosis, presence of a mediastinal mass or lymphoma syndrome, extramedullary disease at or prior to relapse, the intensity of the front-line therapy, the rapidity of achieving initial remission, and the length of first remission all significantly impacted on outcome after relapse. In contrast to those who received intensive maintenance chemotherapy, most of the children with relapsed ALL in this study with favorable prognostic factors have achieved prolonged RFS and have possibly been cured after a matched allogeneic marrow transplant.

K 116 THE ROLE OF LYMPHOKINE ACTIVATED KILLER CELLS AS GRAFT-VS-LEUKEMIA EFFECTORS AFTER T-CELL DEPLETED BMT, M. Hauch, C. Bordignon, I. Cunningham, J. Brochstein, R.J. O'Reilly and C.A. Keever, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.
The removal of mature T-cells from donor bone marrow with soybean agglutinin and SRBC's has markedly reduced both the incidence and severity of graft-vs-host disease (GvHD) post bone marrow transplantation (BMT). In some types of leukemia (i.e. ALL) SBA$^-$E$^-$BMT has been associated with an increased incidence of relapse, possibly due to the lack of GvHD. Such an increase in relapse has not been observed in SBA$^-$E$^-$BM recipients transplanted for chronic myelogenous leukemia (CML). We have tested the ability of lymphokine activated killer cells (LAK) from CML patients post transplantation of SBA$^-$E$^-$BM to mount a graft-vs-leukemia (GvL) response. Ten patients were studied during the first 6 months following matched BMT. None of the patients received GvHD prophylaxis or had >grade I GvHD. PBL from patients and their donors were cultured for 3—5 days in medium containing 100U/ml recombinant IL-2 to generate LAK cells which were used as effectors in 4 hr ^{51}Cr release assays against cultured K562 cells and host and/or allogeneic CML targets frozen prior to BMT. All of the patients and donors tested had normal lytic activity to K562 and LAK cells from 5 patients killed host and/or allogeneic CML targets. Three of the 5 patients whose LAK cells failed to kill CML targets early after BMT relapsed with their leukemia while the remaining two patients died of infectious complications 3 months post transplant. CML targets from these patients could be lysed by LAK cells from controls or from the non-relapsed group. The anti-CML activity of LAK cells from both donors and patients was higher against allogeneic targets (p= 0.007), suggesting a possible MHC restricted inhibitor within the IL-2 activated PBL. We suggest that LAK cells regenerating early after SBA$^-$E$^-$BMT may have a significant role in leukemia resistance in the absence of GvHD.

K 117 FACTORS INFLUENCING SPEED OF ENGRAFTMENT FOLLOWING MATCHED ALLOGENEIC BONE MARROW TRANSPLANTS (BMT) G. Helenglass, S. Milan, S. Milliken, R.L.Powles, Leukaemia Unit, Royal Marsden Hospital, London, U.K.
Unmanipulated marrow from HLA-identical siblings was infused into 211 patients (pts) aged 2 to 48 years. Cyclosporin was given to all, 148 pts with acute myeloid leukaemia (AML), 38 pts with acute lymphoblastic leukaemia and 25 pts with chronic myeloid leukaemia, who were conditioned with either cyclophosphamide 3.6G/m^2 (157 pts) or melphalan 110mg/m^2, followed by total body irradiation (TBI) at 0.03Gy/min to a total mid-plane dose of 9.5Gy (98 pts), 10.5Gy (80 pts) or 11.5Gy (33 pts). Factors significant on univariate analysis, $p<0.05$, included: diagnosis and remission status, age and sex of recipient, donor/recipient sex match, blood group of patient, gut decontamination, TBI dose and infection. A Cox proportional hazards model was used to determine which factors predicted time to engraftment and the following proved to have independently significant effects: AML pts in CR-1 engrafted faster than more advanced stage pts and other leukaemias. Male→Female grafts were faster than sex matched which were faster than Female→Male. Infection immediately preceding BMT is associated with delayed engraftment. Certain variables may be modified to improve engraftment. Analysis of a large co-operative study of these factors would likely prove enlightening and identify patient cohorts where biological modifying therapies are likely to be of greatest benefit.

Variable	X^2 for inclusion	P Value	Hazards Ratio
AML 1st Remission (CR-1)	20.90	< 0.001	0.54
Sex Match donor/recipient	9.80	< 0.002	Male→Female 0.77 Female→Male 1.49
Infection	10.36	< 0.001	2.20

K 118 SECOND OR SUBSEQUENT REMISSION INTENSIFICATION IN ACUTE LEUKEMIA WITH HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS BONE MARROW TREATED WITH DRUGS *EX VIVO*. **LJ Horwitz, G Spitzer, M Auber, S Jagannath, MJ Keating, KB McCredie, KA Dicke, M. D. Anderson Hospital & Tumor Institute, Houston, TX 77030.**

Percoll-separated bone marrow mononuclear cells were incubated at 37° C for 60 minutes with 5 μg/ml of 4-Hydroperoxycyclophosphamide (4HC) and either 1 or 5 μg/ml of Vincristine (VCR) at 5 x 10^6 cells/ml. Patients were in second or subsequent remission (CR) of acute myeloblastic (AML), lymphoblastic (ALL) and promyeloblastic (PML) leukemia. Conditioning regimen was cyclophosphamide (6 g/m^2), BCNU (300 mg/m^2), and VP-16 (750 mg/m^2). Results to date are as follows:

	CASE# (DX)	1 (ALL)	2 (ALL)	3 (ALL)	4 (AML)	5 (AML)	6 (APL)	7 (Mixed Lineage)	MEAN
	CR #	2	3	2	2	3	2	2	--
	CR1 duration, mos	27	13	2	16	5	17	33	16
	VCR μg/ml	1	1	5	1	1	5	1	--
Recovery (Days)	500 granulocytes	31	23	48	20	35	32	30	31
	1,000 granulocytes	43	24	54	29	38	48	64	43
	50,000 platelets	36	21	48	56	-	40	30	40
	100,000 platelets	43	24	69	194	-	54	64	64
	CR mo post-transplant	4	2	2	11+	2	5	11+	4

Complications included arrhythmias and diverticular abscess in #5, a perirectal abscess and persistent Epstein-Barr viremia in #1, and prolonged cytomegalovirus infection in #4. There were no transplant-related deaths. Addition of VCR to 4HC for *ex vivo* treatment does not impair bone marrow recovery or result in any undue complications, provided that the growth of CFU-GM on a reference-thawed specimen exceeds 5,000/kg. Only #5 had <5,000 CFU-GM/kg. The benefit of adding VCR to 4HC in treatment of CR2 + bone marrow is still undetermined.

K 119 SUCCESSFUL BONE MARROW TRANSPLANT (BMT) FOR CHILDHOOD LEUKEMIA IN A GENERAL PEDIATRIC HOSPITAL, A.L. Kent-Ogden, C.P. Steuber, D.H. Mahoney, Z. Rightmire, and D.J. Fernbach, Baylor College of Medicine, Houston, TX 77030.

Historically, BMT has been done in rigid isolation chambers (laminar flow rooms). Between 1979-86, 29 children were transplanted in a pediatric hospital using routine reverse isolation (surgical masks and strict handwashing). Room air filters were changed prior to BMT. Prophylactic antibiotics and gut sterilization were not used. After 1982 Acyclovir prophylaxis was given to pts with previous HSV exposure. Diagnoses were ALL (16 pts), AML (10 pts) and CML (3 pts). Pretransplant conditioning consisted of CTX (14 pts), AraC/CTX (4 pts) or AraC (11 pts). TBI was given: 750 rad x 1 (12 pts) or 200 rad x 6 (17 pts). All pts had fever (T>101°). The avg fever days(fd)/pt was 9.8 (range 1-59) and avg hospital days(hd)/pt was 47 (range 23-112), or a total of 284 fd/1360 hd. There were 12 cases of documented bacteremia: S. auricularis (1), S. aureus (1), S. epidermitis (5), E. coli (2), and pseudomonas (3). One pt had 4 septic episodes. There were 10 cases of localized infection with fever: otitis media (1), UTI (1), pneumonia (1), herpes stomatitis (3), perianal fissure (2), adenitis (2) and decubitus ulcer (1). Two pts had localized skin infection with pseudomonas or S. aureus without fever. Empiric Amphotericin was given to 5 pts. One pt died of pseudomonas sepsis at day 18 post-BMT and 1 pt with graft failure died of E. coli sepsis and aspergillosis at day 62 post-BMT. Acute GVHD was seen in 16/29 pts (55%), severe (grade 3-4) in 4/29 pts (13.8%). 27 pts were discharged 18-102 days post-BMT. Overall survival is 41% (12/29 pts). Pediatric BMT can be done safely in the general hospital setting without an increase in infectious complications.

K 120 PHASE I/II TRIAL OF HIGH DOSE CYTOXAN, CONTINUOUS INFUSION VINCRISTINE, ESCALATING DOSES OF VP-16 AND TBI WITH AUTOLOGOUS BONE MARROW TRANSPLANT (ABMT) FOR CHILDREN WITH SOLID TUMORS. Morris Kletzel, David L. Becton, Laura Hutchins. University of Arkansas for Medical Sciences, Little Rock, Arkansas 72202.

We have performed 10 ABMT in patients with relapse or 2nd remission solid tumors; (6 Neuroblastoma (NB), 1 Ewing's sarcoma (ES), 1 Wilm's tumor (WT), 1 Hodgkin's disease (HD) and 1 non-Hodgkin's lymphoma (NHL)). The conditioning regimen was cyclophosphamide 1200 mg/m^2 daily for 3 days, VP-16 400, 600, or 800 mg/m^2) daily x 3 days, Vincristine 0.5 mg/m^2 IV push followed by 0.5 mg/m^2 daily x 3 days as a continuous infusion not to exceed 1mg per day; 150 cGy per fraction x 6 fractions in 3 days. Patients were infused with unpurged marrow after evaluation with histologic techniques capable of detecting 1 tumor cell among 10^5 cells. By this method they were determined to be "tumor free." Toxicity included severe stomatitis 10/10, mild SIADH 10/10, and grade 4 myelosuppression 10/10, constipation 2/10, diarrhea > 50 ml/kg 8/10, fever 10/10. One toxic death from monilia sepsis and 1 death from progressive disease while awaiting recovery. The dose limiting toxicity was mucositis. 5/6 patients with neuroblastoma achieved remission with 1 patient remaining free of disease 9+ mo. The remaining 4 have relapsed (median 3.2 mo.). The patients with ES and WT have also relapsed with a mean 4.5 mo. The patient with NHL is alive and in remission 1+ mo. We found this regimen to be effective in eliminating residual disease in NB, NHL and ES even though the patients relapse early, ABMT with this regimen should be used earlier in the course of the disease preferably in the 1st remission. The use of TBI is controversial in this group and may add toxicity.

K 121 BONE MARROW TRANSPLANTATION FOR LEUKEMIA IN ZAGREB, YUGOSLAVIA, Labar B., Bogdanic V., Nemet D., Pavletić Ž., Mrsić M., Kaštelan A., Vrtar M., Grgić-Markulin Lj., Dobrić I., The University Hospital Centre, Zagreb, Yugoslavia

From April 1984 to November 1987, 21 patients with acute myelogenous leukemia (AML), 19 patients with acute lyphoblastic leukemia (ALL) and 12 patients with chronic myelocytic leukemia (CML) were treated with bone marrow transplantation from HLA-compatible sibling donors. The median age was 28 years in AML group, 22 years in ALL group and 28 years in CML group, ranging from 5 to 42 years, from 11 to 41 years and from 17 to 37 years respecitevely. Thirty patients (16 with AML and 14 with ALL) have been transplanted in first complete remission (CR), while 10 patients with CML in chronic phase. Three patients with ALL and one with AML were treated in second CR, while 7 others (5 with AML and 2 with ALL) in relapse. Two patients with CML have been in accelerated phase. All patients were conditioned with cyclophosphamide and fractionated total body irradiation. Prophylaxis for graft-versus-host disease (GVHD) was cyclosporine in 28, methotrexate in 2 and cyclosporine plus methotrexate in 22 patients. Fifteen patients with AML, 13 patients with ALL and 6 patients with CML survived in CR from 3 to 39 months, from 3 to 36 months and from 3 to 30 months respectively. Of the 52 patients who underwent BMT 17 died. The causes of death were relapse in 5, acute GVHD in 5, infections in 3, two encephalopathies, one cerebral bleeding and one heart failure. Overall 36(69%) infectious episodes occured, including 23 bacterial and 13 mycotic sepsis. Nine patients with AML, 8 with ALL and 6 with CML have had an acute GVHD grade I. Eleven patients had a grade II, and 6 patients had a grade III/IV. The probability of survival is 67% at 39 months in patients with AML, 63% at 36 months in patients with ALL and 55% at 30 month in patients with CML.

K 122 **RECOMBINANT HUMAN GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (rh-GM-CSF) FOR HEMATOLOGICAL RECONSTITUTION AFTER BONE MARROW TRANSPLANTATION: FIRST CLINICAL RESULTS**

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The issue of this ongoing study is to assess the effects of rh-GM-CSF on hematological regeneration after autologous and allogeneic BMT. Rh-GM-CSF was administered daily by continuous 24 hrs infusion from day 0-28 post BMT at a dose of 500 µg/m² until neutrophil counts reached 1000/µl, then reduced by ½ at 3 day intervals to a final dose of 50 µg/m², provided neutrophils remained >1000/µl. To date six pts are enrolled in this study. 3 pts with neuroblastoma, 1 with Hodgkin's disease, 1 with ALL received autologous and 1 with ALL allogeneic bone marrow after conventional conditioning. As of 11/11/87 data of the first 4 patients are evaluable. Granulocyte counts reached >500/µl at day 7, 10, 11, 12 and >1000/µl at day 8, 12, 13, 16 respectively. The percentage of bands, immature myeloid cells, monocytes and eosinophils was increased in the peripheral blood. In one patient tested so far, chemotaxis of granulocytes as assessed by leukotriene B4 and FMLP continuously improved, whereas bactericidal activity and phagocytosis remained unaffected. There was no toxicity clearly due to the treatment with rh-GM-CSF. Our first results show, that rh-GM-CSF enhances regeneration of myelopoiesis after bone marrow transplantation.

K 123 RESPIRATORY VIRUS INFECTIONS IN MARROW TRANSPLANT RECIPIENTS,

Per Ljungman, Curt A. Gleaves, Joel D. Meyers, Fred Hutchinson Cancer Research Center, Seattle, Wa 98104

Seventy-eight marrow transplant patients were prospectively evaluated for infection with respiratory viruses including adenovirus, respiratory syncytial virus, parainfluenza viruses 1 and 3 and influenza viruses A and B. Fifteen (19%) patients had a respiratory virus isolated. Parainfluenza 1 was detected in six patients, adenovirus in five, parainfluenza 3 in two, and influenza A and respiratory syncytial virus in one each. Twelve patients had infection before transplant and 11 of these had upper respiratory symptoms. In two of these 12 the virus was isolated again after transplant. Three patients had virus isolated only after transplant. Both patients with parainfluenza 3 infection developed pneumonia. A third patient died with disseminated adenovirus infection. Infections with respiratory viruses are frequent and often symptomatic in marrow graft recipients. Since antiviral treatment is available for some of these viruses, early viral diagnosisis is of clinical importance after marrow transplantation.

K 124 BONE MARROW PURGING OF LEUKEMIA WITH LYMPHOKINE-ACTIVATED KILLER CELLS. G.S. Long, D.V. Cramer, J.C. Hiserodt and R.B. Herberman. University of Pittsburgh School of Medicine and the Pittsburgh Cancer Institute. Pittsburgh, PA 15261.

We have investigated the effectiveness of lymphokine-activated killer (LAK) cells as an in vitro method to purge neoplastic cells from bone marrow prior to autologous transplantation. LAK cells represent activated natural killer cells that have acquired the ability to lyse a wide range of neoplastic cells but not normal cells, including bone marrow. Spleen cells from normal F344 rats are cultured in vitro for five days with recombinant interleukin-2 (rIL-2) to generate highly cytolytic LAK cells. The activated LAK cells are incubated with normal bone marrow and/or cells from the F344 rat leukemia line CRNK-16. The bone marrow treated with LAK cells retains the ability to reconstitute lethally conditioned animals. In naive animals, small numbers of CRNK-16 cells (10³) injected intravenously into syngeneic recipients are uniformly fatal in approximately 30 days. Incubation of increasing numbers of CRNK-16 cells (10³ to 10⁶) with 50 x 10⁶ normal bone marrow cells and 50 x 10⁶ LAK cells (1:1 ratio) significantly reduces the ability of the tumor cells to induce a fatal leukemia in normal animals. The cytolytic LAK cells are capable of preventing leukemia in animals that have received 10-100 times the number of neoplastic cells necessary to produce a fatal leukemia in naive animals.

K 125 LONGTERM SURVIVAL OF BMT RECIPIENTS WITH AML, ALL AND CML AFTER PREPARATION WITH CHEMOTHERAPY AND TOTAL BODY IRRADIATION (TBI) OF 500 cGy DELIVERED AS A SINGLE DOSE H.A.Messner, G.Fyles, J. Meharchand, M.D.Minden, J.E.Curtis, G.Lockwood and D.Tritchler Ontario Cancer Institute, Toronto, Ontario CANADA

A total of 166 patients with AML (n=59), ALL (n=37) and CML (n=70) were transplanted following TBI from a cobalt source using 500 cGy in a single fraction delivered at 50-85 cGy/min. In addition, patients with AML and CML received 100 mg/m²/d x 5 Ara-C and 60 mg/kg/d x 2 CTX. Patients with ALL were treated with 2 mg VCR, 100 mg/d x 5 prednisone, 30,000 units/d x 5 L-Asparaginase and 60 mg/kg/d x 2 CTX. The ages ranged from 16-47 years with a median of 29 years. The followup varied from 15-100 months with a median of 49 months. The actuarial disease-free survival (DFS) at 49 months for the whole group was 35% with a relapse rate of 40%. Patients with AML and ALL transplanted during first remission without CNS disease and patients with CML in chronic phase (for whole group n=69) showed a 52% DFS in comparison to 25% DFS for patients transplanted with more advanced disease (n=97). The corresponding actuarial relapse rates at 49 months were 18% and 56%.
We concluded that preparation of good risk patients with chemotherapy and a single fraction of TBI using 500 cGy resulted in DFS and relapse rates similar to that observed in ablative schedules with fractionated TBI at a higher total dose reported by other centres. The rate of idiopathic interstitial pneumonitis was less than 4%.

K 126 GENETICS OF BONE MARROW ENGRAFTMENT IN MICE, James Michaelson, Mark Lawrence, Martin Dorf, Peter Mauch, James Ferrara and Sumiko Miller, Harvard University and the Center for Blood Research, 800 Huntington Avenue, Boston, MA 02115.

In comparison to graft-versus-host disease, the genetics of long-term bone marrow engraftment in mice is poorly understood. We examined the fate of long term bone marrow grafts from 20 different strains transplanted into $(B6xA)_{F1}$ hosts which had received 884 cGy. Engraftment of erythroid cells was assessed by isoelectric focusing of allelic forms of carbonic anhydrase, lymphoid engraftment by glucose phosphate isomerase isoenzymes. The 20 donor strains tested could be ranked according to the number of bone marrow cells necessary to engraft 50% of $(B6xA)_{F1}$ hosts. Strains tested (and relative number of cells required to cause engraftment) are: A/J (1.4×10^5), B10.A (<1.6), $(C57BL/10 \times B10.A)_{F1}$ (<1.6), D1.LP (1.6), B10.BR (4.3), B6.AKR-H-2^k (6.2), AKR (7.9), C57BL/6 (9.9), $(BALB \times A)_{F1}$, C3H.SW (12), C57BL/10 (14), $(C57BL/10 \times B10.BR)_{F1}$, P (43), DBA/2 (45), B6-C-H-2^d (50), C3H (70), DBA (72), A.SW (>100), BALB (>100), SJL (>100). These data indicate that MHC genotype alone is a very poor predictor of the outcome of engraftment and that non-MHC background and gene dosage are critical factors in the fate of bone marrow engraftment.

K 127 COMPUTER ASSESSMENT OF BONE MARROW CYTOREDUCTION DURING INDUCTION THERAPY FOR ANLL, K.B. Miller, A.S. Tischler, H.D. Priesler, W.R. Vogler, A. Gottlieb, J. Goldberg, H. Grunwald, D. Larson, G. Growman, J. McKenna, Tufts-New England Medical Center, Boston, MA 02111, and The Leukemia Intergroup, Buffalo, NY.
The cellularity of bone marrow biopsies from patients with ANLL undergoing remission induction therapy was determined by a semiautomated microcomputer based image analysis system. The microcomputer system determined the absolute cellularity by counting the number of pixels overlying cells as a percentage of the total area of the home marrow biopsy. The pretherapy, day 6 and day 17 biopsies were blindly analyzed by the computer without knowledge of the clinical events. Twenty-eight patients were treated with a single cycle of induction therapy consisting of 3 days of adriamycin and 10 days of continuous infusion ara-c without maintenance or consolidation therapy. Fourteen patients attained a complete remission. There was no correlation between the computer determined pretherapy, day 6 or day 17 bone marrow cellularity and remission outcome. However, in the patients attaining a complete remission there was a direct correlation between the slope of the cytoreduction and remission duration (r.=.72). The greater the slope of the cytoreduction the longer the unmaintained remission. The pathologist's interpretation of the bone marrow cellularity and the slope of the cytoreduction did not correlate with remission duration (r.=.38). The computer assessment of changes in bone marrow cellularity during induction chemotherapy may provide a rapid and precise model for determining sensitivity to therapy in patients with ANLL.

K 128 PREVENTION OF CMV INFECTION BY BLOOD PRODUCTS: A RANDOMIZED TRIAL, W. Miller, J. McCullough, H.H. Balfour, Jr., R. Haake, N.K.C. Ramsay, A. Goldman, R. Bowman, J. Kersey, University of Minnesota Bone Marrow Transplant Team, Minneapolis, MN 55455.
From 1983 to 1987, 130 CMV seronegative allogeneic BMT recipients were randomized to receive screened (S) seronegative (n=66) or unscreened (U) (n=64) blood products. Data regarding incidence of CMV infection (four-fold rise in CMV titer, culture positivity, tissue evidence of CMV infection) and survival were prospectively collected. CMV infection occurred in 30% (KM estimate) in the S vs. 41% in the U arm (p = .09). However, only 2 of 66 in the S arm and 7 of 64 in the U developed culture or biopsy-proven CMV infections (p=.08). Donor seropositivity was associated with an increased risk of developing CMV infection (30% with seronegative vs. 63% with seropositive donor) (p=.02). Neither recipient age nor acute GVHD independently influenced this risk. Interestingly, overall survival was not improved by screening: 36% (S) vs. 48% (U). This was not due to relapse since 11 patients in the S group and 12 in the U suffered relapse, and one year survival censored for relapse was 52% (KM) vs. 69%, respectively (p=.07). In univariate analysis, the following influenced survival: CMV infection (relative risk of death = 2.0, p=.03), gram negative bacteremia (RR = 2.0, p=.03), and donor serology (67% survival with seronegative vs. 37% with seropositive donor (p=.0005)). Gram negative bacteremia complicated BMT in 21 of 66 receiving screened and 9 of 64 receiving unscreened blood products (p=.05). By multivariate analysis, donor CMV seropositivity (RR=2.0, p=.014) and gram negative bacteremia (RR=1.8, p=.06) appeared to negatively influence survival. Unscreened blood products were associated with a slightly improved survival (RR=.64, p=.08), and CMV infection did not independently predict decreased survival (p=.14). Use of seronegative blood products was not associated with decreased mortality despite a reduction in CMV infection. The effect of this method of CMV prophylaxis on survival may vary from center to center but certainly deserves further study.

K 129 COMPARATIVE ANALYSIS OF THE OUTCOME OF TREATMENT OF B-CELL (SIg+) ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) WITH EITHER HLA-MATCHED ALLOGENEIC BONE MARROW TRANSPLANTS IN REMISSION OR INTENSIVE CHEMOTHERAPY. Sharon B. Murphy, on behalf of the writing committee, Anna Butturini, Mortimer Bortin, Mary M. Horowitz, A. John Barrett, Alfred A. Rimm, Hansjörg Riehm, and Robert Peter Gale, St. Jude Children's Research Hospital, Memphis, TN, UCLA School of Medicine, Los Angeles, CA, International Bone Marrow Transplant Registry (IBMTR), Milwaukee, WI, Westminster Hospital, London, England, and Kinderklinik der Medizinischen Hochschule, Hannover, Federal Republic of Germany.
B-cell (FAB-type L3, surface immunoglobulin-positive) ALL historically has had a grave prognosis with fewer than 10% long-term survivors treated with chemotherapy prior to the 1980's. Since then, with the introduction of intensive regimens, particularly incorporating high-dose cyclophosphamide, progress has been realized and improved survival rates have been reported. With the goal of determining optimal therapy, we analyzed reports of modern chemotherapy trials for B-ALL and transplantation data collected from the IBMTR. Chemotherapy results from the BFM study group ALL trials 81/83 and 83/86 (n=46), the Pediatric Oncology Group studies 8106 and 8617 (n >30), and the St Jude experience are compared to 21 cases collected from the IBMTR who received HLA identical allogeneic transplants in first remissions and analyzed for initial risk factors (eg, CNS disease) and outcome. Results will be presented which indicate that transplantation is not altogether convincingly superior to the best results of modern chemotherapy.

K 130 CLONAL ANALYSIS OF MARROW GRAFTS USING MOLECULAR PROBES, Richard Nash, Rainer Storb, Paul Neiman, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.
Retrovirus-mediated gene transfer studies in mice show successful reconstitution of marrow with a limited number of pluripotent stem cells. Oligoclonal reconstitution in human transplantation could help explain some clinical problems in BMT patients. Clonal analysis of granulocytes and T cells isolated from blood of BMT patients with female donors was done using RFLP's present on the X chromosome. The difference in the methylation pattern between the active and inactive X chromosome can be distinguished using a methylation sensitive restriction endonuclease, Hpa II. Forty-two allogeneic BMT patients with counts adequate for isolating DNA from leukocytes were screened. Blood was collected either between days 50-100 or on their long-term follow-up (>1 year). Heterozygosity for Bgl I polymorphism in the PGK gene was present in 16/41 (39%) of cases and heterozygosity for Bam HI polymorphisms in the HPRT gene was present in 5/42 (12%) of cases. Heterozygosity for both polymorphism was present in one patient. We have been able to obtain donor blood samples from 14 of our 20 heterozygous cases. We have currently analyzed granulocyte DNA from 12 donor-patient pairs and 2 patients without a donor sample. The pattern for all patients was polyclonal except for one patient who had a monoclonal pattern. In this case, the same monoclonal pattern was present in the donor suggesting a skew in the random inactivation of the X chromosome of donor marrow towards one allele. The band intensities of the alleles after Hpa II digestion between the paired donors + patients were similar allowing us to state that oligoclonal reconstitution is unlikely. Therefore, multiple stem cells appear to be involved in hematopoietic recovery after human BMT.

K 131 THE EFFECTS OF ANTITHYMOCYTE GLOBULIN (ATG) ON NATURAL KILLER (NK) CELL SUPPRESSION OF CFU-GM. S Neudorf and M Jones, Children's Hospital, Cincinnati, OH 45229.
ATG is effective therapy for some patients with aplastic anemia. The effects of ATG on hematopoiesis is not well understood. Since NK cells may be involved in the pathogenesis of some cases of severe aplastic anemia as well as in the regulation of normal hematopoiesis, we determined whether ATG affected NK and γ-interferon (INF) mediated suppression of CFU-GM. PBMC were treated 45" with 1.0 mg/ml ATG prior to coculturing with BM cells (E:T 10:1) for 18 hrs and plating for CFU-GM. Colonies were scored on d14. BM alone showed 108±33 colonies/10^5 cells (n=6). BM+untreated PBMC showed 45±18 colonies (58% suppression). ATG treated PBMC did not suppress CFU-GM (124±32 colonies). Similarly, PBMC treated with Leu 11b+complement (C') did not suppress CFU-GM (129±36 colonies). In contrast, PBMC treated with OKT3+C' produced 37±19 colonies (66% suppression) confirming that effector cells were NK cells. ATG activity against NK cells did not require C'. In addition incubation of PBMC with ATG for 16 hrs at 37°C did not result in loss of the anti-NK effects of ATG. We asked whether the suppression of CFU-GM by INF was due to augmentation of NK activity. 10^3 U/ml INF suppressed colony formation 93% (n = 3). Pretreatment of BM with Leu 11b and C' or ATG (1 mg/ml) prior to adding INF did not abrogate suppression of CFU-GM. We conclude that 1) ATG at 1 mg/ml can eliminate NK cell suppression of CFU-GM and the effects of NK cell activity did not require C' lysis and did not modulate 2) leu 11b or ATG did not abrogate INF mediated suppression of CFU-GM suggesting that the suppression of CFU-GM by INF is not due to augmentation of NK cell activity.

K 132 INCREASED RISK OF RELAPSE IN CYCLOSPORIN TREATED BONE MARROW TRANSPLANT RECIPIENTS WITH HEMATOLOGICAL MALIGNANCIES. LONG TERM RESULTS OF A RANDOMIZED TRIAL. O. Ringdén, L. Bäckman, J. Tollemar and B. Lönnqvist. Departments of Clinical Immunology, Transplantation Surgery and Medicine, Karolinska Institute, Huddinge Hospital, Stockholm, Sweden
Recipients of HLA identical marrow with hematological malignancies were randomized to treatment with cyclosporin (CSA, n=30) or methotrexate (MTX, n=29). The number of patients with leukemia being in first remission or first chronic phase was 16 in the CSA group and 17 in the MTX group. The median age was 19 and 18 years in the two groups, respectively. All patients were conditioned with 120 mg of cyclophosphamide/kg followed by 10 Gy of total body irradiation (9 Gy towards the lungs). Follow-up time was from 27 to 66 months with a median of 45. As previously reported the CSA patients had a faster engraftment, and less mucositis compared to the MTX patients. Transfusions, hospitalization and early septicemia did not differ between the two groups. Acute graft-versus-host disease (GVHD) grade II-IV occurred in 40% of the CSA patients and 26% of the MTX patients (n.s.). The cumulative incidence of chronic GVHD was 42% in both groups. The overall incidence of cytomegalovirus (CMV) infections was 60% in the CSA group and 67% in the MTX group. The incidence of CMV pneumonitis was 13% in the CSA patients and 39% in the MTX patients (p=0.1). The cumulative risk of relapse was 47% in the CSA patients, which was significantly higher than 10% in the MTX patients ($p<0.05$). The actuarial patient survival after 4 years was 53% for the CSA patients and 56% for the MTX patients. However, disease-free survival was 59% and 38% respectively (n.s.).
Conclusion. The incidence of acute and chronic GVHD did not differ in patients treated with CSA compared to those randomized to MTX. However, patients treated with CSA had an increased probability of relapse.

K 133 INTENSIVE INDUCTION FOLLOWED BY BONE MARROW TRANSPLANTATION AS CONSOLIDATION IN ADVANCED HEMATOLOGIC MALIGNANCIES. W.B. Rybka, L. Paszat, G. Blake, C.R. Freeman, J.L. Hutchison, J. Leclerc, N.B. Whittemore, M. Sternbach. The Montreal General Hospital, Montreal, Quebec, Canada, H3G 1A4.

Relapsed disease continues to be a major complication following bone marrow transplantation (BMT) for advanced hematologic malignancies (AHM). To develop a strategy for limiting relapse, a regimen of initial intensive induction followed by BMT as consolidation was developed. Initial salvage was attempted with VP-16 500 mg/m^2 daily days 1-3 followed by cyclophosphamide 60 mg/kg daily days 4-5 without additional stem cell support. Of 13 patients treated for AHM (Hodgkin's Disease - 6, Non-Hodgkin's Lymphoma - 4, Acute Lymphoblastic Leukemia - 1, Acute Non-Lymphoblastic Leukemia - 2) 2 were in remission, 10 with measurable disease showed rapid tumour response and 1 failed to respond. Three patients died in initial aplasia. Hematologic reconstitution occured at a median (range) of 30 days (25-46) for neutrophils >500/ul and 27 days (19-47) for platelets >50,000/ul following initiation of treatment. Of 8 patients with hematologic reconstitution to date, 6 sustained complete remission and 2 partial remission. Seven patients went on to BMT as consolidation at a median of 95 days (52-140). Three received total body irradiation (TBI) (200 cGy twice daily X3) on days 1-3 and phenylalanine mustard 110 mg/m^2 day 5. In 4 patients with extensive prior irradiation, busulfan 1 mg/kg 4 times daily days 1-4 was substituted for TBI. The stem cell source was autologous in 3 and allogeneic in 4. Survivals following initiation of salvage therapy are >8, >11, 13, 17, 25, >59, >109, >112, >114, 146, >150, >165, >216 days. The feasibility of this regimen has been demonstrated.

K 134 ENHANCED ANTILEUKEMIC EFFECT OF FRACTIONATED TOTAL BODY IRRADIATION PLUS HIGH DOSE ETOPOSIDE IN POOR RISK LEUKEMIAS N. Schmitz, W. Gassmann, W. Heit, M. Wiesneth, M. Suttorp and H. Löffler. Departments of Internal Medicine and Pediatrics, University of Kiel, D 2300 Kiel, and Department of Internal Medicine III, University of Ulm, D 7900 Ulm, FRG

Forty patients (pts) have received a new conditioning regimen consisting of fractionated total body irradiation (6 x 2 Gy, day -7 to day -5) followed by a single infusion of etoposide (60-70 mg/kg) on day -3. Bone marrow transplantation (BMT) from HLA genotypically identical sibling donors (38 pts), a phenotypically identical mother, or a haploidentical brother (1 pt each) was carried out on day 0. The pts ranged in age from 4 to 48 years (median 22 yrs). The majority of pts had acute non-lymphoblastic leukemia (n=18) or acute lymphoblastic leukemia (n=18). Two pts suffered from CML in accelerated phase, 1 pt each had high-grade NHL in 6. complete remission (CR) or myelodysplastic syndrome. Of the 36 pts with acute leukemias 8 pts were refractory to conventional chemotherapy, 9 pts were in relapse, 6 pts were in 2./3. CR of ANLL, and 2 pts were in 3. CR of ALL before preparation for BMT was begun. 6 pts transplanted for ALL in 2. remission and 5 pts with ANLL in 1. CR were carrying a high risk of relapse because one or more of the following characteristics were present: prior MDS, extramedullary leukemia, monosomy 7, relapse on maintenance therapy. 26/40 pts are alive and free of disease for 3 - 30 months (median: 12 months) after BMT. Actuarial disease-free survival is 63 $\pm$ 5 %. Two pts with HLA-identical donors have rejected their grafts and subsequently died of pneumonia. 9 pts have died of other BMT related complications (i.e. interstitial pneumonia, sepsis, bleeding). Only 3 pts have relapsed between 91 and 151 days after BMT, 2 of whom have subsequently died. The actuarial relapse rate is 12 $\pm$ 5 %.

We conclude, that fTBI/etoposide has a major antileukemic effect and should be considered as an alternative preparatory regimen for patients grafted for acute leukemia.

K 135 ORAL ACYCLOVIR PROPHYLAXIS OF HERPES SIMPLEX INFECTIONS AFTER BONE MARROW TRANSPLANTATION: CLINICAL AND CLINICAL-PHARMACOLOGICAL STUDY, K. Schüch, A. Vallbracht, K. Schüch, I. Kumbier, R. Dopfer, H. Schmidt, P. Ostendorf, G. Ehninger, Medizinische Klinik und Hygiene-Institut, Tübingen, FRG.

Viral infections are one of the major complications after bone marrow transplantation with high mortality and morbidity. Fourty-six patients between 3 and 48 yrs. old (median 15 yrs.) received orally 400 mg (under age of 6: 200 mg) acyclovir four times daily for from day -12 to day 84 after BMT. All patients were isolated in laminar-airflow- units for at least 23 days with total-enteral decontamination. They were concomitantly treated with anti-CMV-hyperimmunoglobulin and cotrimoxazol. During acyclovir prophylaxis 7 pts. had herpes simplex virus infections, all of them were seropositve before BMT. Acyclovir plasma concentrations were measured by use of a new HPLC method. No acyclovir was present (detection limit 40 ng/ml) in the plasma of 5 out of 6 pts. with HSV-infections. Three of them had non compliance, a lack of acyclovir resorption developed in 2 pts. under conditioning regimen. No drug related side effects were observed. Laboratory tests did not show liver or renal toxicity. Take and hematologic reconstitution were unchanged. In our study oral acyclovir reduced the incidence of herpes simplex infections after bone marrow transplantation. Herpes infections only occurred in patients with non compliance or lack of acyclovir resorption.

K 136 FECAL SODIUM AS AN INDICATOR OF GI TOXICITY FOLLOWING BONE MARROW TRANSPLANTATION. A. Taveroff, A.H. McArdle, M. Alton-Mackey, W.B. Rybka. The Montreal General Hospital, Montreal, Quebec, Canada, H3G 1A4.

Intestinal toxicity is a serious complication of chemotherapy (CT) and total body irradiation (TBI) used in bone marrow transplantation (BMT), yet difficult to assess objectively. To determine if fecal nitrogen (N), potassium (K+) or sodium (Na+) could be used as a measure of intestinal injury following CT and TBI, these parameters were measured in 5 patients receiving BMT (including TBI) and 5 patients receiving a high dose CT regimen of VP-16 and cyclophosphamide. Twenty-four hour stool collections were begun 1 day prior to treatment and continued daily for 21 days. Daily fecal losses of N, K+ and Na+ were averaged (Mean±SEM) over 4 periods and compared to baseline (a=p<.05,b=p<.01,c=p<.001):

Regimen	Parameter	Baseline	Day 1-7	Day 8-13	Day 14-20
CT only	Fecal N(g/d)	1.3±0.5	1.1±0.2	1.0±0.2	1.2±0.2
	Fecal K+(mmol/d)	8.1±4.1	6.4±1.1	8.8±1.5	6.2±1.4
	Fecal Na+(mmol/d)	6.0±2.1	16.7±4.9(a)	9.5±2.1(a)	11.1±3.8
CT+TBI	Fecal N(g/d)	2.1±0.6	1.8±0.3	2.9±0.6	4.2±1.1(a)
	Fecal K+(mmol/d)	13.1±3.6	18.4±3.3	11.4±2.2	24.5±4.2(b)
	Fecal Na+(mmol/d)	14.5±5.6	63.8±15.9(b)	72.0±14.7(c)	118.6±22.3(c)

Of the three parameters, fecal Na+ loss changed most dramatically and most rapidly. When compared to World Health Organization criteria of GI toxicity, it exhibited a sensitivity of 89%, specificity of 100% and accuracy of 93%. Fecal Na+ loss provides an objective and non-invasive measure of intestinal injury from CT and TBI.

K 137 PROGNOSTIC FACTORS IN MARROW TRANSPLANT RECIPIENTS WITH LEUKEMIA. J. Tollemar, O. Ringdén, B. Sundberg, B. Lönnqvist, G. Gahrton and B. Nilsson. Departments of Clinical Immunology, Transplantation Surgery and Medicine, Karolinska Institute, Huddinge Hospital and Department of Cancerepidemiology, Karolinska Hospital, Stockholm, Sweden.

Until June 1987, 139 patients with leukemia underwent allogeneic bone marrow transplantation. Conditioning included 120 mg/kg of cyclophosphamide and 10 Gy of total body irradiation (the lungs received 9 Gy). Among the donors were 116 HLA identical siblings, 4 twins and 19 mismatches. Among HLA identical siblings the 5-year survival in 30 patients with acute myeloid leukemia in 1st remission was 71%. All 11 children survived compared to an actuarial 5-year survival of 52% in 19 adults (p=0.01). None of these patients had a relapse. Among 15 patients with acute lymphoblastic leukemia in 1st remission the actuarial 5-year survival was 73% compared to 31% for those (n=25) in 2nd-4th remission. The probability of relapse in these two groups was 9% and 45%, respectively (p<0.05). Patients with chronic myeloid leukemia (CML) in first chronic phase (n=27) had a survival plateau from 1-6 years of 65%. In 12 patients with relapse (>25% blasts in the marrow) or accelerated phase of CML there was only one survivor. The relapse patients were excluded in a Cox's multivariate regression analysis. In this analysis, improved survival was associated with grade 0-I acute graft-versus-host disease (GVHD, p<0.0001), HLA-matched siblings (p<0.0008), recipient age ≤17 years (p=0.02), 1st remission and 1st chronic phase (p=0.03), cytomegalovirus (CMV) seronegative recipients (p=0.04) and absence of symptomatic CMV infection (p=0.04). A decreased risk of relapse in bivariate analysis was seen in patients in 1st remission or 1st chronic phase (p<0.001) and in patients with asymptomatic CMV infection (p=0.03). In multivariate analysis the p-values for these two groups were <0.01 0.09, respectively.

Conclusion: Acute GVHD was the major obstacle to successful outcome in these leukemic patients.

K 138 SUSPECTED DONOR RELAPSE SHOWS CHIMERIC RFLPs. Peter A. Zimmerman, Wendy L. Golden, Mary Kochera, Jerry Stein, Naomi Lang-Unnasch, Marcia Simon, Sarah Strandjord, Phyllis Warkentin, Bruce Blazer and Peter Coccia,
Case Western Reserve University, Cleveland, Ohio 44106.

In February, 1984, a 4 year old boy, diagnosed with Acute Lymphocytic Leukemia (ALL), was presented for genetic marker analysis, prior to bone marrow transplantation (BMT). He was transplanted with marrow from his HLA-identical MLC-compatible sister, following $3g/m^2$ ARA-C (X12 doses) and total body irradiation of 12 Gy. Successful engraftment was confirmed at 3 months post-transplant by observation of normal 46,XX cells in the bone marrow and peripheral blood. Graft versus host disease was evident. In July, 1986, cytogenetic analysis revealed 11.4% abnormal polyploid cells (out of 35 cells) in the recipient's bone marrow, the peripheral blood was 100% normal female. Cytogenetically, the abnormal polyploid cells are considered to be of female origin by absence of the Y-chromosome and by G-band polymorphism of chromosome 9. Molecular analysis, using highly informative DNA probes, detects presence of recipient autosomes 5 and 21 and the X-chromosome. To date, mixed chimeric RFLPs have not been demonstrated to appear among cells which are assessed cytogenetically to be of donor origin.

Aplasitc Anemia, Immune Deficiency, and Genetic Diseases

K 200 USE OF RECOMBINANT GRANULOCYTE/MACROPHAGE COLONGY STIMULATING FACTOR (rGM-CSF) FOR ACCELERATION OF ENGRAFTMENT IN BONE MARROW TRANSPLANTATION (BMT): MOUSE AND MAN. BR Blazar, MB Widmer, S Gillis, NKC Ramsay, JH Kersey, PB McGlave, DA Vallera. University of Minnesota, Minneapolis, MN and Immunex Corp., Seattle, WA
We used rGM-CSF to promote engraftment in an allogeneic murine model of graft failure based on donor T cell depletion (TCD) We found that preincubation of TCD marrow with rGM-CSF (13 μg/ml) (n=31) significantly ($p<0.05$) improved engraftment (57% engrafted) as compared to controls (29% engrafted) (n=28) (Blood, in press, 1987). Subsequently, we have tested rGM-CSF administered in the post-BMT setting. Over a range of doses, we observed no graft-promoting effects in recipients of 14 single daily i.p. injections of rGM-CSF (up to 6.5 μg/day) vs. saline. We also tested the effect of a continuous 14 day delivery system (1 μg/day). Mice that received rGM-CSF (n=60) (vs. albumin: n=65) had significantly ($p<0.01$) superior actuarial survival rates to day 100, but a higher degree of host repopulation than controls. Recipients of continuous infusion rGM-CSF (compared to controls) had significantly ($p<0.03$) lower donor cell numbers (72% vs. 51%), higher mean host cell numbers (23% vs. 43%) and a higher incidence of graft rejection (8% vs. 37%). Our working hypothesis is that ex vivo GM-CSF (in high doses) acts on the donor stem cell pool, while lower doses in vivo administration promotes host immunocompetence and graft rejection. In a phase I/II study, we have infused rGM-CSF into 14 human recipients with ALL that have received autologous purged marrow grafts. Recipients of 14 day infusions of low dose rGM-CSF (32-64 $\mu g/M^2/d$) did not have an absolute neutrophil count (ANC)>1000/mm^3 during the study period of 28 days. In contrast, recipients of 21 days of higher doses of rGM-CSF (120-240 $\mu g/M^2/d$), had ANC>1000/mm^3 by day 16 post-BMT.Toxicities have been mild, consisting of backpain, headaches, and nausea/vomiting temporarily related to the infusion.

K 201 ALTERNATIVE BONE MARROW DONORS FOR CHILDREN WITH APLASTIC ANEMIA, B. Camitta, J.T. Casper, J. Hunter, N. Bunin, J. Menitove, L. Lum, R. Ash, Medical College of Wisconsin, Children's Hospital of Wisconsin and the Blood Center of Southeastern Wisconsin, Milwaukee, WI 53233.
Bone marrow transplants from histocompatible sibling donors cure 80-90% of untransfused and 70% of transfused patients with severe aplastic anemia (SAA). Use of partially matched sibling donors or unrelated donors has been unsuccessful because of high incidences of graft rejection and graft vs. host disease (GvHD). Ten children (ages 2-13, median 4 years) with SAA received marrow transplants at our center using alternative donors (sibling 3, parent 4, unrelated 3). Conditioning for the first three patients included cyclophosphamide (CYCLO) ± irradiation, no T-depletion of the marrow, and methotrexate for GvHD prophylaxis. Subsequent patients received: CYLO + cytosine arabinoside + TBI, monoclonal antibody T-cell depletion of the marrow, and methylprednisolone + cyclosporin for GvHD prophylaxis. Three patients failed to engraft and died; all had been heavily pretransfused. Seven patients engrafted. Acute GvHD was >grade 2 in only one patient (non T depleted); this patient is the only one with chronic GvHD. Two engrafted patients died (pneumocystis pneumonia, day 127; systemic parainfluenza, day 116). The remaining five children are alive 99+ to 2206+ days (median 453+). Donors for the survivors were: siblings 2, parents 1, unrelated 2. Unrelated or partially matched family donor transplants should be considered early in the course of SAA before sensitization to/by blood products occurs. With intensive preparation engraftment is expected and GvHD is mild.

K 202 UNRELATED BONE MARROW TRANSPLANTATION IN CHILDREN. J. Casper, N. Bunin, J. Hunter, K. Murray, L. Lum, R. Truitt, J. Menitove, R. Aster, B. Camitta, R. Ash. Medical College of Wisconsin, Children's Hospital and The Blood Center, Milwaukee, WI
Only one-third of children requiring marrow transplants have a histocompatible family donor. We have performed 13 transplants utilizing matched and mis-matched unrelated donors. Patient characteristics were: ages 1-14 (median 5); 9 male, 4 female; severe AA-2, SCID-1, JCML-2, preleukemia-3, ALL-3, AML-1, CML-1. Donor and recipient were HLA A, B, DR serologically identical in 4, A mismatched in 3, B mismatched in 4, and DR mismatched in 2. Relative responses were greater than 14% in 8 of 12 evaluable MLC assays. Supportive care included acyclovir, trimethoprim-sulfa, IV gammaglobulin, and CMV negative blood products. The conditioning regimen included: busulfan 4 mg/kg/day x 2; cytosine arabinoside 3 gm/m^2 q12hr x 6; cyclophosphamide 45 mg/kg/day x 2, methylprednisolone 1 gm/m^2 q12hr x 4 and total body irradiation 14 Gy/9 fractions/3 days with lung shielding at 11.5 Gy (AA patients did not receive busulfan; SCID pt received 4 doses of cyclophosphamide only). IV cyclosporine 3 mg/kg/day was started on day-2. All marrows were T-cell depleted using a CD3 antibody and normal rabbit serum. Twelve pts engrafted (1 too early). Median days to PMN > 500/mm^3 and platelets > 25,000/mm^3 were 18 and 12 respectively. GVHD > grade II was not seen. Eleven of 13 pts are alive and disease-free 1-397 days post-transplant (median 99 days). One death was due to CMV (d 76) and one to P. Carinii (d 127). Post-transplant immune parameters appear comparable to those seen in T-depleted matched sibling transplants. While follow-up time is short we have demonstrated the feasibility of using even mis-matched unrelated donors for transplanting children with otherwise incurable diseases.

K 203 IMMUNOSUPPRESSIVE THERAPY OF APLASTIC ANEMIA: A RANDOMIZED STUDY COMPARING ANTI-HUMAN THYMOCYTE GLOBULIN (ATG) AND HIGH DOSE STEROIDS TO ATG AND LOW DOSE STEROIDS. Kristine C. Doney, Rainer Storb, C. Dean Buckner, Robert P. Witherspoon, Keith M. Sullivan, Frederick Appelbaum, Jean Sanders and E. Donnall Thomas, Fred Hutchinson Cancer Research Center and the University of Washington School of Medicine, Seattle, WA 98104.
Between July 1984 and June 1987 49 patients (pts) with moderate (N=12) or severe (N=37) aplastic anemia (AA) were treated with ATG, 15 mg/kg/day i.v. for 10 days. Twenty-five pts received low dose methylprednisolone (LDM) (0.5 mg/kg/day during the ATG infusion), and 24 pts received high dose methylprednisolone (HDM) (20 mg/kg/day x 4, 2 mg/kg/day x 8, 1.5 mg/kg/day x 8, 4 mg/day x 14). Forty-eight pts also received oxymetholone, 3 mg/kg/day p.o. for 3 months. Pts in each of the two treatment groups were comparable with respect to age, sex, disease duration, etiology of AA, and severity of disease. Responses in the two groups (HDM vs. LDM, respectively) were as follows: complete, 2 vs. 4; partial, 3 vs. 5; minimal, 5 vs. 1; and no response, 13 vs 15. Three pts in the LDM group had evidence of recurrent aplasia. Acute leukemia evolved in 3 pts, all of whom were treated with HDM. Twelve of the HDM pts are surviving compared to 17 of the LDM pts. Causes of death in each group were comparable. Based on responses and survival to date, there is no advantage to giving ATG + HDM compared to ATG + LDM as immunosuppressive therapy for AA.

K 204 EFFECT OF FRACTIONATED AND LOW DOSE-RATE TOTAL BODY IRRADIATION ON BONE MARROW ENGRAFTMENT. J.D. Down and P.M Mauch, Harvard Medical School, Boston, MA 02115.

The modification of total body irradiation (TBI) treatment with fractionation and low dose-rate is currently being sought in a number of bone marrow transplant centers with the hope of preventing normal tissue side-effects such as pneumonitis. In patients receiving T-cell depleted allogeneic bone marrow, graft rejection is also a major problem where the influence of modifying TBI has not been elucidated.

We have developed an experimental system to investigate engraftment of allogeneic marrow from LP mice into treated C57BL/6 recipients. These two strains have different hemoglobin electrophoretic patterns enabling us to determine the extent of erythroid engraftment in recipient animals.

Dose-response relationships for acute dose (100 cGy/min), protracted (4 cGy/m) and fractionated (2 Gy per fraction) TBI treatment were obtained from this end-point. While 9 Gy as a single acute dose was required to achieve 100% donor chimerism, fractionated TBI only allowed complete engraftment at a total dose of 22 Gy. TBI at 4 cGy /min also required higher doses but to a lesser extent. This dose sparing was not reflected in the CFUs assay.

These results suggest that death of separate target cells determine the efficiency of engraftment and can be distinguished from the CFUs population by their ability to repair and/or repopulate during extented TBI treatments.

K 205 T CELL CLONES WHICH RECOGNIZE AND SUPRESS CYCLING PROGENITOR CELLS: A CELLULAR BASIS FOR BONE MARROW TRANSPLANT GRAFT SUPPRESSION AND REJECTION. Stephen G. Emerson, Shanti Thomas, Susan Guba, University of Michigan, Ann Arbor, Michigan 48109.

Clinical trials performed worldwide using T cell depleted bone marrow transplants have demonstrated an alarming rate of both early and late graft rejection. The cause of this phenomenon is probably multifactorial, and may include contributions from lost T cell stem cell growth factors. However, a major cause appears to be cell-mediated immune rejection of donor stem cells by residual host T cells. To define the cellular and molecular basis for this phenomenon, we have isolated and cloned autoreactive T cells which recognize and regulate progenitor cells *in vitro*. Bone marrow progenitor cells were enriched and isolated by panning, irradiated with 20Gy, and incubated with autologous isolated peripheral blood T cells. The irradiated autologous progenitor cells elicited a strong autologous proliferative lymphocyte response (APLR), which was maximal at progenitor cell:T cell ratios of 1:20 and which peaked at 8 days of culture. By repeating the autologous stimulation protocol three times, a $T3^+T11^+DR^+$ line was generated which proliferated maximally only to autologous enriched progenitor cells. From this T cell line individual autoreactive clones were isolated and expanded by limiting dilution on irradiated autologus progenitor stimulator cells. In the initial cloning eight clones were isolated which maintained their proliferative specificity. When incubated with autologous progenitor cells these clones demonstrated a variety of hematotrophic phenotypes, but when added as the bulk parent line the net effect of these autoreactive cells was to suppress *in vitro* 60-90%. Unregulated expansion of such autoreactive T cells may underlie graft rejection *in vivo*.

K 206 HAPLOIDENTICAL BONE MARROW TRANSPLANTATION FOR IMMUNODEFICIENCY. IN VIVO CONDITIONING WITH MONOCLONAL ANTIBODIES. A. Fasth[1], O. Porras[2], W. Friedrich[3], G. Morgan[4], R. Levinsky[4], G. Hale[5], [1]Dept of Pediatrics, Univ of Göteborg, Sweden, [2]Natl Children's Hospital, Costa Rica, [3]Dept of Pediatrics, Univ of Ulm, FRG, [4]Inst of Child Health, Univ of London, U.K., [5]Dept of Pathology, Univ of Cambridge, U.K.

We report our combined experience in haploidentical bone marrow transplants for various immunodeficiency disorders. All the 13 patients received 16 mg/kg Busulphan and 200 mg/kg Cyclophosphamide. T cell depletion was done by lysis with Campath-1M and human complement achieving >3log T cell depletion. In 9 patients Campath-1M and Campath-2 (CD7) were given intravenously over the last 3 days of the conditioning regime. In another 2 patients Campath-1G was used intravenously for 5 consecutive days from day -8 to day -4. Out of the 11 patients stable enfraftment was achieved in 6. No difference was seen between the two regims. In 2 patients late rejection occurred at 9 and 14 months. Because of the poor results, the conditioning regimen was altered to give Compath-1G earlier from day -12 to day -7. Of the patients one has stable engraftment 9 months later, the other rejected following immunosuppression for acute GvHD.

Although with the latter regime all lymphoid cells were depleted from the circulation at the end of the antibody infusion, some further modification is required to overcome the rejection capacity.

K 207 ANTI-LFA-1 IN VIVO IMPROVES SURVIVAL AFTER T CELL DEPLETED BMT, James Ferrara, Julian Down, Pim van Dijken, Peter Mauch and Steven Burakoff, Dana Farber Cancer Institute and Joint Center for Radiation, Boston MA 02115.

We have studied the use of anti-LFA-1 as an addition to total body irradiation (1100 cGy) in the preparation of recipients for bone marrow transplantation (BMT) in a murine model. A/J mice were given 1100 cGy TBI in a split dose and were then transplanted with 0.63 x 10^6 or 2.5 x 10^6 C57BL/6 bone marrow cells that had been depleted of T cells with anti-Thy 1.2 and complement. Mice then received five daily intraperitoneal injections of saline or 0.1 mg anti-LFA-1. Animals receiving anti-LFA-1 had significantly improved survival when compared to saline controls at both marrow doses. None of the mice in either group showed signs of graft versus host disease. Donor marrow engraftment was greater than 90% in all groups. These studies indicate that administration of anti-LFA-1 immediately after transplantation may have a beneficial effect on survival and engraftment of T cell depleted bone marrow. Further studies will explore the mechanisms of this phenomenon.

K 208 PREVENTION OF GRAFT FAILURE BY AN ANTI LFA1 MONOCLONAL ANTIBODY IN HLA INCOMPATIBLE MARROW TRANSPLANTATION, A. Fischer*, S. Blanche*, F. Le Deist*, C. Gaud*, F. Veber*, M. Debré*, P. Hervé**, P. Bordigoni***, W. Friedrich****, M. Lopez*****, M. Delaage******, C. Griscelli*, * UIH, Enfants Malades, Paris, ** CTS Besançon, *** Nancy, **** Ulm, ***** CNTS Saint Antoine, ****** Immunotech Marseille.

Two multicenters trials are presently conducted testing the ability of an anti LFA-1 monoclonal antibody (mouse anti CD11a-IgG1 isotype) to prevent graft failure following HLA incompatible marrow transplantation. The first includes to date 25 patients with inherited diseases (1 month - 4 years old) Wiskott-Aldrich WAS 9, Combined Immunodeficiency CID 5, Osteopetrosis (OP) 7, Gaucher 1, Metachromatic Leukodistrophy (MLD) 1, Fanconi anemia 1 and the second, leukemic patients (8 evaluable patients (2-36 years old) ALL RC2 5, CML CP 3. All but one received a marrow form a related donor with a least 2 HLA antigens mismatched (1 ag = 1, 2 ag = 12, 3 ag = 20). Patients received in addition to chemotherapy or chemoradiotherapy the anti LFA-1 antibody for 10 days (-3 to +6 at a dose of .2mg/kg/daily). Bone marrow inoculum was T cell depleted. Results are the following. Group 1 (n = 25) early death 1 sustained engraftment 18, alive with functional graft 14 (3-26 months follow up -mean 11). Groupe II leukemia (n = 8) sustained engraftment 6, alive in remission 4 (3 - 7 months). Early bacterial and late (2-5 months) viral infections were frequent and severe, causing death in 6 patients. This was related to the peristance of a T and B cell immunodeficiency for 6 months post transplant. These results indicate that anti LFA-1 antibody contributes to the sustained engraftment of T cell depleted incompatible bone marrow. Secondary infections have to be considered although the consequences of the transient immunodeficiency can be overcome in most of the cases.

K 209 BONE MARROW TRANSPLANTATION (BMT) IN TWITCHER MICE: EFFECTS IN THE CENTRAL NERVOUS SYSTEM (CNS). P.M. Hoogerbrugge[1-3], B.J.H.M. Poorthuis[1-3], G. Wagemaker[1-3], D.W. van Bekkum[1-3] and K. Suzuki[4].
[1]Radiobiological Institute TNO, Rijswijk, The Netherlands; [2]Dept. of Pediatrics, State Univ. Leiden, The Netherlands, [3] Dept. Radiobiology, Erasmus University, Rotterdam, The Netherlands and [4]Univ. of North Carolina, Chapel Hill, U.S.A.
The effect of BMT was studied in the galactosylceramidase deficient twitcher mouse (globoid cell leukodystrophy). Twitcher mice have severe neurological involvement, resulting in a life span of approximately 30-40 days. BMT in 7-12 days old twitcher mice resulted in prolonged survival, prevention of hind leg paralysis and increased galactosylceramidase activity in visceral organs. A gradual increase in galactosylceramidase activity in the CNS to approximately 15% of donor level and a concomitant decrease in psychosine accumulation in the CNS occurred. On light and electron microscopic examination carried out on twitcher mice at day 100 after BMT, lipid loaden, foamy macrophages were found in the white matter of the cerebellum and spinal cord. By immunohistochemical anlysis using donor specific markers, the donor origin of the foamy macrophages was demonstrated. Degeneration of myelin was rare and many thinly myelinated nerve fibers, indicative for remyelination, were observed.

K 210 APPEARANCE OF THYMULIN IN PLASMA FOLLOWS LYMPHOID CHIMERISM AND PRECEDES DEVELOPMENT OF IMMUNITY IN PATIENTS WITH SCID TRANSPLANTED WITH T CELL-DEPLETED HAPLOIDENTICAL MARROW, Genevieve S. Incefy, Neal Flomenberg, Trudy Small, Nancy A. Kernan, Joel Brochstein, Dahlia Kirkpatrick, Neena Kapoor, Susan Groshen and Richard J. O'Reilly, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.
Levels of thymulin activity (FTS-Zn), a thymic peptide that circulates in peripheral blood, were analyzed in 15 patients with severe combined immunodeficiency (SCID) who were treated with transplantation of HLA-haplotype mismatched parental bone marrow depleted of T-cells by differential agglutination with SBA and E-rosetting (SBA^-E^- BMT). Before transplantation, 14 of 15 patients demonstrated undetectable or low plasma thymulin levels for their age and only one had a level in the normal range. After SBA^-E^- BMT, however, thymulin became detectable in the plasma of 13 of 14 evaluable patients and reached normal or near normal levels between 21-125 days post-transplant. In those patients in whom the timing of engraftment could be established by emergence of donor lymphocytes, thymulin appeared in the plasma at approximately the same time as chimerism was detected, and in all patients who were engrafted and immunologically reconstituted, the increment in thymulin levels preceded development of immune functions. These studies further support the concept that marrow derived cells induce thymic epithelial secretory function. Furthermore, following T cell-depleted graft, immunologic reconstitution is not seen unless and until recovery of thymus function has been observed.
Supported by PHS grants CA33168 and CA33050, DHHS.

K 211 MARROW TRANSPLANTATION FOR RETICULAR DYSGENESIS. F Leonard Johnson, Henry G Herrod and Victoria Turner. St. Jude Children's Research Hospital and the Universities of Tennessee, Memphis, TN 38101 and Chicago, Chicago, IL 60637.
A three month old male infant was referred for treatment of severe combined immune deficiency and complete absence of circulating neutrophils (reticular dysgenesis). The patient demonstrated excessive suppressor cell activity in both mixed lymphocyte cultures and assay of <u>in vitro</u> immunoglobin synthesis. Circulating T-cells bore the maternal HLA haplotype. He was treated without pretransplant immunosuppression by allogeneic marrow tranplantation ($7.8x10^8$/kg nucleated cells) from his histocompatible 5 year old sister. The maternal T-cell haplotype was not detectable by 1 week and excessive suppressor cell activity disappeared by 3 weeks. Immune recovery returned to normal within 9 months. The patient remained severely neutropenic with a neutrophil count of $<0.1x10^9$/l 15 months following transplantion. Cytogenetic analyses revealed the XX karyotype in peripheral blood and XY karyotype in marrow. A second transplant was performed 16 months following the first, using the same donor and following immunosuppression with busulfan and cyclophosphamide. His recovery from this transplant was uncomplicated with rapid reconstitution of all 3 hemopoietic cell lines and immune function. Three years following the second transplant he is growing normally with normal blood counts and immune function. Cytogenetic analysis of peripheral blood and marrow show the XX karyotype. Marrow transplantation without preparation can restore immune function in reticular dysgenesis but in some patients additional immunosuppression appears necessary for complete myeloid reconstitution.

K 212 USE OF LYMPHOKINE-ACTIVATED KILLER (LAK) CELLS TO PREVENT REJECTION OF TRANSPLANTED BONE MARROW STEM CELLS. Eicchi Azuma and Joseph Kaplan, Wayne State University School of Medicine, Detroit, MI 48201

We have found that lymphokine-activated murine bone marrow and spleen cells with properties of LAK cells exhibit both veto and nonspecific suppressor activity. Prompted by these findings, we have tested the the ability of LAK cells to prevent rejection of bone marrow transplants. Irradiated (900 Rad) B6 mice were given 5 x 10^5 bone marrow cells i.v. from syngeneic B6, semiallogeneic B6D2, or allogeneic C3H mice either alone or together with equal numbers of B6D2 LAK cells (bone marrow cells cultured in lymphokine-rich medium). The numbers of splenic foci generated by day 7 in B6 recipients of B6D2 and C3H marrow alone were 32±17% and 21±5% of those generated in recipients of syngeneic B6 marrow. By contrast, the numbers of splenic foci generated in B6 recipients of B6D2 and C3H marrow coinjected with B6D2 LAK cells were 133±43% and 84±33% of those generated in recipients of syngeneic B6 marrow. These findings indicate that LAK cells markedly inhibit resistance to semiallogeneic and allogeneic marrow in mice, and raise the possibility that such cells might be useful in preventing graft rejection in human recipients of allogeneic bone marrow transplants.

K 213 LONG TERM MARROW SELF RENEWAL DEFICIT FOLLOWING TRANSPLANTATION OF DIFFERING QUANTITIES OF SYNGENEIC MARROW, Peter M.Mauch and Samuel Hellman, Department of Radiation Therapy, Harvard Medical School, Boston, Mass, and Memorial Sloan Kettering Cancer Institute, New York, NY.

Male C3H/HeJ mice were transplanted with varying doses of syngeneic marrow following 1250 cGy total body irradiation. By 30 days post transplant bone marrow death was seen in 100%, 69%, 13%, and 4% of mice receiving 10^4, 10^5, 10^6, and 10^7 syngeneic marrow cells, respectively. Surviving animals were evaluated at 3, 6, and 9 months post transplant for peripheral blood counts (PBC), and bone marrow cellularity, CFUs content, and Rs, a measure of CFUs self renewal capacity. No differences in PBC or in marrow cellularity were seen. Mean CFUs content per hind limb (HL) was significantly lower for the 10^5 group (1744 CFUs/HL) as compared to the 10^6 (2385 CFUs/HL), $p = 0.01$, 10^7 (2633 CFUs/HL), $p = 0.001$, or non-transplant groups (2990 CFUs/HL), $p = 0.001$. CFUs self renewal capacity (Rs) was also significantly lower for the 10^5 group (1.7 ± 0.4) as compared to the 10^6 (7.4 ± 1.8), 10^7 (16.4 ± 5.7), or non-transplant groups (34.6 ± 4.6), $p = 0.001$. The 10^6 and the 10^7 groups were also significantly different from each other, $p = 0.001$, and from non-transplant groups. These differences persisted out to the 9 months evaluated. A significant decrease in animal survival as a function of decreasing donor marrow cell dose was also seen over this time period. These data demonstrate a persistent and cell dose dependent deficit in CFUs/HL and in bone marrow self renewal capacity following bone marrow transplantation that cannot be appreciated through PBC and marrow cellularity. This adds evidence for the limited renewal capacity of the marrow compartment in the transplant setting.

K 214 UNRELATED DONOR (URD) BONE MARROW TRANSPLANTATION (BMT) AT THE UNIVERSITY OF MINNESOTA, Philip McGlave, Diane Arthur, Bruce Blazar, Alexandra Filipovich, William Krivit, Jeffrey McCullough, Norma Ramsay and John Kersey, University of Minnesota, Minneapolis, MN 55455. Fourteen patients with leukemia, 1 with combined variable immunodeficiency (CVID), and 1 with Hurler's syndrome underwent URD BMT. All 16 donor/recipient combinations were HLA DR identical and MLC nonreactive. Of 14 leukemia cases, 12 had CML (8 1CP; 2 AP; 1 BC; 1 2CP) and 2 had ANLL (1 CR; 2CR). Median age was 29 years (range = 13-43). Six donor/recipient pairs were identical at the HLA A and B loci; 7 differed at 1 A antigen; 1 differed at 1 B antigen. All leukemic patients were prepared for BMT with cyclophosphamide (Cy), 60mg/kg/day (day -7, day -6) and 1320cGy fractionated TBI given in 8 doses over 4 days (day -4,-3,-2,-1) and received non-T lymphocyte depleted donor marrow. Thirteen of 14 patients developed peripheral blood evidence of engraftment at a median of 31 days (range = 23-48 days). One patient died without evidence of engraftment at day +44. Nine of 11 evaluable patients developed grade II-IV acute GVHD, 6 of 8 developed extensive chronic GVHD. Eight of 14 leukemic recipients have died, including 6 recipients with donors mismatched at the A or B loci and 2 recipients with HLA matched donors. Six CML recipients (5 CP; 1 AP) survive at 82-908 days (median = 524 days). Four of 6 survivors had HLA identical donors. Karnofsky activity assessment of all survivors is 100%. A 13 year old male with CVID received URD marrow mismatched at both HLA A and B loci. This recipient was prepared for transplant with 850cGy single dose TBI (day -5) and Cy, 50mg/kg/day (day -4,-3,-2,-1) and received T lymphocyte depleted marrow. Donor engraftment was verified by RFLP at day +15. The patient died at day +32 of a CNS bleed. Finally, a 1 year old boy with Hurler's syndrome was prepared with busulfan, 4.5mg/kg/day (day -11,-10,-9,-8) and Cy, 60mg/kg/day (day -5,-4,-3,-2) followed by infusion by T-lymphocyte depleted URD marrow mismatched at the B locus. Donor marrow did not engraft and the patient is undergoing retransplant. URD is useful in the treatment of CML and may be effective in the therapy of other lethal bone marrow stem cell disorders.

K 215 BONE MARROW TRANSPLANTATION FOR CONGENITAL AND ACQUIRED IMMUNODEFICIENCY SYNDROMES, Robertson Parkman, Carl Lenarsky, Donald Kohn and Kenneth Weinberg, Division of Research Immunology/Bone Marrow Transplantation, Children's Hospital of Los Angeles and the University of Southern California School of Medicine, Los Angeles, CA 90027

Allogeneic bone marrow transplantation (BMT) is the treatment of choice for congenital immune deficiency states, which involves either lymphoid and/or hematopoietic stem cells. All forms of severe combined immune deficiency (ADA deficiency, IL 2 deficiency, NP deficiency, etc.) except IL 1 deficiency can be corrected by the replacement of the abnormal lymphoid stem cells with normal histocompatible or haploidentical lymphoid stem cells with the persistence of hematopoiesis of recipient origin. Other defects of T lymphocyte function (Wiskott-Aldrich syndrome, gpL-115 deficiency, Chediak-Higashi syndrome) can be treated by histocompatible BMT although the results with haploidentical BMT are less successful. At present, BMT is not used to treat immune deficiency states due to defects in B lymphocyte differentiation or function. Defects of myeloid differentiation and function (infantile agranulocytosis, chronic granulomatous disease, actin deficiency, etc.) can be corrected by the replacement of the abnormal recipient hematopoietic stem cells by normal hematopoietic stem cells. Ablation of the recipient's normal lymphoid stem cells is necessary to assure stable donor engraftment. BMT for acquired immune deficiency syndrome has been limited. BMT for the acquired immune deficiency syndrome will have its greatest likelihood of success if 1) the number of virally infected cells is reduced prior to transplantation; 2) the patients are transplanted before opportunistic infections occur; and 3) recipient lymphoid and hematopoietic stem cells are ablated to permit maximal donor stem cell engraftment.

K 216 THE ROLE OF DONOR T AND NON-T CELLS IN PROMOTING STEM CELL ENGRAFTMENT IN ANTIBODY-FACILITATED BONE MARROW TRANSPLANTATION, M.W.J. Sadelain, M. Voralia and T.G. Wegmann, Dept. of Immunology, Univ. of Alberta, Edmonton, Canada T6G 2H7

We have previously reported a murine model of bone marrow transplantation in which complete engraftment of donor stem cells is facilitated without irradiation of the host or cytoreductive drug therapy. Syngeneic or semi-allogeneic normal adult hosts are conditioned by a single injection of monoclonal antibody against host major histocompatibility-encoded molecules. We have shown that donor chimerism levels are higher in the $P \rightarrow F_1$ than in the syngeneic $F_1 \rightarrow F_1$ donor-host combinations. These observations prompted us to investigate the optimal composition of the donor cell inoculum, with special reference to whether graft-versus-host reactive cells can increase the engraftment of donor stem cells.

Pretreatment of donor bone marrow with antibodies to T cell markers and complement did not reduce the capacity to engraft, as compared to non-depleted grafts. Moreover, bone marrow from donors tolerized to host alloantigens engrafted as efficiently as that of normal donors. We conclude that antibody-dependent engraftment is indeed donor T cell-independent, in contrast to results seen in some other models. Nevertheless, an accessory role in enhancing engraftment was found for $L3T4^-$, $Ly2^-$, asialo GM_1^+ cells present in the donor inoculum, suggesting that a non-T cell mediated graft-versus-host reaction could play a role in donor stem cell engraftment. We will discuss the possibility that two alternate pathways of engraftment may exist, with respect to T cell involvement.

K 217 ENGRAFTMENT OF FULLY ALLOGENEIC BM WITHOUT LETHAL IRRADIATION. Yedida Sharabi and David H. Sachs, National Institutes of Health, Bethesda, MD 20892

A major problem in applying animal models of mismatched bone marrow transplantation (BMT) to the clinical situation is the need in such models for high levels of irradiation to ensure engraftment. We therefore have attempted to abrogate host resistance without lethal irradiation, using anti-T cell monoclonal antibodies (mAbs) in vivo, with or without a low dose of total body irradiation (TBI). B10 mice were injected 5-6 days before BMT with GK1.5 (anti-L3T4) and 2.43 (anti-Lyt2), or with 30-H12 (anti-Thy1.2), or with a combination of all three mAbs. Although such treatments depleted most host T cells, they did not permit engraftment of fully allogeneic (B10.D2) BM. However, animal pretreatment with GK1.5 and 2.43, plus a low dose of TBI (300R) on the day of BMT, led to a transient state of chimerism with donor cells detectable 2 weeks after BMT but absent by 5 weeks. Addition of irradiation of the host thymus (700R) to this regimen greatly improved alloengraftment, allowing maturation of donor T cells and prolonging the chimeric state. Using this regimen 2 of 7 mice remained chimeric longer than 96 days, and 1 animal developed specific tolerance to a donor skin graft. Three of 4 control animals receiving mAbs, 300R TBI and thymus irradiation but not BM survived. In an attempt to further increase the incidence of stable engraftment we administered 30×10^6 donor spleen cells intravenously 2 weeks after BMT. This treatment resulted in development of fully allogeneic chimerism in 5 of 6 mice and mixed chimerism in the remaining animal. Three of these 6 mice demonstrated long term survival with no clinical evidence of GVHD. This study demonstrates that long term engraftment of fully allogeneic BM and donor specific tolerance can be achieved without a lethal host conditioning regimen.

K 218 RECOVERY OF THE IMMUNE RESPONSE AFTER TRANSPLANTATION OF BONE MARROW DEPLETED OF T LYMPHOCYTES BY IT-101, Gwendoline M. Spurll and Axel A. Fauser, Royal Victoria Hospital, PQ, H3A 1A1, Canada.
The removal of mature T lymphocytes from transplanted marrow decreases the incidence and severity of GVHD. However the effect of T cell depletion on immune reconstitution is unknown. We have used T101 linked Ricin A chain remove T cells from the marrow to be grafted, and have studied the recovery of the B and T cell subsets, and of the ability of T lymphocytes to support immunoglobulin synthesis. The return of B cells, T3 cells, and T4 cells was similar to that in patients receiving autologous transplants. In contrast, the allogeneic non-T cell depleted patients have higher numbers of T3 and T4 cells in the first months after transplantation, perhaps representing activation of these subsets by allogeneic recognition. T8 cells increased after allogeneic transplantation, whether T cell depleted or not, but increased later with T cell depletion. In vitro IgG and IgM production were normal within 2-3 months after allogeneic transplantation whether or not T cells were depleted. In summary, the course of lymphocyte reconstitution after T-cell-depleted allogeneic bone marrow transplantation is more similar to that seen in autologous transplantation than is non-T cell depleted, and the recovery of antibody production is similar whether T cell depletion is performed or not.

K 219 INFLUENCE OF RECIPIENT NK CELL DEPLETION ON SURVIVAL AND HEMOPOIETIC ENGRAFTMENT AFTER LETHAL IRRADIATION AND BONE MARROW TRANSPLANTATION (BMT), Pierre Tiberghien*, John Wine#, Dan L. Longo#, and Craig W. Reynolds#, *BCDP, Program Resources, Inc. and #Biological Response Modifiers Program, NCI-FCRF, Frederick, MD 21701-1013.
In vivo administration of an antiserum against asialo-GM_1 (asGM_1) abrogates NK activity as well as resistance to hemopoietic allograft. However, this effect of NK cells on hemopoietic resistance has been essentially investigated at short periods immediately following BMT by spleen colony formation or IUDR incorporation assays, thus measuring only the effects of the very early steps of hematopoietic reconstitution. In order to investigate the long-term effects of NK depletion in BMT recipients, we decided to examine survival and chimerism at a 2 month endpoint in lethally irradiated, anti-asGM_1 treated BMT recipients. The donor bone marrow (BM) cells were from nude mice (BALB/c) in order to eliminate GVHD and allow correlation of survival with immuno-hematopoietic reconstitution. Different strains of mice were used in order to examine syngeneic and allogeneic combinations. Various doses of donor BM cells were used to quantitate the effects of NK depletion. Initial results demonstrated that anti-asGM_1 treatment of the recipient prior to BMT confers a significant survival advantage to the BMT recipient. This advantage varied within the strains used and was most dramatic in the BALB/c (nude)-B6 combination. Furthermore, results in the allogeneic settings showed evidence of an increased percentage of donor lymphocytes in BMT recipients treated with anti-asGM_1 antiserum. Taken together, these results confirm the previous hypothesis that NK cells contribute significantly to the immune response leading to tolerance or rejection of foreign BM cells in lethally irradiated recipients.

K 220 **CORRECTION OF HUNTER'S SYNDROME WITH BONE MARROW TRANSPLANTATION: A THREE YEAR FOLLOW UP REPORT.** Phyllis I. Warkentin, Sarah E. Strandjord, Chester B. Whitley, Morris S. Dixon, Irwin Schafer and Peter F. Coccia, Case Western Reserve University, Cleveland, Ohio and The University of Nebraska Medical Center, Omaha, Nebraska 68105.
The long term potential of bone marrow transplantation (BMT) to metabolically and clinically correct mucopolysaccharidosis (MPS) is unknown. We report the 38+ mo follow up of a boy with Hunter's Syndrome (MPS II) treated at 7.5 yrs of age with allogeneic BMT from his HLA-MLC identical sister after preparation with busulfan 4 mg/kg/d X 4 d followed by cyclophosphamide 50 mg/kg/d X 4 d. Sustained engraftment is documented by donor sex chromosomes and ABO group on several occasions. Iduronate sulfatase was undetectable pre-BMT in leukocytes or serum; normal leukocyte levels and low but definite serum levels were present on multiple determinations 2.5-38 mo post-BMT. Urinary glycosaminoglycan (GAG) excretion markedly decreased toward normal. Pre-BMT hepatomegaly has resolved; hepatic storage lysosomes disappeared and hepatic GAG content normalized 24 mo post-BMT from 2203 ug/gm liver to a mean of 160 ug/gm (nl=42-293 ug/gm). CSF GAG was normal 24 mo post-BMT. Progressive clinical improvement has been apparent since 8 wks post-BMT. Subcutaneous nodules and storage material in skin have disappeared; hursuitism and coarse facial features have diminished; joint contractures are markedly decreased; and fine motor skills have improved. He has grown > 14 cm in height. He is doing well in 4th grade; his performance IQ is unchanged from pre-BMT; his verbal skills have decreased slightly. His temperament is less aggressive. We conclude that BMT has successfully reversed many of the features of Hunter's Syndrome in this patient and at least stabilized his CNS.

K 221 ENHANCED RETROVIRAL GENE TRANSFER INTO PRIMATE BONE MARROW PROGENITOR CELLS ENRICHED BY DISCONTINUOUS ALBUMIN GRADIENTS, Robert Wieder[1], James A. Zwiebel[1], Gerard Wagemaker[2] and W. French Anderson[1], [1]Laboratory of Molecular Hematology, NHLBI, NIH, Bethesda, MD 20892 and [2]Radiobiologisch Instituut TNO, 2280 HV Rijswijk, The Netherlands.

Before human gene therapy trials can begin, the efficiency of retrovirally mediated gene transfer into primate bone marrow progenitors ought to be improved, potentially by enrichment of infectable progenitor cells. Bovine serum albumin (BSA) step gradient centrifugation has been shown to enrich CFU-GM and BFU-E precursors in rhesus monkey bone marrow. We suspended rhesus mononuclear marrow cells in a 17% BSA solution and layered them upon strata of 21%, 22%, 23% and 25% BSA solutions forming four interfaces which we called 1, 2, 3 and 4 in descending order. The gradients were centrifuged at 1000xG for 30 minutes at 20°C. Unmanipulated cells and cells from each interface were either cultured in methyl cellulose, or were infected with N2, an amphotropically packaged Moloney based retroviral vector coding for neomycin resistance. The infected cells were cultured in cellulose in the presence or absence of G418 (a neomycin analogue). The enrichment in CFU-GM precursors was 3-fold in interface 3 and reached a maximum of 5-6-fold in interface 4. The increased frequency of G418 resistant colonies reached a maximum of 15-fold in interface 3 and was 11-fold in interface 4. The method appears to be useful for enriching CFU-GM precursors as well as infectable progenitors. These two categories of cells appear to separate on gradients, either suggesting that only a subset of progenitors is infectable or that a potential inhibitor of infection migrates with enriched progenitors.

Graft vs. Host

K 300 CYCLOSPORINE AND METHOTREXATE VERSUS CYCLOSPORINE ALONE FOR PREVENTION OF ACUTE GRAFT VERSUS HOST DISEASE AFTER BONE MARROW TRANSPLANTATION FOR LEUKEMIA

Bogdanić V., Mrsić M., Nemet D., Plavšić F., Pavletić Ž., Labar B., Department of Medicine and Department of Clinical Laboratory Diagnostics, University Hospital Centre, Zagreb, Yugoslavia

From May 1985 to July 1987, 28 patients with acute leukemia (16 acute myelogenous leukemia and 12 acute lymphoblastic leukemia) and 6 patients with chronic myelocytic leukemia were entered into a study for prevention of acute graft-versus-host disease (GVHD). All patients were conditioned with cyclophosphamide and fractionated total body irradiation followed by marrow infusion from HLA-compatible sibling donors. The patients were randomized on order to receive cyclosporine (CSP) plus methotrexate (MTX)(N=17) or CSP alone (N=17). Assesment and grading of acute GVHD were based on the experience of the Seattle bone marrow transplant team. All patients had evidence of sustained engraftment. The incidence of acute GVHD is significantly lower in the CSP plus MTX group (27%) compared to CSP group (47%) (p=0.024). The incidence of severity of acute GVHD was also lower in the CSP plus MTX group. Fiftheen out of 17 patients on CSP plus MTX and 12 out of 17 patients on CSP were alive from 2 to 26 months (median 7 months). The probability of survival in CSP plus MTX group and CSP group were 61% and 51% respectively. Our preliminary results are in accordance with the previous reports that combination of CSP plus MTX is superior thanCSP alone in the GVHD prevention.

K 301 **HUMAN CLINICAL TRIALS WITH A PAN-T-LYMPHOCYTE MONOCLONAL ANTIBODY-RICIN A CHAIN IMMUNOTOXIN IN STEROID REFRACTORY GRAFT VERSUS HOST DISEASE(GVHD). V Byers, P Henslee, N Kernan, B Blazar, R Gingrich, G Phillips, J Antin, R Mischak, R O'Reilly, P Scannon. Xoma Corp., Berkeley, CA, U Kentucky, Sloan-Kettering Cancer Center, New York, NY, U Minnesota, U Iowa, Vancouver General Hospital, Harvard U.**

In a phase I/II trial 46 patients with steroid resistant acute or chronic GvHD were treated up to 14 days with an anti-pan-T-lymphocyte(CD5)-ricin A chain immunotoxin (XomaZyme^{R}-H65), in doses escalating from 0.05 to 0.33 mg/kg daily. Approximately 80% had acute GvHD, most with grade III-IV, and all had failed at least 5 days of steroids. Efficacy was evaluated in patients who received 7 doses and were alive at day 15 (24 patients). Ninety percent of evaluable acute GvHD patients showed clinical response. The most responsive organs were the skin and GI tract, although 45% of patients with liver involvement also showed improvement in that organ. Chronic patients also had responses, with clearance of ulcers, decrease in skin thickening and desquamation, and resolution of systemic symptoms. Complete response was associated with a shorter time between BMT and immunotoxin therapy. A drop in the CD5 bearing T lymphocytes occurred between study days 1 and 6 to less than 20% of starting value, and in most cases remained low for 1-3 weeks post therapy. Formation of antimurine antibody was minimal. Hypoalbuminemia, correctable with albumin infusions, was the most prominent clinical observation. BUN and creatinine abnormalities occurred in a minority of patients on concomitant nephrotoxic medications, and usually with a pre-renal component. Further clinical studies are in progress.

K 302 ALLOGENEIC BONE MARROW ENGRAFTMENT IN NEWBORN MICE TREATED PRE AND POSTNATALLY WITH ANTI-THY 1.2 MONOCLONAL ANTIBODY. Ken Culver, Wayne Smith and Morton Cowan. Department of Pediatrics. University of California, San Francisco, 94143.

The mouse is immunologically incompetent at birth, comparable to the human fetus at 14-16 weeks gestation. Using the newborn mouse, we have investigated the ability of monoclonal antibody (MoAb) to potentiate tolerance to allogeneic cells transplanted at birth as a model for human *in utero* bone marrow transplantation. C57BL / 6 ($H\text{-}2^b$;$Thy\ 1.2^+$) pregnant mothers were injected IV with 1.0 mg anti-Thy 1.2 or saline 3 days prior to delivery. The offspring were then given 250 mcg of anti-Thy1.2 or saline with 10 x 10^6 H-2 incompatible C3H ($H\text{-}2^k$;$Thy\ 1.2^+$) marrow cells IP within 24 hours of birth. Splenocytes and marrow cells were evaluated at 2, 4, and 6 months for the presence of donor cells using an anti-H-$2D^kK^k$ class I MoAb.

		2 Months	4 Months	6 Months
$10x10^6$ C3H Cells at birth	Spleen	1.0 ± 0.5 (2/4)*	2.0 ± 1.7 (2/4)	2.5 ± 1.0 (4/5)
	Marrow	ø (1/6)	ø (0/4)	ø (0/4)
Pre and postnatal Thy1.2 + $10x10^6$ C3H Cells at birth	Spleen	6.2 ± 4.0 (4/6)	23.2 ± 7.9 (4/4)	7.9 ± 1.6 (4/4)
	Marrow	2.1 ± 0.9 (3/6)	ø (0/4)	ø (0/4)

* % donor cells ± SEM (No. positive / total number mice)

Donor cells were consistently demonstrated in the spleen at 2, 4 and 6 months post-transplant and associated with a markedly decreased mixed lymphocyte reaction (MLR) to donor cells in vitro. Donor T-cells accounted for 3.7 ± 0.7 % of the cells in the spleen of the anti-Thy 1.2 treated mice, while no donor T-cells were noted in the group treated with marrow alone. There was no increase in mortality or other clinical features of graft-versus-host disease noted. These experiments demonstrate that anti-Thy 1.2 administered pre and postnatally is effective in potentiating tolerance to allogeneic cells in the newborn mouse which persists for at least 6 months post transplantation.

K 303 INTRACELLULAR CALCIUM MOBILIZATION AND PROLIFERATIVE CAPACITY OF LYMPHO-HEMOPOIETIC CELLS AFTER ULTRAVIOLET (UV) IRRADIATION H. J. Deeg, H. Spielberg, N. Cereb, L. Bazar, M. Cottler-Fox, C. June, Georgetown University, Washington, D.C. and Navy Medical Research Institute, Bethesda, MD

We studied the effects of gamma and UV irradiation on intracellular calcium [Ca^{2+}] mobilization, an early step in cell activation. Peripheral blood leukocytes isolated on Ficoll-Hypaque were loaded with acetoxymethyl ester of indo-1 to give intracellular indo-1 concentrations of 20-50 uM. Cells were then prepared for flow cytometric analysis by UV excitation at a flow rate of 400-500 cells/sec. With UVC doses of 0.5-20 mJ/cm^2 there was a dose dependent increase of baseline $[Ca^{2+}]_i$ which reached a maximum 2 hours following exposure. No such change was observed with 2000 rad gamma irradiation. When stimulated by PHA there was no further increase of $[Ca^{2+}]_i$ in UV exposed cells while there was a normal response in gamma irradiated cells. $CD8^+$ cells were significantly more sensitive than $CD4^+$ cells to UVC irradiation. Furthermore, doses of 0.5-2 mJ/cm^2 of UVC which completely abrogated the ability of lymphocytes from peripheral blood and bone marrow to respond to lectin stimulation, still allowed growth of CFU-GM and CFU-GEMM in culture. We conclude that UV exposure interferes with $[Ca^{2+}]_i$ mobilization. There is a differential cell sensitivity which may allow for selective inactivation of certain lymphocyte subsets while still allowing for normal functions in other lymphocytes and hemopoietic precursor cells.

K 304 SUCCESSFUL THERAPY OF ACUTE GRAFT VERSUS HOST DISEASE (aGVHD) WITH HIGH DOSE METHYLPREDNISOLONE (MP), Gerald Elfenbein, David Oblon, Theresa Goedert, Roy Weiner, John Graham-Pole, and Samuel Gross. University of Florida and the Gainesville VA Medical Center, Gainesville, FL 32610.

We have treated 39 consecutive patients (pts) with grade II-IV aGVHD with escalating doses of intravenous MP. The regimen for MP [5 mg/kg/d x 4 days (d), responders continued; non-responders escalated to 10 mg/kg/d x 4 d, responders continued; non-responders escalated to 20 mg/kg/d]. For responders, doses were reduced 20% Q8d. The patients had a median age of 17.5 years (range 0.4-44) with 26 males and 13 females. Underlying diseases included acute myelogenous leukemia 14, acute lymphocytic leukemia 4, chronic myelogenous leukemia 11, aplastic anemia 4, and others 6. The incidence of aGVHD in 94 consecutive allogeneic BMT was 49% (46/94) and for grade II-IV aGVHD was 41% (39/94). The occurrence of aGVHD was independent of underlying disease, prophylactic regimen, and sex of the donor and recipient. The overall response rate of aGVHD to our regimen was 90% (35/39) with confidence limits of 80-100%. 74% (29/39) of pts responded to the initial dose level; 56% (5/9) of escalated pts responded to MP at 10 mg/kg/d. Only 1 of 4 patients responded to MP at 20 mg/kg/d. The high response rate did not allow identification of any factors that are predictive of response/failure to our regimen. There was no relationship between the development of aGVHD and incidence of chronic GVHD nor interstitial pneumonitis. Survival (minimum follow-up 6 mo.) for grade 0-1 is 36% (20/55); for grade II-IV is 41% (16/39). The rate of relapse of leukemia was significantly higher in pts without aGVHD (6/35) versus pts with aGVHD (1/36) at $p<.05$ level. We conclude that high dose MP is effective therapy for aGVHD.

K 305 T CELL DEPLETION WITH RICIN-A CHAIN T101 IN ALLOGENEIC BONE MARROW TRANSPLANTATION TO PREVENT ACUTE GRAFT VERSUS HOST DISEASE, Axel A. Fauser, Chaim Shustik, Adrian Langleben, Guy Laurent, Paul D. Ahlgren, and Bernard A. Cooper. McGill University, Montreal, and CLIN-MIDY, Montpellier, France.

Bone marrow cells from 12 marrow transplant donors were treated with an immunotoxin, which couples A chain of ricin with a monoclonal anti-T-cell antibody T_{101}, to prevent graft versus host disease by the elimination of mature T cells. Donor marrow cells treated with the anti human T cell immunotoxin (IT_{101}) were cultured for erythropoietic colonies, granulocytic colonies and multilineage hematopoietic colonies (CFU-GEMMT) containing myeloid cells and T cells, and optimal conditions were defined for the elimination of T cells present in the harvested donor marrow prior to marrow transplantation. Marrow samples purged with IT_{101} were examined for residual T cells by fluorescence activated cell sorting using anti-T-cell antibodies, ^{3}H thymidine incorporation after PHA stimulation, and an assay for clonogenic T cells. The number of T cell colonies observed in the treated marrow was less than 3% of the number in comparable unpurged donor marrows. Treatment with IT_{101} did not alter the plating efficiency of hematopoietic colonies compared to untreated donor marrow cells. These data suggest that multilineage progenitors responsible for the reconstitution of the recipient hematopoietic system are not affected by marrow IT_{101} purging. Engraftment was achieved in all 12 transplanted patients. We did not observe delayed engraftment or rejections of the graft. In 3 out of 12 patients acute graft versus host disease has been observed (1 grade I, and 2 grade II). The clinical data on 15 patients indicate that the IT_{101} depletion in the donor marrow is effective in decreasing the incidence and severity of acute graft versus host disease.

K 306 GRAFT VS. HOST DISEASE (GvHD) PROPHYLAXIS WITH CD5 (T101) RICIN IMMUNOTOXIN (IT) EX VIVO T CELL PURGING: THE CORRELATION BETWEEN LABORATORY STUDIES QUANTITATING T DEPLETION AND CLINICAL OUTCOME. A.H. Filipovich, D. Polich, P. McGlave J.H. Kersey, D. Vallera. University of Minnesota, Minneapolis, MN 55455.

T101 (CD5) ricin IT was used to T cell deplete bone marrow (bm) from 28 patients (pts.) with histocompatible donors and 9 pts. with histoincompatible donors. IT doses were increased sequentially from 300 (11 pts.) to 600 (9 pts.) to 1000 (17 pts.) ng/10^7 mononuclear cells, attempting to achieve better T depletion. T depletion of bm was monitored by a limiting dilution assay (LDA) quantitating precursors of proliferating lymphocytes (pPTL), and phenotypic analyses of viable lymphocytes in IT-treated bm after 16 day culture with interleukin-2 (IL-2). A significant decrease in time to acute GvHD ($\geq$ grade II) was observed in histocompatible recipients of 1000 ng treated bm (median log depletion pPTL:2.1) compared with recipients of 300 ng treated bm (median log depletion pPTL:1.5) ($p = .003$). So far, no correlation between T cell dose and development of GvHD has been seen in histocompatible transplants monitored by LDA (18). However, lymphocyte phenotyping after IL-2 culture has revealed an unexpected finding: outgrowth of CD3+ and/or CD2+, but CD5- lymphocytes in 10/31 samples studied: 300 ng (3/4 - 75%), 600 ng (3/9 - 33%), 1000 ng (4/18 - 22%). Furthermore, IL-2 mediated outgrowth of CD3+ and/or CD2+, CD5- cells correlated with subsequent GvHD: 7/10 pts. with GvHD vs. 1/8 pts. without GvHD ($p=.02$). We are in the process of functionally characterizing the IL-2-responsive CD3+ and/or CD2+, CD5- lymphocytes which may be associated with GvHD following IT-mediated GvHD prophylaxis.

K 307 COMPARISON OF ETOPOSIDE, CYCLOPHOSPHAMIDE, CYTARABIN AND TOTAL BODY IRRADIATION (TBI) AS IMMUNOSUPPRESSIVE AGENTS FOR BONE MARROW TRANSPLANTATION (BMT) W. Gassmann, L. Uharek, H.-O. Wottge, W. Mueller-Ruchholtz, II. Medical Department and Department of Immunology, University of Kiel, D 2300 Kiel, FRG

Etoposide has been shown to have high antileukemic activity. However, data concerning its immunosuppressive potency in the context of BMT are scarce. Therefore, we tested the effectiveness of etoposide regarding the prevention of graft rejection in comparison to more classical agents used in this setting. Experimental model: LEW-rats were given a marrow ablative dose of oral busulfan (35 mg/kg). Twenty-four hours later they received F1 (CAP/LEW) bone marrow which is unable to induce a GVHR. Since a lethal dose of busulfan is not sufficiently immunosuppressive, graft rejection ensues unless further immunosuppression is added. Acceptance or rejection of the transplanted marrow was monitored by determining hematocrit, leukocyte count and differential blood count twice weekly, in order to distinguish deaths resulting from rejection (Hkt < 30 %, granulocyte count < 500/µl) or from other causes. Results: The following rejection rates were observed after various doses of TBI or cytostatic agents given in addition to busulfan prior to BM-grafting. TBI: 1.5 Gy 100 %, 3 Gy 40 %, 4,5 Gy 0 %, 6 - 9 Gy 0 %. Cyclophosphamide: 10 mg/kg 100 %, 30 mg/kg 85 %, 60 mg/kg 29 %, 90 - 480 mg/kg 0 %. Etoposide: 30 mg/kg 90 %, 45 mg/kg 60 %, 60 mg/kg 7 %. Cytarabine: 8 x 75 mg/kg (8 x 3 g/m^2) 75 %, 12 x 75 mg/kg 75 %, 16 x 75 mg/kg 25 %. Discussion: These data show that approximately 3 Gy TBI, 60 mg/kg cyclophosphamide, 45 mg/kg etoposide, and 12 - 16 x 75 mg/kg cytarabin exhibit equivalent immunosuppressive potency when used as conditioning agents for allogeneic BMT.

K 308 ALTERNATIVE DONOR SOURCES IN HLA-MISMATCHED MARROW TRANSPLANTATION: T CELL DEPLETION OF CADAVERIC MARROW, R. Gress, R. R. Quinones, R. D. Moses, H. Nakamura, and P. J. Lucas, Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.
Evaluation of alternative marrow donor sources and methods of marrow harvest is important in considering the application of allogeneic bone marrow transplantation (BMT) across increasingly greater HLA disparities. For BMT with minimal HLA matching, we have evaluated surgically resected cadaveric vertebral body marrow for T cell content and for cell yield after T cell depletion by a pool of monoclonal antibodies and complement. T cell content was evaluated by limiting dilution analysis which was specific for detecting T cells, had an efficiency of about 50%, and which had a specificity sufficient to detect on the order of one T cell in 10^5 marrow cells. In a series of twenty surgical harvests, the mean initial cell yield was 4.4×10^9 per vertebral body with 4-6 vertebral bodies per harvest. Passing cells over Ficoll decreased this yield by 24%. Depletion of T cells further reduced cell numbers by 36% for a final cell yield of 9.2×10^9 per harvest. The T cell content of these marrows was decreased by more than three logs by the depletion procedure to a level of <0.001%. By current information, this level of T cell content is insufficient to cause graft-versus-host disease, and the cell yields are of a magnitude comparable to those used in routine allogeneic BMT.

K 309 REDUCTION OF BONE MARROW MYELOID PROGENITORS AND COLONY STIMULATING FACTOR PRODUCTION IN GRAFT-VS-HOST REACTION, Frances T. Hakim, Dov H. Pluznik and Gene M. Shearer, Immunology Branch, NCI, NIH, Bethesda, MD 20892.
Injection of parental C57Bl/10 spleen cells into unirradiated, immune-competent (B10 x B10.BR)F1 hosts results in an immune-suppressive graft vs host reaction (GVH). Myeloid colony forming units (CFU-GM) in the bone marrow were assessed by culturing in vitro with granulocyte and macrophage colony stimulating factors (CSF). After induction of GVH, myeloid stem cells in the total leg bone marrow initially doubled, then during the third-fifth week, decreased by 15 to 20 fold. During the same period, splenic immune functions including Concanvalin A stimulated production of Interleukin 3 and GM-CSF were suppressed by more than 90%. Furthermore, anti-host stem cell effectors could be demonstrated in GVH marrow by coculture with normal F1 marrow, resulting in a 15 to 40% decrease in CSF stimulated F1 marrow proliferation. Stem cell populations subsequently returned to normal levels, coincident with repopulation of marrow and spleen with parental cells. Thus GVH may affect myeolpoiesis by direct attack on host stem cells and by suppression of CSF production, contributing to the pancytopenia of GVH disease.

K 310 FUNCTION OF F1 DONOR B-CELLS IN RFM HOSTS WHICH DEVELOP HOST VERSUS GRAFT DISEASE (HVGD) AND IN T6T6 HOSTS WHICH DO NOT, Richard C. Hard, Jr., Medical College of Virginia/VCU, Richmond, VA 23298.
HVGD is the immunodeficiency syndrome induced by the perinatal inoculation of F1 hybrid spleen cells into susceptible strains of related inbred mice. T-cells are severely depleted, but there is polyclonal hyperplasia of F1 donor B-cells,hyperglobulinemia and nephropathic immune complexes. The function of F1 donor B-cells sensitized to glucose oxidase (GO) was studied in tolerant, healthy T6T6 hosts and in RFM hosts, treated or not to prevent HVGD. Titers of antibodies (Ab) to GO and the numbers of F1 donor B-cells (GOBC) were quantitated. In RFM/(T6xRFM)F1α-GO chimeras, there was a pattern of peak and decline in anti-GO Ab's and numbers of GOBC. In T6/(T6xRFM)F1α-GO chimeras, the titers of Ab and numbers of GOBC rose, then plateaued rather than declined. These results indicate that the effects of the allogenic HVG reaction on F1 donor B-cells is not sufficient to explain why HVGD develops in RFM but not T6T6 chimeras. The inoculation of adult RFM spleen cells into 14 day old RFM/(T6xRFM)F1α-GO chimeras had a suppressive effect on anti-GO Ab titers, but more importantly, prevented renal disease. NK cells and NK cells activated in vitro with IL-2 had highly variable effects on anti-GO Ab titers that could not be correlated with their failure to prevent renal disease. Taken together, these results suggest that tests of F1 donor B-cell function in parent/F1 chimeras measure the vigor of the T-dependent allogenic HVG reaction, but not the processes by which nephropathic immune complexes are formed. Supported by NIH Grant #AI-20208.

K 311 EFFECT OF GNOTOBIOTIC MEASURES ON INFECTIONS AND GRAFT-VERSUS-HOST DISEASE AFTER BONE MARROW TRANSPLANTATION IN CHILDREN, Jaak M. Vossen[1] Peter J. Heidt[1,2] and Dirk W. van Bekkum[2], 1: Department. of Pediatrics, University Hospital, Leiden and 2: Radiobiological Institute TNO, Rijswijk, the Netherlands.

In a period of 14 years, 65 consecutive pediatric patients with severe aplastic anaemia or leukemia were treated with bone marrow transplantation (BMT) from MHC genotypically identical siblings. All were nursed in strict reverse isolation. Forty-five patients underwent complete gastrointestinal decontamination (C-GID); 20 were selectively decontaminated (S-GID). Criteria for a successful decontamination were strictly defined. None of the patients developed new infections with Gram-neg. bacteria. The only new infections observed after BMT were catheter-septicaemias caused by Gram-pos. organisms and septicaemias caused by alpha-haemolytic streptococci, the latter being associated with long lasting granulocytopenia. Only one patient developed interstitial pneumonia associated with a positive serology for cytomegalovirus. The influence of the different types of decontamination on Graft-versus-Host disease (GvHD) could be evaluated in 58 patients. Successful C-GID prevented acute GvHD significantly more than did successful S-GID ($p < 0.05$). Also unsuccessful C-GID prevented acute GvHD significantly more than did unsuccessful S-GID ($p < 0.05$). From these observations it can be concluded that as a method to prevent acute GvHD, C-GID is preferable to S-GID.

K 312 SUCCESSFUL *EX VIVO* AND *IN VIVO* IMMUNOMODULATION OF HUMAN MARROW IN BONE MARROW TRANSPLANTATION(BMT) ACROSS HISTOCOMPATIBILITY BARRIERS, A CASE REPORT. PJ Henslee, JS Thompson, E Marciniak, JS Macdonald, MJ Messino, EH Romond, CD Jennings, PJ Scannon & VS Byers. *From the University of Kentucky, Department of Medicine and Pediatrics,* Lexington, Ky and the XOMA Corporation, Berkeley, Ca. (Supported in part by grant NO1-A1-42539.)

Major complications of histo-incompatible marrow grafting include failure of sustained engraftment and development of severe acute and chronic graft versus host disease (GVHD). Proliferating donor lymphocytes have been shown to be responsible for GVHD in both animal and human marrow transplantation. Various techniques employed for T-cell depletion of disparate marrow grafts correlate with failure of either engraftment or GVHD prophylaxis dependent upon the degree of T lymphocyte reduction. A Phase I Trial has been initiated to examine the efficacy of partial (1.5 log) *ex vivo* T-cell depletion of histo-incompatible donor marrow with an anti-CD-3 monoclonal antibody(MoAb), T10B9, in combination with post grafting *in vivo* immunomodulation by a 7-day infusion of ricin A chain-conjugated, anti-CD-5 MoAb, XomaZyme®-H65. The results obtained in the first patient lend support to the hypothesis that graft immunocompetence is essential to secure disparate marrow engraftment however, GVHD can be successfully aborgated through *in vivo* cleansing of T-cells early in the initiation phase of GVHD. Briefly, the patient is a 43 year old male with chronic myelogenous leukemia in blast crisis conditioned with total body irradiation and high dose VP-16, Cytosar, and Cytoxan in preparation for a haploidentical marrow graft from a son mismatched for B and Dr human leukocyte antigens with significant bidirectional reactivity in mixed lymphocyte culture. Normal marrow production developed during infusion of XomaZyme®-H65 between day +10 to +17 during which time initial, minimal, clinical and histologic GVHD of skin and gastrointestinal tract resolved. At day +93 the patient enjoys remission with continued normal, donor-derived, marrow production, no recognizable GVHD, and evidence of immunoreconstitution. This promising treatment approach warrants further investigation.

K 313 INCOMPLETE T-CELL REMOVAL WITH ANTI-LEU-1 DUE TO $CD3^+/CD5^-$ T-CELL POPULATION, J. Jansen, E. Srour, C. Cook, D. Walker and T. Leemhuis, Indiana University School of Medicine, Indianapolis, IN 46223.

Twenty-three patients received bone marrow transplants that had been treated in-vitro with two rounds of monoclonal antibody anti-Leu-1 (CD5) and baby rabbit complement ($\bar{C}$). No post-transplant GVHD prophylaxis was used. The depletion procedure removed 53.0% (0.5 - 74.0) (median; range) of total cells, 39.6% (-27.5 - 77.1) of CFU-GM, 33.2% (-67.5 - 74.2) of BFU-e, and -21.0% (-204.3 - 79.5) of CFU-GEMM. In two cases, poor T-cell depletion was obtained (29 and 68%) in spite of the use of active complement. In the other 21 cases, the first round of anti-Leu-1 and $\bar{C}$ resulted in depletion of 87.9% (14.8 - 98.7) and 94.5% (21.8 - 100) T cells as documented with fluorescence with Leu-4 (CD3) and Leu-3a + Leu-2a (CD4 + CD8), respectively. After two rounds of treatment, the depletion was 96.9 (87.7 - 100) and 97.9 (80.7 - 100) respectively. The fact that the depletion was incomplete was largely due to the presence of a $Leu\text{-}4^+/Leu\text{-}1^-$ ($CD3^+/CD5^-$) population which made up 9.5% (3.9 - 29.2) of the $Leu\text{-}4^+$ lymphocytes. In spite of the incomplete T-cell depletion, none of the successfully T-cell depleted patients developed more than Grade I aGVHD. Limiting dilution assays (LDA) documented a better T-cell depletion: 98.9% (88.7 - 99.8). This difference can be explained by the poor in-vitro proliferative capacity we found for the $CD3^+/CD5^-$ subset. These data show that complete T-cell depletion may not be necessary for a good anti-GVHD effect in HLA-identical sibling transplants. Furthermore, the degree of T-cell depletion detected is very much dependent upon the test system used.

K 314 ABNORMAL INDUCTION OF Ly6+ THYMOCYTES AND Lyt-2/L3T4+ CELLS IN THE PERIPHERY DURING GRAFT VS. HOST REACTIONS (GvHR's), Robert B. Levy, M.V. Jones, A. Cotterell and T.M. Malek, University of Miami School of Medicine, Miami, FL, 33101

The inoculation of immunocompetent parental lymphocytes into non-irradiated F_1 recipients is a well characterized murine model to study GvHR induced immune abnormalities. Class I/II 'suppressive' [B6 → BDF_1; B10.A → (B10 x B10.A)F_1)] and 'stimulatory' (DBA/2 → BDF_1), and class II [B6 → (B6 x B6.bm12)F_1] GvHRs were initiated to investigate conditions under which alterations in the expression of thymocyte cell surface markers would occur. Within two weeks following the induction of class I/II suppressive and class II GvHR's, in contrast to the decrease in the % and numbers of Lyt-2+ and L3T4+ thymocytes, the % (80-100% in class I/II suppressive and 10-20% in class II GvHR's) and numbers of Ly-6+ cells increased in the host thymus. Two color fluorescent analysis during acute class I/II suppressive GvHR's demonstrated that greater than 85% of Ly2+ and 85% of L3T4+ thymocytes expressed Ly-6. Notably, the numbers of double positive Ly2/L3T4 cells decreased to 10-15% in suppressive but not stimulatory GvHR thymuses.

Ly-6 expression was also increased in the periphery of both suppressive and stimulatory GvHR's. Examination of T-cells in the spleens indicated that 10 days following induction, Ly2/L3T4+ (10-15%) cells were detected during class I/II suppressive, but not in class I/II stimulatory GvHR or allo-antigen primed mice. These studies have demonstrated that intrathymic activation of lymphocytes as determined by induction of Ly-6A/E expression, as well as decreased numbers of Ly2/L3T4+ thymocytes occurs during selected types of allogeneic cell interactions. An interesting correlation was noted between GvHR's expressing such thymic involvement and the presence of significant numbers of Ly2/L3T4+ cells in the periphery.

K 315 PGE2 LEVEL IN PATIENTS RECEIVING BONE MARROW TRASPLANT AND THE EFFECTS OF PLACENTAL GAMMA-GLOBULIN (PGG) ON PGE2, JIANG Bin, LU Dao-pei and ZHONG Rui-kun, Institute of Hematology, Beijing Medical University, Beijing, China

In an attempt to elucidate the role played by prostanglandin E2 (PGE2) during chronic Graft-Versus-Host Desease (cGVHD), PGE2 level was determined in the supernatant of mononuclear cell culture from the recipients of bone marrow trasplant (BMT). Blood mononuclear cells were obtained from 7 recipients of BMT (4 with cGVHD, 3 without cGVHD). Recipients before BMT and normal individuals were used as control. Blood mononuclear cells of the recipients with cGVHD produced more PGE2 (mean 513.5±50.1 pg/ml)than those without cGVHD (mean 91.1±15.9 pg/ml, $p<0.01$).

PGG has been used at our hospital to prevent GVHD. In order to ascertain whether it can inhibit the PGE2 secreting function of monocyte-macrophage, Mononuclear cells were incubated with PGG at the concentration of 2 mg/ml. After 3 days of incubation, PGE2 in the supernatant was determined by radioimmuno assay. PGE2 in the supernatant of mononuclear cells from cGVHD was significantly less in the presence of PGG (111.1±50.8 pg/ml) compared to in the absence of PGG (513.5±50.1 pg/ml, $p<0.05$).

This study suggests that PGE2 might play a role in the produce of cGVHD and that PGG could inhibit the high PGE2 level produced by mononuclear cells from patients with cGVHD.

K 316 ALPHA PARTICLE EMITTING IMMUNOCONJUGATES FOR MARROW TRANSPLANTATION, R. Macklis, B. Kinsey, A. Kassis, J. Ferrara, W. Kaplan, R. Atcher, J. Hines, N. Coleman, J. Adelstein and S. Burakoff; Harvard Medical School.

Alpha particles are highly cytotoxic, high energy ions which deposit all their energy over a short (40-80 micron) path length. We have prepared alpha particle emitting murine anti-T cell radioimmunoconjugates (RICs) by chelating anti-Thy 1.2 IgM MoAbs with the short half-life alpha emitter Bismuth-212. The resulting RIC demonstrates antigen-selective cytotoxicity in vitro against normal T cells and Thy 1.2+ tumor cells. Approximately 3 alpha-emitting RICs per target cell reduce 3H-TdR incorporation to background levels. Mice given allogeneic marrow transplants using bone marrow purged of T cells by in vitro exposure to the alpha emitting anti-Thy 1.2 RIC showed rapid hematopoietic reconstitution without the development of graft-vs-host disease. Mice inoculated with a Thy 1.2+ experimental ascites showed antigen-selective cure after treatment with 180-230 uCi/mouse of the RIC. Alpha particle emitting RICs show great promise for antineoplastic and immunosuppressive radioimmunotherapy in the setting of bone marrow transplantation.

K 317 NATURAL SUPPRESSOR (NS) CELL ACTIVITY INDUCED DURING CHRONIC GVHD IN A MURINE MODEL OF BONE MARROW TRANSPLANTATION, Tom Maier, James H. Holda and Henry N. Claman, University of Colorado School of Medicine, Denver, CO 80262.

Severe immunosuppression occurs in chronic murine GVHD across minor histocompatibility barriers (a model for human bone marrow transplantation). The spleens of these GVHD mice contain cells which potently suppress immunological responses. The cells responsible for this suppression are natural suppressor (NS) cells. NS cell activity is the ability of apparently nonprimed "null" cells to nonspecifically inhibit immune responses. Neither T cell enriched nor T cell depleted spleen cells can produce this GVHD suppression, but injection of a mixture of the two induces NS-mediated suppression. Early after induction (10-20 days), GVHD spleen cells can inhibit normal spleen cell responses to all immunologic stimuli including the T and B cell mitogens Con A and LPS. With time, this NS activity begins to wane (40-60 days). However, the ability to suppress an LPS response wanes sooner than the ability to suppress a Con A response. If Con A supernate (CAS) is added to the LPS suppression assay, then the NS cells are activated to inhibit the LPS response similar to the Con A suppression. The addition of either IL-2 or IFN-γ will replace the CAS in activating the GVHD NS cells. The addition of anti-IFN-γ antibody removes the ability of the IL-2 as well as the IFN-γ to activate the NS cells, and thus it appears that the IL-2 is working via the stimulation of IFN-γ. Anti-INF-γ also removes the ability of the GVHD NS cells to suppress a Con A response. Therefore, it appears that GVHD NS cells require T cell signals (IFN-γ) both in vivo and in vitro to express their suppressive activity. Supported by NIH grants AI-12685 & AI-07035, the Leukemia Society and the Arthritis Foundation.

K 318 RISK FACTORS OF ACUTE GRAFT-VERSUS-HOST DISEASE IN PATIENTS WITH HLA-IDENTICAL BONE MARROW TRANSPLANTS, Jean-Yves Mary, Martine Bagot*, Michele Heslan*, Mathieu Kuentz+, Catherine Cordonnier+, Jean-Paul Vernant+, Louis Dubertret*, Jean-Paul Levy°, INSERM U263, Paris, *INSERM U312, Creteil, +Hôpital Henri Mondor, Creteil, °INSERM U152, Paris, France.

Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality in patients receiving bone marrow grafts from HLA-identical siblings. Depletion of mature T lymphocytes decreases GVHD incidence but gives rise to severe complications, graft failures or rejections. It appears to be of clinical relevance to predict donor-recipient pairs at low risk of GVHD, for whom T-cell depletion should be avoided. 37 patients with haematological malignancy were entered into the study from 1984 to 1986. They all received a non-depleted allogeneic bone marrow transplant from an HLA-identical sibling donor, with standardized conditioning regimen and GVHD prophylaxis using methotrexate only. The following factors were investigated through non-parametric statistics for their influence on acute grade II-IV GVHD occurrence: diagnosis, recipient and donor age and sex, donor previous pregnancy(ies), sex-mismatch, mixed epidermal cell-lymphocyte reaction (MECLR/MLR). Increasing recipient and donor ages, donor previous pregnancy(ies) and CML diagnosis were correlated with acute GVHD. Taking into account relationships between these factors, stepwise multiple linear discrimination and logistic regression selected the MECLR/MLR index, donor previous pregnancy(ies) and CML diagnosis as primary factors of occurrence of acute GVHD. Using jackknife procedure, acute GVHD occurrence was correctly predicted in 33/37 patients. Clinical implications of these interacting factors were derived. In particular, a MECLR/MLR index lower than 3 was of good prognosis in acute leukemia patients grafted with bone marrow from a male donor or a female donor with no previous pregnancy. This method allowing to evaluate the risk for a recipient to develop an acute GVHD deserves a further evaluation of its applicability on larger series of patients.

K 319 DECREASED INCIDENCE OF ACUTE GRAFT VERSUS HOST DISEASE (aGVHD) IN ALLOGENEIC BONE MARROW TRANSPLANT (BMT) RECIPIENTS RECEIVING PROPHYLAXIS WITH METHOTREXATE (MTX) AND CYCLOSPORINE A (CyA) IN COMPARISON TO MTX AND PREDNISONE (Pred), J.M. Meharchand, G.M. Fyles, M.D. Minden, J.E. Curtis, G. Lockwood, D. Trichler and H.A. Messner, Ontario Cancer Institute and University of Toronto.

Two sequential prophylactic regimens for aGVHD were compared. 103 consecutive patients undergoing allogeneic BMT for aplastic anemia or hematologic malignancies received 4 doses of MTX (15mg/m^{2}x1, 10mg/m^{2}x3) and Pred (40mg/m^2 for 6 weeks). Patients with aGVHD grades II-IV were treated with CyA (12.5mg/kg/d po or 5mg/kg/d iv). This group of patients (GrpI) was compared with 32 consecutive patients receiving 4 doses of MTX (15mg/m^{2}x1, 10mg/m^{2}x3) plus CyA (12.5mg/kg/d po or 5mg/kg/d iv) prophylactically and Pred (40mg/m^2) for treatment of grades II-IV aGVHD. The median age in both groups was similar (29 yrs). The incidence of aGVHD grades II-IV was 49% in Grp I and 28% in GrpII ($p<.05$). No cases of grade IV aGVHD were observed in GrpII. In GrpI with a median follow-up of 35 months, there was no significant difference in survival between patients with grade 0-I aGVHD compared to patients with grade II-IV aGVHD. Similarily in GrpII with a median follow-up of 7 months, there was no signficant difference in survival between patients with grade 0-I aGVHD compared to patients with grade II-IV aGVHD. The median follow-up in the second group is too short to allow a meaningful comparison with the survival of patients in the first group. In neither group was age found to be a significant risk factor for survival. This may suggest that CyA used either prophylactically or therapeutically influences the effect of age on survival following aGVHD.

K 320 THE ABILITY OF MURINE CTL CLONES TO REJECT ALLOGENEIC MARROW GRAFT WITH SPECIFICITY FOR MHC GENE PRODUCTS. H. Nakamura and R.E. Gress. Immunology Branch, NCI, Bethsda 20892.

The rejection of marrow grafts is a significant complication of clinical allogeneic bone marrow transplantation, especially in the setting of T cell depleted donor marrow. The presence of host cytotoxic T lymphocytes (CTL) in association with such graft rejection has provided a basis for implicating these cells in the rejection process in both the clinical setting and in experimental marrow transplantation models. To directly assess the ability of murine CTL to reject allogeneic marrow grafts, lymphocytes were isolated and cloned from spleens of B6 ($H\text{-}2^b$)mice 7 days after 650R irradiation and inoculation with either BDF1 ($H\text{-}2^{b/d}$) or B6C3F1 ($H\text{-}2^{b/k}$) marrow grafts depleted of T cells by R@MB+C'. Six clones were isolated which were Thy 1^+, $Lyt2^+$, $L3T4^-$ and cytolytic with specificity for the MHC disparity of donor marrow; NK activity was not detectable. B6 mice were then irradiated with 1025R and injected with varying numbers of cloned CTL (or no cloned cells) followed 4hr later with either BDF1 or B6C3F1 marrow. Five days later rejection of BM was assayed by ^{125}IUdR uptake of host spleens. The uptake in spleens from animals injected with BDF1 BM was significantly lower in those recipients receiving $H\text{-}2^d$ specific, but not $H\text{-}2^k$ specific, cloned CTL ($p<0.05$). Conversely, in those animals receiving B6C3F1 marrwow, splenic uptake was significantly lower in animals also inoculated with $H\text{-}2^k$ specific, but not $H\text{-}2^d$ specific, CTL clones ($p<0.05$). Animals receiving either the clone with an inappropriate MHC specificity or no cloned cells had no significant difference in ^{125}IUdR uptake. These results directly demonstrate an ability of CTL to mediate marrow graft rejection in vivo with specificity for MHC gene products.

K 321 SYMPTOMATIC CYTOMEGALOVIRUS INFECTION AFTER BONE MARROW TRANSPLANTATION. IMPORTANCE OF HIGH MARROW CELL DOSE. T. Paulin and O. Ringdén. Departments of Clinical Immunology and Transplantation Surgery, Karolinska Institute, Huddinge Hospital, Stockholm, Sweden.

Until September 1987, 180 patients underwent allogeneic bone marrow transplantation (BMT). The total incidence of cytomegalovirus (CMV) infections was 58% (104/180 patients). Sixty-three patients had symptomatic CMV infection whereof 21 had interstitial pneumonitis. In Cox regression multivariate analysis including all patients, symptomatic CMV infection was associated with a low marrow cell dose ($p<0.01$), grade II-IV acute graft-versus-host disease (GVHD)($p=0.01$), male donors ($p=0.03$), a positive recipient CMV serology ($p=0.05$), and high recipient age ($p=0.04$). Age and marrow cell dose were analyzed as continuous variables. Symptomatic CMV infection was not correlated with donor CMV serology, donor age, recipient sex, remission status and HLA match. When the analysis was restricted to leukemic patients only, low marrow cell dose was still significantly correlated to symptomatic CMV infection ($p=0.02$). Patients with acute myeloid leukemia (AML) had a significantly higher incidence of symptomatic CMV infection compared to patients with acute lymphocytic leukemia (ALL) and chronic myeloid leukemia (CML) taken together ($p=0.05$). In spite of a small number of observations in each diagnostic subgroup low marrow cell dose was still associated with symptomatic CMV infection in patients with AML ($p=0.03$) whereas no correlation was seen in patients with ALL and CML.

Conclusion: In multivariate analysis low marrow cell dose, acute GVHD, male donors, positive recipient CMV serology and high recipient age were associated with symptomatic CMV infection after BMT. In patients with AML, in contrast to other leukemias, low marrow cell dose was associated with symptomatic CMV infection.

K 322 MISMATCHED FAMILY DONORS AND MATCHED UNRELATED DONORS FOR ALLOGENEIC BONE MARROW TRANSPLANTATION USING FIXED LOW NUMBERS OF T-CELLS. C.H. Poynton, J.A. Whittaker, R. Bailey-Wood, D. Geddes and M. Worwood. University of Wales College of Medicine, UK

Introduction: A safe balance between graft vs host disease (GVHD) and marrow rejection is particularly difficult in mismatched and unrelated transplants. We have attempted to see if there is an intermediate T-cell dose that allows stable engraftment without severe GVHD.
Patients: 6 patients have been entered into the study - 3 haplotype mismatched family donors and 3 unrelated phenotypically matched donors. 3 patients had high grade lymphomas in remission and 3 patients were in chronic phase PhI+ CGL.
Procedure: Standard conditioning therapy was used including 10Gy fractionated TBI in 5 patiemts and chemotherapy alone in one. 4×10^8 cells/kg were harvested. T-cell depletion with CAMPATH-1 was monitored by FACS analysis and by T-cell proliferation assay. After marrow infusion donor peripheral blood containing 2×10^5 T-cells/kg was infused.
Engraftment: Median time to 1×10^9 neutrophils was 17 days. Cytogenetics and minisattelite probes were performed on marrows at days +14, 100 and 200. All patients engrafted rapidly.
GVHD: Grade I mild skin GVHD was seen in 5 patients and Grade III skin GVHD with CMV interstitial pneumonitis in one patient. The T-cell proliferation assay suggested poor depletion in the patient with severe GVHD. Only the patient who did not receive TBI has evidence of unstable mixed chimerism at 6 months but with normal blood counts.
Survival: All patients are alive at 1-8 months after BMT in haematological remission.
Conclusion: Variability in the effectiveness of T-cell depletion results in difficulty in giving a standard T-cell dose. Results so far suggest that it may be possible to reduce the risk of serious GVHD and get rapid engraftment of donor marrow. Follow up is too short to draw any conclusions about the stability of these grafts.

K 323 LFA-1 FUNCTION IN THE ABSENCE OF TARGET CELL LIGANDS. Ralph Quinones, Pierre Henkart, David Segal and Ronald Gress, Immunology Branch, NCI, Bethesda, MD 20892.

A target cell independent assay to directly quantify activation of human CTL effector function by measuring secretion of a granule enzyme was developed to explore the function of accessory molecules and any role of ligands on target cells. Secretion was triggered with anti-CD3 monoclonal antibody (MAb) on polystyrene beads and was profoundly inhibited by anti-LFA-1 MAb (CD18), but not by anti-CD2 MAb. Suboptimal secretion could be activated by soluble anti-CD3 MAb, and was inhibited by anti-LFA-1 MAb. Secretion was also induced by ionomycin +/- PMA, but was not inhibited by anti-LFA-1 MAb, therefore demonstrating that anti-LFA-1 treatment did not affect the cells' ability to secrete. These data imply that the LFA-1 molecule function may be complex and involve more than conjugate formation by a receptor-ligand interaction. It is therefore also possible that anti-LFA-1 MAb inhibit alloaggressive human cells by more than one mechanism.

K 324 BREAKING OF TOLERANCE IN LONG-TERM MURINE RADIATION BONE MARROW CHIMERAS: ROLE OF T-CELL-DEPLETION OF INITIAL BONE MARROW INOCULUM, David H. Sachs, Michael A. Sheard, and Megan Sykes, National Institutes of Health, Bethesda, MD 20892.

Lethally irradiated mice reconstituted with T-cell-depleted (TCD) fully allogeneic bone marrow (BM), whether alone or in combination with TCD syngeneic BM, demonstrate stable, permanent chimerism with specific tolerance to donor alloantigen. We challenged this tolerance in established long-term chimeras using intravenous host-versus-graft (HVG) inocula containing normal (non-tolerant) recipient-strain splenocytes, and examined the role of T-cell-depletion of the original BM inocula in determining the outcome. B10 mice were lethally irradiated and reconstituted with TCD syngeneic plus TCD or non-T-cell depleted (NTCD) allogeneic (B10.D2) BM or with TCD allogeneic BM alone. All animals appeared healthy with no clinical evidence of GVHD, and chimerism was documented using flow microfluorimetry before administration of HVG inocula. $20\text{-}30 \times 10^6$ B10 splenocytes were sufficient to break tolerance in long-term chimeras originally reconstituted with TCD allogeneic BM, whereas tolerance in recipients of NTCD allogeneic plus TCD syngeneic BM was completely resistant to such inocula. Similar studies in an F1 into parent combination, in which T-cells in the reconstituting BM inoculum were genetically incapable of graft-versus-host (GVH) reactivity, indicated that such resistance to the effects of HVG inocula requires the genetic capacity for GVH reactivity from the allogeneic BM inoculum. These findings may be related to the clinical observation that administration of TCD allogeneic BM is associated with an increased incidence of delayed graft failure.

K 325 CORRELATION OF SERUM CSA CONCENTRATION AND SEVERITY OF ACUTE GVHD, H. Schmidt*, G. Ehninger*, R. Dopfer&, R. Naumann*, H. Einsele*, M. Haen*, K. Schüch*, D. Niethammer&, H.D. Waller*; *Department of Internal Medicine II, Department of Haematology and Oncology, D7400 Tübingen, FRG; &Children Hospital, Department of Haematology and Oncology, D7400 Tübingen, FRG

Between 1982 and 1986 51 patients were treated with ciclosporin (CSA) to prevent graft versus host disease (GvHD) after bone marrow transplantation. 12 patients were suffering from acute lymphocytic leukemia, 15 from acute non lymphocytic leukemia, 14 from chronic myeloic leukemia and 12 from severe aplastic anemia. As described before major side effects of CSA were tremor, hypertension, hepatotoxicity and nephrotoxicity. Nephrotoxicity was even enhanced as soon as cotrimoxacol was added to CSA. Acute GvHD O° to II° occurred in 80 % of the patients, GvHD III° and IV° in 20 % starting 8 to 20 days after BMT. Two to 4 days before the onset of GvHD CSA serum levels were on average significantly (p-value < 0.05) lower in patients who got GvHD III° and IV° (130 - 190 ng/ml) compared to the others (350 to 450 ng/ml). Our data indicate that high enough serum concentrations of CSA (200 to 400 ng/ml) during the first months after BMT may be important to reduce severity of acute GvHD.

K 326 POST BONE MARROW TRANSPLANT (BMT) B CELL LYMPHOPROLIFERATIVE DISEASE (BLPD) R.S. Shapiro, K. McClain, B. Blazar, K. Gajl-Peczalska, J. Kersey, G. Frizzera, and A.H. Filipovich, University of Minnesota, Minneapolis, MN 55455.
Eight patients (pts.) developed BLPD 1-47 months following BMT for leukemia (5) or immunodeficiency (3). Risk for BLPD is greatest for recipients of mismatched T depleted bone marrow (6/28 (21%)), followed by unrelated non-T depleted BMT (1/17 (6%)). This sharply contrasts to matched non-depleted related BMT (1/478) or matched T-depleted related BMT (0/56). Presentation of BLPD included fever (8), anorexia (8), hepatitis (7), abdominal pain (7), lethargy (6), lymphadenopathy (5), CNS changes (4) and pharyngitis (3). EBV serology consistant with primary (3) or past (5) infection and EBV-specific DNA hybridization demonstrating 5-50 genome copies/10ug tumor DNA (7/7), implicate EBV as an associated etiologic agent. Restriction fragment length polymorphism and cytogenetic analysis of tumors showed donor (5) or host (2) origin. Monoclonal foci were found by immunophenotyping (6/8) and/or immunoglobulin heavy chain C_{μ} and JH gene rearrangement (4/6). Although clonality and morphology were highly correlated there were instances of discordance. Histology ranged from polymorphic B cell hyperplasia to polymorphic B cell lymphoma and in several pts. varied in consecutive samples or samples from multiple sites. Acyclovir prophylaxis did not appear effective since 6/8 developed BLPD while receiving this drug. Six pts. died of BLPD 13-109 days after diagnosis despite therapy with antiviral, immunologic +/or chemotherapy. Two pts. appeared to respond to interferon alpha. One had no progression of BLPD but died of CMV pneumonitis, the other had complete resolution and is disease-free off therapy >1 year.

K 327 EFFECT OF CYCLOSPORIN A ON L3T4 AN LYT 2 T CELL FUNCTION. Gene M. Shearer and Masahiro Fukuzawa, Immunology Branch, NCI, NIH, Bethesda, MD 20892.
Mice were injected intraperitoneally with 15 mg, 30 mg or 75 mg/kg/day of Cyclosporine A (CsA) for 20 days. One day after the last injection, spleens of the mice were tested for L3T4 T helper and Lyt 2 T helper and effector function. A selective defect was observed in L3T4 T helper cell function, but not in Lyt 2 T helper or effector function at the 15 or 30 mg/kg doses. A dose of 75 mg/kg abrogated both L3T4 and Lyt 2 T cell function. Co-cultures of spleen cells from control and CsA-treated mice indicated activation of suppressor cells that were selective for L3T4 T helper, but not for Lyt 2 function at 15 or 30 mg/kg CsA, and activation of suppressor cells that abrogated both L3T4 and Lyt 2 function at 75 mg/kg CsA. The suppressors activated at 15 or 30 mg/kg, but not those activated at 75 mg/kg, were sensitive to anti-Thy 1 and Lyt 2 antibodies and complement. These effects were not detected if the mice were rested for two weeks between CsA administration and testing. These findings indicate that in vivo treatment with CsA can activate different populations of suppressor cells depending on the doses used.

K 328 NEW APPROACHES FOR AMPLIFICATION OF GRAFT VS LEUKEMIA (GVL) EFFECTS WITHOUT SEVERE GVHD IN ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT). Shimon Slavin, Lola Weiss, Elizabeth Naparstek, Reuven Or. Dept of Bone Marrow Transplantation & Cancer/Immunobiology Research Laboratory, Hadassah Univ Hospital, Jerusalem, Israel.
The immunological effects of graft-derived cells against residual tumor cells continue to represent an important component in the overall effects of BMT. Our goal was to develop a protocol for reducing graft rejection, improving GVL without severe GVHD and transfering donor immunocompetence to BMT recipients. We have previously shown that potent GVL effects against the BALB/c murine leukemia (BCL1) can be mediated by allogeneic BM cells tolerant of host's alloantigens, suggesting that GVL may be independent of GVHD. Effector cells of GVL ($H\text{-}2^{b}$ vs $H\text{-}2^{d}$) were shown to be Thy 1.2^{+} Lyt-2^{+} L3T4^{-}. Durable C57BL/6→(BALB/c x C57BL/6)F1 chimeras prepared using total lymphoid irradiation (TLI) developed resistance to GVHD; at 6 months, no GVHD developed following repeated (x6) inoculation of $30x10^{6}$ C57 spleen cells. Similarly, F1 recipients of T-depleted C57 cells developed time-dependent resistance to increasing numbers of C57BL/6 spleen cells. Consequently, 25 patients undergoing allogeneic BMT for leukemia, aplastic anemia and thalassemia received 10^{3},10^{4},10^{5},10^{5} & $2.5x10^{5}$ T-cells/kg from donors' blood at 1,6,13,20 & 27 days post-BMT. Only patients with aplastic anemia received cyclosporine A $\leq$ 90 days. no anti-GVHD prophylaxis was used in other patients. Our pilot results suggest that donor T cells may be safely administered post-BMT with minimal or no GVHD.

K 329 CLINICOPATHOLOGICAL ASSOCIATIONS OF KERATINOCYTE HLA-DR EXPRESSION AFTER ALLOGENEIC MARROW TRANSPLANTATION, John P. Sloane and Caroline J. Elliott, Royal Marsden Hospital, Sutton, U.K.
Immunohistological techniques were used to study HLA-DR expression in skin biopsies from recipients of allogeneic marrow. Positivity was found not only in biopsies exhibiting graft versus host disease (GvHD) but also in many specimens where the diagnosis could not be made on histological grounds. In these cases, keratinocyte staining was strongly associated with the presence of a clinical rash and the subsequent development of GvHD in other sites. Positivity was not seen in recipients of T-cell depleted marrow even when they had rashes. Although this indicates a crucial role for T-cells, sequential studies indicated that keratinocyte HLA-DR positivity precedes lymphocytic infiltration of the epidermis in GvHD. Increased HLA-DR expression on keratinocytes may result from release of factors (eg. gamma interferon) consequent upon interaction of donor T lymphocytes with recipient upper dermal cells, possibly in the vascular complex.

K 330 PROLONGED SELECTIVE IgG4 DEFICIENCY POST BONE MARROW TRANSPLANTATION, Brian R. Smith, Peter H. Schur, Joel M. Rappeport, Divisions of Hematology and Immunology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA and Department of Medicine, Yale University School of Medicine, New Haven, CT.
Following marrow transplantation a variable but progressive increase in IgG production is noted with the establishment of B cell chimerism. 28 patients were studied for serum levels of IgG subclasses from 41 to 2765 days post BMT. 17 patients underwent BMT for leukemia, 7 for aplastic anemia, and 1 each for Wiskott-Aldrich syndrome, immunodeficiency, PNH, and myeloproliferative disorder. All patients but one received histocompatible BMTs. 3 patients received syngeneic BMTs. 7 received T depleted BMTs. Overall, 1/28 (4%) pts demonstrated IgG1 deficiency, 5/28 (18%) IgG2 deficiency, 3/28 (11%) IgG3 deficiency, but 17/28 (61%) were IgG4 deficient. All IgG1, IgG2, and IgG3 deficient patients were also IgG4 deficient. No correlation of IgG4 deficiency was noted with the presence of GVHD. The IgG4 level was, however, positively correlated with the level of circulating total B cells (CD19+) and Leu1(CD5)+ B cells ($r^2 = 0.43$, $p=0.0002$). Of 19 patients studied greater than 1 year post BMT, 10 were IgG4 deficient. 6 of the 10 IgG4 deficient patients had recurrent infections while only 1 of 9 with normal IgG4 levels suffered recurrent infections. The IgG4 deficient patients had normal total IgG levels. The reconstitution of IgG4 may be severely delayed post BMT and not reflected in total IgG measurements. Moreover, IgG4 deficiency may lead to increased susceptibility to recurrent infections late post marrow transplantation.

K 331 DONOR T-CELLS LEAD TO INCREASED LEVELS OF CHIMERISM IN RADIATION BONE MARROW CHIMERAS INDEPENDENTLY OF THEIR POTENTIAL GVH REACTIVITY, Megan Sykes, Michael A. Sheard, and David H. Sachs, National Institutes of Health, Bethesda, MD 20892.
Lethally irradiated mice reconstituted with a mixture of T-cell-depleted (TCD) syngeneic plus non-T-cell-depleted (NTCD) fully allogeneic bone marrow (BM) repopulate as 100% allogeneic chimeras, whereas recipients of mixtures of TCD syngeneic plus TCD allogeneic BM reconstitute as mixed chimeras. Despite elimination of the host BM-derived cells, the former group appear free of graft-versus-host disease (GVHD). This finding suggested that elimination of host lymphohematopoietic elements might be due to an independent process from that which produces GVHD. We have tested this in a system lacking the genetic potential for GVH reactivity, using similar BM inocula in an F1 into parent combination. B10 mice received lethal whole body irradiation (1000R) followed by an intravenous inoculum containing TCD B10 plus TCD or NTCD (B10xB10.A)F1 BM cells. Peripheral blood lymphocyte phenotype was analyzed 6 weeks later using fluorescence microfluorimetry. Although 100% F1 chimerism was not achieved in any situation, F1 chimerism was markedly greater in recipients of TCD syngeneic plus NTCD F1 (mean 92.0±S.D.1.7.%F1) compared with recipients of TCD syngeneic plus TCD F1 BM (mean 60.1±S.D.6.4 %F1). H-2 typing of Ia-positive cells indicated that increased levels of F1 chimerism reflected repopulation by F1 stem cells rather than proliferation of T-cells from the marrow inoculum. These results suggest that increased levels of alloengraftment observed when T-cells are not removed from donor BM inocula may reflect the activity of a subset of Thy-1-positive cells distinct from that which produces GVHD.

K 332 IN VIVO USE OF MONOCLONAL ANTIBODY IMMUNOTOXINS (ITs) FOR THERAPY OF ACUTE GRAFT-VERSUS-HOST DISEASE (GVHD) ACROSS A MAJOR HISTOCOMPATIBILITY BARRIER IN MICE. Daniel Vallera, Vera Byers, Julie Smith, Dale Snover, Charlotte Chang, Bruce Blazar. University of Minnesota, Minneapolis, MN 55455 and the XOMA Corporation, Berkeley, CA.

Therapy of acute GVHD with an anti-CD5 ricin toxin A chain (RTA) (Xomazyme H65) is yielding promising clinical results in a multi-institutional trial in patients who have failed steroids. To test the efficacy of immunotoxins (IT) in GVHD therapy de novo, we have tested IT in an established murine model of lethal GVHD. 50 x 10^6 marrow cells and splenocytes from C57BL/6 donors were administered to MHC-disparate irradiated (8 Gy) B10.BR recipients. The median survival time (MST) of the recipients was 21 days. Anti-Lyt-1 (a murine equivalent to anti-CD5)-RTA was injected at dose of 10 ug ip beginning day 8 post-BMT at a time when clinical and histopathological evidence of GVHD was present. This treatment strategy prolonged MST (>Day 28). Similar treatments with Thy1.2-RTA showed no histological evidence of GVHD in 4 of 10 (40%) of the treated animals, whereas none of the controls (9 of 9) were GVHD-free. The anti-Thy1.2 RTA, but not anti-Lyt-1-RTA showed non GVHD related toxicity manifested by weight gain, hypoproteinemia, pleural and peritoneal effusions, and liver friability. Experiments are now in progress to investigate the etiology of IT toxicity in the setting of GVHD. In addition, we are modifying schedules, dosages of IT administration as well as investigating the therapeutic index of alternative ITs directed against more restricted cell populations believed to be involved in the effector phase of GVHD. This murine model may prove useful for optimization of therapy and minimization of IT toxicities in the clinic.

K 333 SELECTIVE DECONTAMINATION OF THE DIGESTIVE TRACT OF THE DONOR AGGRAVATES MURINE DELAYED TYPE GRAFT VERSUS HOST DISEASE. D. Veenendaal, F. de Boer and D. van der Waaij, Lab. for Medical Microbiology, University of Groningen, Oostersingel 59, Groningen, NL-9713EZ, The Netherlands.

Recently it has been found that Selective Decontamination of the Digestive tract (SDD) (=elimination of Enterobacteriaceae) changes the proliferation state of bone marrow stem cells and precursor cells in mice (1,2). As the cellular composition of the Bone Marrow (BM) graft influences Graft versus Host Disease (GvHD), allogeneic BM-transplantation was performed on conventional (conv) C3H/HeN and C57Bl/6J mice in both directions. BM-donors were SDD-treated, starting two weeks before BMT. SDD was carried out by oral aztreonam administration in the drinking water (100 mg/l). Weekly cultures of faeces yielded absence of Enterobacteriaceae during SDD.

In comparison to controls, SDD of C57Bl/6J donors significantly increased Delayed Type (DT) GvHD in conv C3H/HeN recipients, whereas no change in the occurrence of DT-GvHD was observed in conv C57Bl/6J recipients. This indicates that the mitigating effect of SDD of the donor was not caused primarily by elimination of Enterobacteriaceae. None of the mice suffering from DT-GvHD died because of septicemia, since heart-blood cultures of these animals were negative.

We conclude that modulation of the intestinal flora of the donor by SDD may change the cellular composition of the BM graft. This change either aggravates or mitigates the degree of DT-GvHD in conv mice. The bacterial composition in the digestive tract of the donor is therefore possibly as important to prevent DT-GvHD as the bacterial composition of the flora of the recipient.

1) Goris H. et al (1985), Infect Imm 50,2:473-441 2) Goris H. et al (1986), J Inf Dis 18:55-63.

K 334 BONE MARROW TRANSPLANTATION WITH A FIXED LOW NUMBER OF T-CELLS IN THE MARROW GRAFT, Leo F. Verdonck, Gijsbert C. de Gast, Hans G. van Heugten and Adriaan W. Dekker, Dept. of Haematology, University Hospital Utrecht, The Netherlands.

Allogeneic bone marrow transplantation (BMT) in man is hampered mainly by graft-versus-host disease (GVHD). Ex-vivo T-cell depletion of the marrow graft has decreased the incidence and severity of GVHD, but has resulted in a higher incidence of graft failure and of relapse of the disease. In order to find an optimal T-cell number which avoids the extreme risks on both sides, we performed BMT's with a fixed low number of T-cells. In 21 patients with 1.10^5 T-cells/kg body weight no graft failure was observed, although 1 patient with severe aplastic anemia is still transfusion dependent 6 months after BMT. In 10/20 evaluable patients (1 died early of veno-occlusive disease) only acute GVHD gr 1 (4 patients) or gr II (6 patients) of the skin was observed, reacting favourably to steroids. Chronic GVHD was seen in 5 patients, only after preceding acute GVHD. No relapse was observed in the 6 leukemic patients transplanted in first remission or chronic phase with a follow-up of at least 6 months. Cytomegalovirus interstitial pneumonia was the main problem, occurring only in seropositive patients. These results suggest that with a fixed low number of T-cells severe acute GVHD and graft failure can be avoided, whereas the risk of leukemic relapse may be low.

K 335 IMMUNODEFICIENCY IN MURINE GRAFT-VERSUS-HOST DISEASE: EVIDENCE FOR AN ANTIPROLIFERATIVE SUPPRESSIVE MECHANISM. D.A. Wall, S.H. Hamberg, S.J. Burakoff, A.K. Abbas, and J.L.M. Ferrara. Pediatric Hematology-Oncology, The Children's Hospital and Dana Farber Cancer Institute, Pathology Dept. Brigham and Women's Hospital, and Harvard Medical School, Boston.

Recovery of immune function was studied in a murine bone marrow transplantation (BMT) model between H-2 identical, minor histocompatibility disparate mice (B10.BR→ irradiated CBA/J) in which severity of graft-vs-host disease (GVHD) was proportional to the number of mature T lymphocytes transferred in the donor marrow inoculum. Mice with mild GVHD had normal phenotypic reconstitution of immune cells but a profound delay in the recovery of the ability of splenocytes to respond to mitogen (LPS, CON A). Isolated mature B cells from the unresponsive populations functioned normally. Splenocytes from the mice with GVHD suppressed proliferative responses of normal lymphocytes in coculture but did not effect lymphokine secretion by T cells. This phenomenon was also seen when a panel of T helper clones were treated with a variety of mitogenic stimuli in the presence of spleen cells from the mice with GVHD. Suppression was reversed and immune function recovered if the spleens from the mice with GVHD were a) depleted of Thy1 positive cells, b) treated with leucine methyl ester, or c) cultured in the presence of a neutralizing anti-interferonγ antibody. Thus there appears to be a non-specific antiproliferative signal generated during the GVHD response that is able to reversibly down regulate the immune response as measured by multiple parameters in vitro. These observations suggest directions for the study of the immunodeficiency associated with GVHD, and potential therapeutic strategies.

Autotransplants

K 400 SEQUENTIAL MYELOABLATIVE CHEMOTHERAPY FOR HODGKIN'S DISEASE. T. Ahmed, D. Ciavarella, E. Feldman, J. Ascensao, C. Engelking, F. Hussain, and Z. Arlin. New York Medical College, Valhalla, NY.

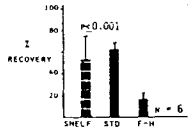
We studied thirty patients with refractory or relapsed Hodgkin's disease. These thirty people had bone marrow stored either at 4° C in the liquid state or frozen at -80° C. All thirty patients were given cytoxan 5 gm/m², etoposide 1500-2000 mg/m² and BCNU 400-600 mg/m². Four of these patients were transplanted again following ThioTEPA 900 mg/m², vinblastine 0.4 - 0.5 mg/kg and high dose cytarabine 3 gm/m². Fourteen patients achieved complete remission and five pacients had partial remission. The probability of achieving complete remission was equal in patients who had $\leq$ 2 regimens or $\geq$ 3 regimens. Pulmonary toxicity was less frequent in patients receiving $\leq$ 450 mg/m² of BCNU. The sequential regimen appeared to be well tolerated. Patients receiving liquid storage bone marrow had faster recovery of platelets than patients undergoing transplantation with cryopreserved bone marrow. Myeloablative chemotherapy in conjunction with bone marrow transplantation may improve the prognosis of patients with refractory Hodgkin's disease. Liquid storage bone marrow appears to be associated with earlier platelet engraftment.

K 401 TRANSPLANTATION OF CD34+ MARROW STEM CELLS IN PATIENTS WITH METASTATIC BREAST CANCER Ronald Berenson, William Bensinger, Robert Andrews, Dale Kalamasz, Roger Hill, C. Dean Buckner, Irwin Bernstein, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

The CD34 antigen is expressed by 1-3% of human (and primate) marrow cells including virtually all hematopoietic progenitors detectable by in vitro assays. We have previously shown that autologous CD34+ marrow cells isolated from baboon marrow by immunoadsorption with the anti-CD34 antibody 12-8 were capable of reconstituting hematopoiesis when transplanted into lethally irradiated animals. Studies have shown that the CD34 antigen is not present on tumor cells from patients with breast cancer. Immunoadsorption with antibody 12-8 was thus used to positively select CD34+ marrow cells free of tumor cells for transplantation in three patients with metastatic breast cancer with a history of, or at high risk of, marrow involvement. A total of 13.0, 16.0 and 14.4 x 10^9 marrow cells were labeled successively with antibody 12-8, biotinylated goat antimouse antiserum and passed over columns of avidin-Biogel. We recovered 50, 170, and 260 x 10^6 column adherent cells (representing 0.4-1.8% of the starting nucleated cell number) containing 73%, 81% and 67% CD34+ cells, respectively. The positively selected cells were highly enriched for colony-forming cells (CFU-GM) and did not contain detectable tumor cells ($<_6$1 in 10,000 marrow cells). Recently, one of the patients was transplanted with 50 x 10^6 positively selected cells (1.0 x 10^6 cells/kg) following marrow ablative therapy and is currently 15 days post-transplant with evidence of myeloid and erythroid engraftment by marrow examination. These studies suggest that CD34+ marrow stem cells can be selected in the absence of most or all tumor cells and may be clinically useful for bone marrow transplantation.

K 402 NEW METHOD OF AUTOMATED PROCESSING OF PEDIATRIC SIZE BONE MARROWS FOR AUTOTRANSPLANTATION: SHORTENED PROCESSING TIME AND INCREASED NUCLEATED CELL RECOVERY, M.S. Cairo, L. Sender, G. Bennetts, C. Toy and C. VandeVen, Dept. of Med., U.C. Irvine, Children's Hospital Orange County, Orange, CA.

Bone marrow transplantation and harvesting from autologous sources is becoming increasingly used in the treatment of malignant diseases. Concentrating the progenitor stem cells in a centrifugal field with a blood cell processor is commonly used. Small volumes of bone marrows are usually too small to be obtained on a cell processor and must be processed by a longer and less efficient ficoll-hypaque (FH) separation technique. This laboratory has developed a unique technique to process bone marrow volumes of as little as 200 mls on the Cobe 2991 cell processor. Volumes of bone marrow from each of 5 adult harvests were split into three fractions: 200 mls was layered onto a FH gradient (d=1.077); 200 mls was layered onto a packed red blood cell "shelf" which had been created by "interphasing" a unit of autologous blood with the Cobe 2991 cell processor. The remainder of the volume (> 400 ml) was processed according to standard protocol using the Cobe 2991. There was no statistical difference in comparing mononuclear cell recovery in standard vs "shelf" (73.2% vs 74.3%) and comparing CFU-C recovery (295±50 vs 300±100 colonies/ml). Significantly greater yields of nucleated cells were recovered in the "shelf" technique than by the FH technique (see graph). Standard method and "shelf" method were compared to control FH. The standard and "shelf" methods took one-third less time to process (2'±10'' vs 6'±15''). This method could prove to be a valuable technique in processing bone marrows in the pediatric populations whereby a higher yield of nucleated cell recovery could be attained with shorter processing time with sterility and viability maintained.



K 403 RECONSTITUTION FOLLOWING TREATMENT OF BONE MARROW WITH LYMPHOKINE-ACTIVATED KILLER CELLS. D.V. Cramer, G.S. Long, J.C. Hiserodt, and R.B. Herberman. University of Pittsburgh School of Medicine and the Pittsburgh Cancer Institute. Pittsburgh, PA 15261.

We have investigated the influence of lymphokine-activated killer (LAK) cells on the survival of stem cell function in normal bone marrow. LAK cells represent activated natural killer cells that have acquired the ability to lyse a wide range of neoplastic but not normal cells. Spleen cells from F344 rats were cultured in vitro for five days with recombinant interleukin-2 (rIL-2) to generate a population of highly cytolytic LAK cells. The activated LAK cells are incubated with normal bone marrow and the survival of pluripotent stem cells assessed by in vivo reconstitution of lethally-conditioned recipients and in vitro CFU colony assays. Bone marrow cultured with LAK cells retains the ability to reconstitute animals lethally conditioned with busulfan and cyclophosphamide. The rate of restoration of peripheral blood parameters in the recipients of the treated marrow is normal. The in vitro generation of granulocyte/macrophage CFU and the generation of CFU-s colonies is suppressed, suggesting that these assays may not accurately measure the ability of the bone marrow to restore hematopoietic function. The lack of effect on LAK cells on bone marrow to reconstitution may provide the opportunity to use this procedure to purge bone marrow of neoplastic cells prior to autologous transplantation.

K 404 AUTOLOGOUS BONE MARROW AND PERIPHERAL BLOOD CELLS TRANSPLANTATION: RAPID AND COMPLETE HEMOPOIETIC RECONSTITUTION FOLLOWING MYELOABLATIVE CHEMO-RADIOTHERAPY. A. M. Gianni, M. Bregni, S. Siena, S. Villa, G.A. Sciorelli, F. Ravagnani, G. Pellegris and G. Bonadonna, Istituto Nazionale Tumori, Milano, Italy.

Nineteen patients were treated according to a high-dose chemo-radiotherapy program consisting of the sequential administration of (a) cyclophosphamide (6-7 g / m^2) on day 0; (b) vincristine (1.4 mg / m^2), methotrexate (8 g / m^2) plus leucovorin rescue, cisplatin (120 mg / m^2) on days +21-25; and (c) total body irradiation (12.5 Gy total), melphalan (120-180 mg / m^2) on days +42-45 . Following melphalan infusion, 13 patients were grafted with autologous bone marrow, and 6 patients with autologous marrow plus peripheral blood leukocytes. For the 'BM only' group compared to the 'BM+PBL' group, there was a significant difference in the number of days with less than 0.5 x 10^9 WBC / l (median, 12 *v* 8, p=0.003) and with less than 0.5 x 10^9 granulocytes / l (17 *v* 9.5, p=0.002); first day to achieve more than 0.5 x 10^9 WBC / l (15 *v* 10, p=0.001) and more than 0.5 x 10^9 granulocytes / l (17 *v* 12, p=0.002); post-transplant day of last platelet transfusion (24 *v* 9, p=0.001) and RBC transfusion (19 *v* 13.5, p=0.008); number of platelet transfusions (5 *v* 1, p=0.002) and of red blood cell transfusions (7 *v* 4, p=0.017); day of discharge (31.5 *v* 19.5, p=0.045). We conclude that upon addition of a small amount of circulating stem cells , hematological recovery is so prompt that myeloid toxicity should no longer be considered the major factor limiting the early application of high-dose chemo-radiotherapy.

K 405 INTENSIVE THERAPY WITH AUTOLOGOUS MARROW TRANSPLANTATION (ABMT) FOR EWING'S SARCOMA(ES):

J. Graham-Pole, R. Marcus, N Mendenhall, D. Springfield, J. Fort, S. Gross, G. Elfenbein, University of Florida (UF), Gainesville, Florida.

Analysis of 53 patients (pt) with ES receiving radiation therapy (RT) and adjuvant chemotherapy (CT) at UF from 1966-81 showed significant differences in disease-free survival (DFS) according to maximum (max) tumor diameter (dia). Since 1982 we have stratified 42 pt by max tumor dia. 15 pt with dia < 8cm (Gp 1) received standard CT for 1 year plus local RT (5040-6000 cGy). 10 pt (Gp 2) with tumor dia > 8cm received identical local RT + CT until wk 40, then melphalan 180mg/m^2 + ABMT. 17 pt (Gp 3) received intensive CT (2 courses Vincristine (V) 2mg/m^2, Cytoxan (C) 1800mg/m^2, Adriamycin (A) 90mg/M^2), RT wk 6-11, 3 more courses of VAC, then VAC + total body irradiation (TBI) 800 cGy + ABMT wk 20.

Results:	Gp 1	2	3
# pt	15	10	17
follow up (median)	33m	19m	21m
# DFS (%)	9(60)	1(10)	13(76)

Conclusions: (1) Tumor size is prognostic in ES; (2) local RT plus non-ablative CT is effective for small tumors; (3) local RT plus ablative CT with TBI and ABMT is effective for large tumors; (4) controlled trials are needed to refine the use of CT, local RT, and ABMT, using such stratifications in ES.

K 406 ACUTE RADIATION NEPHRITIS (HEMOLYTIC-UREMIC SYNDROME) AFTER BONE MARROW TRANSPLANTATION. E.Guinan, N.Tarbell, C.Niemeyer, S.Sallan and H.Weinstein, The Children's Hospital and Dana-Farber Cancer Institute, Boston, MA.

Renal disease has been observed infrequently following bone marrow transplantation (BMT). We report a high incidence of a syndrome compatible with acute radiation nephritis in pediatric patients (pts) after allogeneic or autologous BMT for neuroblastoma (NB)or autologous BMT for acute lymphoblastic leukemia (ALL). Pts were all transplanted between 1980 and 1986 at The Children's Hospital and were considered evaluable if they survived in complete remission for more than three months after BMT. Ten evaluable pts were transplanted for NB after preparation with VM-26, cyclophosphamide (CTX), melphalan, cis-platinum and 1200-1300 cGy fractionated total body irradiation (TBI) given over 3 days in 6 fractions. Seven of ten developed renal disease. Twenty-one evaluable pts were transplanted for CALLA positive ALL after preparation with VM-26, cytosine arabinoside, CTX and TBI. One pt received 850 cGY in a single fraction. The others received 1200-1400 cGy in 6-8 fractions over 3-4 days. Seven of twenty-one pts developed renal disease. All 14 pts with renal disease had microscopic hematuria and elevations of BUN (range 33-81mg %) and creatinine (range 0.7-2.7 mg %) at a median of 5 mos (range 4-7) after BMT. All pts also had a fall in hematocrit with evidence of microangiopathic hemolytic anemia. Two pts with NB and 1 pt with ALL underwent kidney biopsy which revealed mesangiolysis, mesangial proliferation and thickening and splitting of the basement membrane consistent with acute radiation nephropathy. In summary, 14/31 evaluable pediatric pts treated with multiagent conditioning plus TBI prior to BMT, developed anemia and renal insufficiency 4-7 mos post BMT. Perhaps such intensive chemotherapy regimens make the kidney more vulnerable to TBI-mediated nephrotoxicity.

K 407 *TRIPLE LUMEN CATHETERS (TLC) IN AUTOLOGOUS BONE MARROW TRANSPLANT* (ABMT) PATIENTS (Pts). W. Harvey, N. Huth, T. Pick, P. Thomas, A. Potter, R. Solenberger. University of Texas Medical Branch, Galveston, Texas 77550, Brooke Army Medical Center, San Antonio, Texas 78234.

We report here our experience using TLCs in 29 Pts, 13 adults and 16 children who have had ABMT. Median age was 9 years (range 1.8-66 yrs). There were 12 females and 17 males. All Pts had a TLC (Arrow International, Inc., Reading, PA) placed in the central venous system. Childrens lines were placed in the operating room under general anesthesia by a surgeon (RS) with skin tunneling. Adult lines were placed by supervised housestaff at the bedside. TLCS were used to deliver antibiotics, hyperalimentation and blood products as well as to draw most blood specimens. 2 of 29 (7%) TLCs were removed prior to the end of therapy for presumed line sepsis. 5 of 13 (38%) TLCs were accidentally removed by adults. 3 had a second TLC inserted without complications. 2 had unsuccessful attempts at second TLC placement. Overall, 32/34 (94%) TLC placement attempts were successful. There were three complications (9%) related to the 34 attempted placements: one pneumothorax requiring a chest tube, one subclavian artery puncture without consequence, and one retropharyngeal hematoma which resolved without treatment. We believe the TLC is an alternative to Hickmans or Broviac catheters and offers an advantage by having three lumens. We recommend surgical placement with skin tunneling versus percutaneous placement.

K 408 AUTOGRAFT USING PERIPHERAL BLOOD STEM CELLS (PBSC) IN VARIOUS MALIGNANT HEMATOLOGICAL DISEASES, Ph. Henon, A. Debecker, J.C. Eisenmann and M. Lepers - Centre Hospitalier - 68051 Mulhouse Cédex - France.
We have autografted 10 patients with various malignant hematological diseases using PBSC collected by cytapheresis after induction or consolidation chemotherapy and an additional patient will be engrafted in the near future :
A) 8 patients (extreme 14 - 57 y. old) with acute leukemia (A.L.) : a) 4 ALL (1 Calla+, 2 T-ALL, 1 B-ALL). 2 were autografted in 1st CR, the other 2 in 2nd CR. b) 4 ANLL (2 M3, 1 M4, 1 M5), all engrafted not later than 4 months after 1st CR. Each of these 8 patients was conditioned before autografting with TBI + chemotherapy. The median PB CFU-GM dose infused was of 39 x 10^4/kg body weight (range 23-69). The B-ALL patient died 3 days after autograft by chemo-radio toxicity. Early hemopoietic recovery occured from d10 in the others. The 2 ALL patients relapsed off therapy 3 ½ and 7 months post-graft respectively.
B) 2 patients (58 and 64 y. old) with myeloma. PBSC had been previously collected after intensive chemotherapy comprising high doses of Melphalan (HD-M), which obtained CR. The pregraft conditonement was TBI + HD.M. Autograft was performed using both PBSC (5 x 10^4 and 6,4 x 10^4/kg body weight respectively) and products of non purged bone marrow collection. The hemopoietic recovery was similar to the one observed in AL. The 2 patients remain in CR.
C) 1 patient (55 y.old) with CGL. PBSC were collected twice in chronic phase after 2 courses of intensive chemotherapy followed by an almost total eradication of the Ph1 clone. This patients has not yet been autografted, but he will be at the time of the symposium where the data will be reported.

K 409 PHASE I/II STUDY OF ETOPOSIDE (VP16), CYCLOPHOSPHAMIDE (CY) AND TOTAL BODY IRRADIATION (TBI) WITH AUTOLOGOUS MARROW TRANSPLANTATION (AMT) FOR RESISTANT HEMATOLOGIC MALIGNANCY, G. Herzig, J. Fay, R. Herzig, S. Wolff, G. Phillips, D. Frei-Lahr, J. Lowder, R. Brown, Washington University, St. Louis, MO, Baylor University, Dallas, TX, Cleveland Clinic, Cleveland, OH, Vanderbilt University, Nashville, TN, University of British Columbia, Vancouver,B.C.
Fifty-four patients (pts) with relapsed or refractory acute leukemia or lymphoma received VP16, CY, TBI and AMT with sequential dose escalation of each agent. The maximum tolerated combination was VP16-1800 mg/M^2 (26 hr IV infusion), CY-150 mg/Kg (50 mg/Kg/day x3 days) and TBI 1000 cGy (200 cGy bid x5 doses) with no fatal toxicity in 6 patients at risk $\geq$ 3 mos. Increasing VP16 to 2250 mg/M^2 led to fatal toxicity in 2 of 3 pts (1 liver, 1 lung) and increasing Cy to 200 mg/Kg led to severe (non-fatal) skin toxicity in 3 of 4 pts. Increasing TBI to 1200 cGy led to fatal pneumonitis in 4/24 (17%) of pts compared to 1/13 (7%) at 1000 cGy. The regimen demonstrated substantial activity: in preliminary analysis of 30 patients with progressive non-Hodgkin's lymphoma 18 pts (60%) entered CR and 13 (43%) are alive, free of disease at 5-20 (median 11) mos follow-up.

K 410 PHASE I-II STUDIES WITH HIGH-DOSE THIOTEPA AND AUTOLOGOUS MARROW TRANSPLANTATION (AMT) IN PATIENTS (PTS) WITH REFRACTORY MALIGNANCIES. R. Herzig, J. Fay, G. Herzig C.F. LeMaistre, S. Wolff, D. Frei-Lahr, S. Strandjord, P. Coccia, L. Giannone, D. Norris, J. Weick, B. Bolwell, S. Rothmann, J. Lowder. Cleveland Clinic Foundation, Cleve. OH 44106.
In a phase I-II study 179 pts (97 men, 82 women), median age 40 (range: 2-70) yrs, received 180-1575 mg/M^2 thioTEPA with AMT. Pharmacokinetic studies with high-dose thioTEPA were similar to but proportionately higher than conventional doses. Myelosuppression regularly occured, with recovery of granulocytes >500/µl and platelets >20,000/µl at a median (range) of 17(7->80) and 21(6->90) days after AMT. Dose-related nonfatal extramedullary dermatologic and GI (oral mucositis) toxicities were observed. Skin toxicity (erythema, desquamation, bronzing) had not been reported before; the incidence statistically increased after 1125 mg/M^2 and approached 100% in pts receiving 1575 mg/M^2. GI toxicity increased at 1125 mg/M^2, but higher thioTEPA levels did not increase toxicity. CNS toxicity (organic brain syndrome) was dose-limiting: 9/17 pts affected at 1575 mg/M^2 (1 fatal), led to dose-reduction. With more pts entered at lower levels, a maximum dose of 1125 mg/M^2 was established. Antitumor responses were evaluable in 143 pts. A dose-response relationship was seen: 9/37 (24%) pts who received <900 mg/M^2 responded compared to 62/106 (58%) pts who received ≥900 mg/M^2 ($p<0.0005$). Pts who received ≥900 mg/M^2 had responses in breast (5/14), colon (8/10), gastric (1/2), germ cell (3/5), glioblastoma (2/4), lung (1/3), lymphoma (4/6), melanoma (21/37), neuroblastoma (3/4), ovary (6/10), sarcoma (3/5), unknown primary (4/5), Wilm's (1/1). The median (range) duration of unmaintained response was 5(2-31+) months; 18% have had responses longer than 1 year.

K 411 INACTIVATION OF LEUKEMIA CELLS BY SEQUENTIAL EXPOSURE TO MEROCYANINE 540 AND CYTOTOXIC ANTIBODIES, Takeyoshi Itoh, Edward D. Ball and Fritz Sieber, Medical College of Wisconsin, Milwaukee, WI 53201, and Dartmouth-Hitchcock Medical Center, Hanover, NH 03756.

Purging of autologous bone marrow grafts with high concentrations of residual tumor cells may best be approached by treatment with two or more agents whose mechanisms of action is based on different principles. In this communication, we report on the combined effects of merocyanine 540 (MC 540)-mediated photosensitization and complement-mediated immune lysis on HL-60 cells. MC 540 reacts primarily with the lipid portion of the plasma membrane whereas antibodies recognize proteins and carbohydrates. Incubation with the monoclonal antibodies PM-81 (20 ug/ml) and AML-2-23 (20 ug/ml) and complement reduced the concentration of in vitro clonogenic HL-60 cells 210 to 2500-fold, whereas exposure to MC 540 (15 ug/ml) and white light (approx. 15 J/cm²) caused a 15 to 220-fold reduction of in vitro clonogenic cells. Exposing HL-60 cells sequentially to MC 540-mediated photosensitization and the two antibodies reduced the concentration of clonogenic cells 27,000 to 770,000-fold. Similar results (217,000 to 263,000-fold reduction) were obtained when the experiment was performed in the presence of a 20-fold excess of irradiated normal bone marrow cells (simulated autologous remission marrow graft). The overall reduction of tumor cells was the same regardless of the sequence in which the two purging procedures were employed. In summary these results suggest that cytotoxic antibodies and lipophilic photosensitizers may be promising candidates for combination purging protocols.

K 412 A CASE OF BONE MARROW FIBROSIS AFTER ABMT. CAN THE PREPARATIVE REGIMEN (BUS+CY) CAUSE A DAMAGE OF THE MARROW STROMA?

IZZI T, ROSSI G, FERREMI P, RONCOLI B, VERZURA P, CAPUCCI MA, BONFANTI V, CARBONE C, CHIODERA PL, SACCHI G, FRANCESCHINI F, MORETTI L, MARINONE G.
OSPEDALI CIVILI DI BRESCIA E OSPEDALE DI PESARO - ITALY

A 23 year old male patient (G.F.) affected by AML/M2 was treated with two cycles of TAD-9 chemotherapy (German multicenter study). After a complete remission the marrow was harvested and cryopreserved ($2.9x10^8$/Kg). We prepared the patient with BUSULFAN (14 mg/Kg) and CYCLOPHOSPHAMIDE (200 mg/Kg). After the reinfusion of the unpurged bone marrow cells ($2.7x10^8$/Kg) the patient left the isolation at +48. The granulocytes were 500 µl at +48 and the platelets were 20000 µl at +50. The clinical course was without complications, but the finding of a severe marrow fibrosis with reactive hyperostosis at +10 from ABMT. The complete disappearance of the fibrosis at +40 preceded a complete hemopoietic recovery. Our hypothesis is that BUS and CY caused a transient damage in the marrow stroma, mainly because of the short interval between marrow harvest and starting of the preparation to ABMT (10 days).

K 413 AUTOTRANSPLANTS FOR POOR PROGNOSIS NON SEMINOMATOUS GERM CELL TUMORS

J.L. PICO, M. OSTRONOFF, J.P. DROZ, D. BAUME, F. BEAUJEAN*, M. HAYAT. INSTITUT GUSTAVE ROUSSY, VILLEJUIF, FRANCE. * CDTS CRETEIL, FRANCE.

The prognosis of Non Seminomatous Germ Cell Tumors (NSGCT) has improved greatly over recent years. Nevertheless, a percentage of patients relapse and die of tumor progression. The analysis of prognostic factors identifies a group of patients of poor prognosis. High dose chemotherapy (HCD) deserves attention in this population because the patients are young, there is a dose-response relationship and the bone marrow is rarely involved. 41 pts received 44 courses of PEC protocol : ETOPOSIDE (VP) 350 mg/m2/d x 5 - CDDP 40 mg/m2/d x 5 and Cyclophosphamide (CTX) 1600 mg/m2/d x 4 d + Mesna followed by ABMT at d 8. Median age was 29 years (13-50). Initial localisation was : testicular 29, ovary 4, extra-gonadal 8.

Myelosuppression was consistantly observed with a 15 d (6-37) median time of granulocytopenia (< 0,5 x 10^9/l) and a 13 d (3. > 32) median time of thrombopenia (< 20 x 10^9/l). Non hematologic toxicity included vomiting, mucositis, diarrhea and peripheral neuropathy. 4 toxic deaths (9%) occured. Anti-tumor response was high with 20/32 evaluable pts in CR (62,5%) and 30/32 CR + PR (93,7%). For better analysis of the outcome we stratified the 41 pts in three groups according to clinical status. 12 pts with progressive disease (PD) : 2 toxic death, 1 alive with disease, 9 died of tumor progression. 6 pts with non-resistant relapse : 4 in CR (33, 35, 36, 38 m^+), 1 alive with disease, 1 died of tumor progression. 23 pts treated for consolidation of a first CR or PR : 16 in CR with a median duration of 14,5 m^+ (1-26 m^+), 3 alive with disease, 2 toxic deaths, 2 died of tumor progression.
For the PEC protocole, we conclude that the efficacy is high, toxicity was also high but acceptable, making it a good indication for NRR wether or not this approach is valid as consolidation for first PR or CR in poor prognosis pts should be determined by a randomised trial comparing it with conventional therapy.

K 414 AUTOLOGOUS PERIPHERAL STEM CELL TRANSPLANTATION FOLLOWING HIGH DOSE CHEMOTHERAPY- AN UPDATE, Anne Kessinger, James D. Landmark, Douglas M. Smith, Dennis D. Weisenburger and James O. Armitage, University of Nebraska Medical Center, Omaha, NE 68105. Twenty six patients with malignancies refractory to primary therapy were candidates for high dose therapy with autologous bone marrow transplantation except that they had bone marrow metastases or had pelvic bone marrow hypoplasia. Their median age was 35 years and 14 were males. Fifteen patients had Hodgkin's disease, 7 had breast cancer, and 4 had non-Hodgkin's lymphoma. Peripheral stem cells were collected with apheresis without manipulations to increase the number of circulating stem cells. Six to 14 separate 2.5 - 4 hour collections, repeated no more often than 3 times weekly were done for each patient. The collected cells contained a median of 7.2 x 10^8 mononuclear cells/kg patient weight (range 4.9-16.4 x 10^8 mononuclear cells/kg). After cryopreservation and thawing, these cells contained a median of 4.09 CFU-C/kg (range .51-98.6 CFU-C/kg). The high dose therapies given varied according to the disease being treated and included either 120 mg/kg or more of cyclophosphamide or total body irradiation (7 patients) in addition to other chemotherapeutic agents. Six patients had an early death. Circulating white cells reappeared at a median of 8 days (range 3-15 days) following transplantation. Median time to reach 0.5 x 10^9/l granulocytes was 21 days, median time to maintain 10 gm/dl hemoglobin without transfusion support was 21 days and median time to maintain 20 x 10^9/l platelets was 20 days. Autologous peripheral stem cell transplantation is an acceptable alternative to autologous bone marrow stem cells transplantation for accelerating recovery of hematopoiesis following high dose therapy for malignancies.

K 415 COLLECTION AND USE OF PERIPHERAL BLOOD STEM CELLS IN PEDIATRICS, L.C. Lasky, B. Bostrom, J. Smith, X.J. Xu, and N.K.C. Ramsay, University of Minnesota, Minneapolis, Minnesota 55455

Peripheral blood stem cells (PBSC), along with the avoidance of marrow harvest under general anesthesia, offers the potential for this form of "transplant" in patients with disease involving the marrow. Reports of successful PBSC use have been limited to adults. This report describes peripheral blood stem cell collection in extremely small individuals, and documents the successful use of such cells in the case of a 2½ year old boy with progressive neuroblastoma involving the marrow. Collections were performed using a Fenwal CS3000 blood cell processor primed with fresh frozen plasma and red cells, using a low flow rate to avoid citrate toxcity. The transplant was performed using the 4 collections lowest in positivity for neuron specific enolase, of the 5 collected. Three were negative at a level of detection of < 1 in 10^5 cells. Preparation for infusion was carried out with high dose cyclophosphamide, etoposide, and cisplatin. Post-transplant recovery was uneventful, and engraftment was faster than in 4 patients treated with similar preparation and autologous marrow (Table). During six weeks followup, hematopoietic engraftment has endured. Using careful technique, PBSC support in lieu of marrow may be a viable treatment modality in childhood neuroblastoma or other solid tumor, particularly when the marrow is involved with disease, even in tiny children.

Days from Infusion Until:

Group	Engraftment*	ANC> 500/μL	Last Platelet Transfusion	Last Red Cell Transfusion	Sustained Platelet Count >150,000/ul
Marrow (n=4)	24 (16-26)#	25 (17-37)	15 (13-21)	20 (16-23)	25 (22-28+)
PBSC	14	19	13	11	19

*Day WBC exceeded 1000/μL for 3 consecutive days;#median (range); ANC= absolute neutrophil count.

K 416 HIGH-DOSE VP-16, HIGH-DOSE CISPLATIN, AND AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) FOR LUNG CANCER: A PHASE I TRIAL. H.M. Lazarus, T.R. Spitzer. Ireland Cancer Center, Case Western Reserve University, Cleveland, OH 44106.

We began a phase I trial using fixed-dose cisplatin, escalating doses of VP-16, and ABMT for refractory small cell (SCLC) and non-small cell (NSCCL) lung cancer. Twenty patients (pts) representing 23 treatment courses were evaluated. Median age was 57 (range: 38-68) years. Three pts had SCLC and 17 pts had NSCCL. Pts received cisplatin 200 mg/m² over 5 days and VP-16 iv starting dose 600 mg/m²/day for 3 days (total 1800 mg/m²) which was escalated to 800, 1000, 1200, 1400 and 1600 mg/m²/day for 3 days, and ABMT. Toxicities included nausea, vomiting, alopecia, high-tone hearing loss, mucositis, diarrhea, and myelosuppression. Reversible renal insufficiency (peak serum creatinines 6.7, 6.7, and 3.6 mg%) occurred in 3 pts who completed the therapy; one pt had renal dysfunction after 2 doses of VP-16 and is inevaluable (IE). In 3 pts early death (ED) occurred within 4 weeks of ABMT. Two complete (CR) (5, 12 mo duration) and 9 partial (PR) remissions (2-12+ mo) were observed. No response (NR) was noted in 6 pts and tumor progression (TP) occurred in 2 pts. The upper dose limit for VP-16 has not yet been reached. The acceptable toxicity and the encouraging anti-tumor effect suggest this approach may be useful therapy for lung cancer.

No.	VP-16 (mg/m²)	CR	PR	NR	TP	ED	IE
6	1800		2	2		2	
5	2400	1	2		1	1	
4	3000		3	1			
3	3600	1	1	1			
4	4200		0	2	1		1
1	4800		1				
23		2	9	6	2	3	1

K 417 DELAYED REJECTION OF CARDIAC ALLOGRAFTS FOLLOWING EXTENSIVELY T LYMPHOCYTE-DEPLETED AUTOLOGOUS MARROW TRANSPLANTATION IN THE RHESUS MONKEY, R.D. Moses, K.S. Orr, J.D. Bacher, D.H. Sachs, and R.E. Gress, Immunology Branch, NCI, Bethesda, MD 20892

We have extended our studies of cardiac allograft survival in rhesus monkeys conditioned with total body irradiation ([TBI] 600 rads on days -1 and 0) and T cell-depleted (TCD) autologous bone marrow transplantation ([BMT] day 0), then given a MHC-mismatched heterotopic cardiac allograft (day 1). Acute allograft rejection was abrogated in all 9 animals (Rx) receiving extensively depleted marrow (T cell content $\leq$0.12% by limiting dilution analysis). Higher T cell content was associated with acute rejection. Two Rx animals developed heart graft rejection at 5 1/2 and 3 1/2 months post-BMT, the latter also rejecting a cryopreserved donor skin graft more rapidly than a 3rd-party skin graft at 3 months post-BMT. One Rx animal remains rejection-free at 5 1/2 months (marrow T cell content 0.00014%). Peripheral $CD4^+$ helper T cell numbers, IL2 production, mitogen response, and MLR were profoundly depressed for >3 months post-BMT, while $CD8^+$ T cells, $2H7^+$ B cells, $Leu11^+$ NK cells, and IL2-supplemented CML recovered by 2 months. The two late Rx cardiac allograft rejections were correlated with a return of peripheral $CD4^+$ helper T cells. We conclude that 1) cardiac allograft survival is prolonged following TBI and extensively TCD autologous BMT, 2) freedom from acute rejection is associated with profound early nonspecific immunosuppression without demonstrated specific tolerance induction, and 3) late rejection correlates with the return of $CD4^+$ helper T cells in the presence of intact $CD8^+$ T cells and IL2-dependent cytotoxic effector function. The latter observation is consistent with a critical role of $CD4^+$ T cells in organ graft rejection.

K 418 AUGMENTED CYCLOPHOSPHAMIDE (C), BCNU (B) AND ETOPOSIDE (V) = CBV AND AUTOLOGOUS BONE MARROW TRANSPLANTATION (BMT) FOR PROGRESSIVE HODGKIN'S DISEASE (HD), G. Phillips, M. Barnett, N. Buskard, J. Connors, J. Fay, G. Herzig, R. Herzig, P. Klimo, H-G. Klingemann, F. LeMaistre, J. Lowder, J. Moquin, S. O'Reilly, D. Reece, S. Wolff and N. Voss. U. of British Columbia and Cancer Control Agency of BC, Vancouver, BC, Washington U., St. Louis, MO, Cleveland Clinic, Cleveland, OH, Baylor U., Dallas TX, Vanderbilt U., Nashville TN, U. of Texas, San Antonio, TX.

We have evaluated an intensive combination chemotherapy and autologous BMT regimen for patients with progressive HD incurable with conventional therapy. Between 03/85 and 06/87, 42 patients with recurrent HD were entered. Median age was 30 (range 16-56) years; 26 were males. All had previously received multidrug primary regimens that produced complete remission (CR) in 29; 23 had also received radiotherapy (RT). Although all had progressive HD before entry, if patients were not deemed refractory to conventional therapy they received MVPP 1-4 cycles and/or local RT immediately before C (1.8 gm/m^2 daily x 4), B (0.6 gm/m^2 x 1 day) and V (0.4 gm q 12 h x 3 day) and cryopreserved autologous BMT. Five patients died < day +30, 1 with residual HD. CR was achieved in 31/42 (74%) patients. Three patients died in CR, 5 relapsed after BMT. All others remain in CR, median +213 (range +54 to +757). Ten patients died of toxicity: sepsis (4), renal failure (1), and interstitial pneumonia (5). A preliminary statistical analysis reveals that event-free survival is strongly correlated with prior CR. We conclude that augmented CBV and autologous BMT may be administered to most progressive HD patients with a high CR rate, and a low early relapse rate.

K 419 HAEMATOPOIETIC RECONSTITUTION AFTER AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION IN 58 PATIENTS WITH HEMATOLOGICAL MALIGNANCIES. J. Reiffers, S. Castaigne, G. Leverger, H. Tilly, E. Lepage, P. Henon, L. Douay for the France Auto Greffe Group, France.

Fifty-eight patients (median age = 24 years) underwent autologous blood stem cell transplantation (ABSCT). Thirty-three patients (pts) had ANLL, 20 pts had ALL and five pts had non Hodgkin lymphoma or myeloma. At the time of ABSCT, 47 pts were in complete remission while 11 had progressive disease. Fourty three pts were prepared for ABSCT with TBI (1000 - 1200 rads) associated with Cyclophosphamide (120 mg/kg) in 34 cases, with high-dose Aracytine (3 g/m2, 8-12 doses) in 6 cases or with the BEAM regimen in 3 cases. Thirteen other pts were conditioned with Busulfan (16 mg/kg) and Cyclophosphamide (200 mg/kg) (5 pts) or Melphalan (140 mg/m2) (8 pts). Finally, two pts received other conditioning regimens without TBI. For ABSCT, the pts were infused with a median number of 4.9 x 10^8/kg nucleated cells corresponding to a median number of 10.8 x 10^4/kg (0-155) CFU-GM cells. Three patients died shortly after ABSCT and were thus not evaluable for haematological reconstitution. One patient had a complete graft failure and another had no megacaryocyte engraftment. Fifty-three pts successfully engrafted. The median time to reach 500 granulocytes and 50 000 platelets/mm3 was 14 days (8-60) and 26 days (8-120) respectively. In pts infused with more than 15 x 10^4 CFU-GM/kg, haematological reconstitution was observed within one month in every case but three pts had slightly delayed platelet engraftment (30,49 and 50 days). This study confirms that peripheral blood stem cells are able to reconstitute haematopoiesis after supralethal therapy and indicates that the safe minimum number of CFU-GM is 15 x 10^4/kg.

K 420 TUMOR CELLS CULTURED FROM HISTOPATHOLOGICALLY NORMAL MARROW OF PATIENTS WITH LYMPHOMAS AND SOLID TUMORS. J.G.Sharp, S.S. Joshi, J.O. Armitage, L. Bolte, A. Kessinger, S. Mann, W.P Vaughan, D.D. Weisenburger University of Nebraska Medical Center Omaha, NE 68105. We evaluated histologically normal bone marrow of cancer patients referred for high-dose therapy with autologous bone marrow rescue for the presence of tumor cells using long-term culture techniques. Tumor cells were detected in the marrow of 20/39 (48%) of patients with non-Hodgkins' lymphoma and 19/30 (63%) patients with solid tumors. In breast cancer patients the detection rate was 15/19 (79%). Preliminary clinical results suggest that patients with solid tumor cells metastatic to histologically normal marrow do as well as those whose marrow is tumor free. The overall survival in the patients with lymphoma metastatic to marrow is currently better than that of the tumor-free group. The efficiency of the methods of tumor detection has been calibrated by addition of tumor cell lines (Raji, CEM, MCF-7) to normal bone marrow. For uncultured mixtures with lymphoma cells, the average detection limit by the pathologist is 1 tumor cell in 20 normal cells, and for breast 1 in 20,000 normal cells. Culture techniques increase lymphoma detection to 1 cell in 10,000 cells and for breast to 1 in a million cells. In clonal assays of these cultures, hematopoietic cells often cluster around tumor cells suggesting a symbiosis which might be CSF mediated. Progenitor cell maintenance after 12-14 weeks of culture was significantly greater in marrow cultures with metastatic tumor cells. This symbiosis together with the low NK cytotoxicity of bone marrow might account for high frequency of metastasis to this site. (Supported by an Imogene Jacobs Memorial Grant from the American Cancer Society)

K 421 HIGH-DOSE CYCLOPHOSPHAMIDE/VP-16/PLATINUM (CVP) INTENSIFICATION FOR METASTATIC BREAST CANCER (MBC), G Spitzer, F Dunphy, A Buzdar, G Hortobagyi, M Auber, F Holmes, K Jabboury, L Horwitz, S Jagannath, K Dicke, U.T. M.D. Anderson Hospital & Tumor Institute, Houston, TX 77030.

High-dose CVP intensification with autologous bone marrow transplantation (ABMT) was evaluated in 25 patients (pts) with MBC. Pt characteristics at entry were: median age of 42 (range 29-62), 60% pre-menopausal, 40% peri- or post-menopausal, 60% estrogen receptor (ER) status <10 fmol/mg, 24% unknown, 4 ER positive; respective values 14, 16, 19, and 159. Most pts had 2 or more sites of involvement. Disease sites were: lung 56%, breast or soft tissue 40%, nodes 36%, liver 20%, isolated bone involvement 28%. Median disease-free interval was 54 weeks, range 0-756. Pts received 2-6 courses of standard induction chemotherapy, most commonly Adriamycin 60mg/m^2 + Cytoxan 600mg/m^2. Response to induction in evaluable pts was: complete response (CR) 22%, partial response (PR) 65%; overall 87%. 3 pts (13%) showed progressive disease. Induction was followed by 2 courses CVP (cyclophosphamide 4.5g/m^2, VP-16 750-900mg/m^2, platinum 120-150mg/m^2. 2 pts (8%) died of toxicity within 30 days. Restaging of the 2nd CVP showed a CR rate of 65%, PR rate of 27%, overall response rate 92%; 2 PR pts were rendered no evidence of disease following surgery or radiotherapy, for a total of 74%. 9 pts (36%) relapsed and 5 have died. Median follow-up from induction and from CVP intensification is 49 and 27 weeks, respectively. Median survival from induction is estimated at 80+ weeks; survival from 1st CVP is not yet reached. 6 pts are approximately 1 year or greater disease-free. This data suggests that CVPx2 intensification increases impressively the complete remission rates in metastatic breast cancer. Toxicity is acceptable, and the present unmaintained CR of several patients from 1-$1^1/_2$ years appears promising.

K 422 PROGENITOR NUMBERS (CFU-C, CFU-DG, AND CFU-Mix) AND HEMOPOIETIC RECOVERY FOLLOWING AUTOLOGOUS MARROW TRANSPLANTATION, F.M. Stewart, D.L. Kaiser, E. Niskanen, University of Virginia, Charlottesville, VA, 22908.

34 consecutive pts were treated with HD chemotx ± TBI and ABMT. We studied the relationship of CFU-C, CFU-Mix, and CFU-DG to recovery of peripheral blood counts. Tx included CY 60mg/kg, d -7,-6, and TBI 200 r/d, d -5 through 0 (TBI) in 6 pts; CY 1.5 g/m^2/d, d -6 through -3, and VP-16 150 mg/m^2-300 mg/m^2/d, d -6 through -3 (EC) in 15 pts; Adria 45 mg/m^2 IV, days -9, -8, vlb 0.3 mg/kg, d -6, -5, and CY 60 mg/kg, d -3, -2 (CAV) in 7 pts; BCNU 300 mg/m^2, d -6, VP-16 250 mg/m^2/d, d -6 through -3, CY 1.5 g/m^2/d, d-6 through -3 (BEC). Marrow was infused on day 0. Hemopoietic recovery was measured indirectly through determination of daily peripheral counts until resolution of severe neutropenia. Analysis of the mean percent recovery following freezing of CFU-C, CFU-DG, and CFU-Mix shows 47%, 84%, 58% respectively. The 4 treatment regimens were analyzed for probability of achieving graded levels of neutrophil recovery according to time following marrow transplantation. Pts treated with either BEC or TBI had prolonged recovery for both neutrophils and platelets compared to the recovery of patients treated with EC or CAV ($p = <.05$). Correlation coefficients for the number of nucleated cells/kg of marrow harvested or CFU's/kg and hemopoietic recovery were calculated for all pts. In pts txed with BEC or TBI, only a correlation between improved recovery to a neutophil count of 500/cu.mm and CFU-DG pre-freeze was noted ($p=0.005$). This effect was not seen in post-thaw CFU-DG. No other assays (CFU-C, CFU-Mix) showed significant correlation with recovery. The use of progenitor assays such as CFU-DG with or without marrow purging techniques or other progenitor assays deserve further evaluation.

K 423 PHASE 1 STUDY OF HIGH DOSE CARBOPLATIN (CBDCA) AND VP-16 WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN PATIENTS WITH REFRACTORY GERM CELL CANCER, C. Nichols, L. Akard, S. Williams, G. Tricot, P. Loehrer, R. Hoffman, L. Einhorn and J. Jansen, Indiana University School of Medicine, Indianapolis, IN 46223.
Twenty patients (pts) with refractory germ cell cancer have been treated with high dose chemotherapy plus ABMT. All patients had failed two prior cisplatin regimens (one with ifosfamide). Successive groups of pts were treated with a fixed dose of VP-16 (1400 mg/m^2 QOD X 3) and escalating doses of CBDCA at 300 mg/m^2 QOD X 3 to 660 mg/m^2 QOD X 3. Sufficient marrow was harvested for 2 courses of therapy. Responding pts were eligible for the second course of therapy using identical doses of CBDCA/VP-16 and the remaining cryopreserved marrow. Hematologic toxicity was severe with all pts having granulocytopenic fever with each course of treatment (n=31). Extramedullary toxicity included abdominal pain and diarrhea (18 pts), hepatic transaminitis (4 pts) and hyperbilirubinemia (3 pts). Significant oto-, neuro-, or nephrotoxicity was not seen. There were 3 therapy-related deaths at the highest CBDCA doses; all with Candidemia as the principal cause. 15 pts have finished treatment. CR was obtained in 6 pts (1+,1+,3+,4+,4,8+ months); in 4/6 of these pts, the duration of CR has exceeded any previous CR induced by cisplatin-based regimens. 3/6 pts obtaining CR were cisplatin refractory (progression on or within 1 month after cisplatin). Therapy is continuing in the 5 remaining pts. The toxicity of this approach is substantial, and necessitates intense supportive care. Nonetheless, these data provide evidence of true non-cross resistance between high dose CBDCA/VP-16 and conventional cisplatin-based regimens in pts with refractory germ cell cancer.

K 424 RESIDUAL ACUTE LYMPHOBLASTIC LEUKEMIA BLASTS IN AUTOLOGOUS REMISSION MARROW GRAFTS, Fatih M. Uckun, John H. Kersey, Kevin G. Waddick and Norma C. Ramsay, Departments of Therapeutic Radiology-Radiation Oncology, Pediatrics, and the Bone Marrow Transplantation Program, University of Minnesota, Minneapolis, MN 55455.
We used leukemic progenitor cell (LPC) assays in combination with fluorescence activated cell sorting (FACS) to quantify residual leukemic blasts in remission marrow grafts from 12 B lineage ALL patients who underwent autologous BMT. Marrow grafts of B lineage ALL patients were purged with the MoAb BA-1 (anti-CD24), BA-2 (anti-CD9), BA-3 (anti-CD10) + complement (C') + 4-hydroperoxycyclophosphamide (4-HC). Prepurge and postpurge samples of autografts were two-color stained for CD19 (orange) and surface immunoglobulin (sIg) (green). There were 7.2 $\pm$ 3.2% $CD19^+sIg^-$ cells in prepurge samples, and 0.8 $\pm$ 0.5% $CD19^+sIg^-$ cells in postpurge samples. Subsequently, $CD19^+sIg^-$ FACS sorted B cell precursors were assayed for blast colony formation in the presence of low molecular weight B cell growth factor. Colony formation was obtained in prepurge samples from 12 of 12 cases with a mean PE of 0.139 $\pm$ 0.046%. By comparison, postpurge marrow samples grew blast colonies in 5 of 12 cases with a mean PE of 0.513 $\pm$ 0.393%. The mean % of LPC among MNC was 0.008 $\pm$ 0.003% prior to purging and 0.004 $\pm$ 0.003 % after purging. The estimated log kill ranged from 0.0 to 4.3 logs (mean = 1.9 $\pm$ 0.4 logs). To our knowledge, this report represents the first application of LPC assays in combination with FACS techniques for detection of residual ALL blasts in remission marrow samples from B-lineage ALL patients before and after purging.

K 425 HIGH DOSE CYCLOPHOSPHAMIDE (C), BCNU (B) AND ETOPOSIDE (V) WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN RELAPSED HODGKIN'S (HD) AND NON-HODGKIN'S LYMPHOMA (NHL): UPDATE OF A PHASE I TRIAL. C Wheeler, JH Antin, WH Churchill, BR Smith, SE Come, GJ Bubley, DS Rosenthal, JM Rappaport, LE Schnipper, and JP Eder. Beth Israel Hosp., and Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02215.
Since 4/86 31 patients (pts) with histologically negative marrows have been treated in a phase I trial to determine the maximally tolerated dose of CBV administered in divided doses with non-purged ABMT in pts with relapsed lymphoma. B was administered in 4 doses day (d) -7 to -3 at 450 mg/m^2 total dose. Escalating doses of C and V were given bid d -7 to -3. Cryopreserved autologous marrow was reinfused d 0. Pts were treated at 6 dose levels:

	I(n=7)	II(n=4)	III(n=5)	IV(n=9)	V(n=5)	VI(n=1)
BCNU mg/m^2	450	450	450	450	450	450
Cyclophosphamide g/m^2	4.5	6.0	6.0	7.2	7.2	7.2
Etoposide mg/m^2	1200	1200	1600	1600	1800	2000

Median age was 34 (range 18-66). 20/31 pts with NHL had >minimal disease at transplant; 7/18 had disease refractory to salvage therapy. Pts with HD had failed at least 2 chemotherapy regimens. There were 2 toxic deaths: 1 hepatic veno-occlusive disease at level I; 1 interstitial pneumonia at level IV. Toxicity included severe reversible oral mucositis (6 pts), reversible esophageal stricture (1 pt), asymptomatic pericardial effusion (3 pts), and serious bacterial infection (2 pts). Asymptomatic decrease in DLCO occurred in 4/11 evaluable pts. Median time to >500 PMNs was 21 d (range 11-55 d); median time to platelets >20,000 was 24 d (range 13-85 d). 8/18 pts with NHL and 6/12 evaluable pts with HD are in continuous CR at 27+ to 566+ days (median follow-up 133+ d).

K 426 A Phase II Study of Induction Chemotherapy Followed by Intensification with High Dose Chemotherapy with Autologous Stem Cell Rescue (ASCR) in Stage IV Breast Cancer. S. Williams, J. Bitran, R. Desser, J. Golick, J. Beschorner, L. Fullem and H. Golomb. University of Chicago and Michael Reese Medical Centers, Chicago, IL 60637.

This study was designed to determine whether induction chemotherapy (CT) consisting of leucovorin, vincristine, methotrexate, doxorubicin and cyclophosphamide (LOMAC) followed by high dose intensification chemotherapy (ICT), cyclophosphamide 2.5 gm/m^2 and thiotepa 225 mg/m^2 intravenously days -6, -4, -2 and ASCR could increase the complete response rate and survival in women with stage IV breast cancer. From July 1, 1986 to December 1, 1987 22 women, median age 44 yrs (range 24-64) and a median PS of 1 (range 0 to 3, CALGB) were enrolled. Eleven patients (pts) had received prior adjuvant chemotherapy. No pt had received chemotherapy for stage IV disease. One pt expired from sepsis during her first course of LOMAC and one pt had concurrent renal cell cancer leaving 20 pts evaluable for LOMAC response. Of the pts who have completed LOMAC induction, there are 10 PRs (50%), 6 CRs (30%), 3 stable disease, and 1 no response. Of the 19 pts eligible for ICT, 3 refused leaving 16 fully evaluable pts. The response rates among these 16 pts is 11 CRs, 2 PRs and 1 NR. The toxicities have included nausea/vomiting, mucositis, diarrhea, dermatitis and alopecia. All pts completing ICT have recovered normal peripheral counts. The median number of days in hospital for this phase is 39 (range 27-80 days). Induction therapy followed by ICT in new onset stage IV breast cancer can lead to high complete response rates. However, it remains to be determined if there is any benefit in survival.

K 427 **N,N',N''-TRIETHYLENE THIOPHOSPHORAMIDE (THIO-TEPA) (TT) WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) FOR METASTATIC MALIGNANT MELANOMA (MMM). A PHASE I-II STUDY OF THE NORTH AMERICAN BONE MARROW TRANSPLANTATION GROUP, Steven Wolff, Roger Herzig, Geoffrey Herzig, Joseph Fay, Gordon Phillips, Fred LeMaistre, Debra Frei-Lahr, James Lowder, Brian Bolwell, Leonard Giannone, Donna Reece, Vanderbilt University, Nashville TN 37232, Cleveland Clinic Foundation, Cleveland OH 44106, Washington University, St. Louis MO 63110, Baylor University Medical Center, Dallas TX 75246, University of British Columbia, Vancouver BC V5Z 1M9 Canada, University of Texas at San Antonio, San Antonio TX 78284.**

High-dose (HD) TT was administered in a broad phase I-II study with ABMT to patients with MMM. TT was administered over 2 hours on each of 3 consecutive days followed in 3-4 days by ABMT. The total dose varied from 180 mg/m^2 to 1575 mg/m^2. Sixty-seven patients were treated with 69 courses of therapy. Fifty-five patients had received prior cytotoxic therapy and 12 were untreated. Sixty patients had melanoma disseminated to at least one visceral site, 7 patients had skin or lymphatic metastases only. The overall response rate (cr + pr) was 56% with 95% CI of 42-69%. The median response duration was 4 months with a range of 1-31+ months. Ten percent of the responders are tumor free more than 1 year after treatment. In summary, HD TT has demonstrated a high response rate with both partial and complete responses noted. The magnitude of response is provocative considering the poor results with standard chemotherapy. Studies are now in progress to evaluate methods for improving the complete response rate and duration of response for patients with MMM.

K 428 rhG-CSF HASTENS GRANULOCYTE RECOVERY IN HODGKIN'S DISEASE AFTER HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS BONE MARROW TRANSPLANT (ABMT), K. Taylor, G. Spitzer, S. Jagannath, K. Dicke, M. Vincent, L. Souza, U.T. M.D. Anderson Hospital & Tumor Institute, Houston, TX 77030; AMGEN, Thousand Oaks, CA 91320.

High-dose Cytoxan, BCNU and VP-16 chemotherapy (CBV) with ABMT is effective therapy for relapsed/refractory Hodgkin's disease, producing 45% complete remission (CR) rate, 75% of these remissions being durable at 1-5 years (Ann Intern Med 104:163, 1986). Myelosuppression and resultant infection remain the major cause of morbidity/mortality in patients (pts) so treated. We designed a phase II trial employing rhG-CSF to hasten AGC recovery in these pts and lessen the risk of infection. Currently, 9 pts with relapsed/refractory Hodgkin's disease have been treated with Cytoxan 1.5g/m^2/day (days -6 to -3), BCNU 300mg/m^2 (day -6), VP-16 125mg/m^2 q12h (days -6 to -4); followed by ABMT (day 0). rhG-CSF 60μg/kg/day (given by 30-minute infusion) was begun on day 1 for a maximum of 28 days; 2 dose-reductions and cessation of therapy was conducted earlier if AGC reached and was maintained at >2500 for 3 consecutive days. All 9 pts have experienced initial accelerated recovery to AGC >100/mm^3 by day 12, compared with historical controls (median recovery AGC >100/mm^3 15 days [range 7-34]). 5 of 8 pts (62%) have reached AGC >500 by day 13 (historical median 21 days, range 10-51). Platelet recovery has not been delayed, only 1 pt required platelet transfusions after day 22. rhG-CSF has been extremely well tolerated and serious infection has not been seen. Judging from these studies, rhG-CSF may hasten early granulocyte recovery in Hodgkin's disease treated with CBV/ABMT. Further pts are being studied to establish if rhG-CSF will reduce the toxicity of this regimen.