NEW STRATEGIES IN BONE MARROW TRANSPLANTATION

Organizers: Richard Champlin and Robert Peter Gale

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C 001 BONE MARROW TRANSPLANTATION ACTIVITY - THE LAST DECADE.
A. John Barrett, Dept. of Haematology, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 ONN England.

The 1980's have seen an exponential rise in the number of allogenic and autologous bone marrow transplants (BMT) carried out world wide. Optimists emphasise the greater scope for BMT firstly in the widening spectrum of malignant and non-malignant haematological disorders correctable by BMT, secondly, in the gradual increase in the upper age limit for allogenic and autologous transplants to over 50 years and 60 years respectively, and thirdly, in the extension of BMT to patients who do not have a matched sibling donor by the use of autologous marrow, matched but unrelated donors or partially matched family donors.

Pessimists argue that advances claimed for BMT are largely illusory. There has been for example no major change in the survival after BMT for acute leukaemia that cannot be attributed to selection of good risk patients in first remission for the transplant procedure; and similarly advances in treatment of patients with aplastic anaemia by marrow transplantation may derive more from early treatment and selection of high intensity conditioning regimes for sensitized patients than from any fundamental change in treatment approach. The ability to reduce the impact of specific complications such as relapse, graft versus host disease (GVHD), or rejection while impressive, have not contributed to an improvement in the overall disease free survival. Thus, the introduction of T cell depletion to prevent GVHD appears in retrospect as a sideways leap since the improvement in survival associated with reduced incidence and severity of GVHD is almost equally offset by an increased probability of leukaemic relapse.

Comparison of IBMTR analyses from the beginning and the end of the decade using specific disease categories as yardsticks will be made to confirm or refute the evidence of progress as measured by improved disease free survival.

C 002 MAJOR ISSUES IN MARROW TRANSPLANTATION, E.D.Thomas, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

From 1960-1970 great progress was made in chemotherapy and radiation therapy for the treatment of hematologic malignancies, but the benefits from these modalities then reached a plateau. In the 1970's intensive research in many institutions developed regimens of ablative therapy followed by marrow transplantation which resulted in cures for many patients with previously incurable diseases. Unfortunately, this fountain also began to run dry except for a few artifical irrigations achieved by elusive progress based on premature reporting of studies or the removal of bad risk patients from study. Further progress will only be achieved when some or all of the following issues are dealt with realistically: more effective cytotoxic regimens without major organ damage, control or prevention of GVHD, directed cytotoxic therapy usually with monoclonal antibodies, prevention of opportunistic infections, assessment of growth factors and biological response modifiers, detection of minimal residual disease, separation of stem cells, enlargement of the marrow donor pool, identification of the best timing strategy for marrow transplantation including the avoidance of unnecesary transplants and of excessive treatment before referral for transplantation, assessment of the role of autologous marrow grafts and the value of purging, if any. Even more importantly, the fundamental questions of the etiology of leukemia and the immunology of tolerance must be determined.

Transplants in Leukemia

C 003 ALLOGENEIC MARROW TRANSPLANTATION FOR MYELOID LEUKEMIAS, Frederick R. Appelbaum for the Seattle Marrow Transplant Team, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

The outcome of allogeneic marrow transplantation for myeloid malignancies, including myelodysplasia (MDS), chronic myelogeneous leukemia (CML), and acute myelogenous leukemia (AML), is now reasonably well defined. Using genotypically matched donors, standard preparative regimens such as cyclophosphamide-total body irradiation (CY-TBI) or CY-busulfan, and standard graft-vs-host disease prophylaxis, cure rates of 45-70% are consistently reported if transplant is carried out early in the course of the disease and 10-20% if transplant is delayed until patients have end stage disease. A major goal of research efforts at the Fred Hutchinson Cancer Research Center has been to develop preparative regimens with greater tumor ablative effects and less toxicity than those currently in use. One approach has been to combine other drugs (busulfan, cytarabine, or etoposide) with CY-TBI. In a phase I-II study, the combination of busulfan with CY-TBI looks particularly encouraging. Among the first 23 patients treated for recurrent myeloid malignancies with this regimen only 2 have relapsed. The actuarial event-free survival of 67% at 18 months is superior to what might be expected in this high risk group of patients. A randomized phase III study is underway. A more experimental approach has been to use anti-myeloid antibodies conjugated to radionuclides as part of a preparative regimen. Animal models demonstrate that with this approach radiotherapy can be given with relative specificity to the myeloid compartment (eleven-fold greater radiation to marrow than to any other organ). In this model we have also demonstrated that pretreatment with a dose of "cold" antibody before the labeled antibody improves specificity by clearing antigen positive cells from circulation, and that retention of the radionuclide at the target site can be prolonged by inhibiting deiodination either pharmacologically or with the use of alternative labeling techniques. Early results of human trials with antibody-radionuclides will be presented.

C 004 **HOW DO TRANSPLANTS CURE LEUKEMIA**, Robert Peter Gale, UCLA School of Medicine, Los Angeles, CA 90024.

Bone marrow transplantation results in long-term freedom from relapse (? cure) in some situations where chemotherapy is ineffective or less effective. An example is chronic myelogenous leukemia. No persons receiving chemotherapy achieve remission whereas transplants result in a 96 percent freedom from relapse i.e. a 4% risk of relapse. It is unlikely that this increased anti-leukemia efficacy results solely from high-dose chemotherapy and radiation since leukemia relapse is higher in persons without graft-versus-host disease (10%), recipients of twin transplants (45%) and recipients of T-cell depleted transplants (> 50%). These data imply either that immune mechanisms eradicate leukemia cells surviving chemotherapy and radiation or that it is not necessary to eliminate all leukemia cells to achieve long-term freedom from relapse. The latter notion is supported by the finding of residual CML cells for up to 5 years post-transplant in persons without leukemia relapse. These concepts regarding transplants are similar to analyses of persons with acute leukemia receiving chemotherapy. Here also, complete eradication of leukemia cells is probably not needed to achieve long-term freedom from relapse. In some cases of remission in acute leukemia, there is re-establishment of a preleukemia phase. On other instances there is maturation of the leukemia clone. These concepts also apply to observations following transplants in CML since the chronic phase of this disease is best regarded as a preleukemia. In summary complete eradication of the leukemia clone may not be necessary to achieve long-term freedom from relapse following chemotherapy or transplants.

C 005 HIGH RISK ACUTE LYMPHOBLASTIC LEUKEMIA (ALL); AUTOLOGOUS AND ALLOGENEIC TRANSPLANTATION USING ANTIBODIES AND IMMUNOTOXINS,

John Kersey, Fatih Uckun, Daniel Weisdorf, Daniel Vallera, Mark Nesbit, Robert Haake and Norma Ramsay, Bone Marrow Transplantation Program, University of Minnesota, Minneapolis, MN 55455.

Since 1982 patients with high risk ALL who lack a HLA-matched sibling donor have had transplants with autologous monoclonal antibody purged marrow in Minnesota (N=126). Patients with HLA-matched siblings had allogeneic grafts in the same time period (N=73). All patients had high risk features, with more than 90% of patients having had one or more previous relapses; all were in remission at time of transplantation. Sequential studies compared CY + twice daily TBI (Minnesota regimen), ARA-C + TBI, and three times daily TBI + CY (New York regimen). Marrow purging protocols have utilized sequentially BA 1,2,3 (CD 9,10,24) + complement and B43 (CD19)-PAP toxin for B lineage, and CD5 + CD7 ricin toxin for T lineage ALL. Recently, low dose 4HC has been added to the marrow purging protocol based on in vitro clonogenic assays. Autologous marrow recipients have had significantly shorter hospital stay and less peritransplant morbidity and mortality than allogeneic recipients. On the other hand, relapse rates have been higher in autologous recipients. Increasingly, patients at time of transplantation have received very intensive primary therapy and are relatively resistant to chemoradiotherapy. Thus, there is a need for improved in vivo antileukemic therapy in both autologous and allogeneic recipients. Studies in the clonogenic ALL assay suggest that therapies which use different killing mechanisms, eg. antibodies, immunotoxins, and cytokines will potentially be useful for in vivo therapy in these patients.

C 006 AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA

Bob Löwenberg, Dr. Daniel den Hoed Cancer Center,
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The fact that the applicability of allogeneic bone marrow transplantation in patients with acute myeloid leukemia is restricted to a minority of cases has given an impetus to the development of autologous bone marrow transplantation as a variant modality. Autologous bone marrow transplantation takes advantage of similar marrow ablative antileukemic cytotoxic regimens but is not subject to the restrictions of the availability of HLA compatible donors, and its use can be extended to patients up to approximately 60 years of age. Prospective studies have recently been undertaken to assess the value of autologous BMT in patients with AML in first remission. The Dutch prospective study has the longest i.e. a median follow up of four years. The results indicate that autologous bone marrow transplantation can be offered to no more than approximately 1/3 of all complete remittors and allogeneic bone marrow transplantation to approximately 1/5 of those, so that in practice considerable proportion of potential candidates will miss BMT as a realistic therapeutic option. The reasons for CR cases not undergoing transplantation are variable and are mainly related to interfering relapse or problems related to adequate haematological regeneration or difficulties to harvest an adequate autograft. The probabilities of relapse disease- free survival and survival for autotransplant patients are less than for allo BMT recipients most probably due to the absence of the allogeneic antileukemic effect.

C 007 NOVEL APPLICATIONS AND FUTURE DIRECTIONS, George W. Santos, Richard J. Jones, Carole B. Miller. Bone Marrow Transplantation Program, Johns Hopkins University Oncology Center, Baltimore, MD 21205. Relapse of acute leukemia and lymphoma, particularly in autologous bone marrow transplants (BMT), is a major problem. The detection and sensitivity to cytotoxic agents of minimal residual disease (MRD) correlated with clinical relapse in BMT should allow one to design more effective anti-tumor strategies and perhaps even provide approaches to tailoring part of the patient's treatment. In addition the application of effective non-toxic post transplantation therapy to already intensive BMT preparatory regimens may lead to a further decrease in relapse rates. We wish to present our preliminary experience with both of these approaches. We have been able to detect MRD in acute lymphoblastic leukemia (ALL) and acute myelocytic leukemia (AML) using an in vitro cell cloning assay. Clonal rearrangement of heavy chain gene to confirm tumor identity was employed for ALL. The identity of AML cells was judged by circumstantial criteria. Interestingly ALL Cells were cultured from 6 of 11 patients in complete remission (CR) following BMT in CRI. Four of 6 patients culture positive have relapsed and only 1 of 5 culture negative have relapsed. Further, AML cells were cultured from 10 of 18 patients with AML in CRI (no BMT) with minimal follow up of 6 months. Six of these 10 have relapsed but only 1 of 8 with negative cultures have relapsed. In addition the sensitivity of ALL or AML cells grown from remission patients to 4-hydro-peroxyclophosphamide (4HC) predicted relapse after autologous BMT. In addition to predicting the efficiency of purging, the assays may have predicted the in vivo response to Cyclophosphamide (CY) containing BMT preparative regimens. The therapeutic effect of inducing autologous graft-versus-host disease (GVHD) with low dose Cyclosporine (CsA) following autologous BMT is being investigated in Non-Hodgkins lymphoma (NHL) and AML. To date we have been able to induce this syndrome in 13 of 15 patients with NHL without serious toxicity or morbidity. Although there is strong animal and in vitro data to support the therapeutic rationale it is too early to comment on this aspect in the clinical trials.

Aplastic Anemia and CML

C 008 PRE- AND POST-TRANSPLANT IMMUNOSUPPRESSIVE THERAPY WITH BONE MARROW TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA, Richard Champlin, M.D., Division of Hematology/Oncology, UCLA School of Medicine, Los Angeles, CA 90024
Pre- and post-transplant immunosuppressive therapy is designed to prevent graft rejectio and graft-versus-host disease (GvHD), and allow for prompt hematologic and immunologic recovery. A number of approaches have been studied. Recent analysis of the IBMTR and data from individual centers indicate that graft failure occurs in approximately 15% of patients receiving cyclophosphamide conditioning and post-transplant cyclosporine. Intensification of conditioning through the addition of $\geq$ 3 Gy TBI or 6 Gy total lymphoi radiation to cyclophosphamide reduces graft failure to $\leq$ 5% but increases toxicity and other early complications. Overall survival is similar, approximately 70% for cyclophosphamide alone or cyclophosphamide/TLI. A recent study at UCLA of cyclophosphamide plus 3 Gy TLI was associated with graft failure in 4 of 29 previously transfused patients (actuarial rate = 20%), similar to recent results with cyclophosphamide alone. These data indicate that addition of higher doses of radiation will be necessary to reduce graft failure but such therapy also increases early mortality. A randomized, controlled study is necessary to determine if addition of TLI improves transplant outcome. Combinations of cyclosporine/methotrexate or cyclosporine/prednisone have been more effective than single agents to prevent acute GvHD, but no drug therapy has reduced the incidence of chronic GvHD. Innovative forms o immunosuppressive therapy are necessary to reduce rejection and GvHD while improving survival following bone marrow transplantation for severe aplastic anemia.

C 009 NOVEL APPROACHES TO THE THERAPY OF CHRONIC MYELOGENOUS LEUKEMIA, Albert Deisseroth, Moshe Talpaz, Hagop Kantarjian, Emil Freireich, Fred LeMaistre, Jordan Gutterman, Gary Spitzer, Chris Reading, Zack Howard, CV Herst, David Seong and Andrew Feinberg. M.D. Anderson Cancer Center, Houston, TX 77030 and The University of Michigan Medical Center, Ann Arbor, MI 48109. Chronic myelogenous leukemia is a disease which exhibits a transformation from an indolent to an aggressive phase at an average of four years after diagnosis. Two therapeutic interventions have been shown to delay this progression: bone marrow transplantation and alpha interferon. Unfortunately, not all patients affected with CML can derive benefit from these two modalities of therapy. They may be resistant to interferon or there is an unavailability of bone marrow transplantation donors. We are pursuing two strategies which may increase the number of patients who are eligible for this therapy: (1) We have developed a nuclear protein DNA binding assay which has the potential for identifying patients at diagnosis who exhibit interferon resistance. We are studying this assay prospectively to identify patients who are resistant to interferon at diagnosis and to develop in vitro models for formulating therapeutic approaches to the circumvention of interferon resistance; (2) We have adapted the polymerase chain reaction system for the detection of single cells which contain bcr-abl mRNA. We are applying this assay to the analysis isolation of stem cells which belong to the early progenitor population in CML marrow and are bcr-abl negative. This system may provide a model through which populations of early progenitor cells can be collected for biological studies, ex vivo expansion, and autologous reconstitution.

C 010 BONE MARROW TRANSPLANTATION FOR FANCONI ANEMIA, Eliane Gluckman, Hélène Bourdeau, Gérard Socié, Olivier Brison, Agnès Devergie, Jean-Marc Cosset, Roland Berger, Dominique Thierry, Pierre Lehn, Arleen Auerbach and Hal Broxmeyer, Bone Marrow Transplant Unit, Hopital Saint-Louis, Paris, Institut Gustave Roussy, Villejuif, France, Rockefeller University New-York, Walter Oncology Center, Indianapolis USA.
Fanconi anemia (FA) is an autosomal recessive inherited condition in which malformation are associated with bone marrow failure. Spontaneous or DNA breaking agents induced chromosomal breakage of cultured lymphocytes are of great help in establishing the diagnosis. Marrow transplantation may be hazardous in view of the high mortality rate from early acute graft versus host disease (GVHD) and from Cyclophosphamide induced toxicity which is related to the unusual sensitivity of FA cells to alkylating agents. We have shown by preliminary in vivo and in vitro tests that a modified conditioning regimen improved markedly results of BMT. This regimen consisted of : Cyclophosphamide 5mg/kg IV for 4 consecutive days followed by 5Grays thoraco abdominal irradiation with lung and liver shielding. Cyclosporine A alone was given for prophylaxis of GVHD. We have treated 22 patients with this regimen of whom 17 (77%) are currently alive with a median follow-up of 4 years. The main cause of death was GVH and CMV infection. None developed secondary maligancy on long term follow-up. Of note, one patient was successfully transplanted with a matched unrelated donor from the French panel without any modification of the transplant procedure. One patient received cryopreserved cord blood cells from his HLA matched new born sibling. There was some delay of engrafment and no GVH. One year after transplant the patient is doing well. Study of chimerism by molecular probes and cytogenetic analysis showed a prompt myeloid take and a delay of complete lymphoid chimerism for more than 3 months. These results show that BMT in FA can be successfully performed if the conditioning is reduced, HLA typing during the prenatal diagnosis and cord blood cryopreservation could be helpful in this disease or in other inherited disorders.

C 011 AUTOGRAFTING FOR CML: IS THERE A ROLE? John M Goldman, Centre for Adult Leukaemia, Royal Postgraduate Medical School, London, UK.

Hemopoietic stem cells from the marrow or peripheral blood of patients with untreated CML can be collected, cryopreserved and subsequently used to restore hemopoiesis after high dose chemotherapy or chemotherapy administered to the patient in transformation. The majority of patients treated in this manner relapse early with transformed leukemia but a small proportion have remained in second chronic phase for more than one year. There are various theoretical rationales for autografting in chronic phase: (1) It may be possible to reduce the size of the leukemic stem cell compartment and thereby delay the onset of transformation; (2) It may be possible in some cases to restore Ph-negativity and this might be more readily achieved if measures could be developed that favored the survival after autografting of Ph-negative stem cells, and (3) Autografting might induce a clinically useful graft-versus-host leukemia effect. A number of important conceptual questions remain: (1) How should the patient be treated before autografting? (2) Is there a difference between blood- and marrow-derived stem cells? (3) What if any methods are effective for in vivo manipulation to favor survival of Ph-negative stem cells, and (4) Is treatment after autografting, eg with alpha-interferon or IL-2, useful in maintaining the Ph-negative state? Current clinical results from 4 centers suggest that autografting in chronic phase might in some circumstances prolong survival; some patients are restored to Ph-negative hemopoiesis and this can on occasion be prolonged. One may conclude that further clinical studies are warranted.

C 012 BONE MARROW TRANSPLANTATION IN CHRONIC MYELOID LEUKAEMIA (CML), H.A. Messner, Ontario Cancer Institute and University of Toronto.

Bone Marrow transplantation (BMT) has become the treatment of choice for patients with CML. Studies with a follow up of five and more years report a forty to fifty percent event free survival for patients transplanted in chronic phase. There is mounting evidence that patients transplanted during the first year after diagnosis appear to have a significantly better event free survival compared to patients transplanted at a later time in chronic phase. The results in patients transplanted in accelerated phase or blast crisis are less favourable. A five year event free survival of ten to twenty five percent is expected for this group of patients. Cytogenetic evaluation of long term survivors revealed that the majority of patients are free of the Philadelphia chromosome. The absence of any evidence for a residual malignant population was confirmed in studies using the more sensitive bcr-probes. The control of CML following BMT is likely dependent on a number of different mechanisms. These include the choice the preparatory regimen, the cellular composition of the infused bone marrow, and the choice of GVHD prophylaxis. The relapse rate in non T-cell depleted grafts for patients in chronic phase varies from eight to twenty percent. A significantly higher relapse rate was observed for T-cell depleted grafts. Similarly, a higher relapse rate was reported by some centres that have used cyclosporin A as graft-versus-host disease prophylaxis for a prolonged period of time. The relapse rate was significantly reduced when of administration of CyA was reduced to two months. Patients that relapse after a bone marrow transplantation may be treated with conservative approaches. More recently Interferon has been found useful in isolated cases to control or ablate the Philadelphia chromosome positive cell population.

C 013 ALLOGENEIC BONE MARROW TRANSPLANTATION FOR APLASTIC ANEMIA: MAJOR ISSUES

Rainer Storb, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

Results of treating patients with aplastic anemia by either immunosuppressive therapy or allogeneic bone marrow transplants in Seattle are compared to those obtained at other centers. Approaches taken to avoid or overcome the problem of graft rejection and minimize the incidence and severity of acute and chronic graft-versus-host disease are discussed. Attempts at HLA-nonidentical transplants from family members or unrelated donors after failure of immunosuppressive therapy are described.

Biology of Bone Marrow Transplantation-I

C 014 GRAFT-VERSUS-LEUKEMIA REACTIONS FOLLOWING BONE MARROW TRANSPLANTATION

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To determine whether graft-versus-leukemia (GVL) reactions are important in preventing leukemia recurrence after bone marrow transplantation, we studied 2254 persons receiving HLA-identical sibling bone marrow transplants for acute myelogenous leukemia (AML) in first remission, acute lymphoblastic leukemia (ALL) in first remission, and chronic myelogenous leukemia (CML) in first chronic phase. Four groups were investigated in detail: recipients of non-T-cell depleted allografts without graft-versus-host disease (GVHD), recipients of non-T-cell depleted allografts with GVHD, recipients of T-cell depleted allografts and recipients of genetically identical twin transplants. Decreased relapse was observed in recipients of non-T-cell depleted allografts with acute (relative risk 0.68, p=0.03), chronic (relative risk 0.43, p=0.01) and both acute and chronic GVHD (relative risk 0.33, p=0.0001) as compared to recipients of non-T-cell depleted allografts without GVHD. These data support an antileukemia effect of GVHD. AML patients who received identical twin transplants had an increased probability of relapse (relative risk 2.58, p=0.008) compared to allograft recipients without GVHD. These data support an antileukemia effect of allogeneic grafts independent of GVHD. CML patients who received T-cell depleted transplants with or without GVHD had higher probabilities of relapse (relative risks 6.91 and 4.45, respectively, p=0.0001) than recipients of non-T-cell depleted allografts without GVHD. These data support an antileukemia effect independent of GVHD that is altered by T-cell depletion. These results explain the efficacy of allogeneic bone marrow transplantation in eradicating leukemia, provide evidence for a role of the immune system in controlling human cancers and suggest future directions to improve leukemia therapy.

C 015 ROLE OF LYMPHOID CELLS IN ALLOGENEIC MARROW ENGRAFTMENT, Paul J. Martin, Division of Clinical Research, Fred Hutchinson Cancer Research Center, Department of Medicine, University of Washington School of Medicine, Seattle, WA 98104

Clinical trials have shown that removal of T cells from donor marrow decreases the incidence of graft-versus-host disease (GVHD) after allogeneic transplantation but greatly increases the risk of graft failure. Precise causes for graft failure associated with T cell depletion of donor marrow in humans remain difficult to define. Nonetheless, there does exist a body of evidence consistent with the hypothesis that donor T cells help to eliminate residual host lymphocytes surviving the preparative regimen and remaining capable of causing rejection. 1) It has been possible to recover viable host lymphoid cells after administration of the conditioning regimen but before infusion of the marrow. 2) Several clinical studies of T cell-depleted HLA-identical marrow transplantation have shown an inverse relationship between the risk of graft failure and the amount of pretransplant cytoreduction and immunosuppression achieved by increased TBI. These results implicate a partially radiosensitive host element in causing graft failure. 3) Depletion of donor T cells is associated with a high incidence of mixed lymphoid and mixed hematopoietic chimerism (i.e., persistence of host cells) compared to similar patients transplanted with unmodified marrow. 4) Host lymphocytes with antidonor cytotoxic activity have been identified in the blood of patients with graft failure after T cell-depleted HLA-mismatched marrow transplantation. We now have defined conditions where allogeneic engraftment in a murine marrow transplant model depends on the presence of donor lymphoid cells in the graft. The murine marrow transplant model was developed by assessing the tolerance ranges for TBI and GVHD in CB6F1 ($H\text{-}2^{d/b}$) recipient mice and then by determining the amount of TBI that would allow engraftment when donor C3B6F1 ($H\text{-}2^{k/b}$) T cells were present but not when they were absent. In further experiments, we demonstrated that alloreactive T cells were responsible for the graft-facilitating activity and showed that donor CD8 cells could mediate this effect while CD4 cells could not. The CD4 cells not only failed to enhance engraftment of donor cells but also impaired autologous reconstitution by host hematopoietic cells. These results lead to the hypothesis that allorecognition of host lymphocytes is required for a graft-facilitating effect and that allorecognition of antigens expressed only on other tissues is not sufficient for graft-enhancement. Specifically, we propose that CD8 cells facilitate engraftment through recognition of MHC class I antigens on host T cells and that CD4 cells do not show graft-enhancing activity because murine T cells do not express MHC class II antigens. Likewise, we propose that CD4 cells eliminate host hematopoietic cells through recognition of MHC-class II antigens on stem cells. Our results suggest that attempts using exogenous lymphokines as a single approach for overcoming the problem of graft failure associated with T cell depletion are not likely to be successful. We propose that greater attention should be directed towards developing more effective pretransplant immunosuppressive regimens.

C 016 CHIMERISM AND THE INDUCTION OF TRANSPLANTATION TOLERANCE

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The establishment of lymphohematopoietic chimerism via lethal irradiation and bone marrow transplantation (BMT) leads also to the induction of transplantation tolerance to antigens present on the reconstituting bone marrow cells. The major complications of this procedure are the induction of graft-vs.-host disease (GVHD) and, when the BMT is carried out using T-cell depleted (TCD) marrow across an MHC barrier, immunoincompetence and failure of engraftment. Previous studies have shown that reconstitution of lethally irradiated mice with a mixture of TCD host plus donor bone marrow leads to long-term, mixed lymphohematopoietic chimerism, immunocompetence, and resistance to GVHD. Specific tolerance to subsequent skin grafts from the donor strain is also induced, most likely on the basis of clonal deletion. Mixed chimerism and transplantation tolerance have also been obtained using a preparative regimen including monoclonal anti-T cell antibodies, low dose total body irradiation and thymic irradiation. Because this regimen is non-lethal, it may be suitable as a clinical approach to the induction of transplantation tolerance in preparation for organ transplantation.

T cell depletion of allogeneic marrow is not always desirable, because of the anti-leukemic and alloengraftment-promoting effects of T cells. When non-TCD allogeneic marrow is used, however, additional measures to prevent GVHD are needed. While the previously demonstrated anti-GVHD effect of TCD syngeneic marrow was only effective against acute GVHD, we have now found that interleukin 2 (IL-2) administered in high doses the first few days following BMT has a marked protective effect against both acute and chronic GVHD mortality. Co-administration of TCD syngeneic marrow and IL-2 produced the greatest protection, and the timing of IL-2 administration appeared to be critical. Preliminary data indicate that despite the marked protection against GVHD afforded by this protocol, there was still a potent anti-leukemic effect from the allogeneic T cells.

C 017 T CELL TOLERANCE IN BONE MARROW CHIMERAS: INTRATHYMIC VS. EXTRATHYMIC TOLERANCE. Jonathan Sprent, Er-Kai Gao and Susan R. Webb. Department of Immunology - IMM4A, Research Institute of Scripps Clinic, La Jolla, CA 92037.

The role of the thymus in imposing tolerance to MHC (H-2) antigens was examined by transferring T-depleted parent *a* stem cells to (*a* x *b*)F_1 hosts exposed to supralethal irradiation. Despite the rapid disappearance of host antigen-presenting cells, the donor T cells differentiating in the chimeras showed strong tolerance to host H-2 determinants. Tolerance was more marked for $CD8^+$ cells than for $CD4^+$ cells. Based on a variety of assays, including measuring V_β expression on intrathymic vs extrathymic T cells, it was concluded that tolerance in the chimeras is induced within the thymic microenvironment and, at least for $CD4^+$ cells, reflects contact with thymic epithelium. For $CD4^+$ cells, the evidence suggests that thymic epithelium deletes high affinity cells and induces a reversible form of anergy in low-affinity cells.

To examine extrathymic tolerance of T cells, heavily irradiated mice were injected with large doses of H-2 different spleen cells. Provided that the injected cells were enriched for $CD4^+$ cells (which protect mice against lethal graft-versus-host disease), the recipients survived for one year or more. The notable finding was that the donor T cells harvested from the long-term hosts retained strong host H-2 reactivity, implying that the mature donor T cells were resistant to tolerance induction.

C 018 EXPERIMENTAL APPROACHES FOR GVHD THERAPY AND REJECTION IN THE MOUSE. Daniel A. Vallera, Stephen F. Carroll, and Bruce R. Blazar, Departments of Therapeutic Radiology and Pediatrics, University of Minnesota, Minneapolis, MN 55455 and Xoma Corporation, San Francisco, CA 94710.

We and others have studied ex vivo and in vivo approaches for treatment of graft-versus-host disease in murine recipients of histoincompatible bone marrow transplants. One of the most pressing problems in the field of T-cell depletion has been the association of donor T-cell removal with increased engraftment problems in numerous bone marrow transplant centers. We will discuss a murine model which promotes engraftment problems in the face of donor T-cell depletion with the monoclonal antibody anti-Thy1.2+complement. The dipeptide L-Leucyl-L-Leucine Methyl Ester (LLME) selectively eliminates cytotoxic cells and their precursors, natural killer cells, and macrophages. Ex vivo treatment of C57BL/6 ($H\text{-}2^d$) donor cells completely protected 30 B10.BR ($H\text{-}2^k$) recipients of fully allogeneic donor marrow from GVHD without depressing donor cell engraftment. In contrast, anti-Thy1.2+C' treatment prevented GVHD, but at the expense of donor cell engraftment. We will discuss other models and approaches directed at preventing GVHD without affecting engraftment. We also tested whether LLME pretreatment could distinguish GVHD-causing cells from cells mediating graft-versus-leukemia (GVL) effects. Injection of EL4, a thymic leukemia/lymphoma, into allogeneic bone marrow transplant recipients show that LLME pretreatment eliminates cell populations responsible for GVHD and GVL.

We have also tested monoclonal antibodies linked to ricin toxin A chain, a catalytic inhibitor of protein synthesis, for their potential in eliminating GVHD effector cells in vivo. IT was administered to mice with established GVHD 8 days after bone marrow transplant. Anti-Ly1-RTA transiently protected mice from ongoing graft-versus-host disease. However, Anti-Ly1-RTA were hepatotoxic and significantly elevated peripheral blood neutrophil levels at higher dosages. Anti-Thy1.2-RTA caused a histopathological protection from graft-versus-host disease. However, it also caused a precipitous increase in weight and significant decrease in total plasma protein reminiscent of capillary leak syndrome not observed in mice given anti-Ly1-RTA. Although the toxic effects of anti-Thy1.2-RTA were too severe to show a survival advantage, histopathology studies showed a substantial anti-GVHD effect. Dose levels and schedules that were toxic to our irradiated mice were not toxic to unirradiated mice, suggesting that toxicity is exacerbated by irradiation. Novel experimental approaches for treatment of GVHD and rejection using monoclonal antibodies from other laboratories will be discussed.

Biology of Bone Marrow Transplantation-II

C 019 PRODUCTION OF GROWTH FACTORS FOR THE HEMOPOIETIC AND IMMUNE SYSTEMS AFTER MARROW TRANSPLANTATION, Kerry Atkinson, Department of Hematology, St Vincent's Hospital, Sydney, NSW 2010 Australia.

The genes for seven human interleukins (IL 1-7) and four other hemopoietic growth factors (G-CSF, GM-CSF, M-CSF, and erythropoietin) have been molecularly cloned and their products expressed. A number of these products are now in clinical use in attempts to accelerate recovery of the immune and hemopoietic systems after marrow transplantation. Our laboratory has explored the production of these factors by recipients of bone marrow transplants. IL 1 production by LPS-stimulated monocytes returns to the normal range early after transplant (as do other monokines such as prostaglandins E_2). IL 2 production from mitogen-stimulated peripheral blood mononuclear cells (PBMC) is markedly impaired early post transplant and this impairment can be long lasting. IL 2 production is not corrected by stimulation with lonomycin plus phorbol ester, suggesting that the production defect is not due to an accessory cell defect or to a defect of T cell surface receptor signal transduction. Additionally, the responsiveness of PBMC to exogenous IL 2 is diminished post transplant. IL 3 production (mRNA expression from mitogen-stimulated PBMC) is also impaired significantly in recipients of allogeneic marrow transplants. Similar data will be presented for IL 4, IL 5 and IL 6. GM-CSF mRNA expression from mitogen-stimulated PBMC is also significantly impaired and levels may be non-detectable for as long as 1750 days post transplant. Production of IL 2, IL 3 and GM-CSF is not affected by the presence of graft-versus-host disease, but rather by the use of post transplant immunosuppressive therapy. Erythropoietin serum levels are decreased in recipients of both autologous and allogeneic marrow transplants, but allograft recipients have a more severe decrease and levels are inappropriately low for the concurrent haemoglobin concentration. Finally, two other cytokines produced primarily by T lymphocytes have been found to return to the normal range early post allograft: these are gamma interferon and macrophage procoagulant inducing factor. A cytokine profile can thus be generated for marrow transplant recipients. In general, monokines return to normal levels early post transplant, while levels of most T lymphokines remain depressed for longer.

C 020 EFFECTS OF HEMATOPOIETIC GROWTH FACTOR ADMINISTRATION ON IRRADIATED MICE Bruce R. Blazar, Michael B. Widmer, Daniel A. Vallera, Departments of Pediatrics and Theraputic Radiology, University of Minnesota, Minneapolis, MN 55455 and Immunex Corporation, Seattle, WA 98101.

Recombinant cytokines have been used to accelerate hematopoiesis in a variety of animal species. This review will focus on the in vivo administration of recombinant cytokines in monkeys, dogs, and mice that have received lethal or sublethal doses of total body irradiation and/or chemotheraputic agents. Studies of granulocyte/macrophage colony-stimulating factor (GM-CSF) or granulocyte (G)-CSF given by repeated injections or infusions indicate that these cytokines can shorten the duration of the neutrophil nadir and/or enhance survival of recipients of sublethal or lethal irradiation without bone marrow rescue. Similar beneficial results of GM-CSF or G-CSF are observed in recipients of autologous marrow grafts. Likewise, IL-1 adminstered prior to or shortly after TBI conferred a radioprotective effect to irradiated murine recipients. Our laboratory has investigated the use of cytokines in a murine allogeneic BMT model in which T-cell depletion with anti-Thy plus complement results in a persistence of residual host cells. Therefore, the effects cytokines can be evaluated for their impact on survival, hematological recovery, and donor cell engraftment. We have tested a number of cytokines in an ex vivo incubation of the T-cell depleted donor graft as a means of providing selective donor marrow stimulation/preactivation prior to bone marrow infusion. By a single brief incubation with GM-CSF prior to BMT, we were able to improve alloengraftment without affecting survival or hematological recovery as compared to recipients of sham-treated marrow. In contrast, preincubation of the donor graft with IL-3 decreased alloengraftment and leukocyte recovery. IL-1 preincubation did not affect alloengraftment or hematological recovery but did have a mild beneficial effect on survival post-BMT. These data differ, in part, from our experience with the in vivo adminstration of cytokines. Using a 14 day miniosmotic pump, we have noted increased survival in recipients of GM-CSF or G-CSF. A marked neutrophil stimulation was observed in recipients of G-CSF but not GM-CSF in this setting. Alloengraftment was not affected by G-CSF infusions but was decreased in recipients of GM-CSF. More recently, we have studied the in vivo adminstration of IL-1, IL-4, and IL-2. Studies currently in progress will be discussed v/v the above described parameters of efficacy. In this review, we will summarize the results of cytokine administration in animal models.

C 021 THE USE OF INTERLEUKINS AFTER BONE MARROW TRANSPLANTATION.

M.K. Brenner, D.J. Gottlieb, H.E. Heslop, C. Bello-Fernandez, J.E. Reittie, A.V. Hoffbrand, A.B. Mehta, H.G. Prentice, Department of Haematology, Royal Free Hospital, London, U.K.

There is increasing evidence that the lower relapse risk associated with both autologous and allogeneic transplantation compared with chemotherapy alone is associated with marrow derived anti-tumour effector mechanisms. These are in part mediated by CD3+ CD16- and CD3- CD16+ MHC unrestricted activated killer cells which are directly cytotoxic to clonogenic leukaemia blasts and which also secrete inhibitory cytokines such as TNF and gamma interferon. Infusion of Interleukin 2 enhances these effector mechanisms after autologous transplantation and induces them after chemothrapy for acute myeloid leukaemia. Additionally in both patient groups IL2 enhances neutrophil regeneration by induction of IL3 and GM-CSF. Finally IL2 induces down-regulatory cytokines, in particular IL 4. Manipulation of this negative feedback loop may increase and prolong the cytotoxic effector function generated so that low dose IL2 may be sufficient to induce substantial levels of cytotoxic cell activation. The effects of IL2 on humoral immunity after BMT are less desirable, as the agent abrogates responses to vaccine antigens and may increase the risks of sepsis.

C 022 HEMATOPOIETIC GROWTH FACTORS, David W. Golde, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024

The hematopoietic growth factors are a family of glycoprotein hormones that regulate hematopoietic cell proliferation and mature blood cell function. The list of colony-stimulating factors and interleukins known to stimulate blood cell production is now quite long; these hormones are produced in quantity by recombinant molecular methodologies and most have entered clinical trials. We have performed trials of G- and GM-CSF in a number of disorders of hematopoiesis including those associated with HIV infection, bone marrow transplantation, hairy-cell leukemia, and aplastic anemia. Our results and those of others show great promise for the use of the hematopoietic growth factors in disorders associated with impaired hematopoietic cell function. The colony-stimulating factors also have profound effects on mature myeloid cells. They augment adherence, chemotaxis, phagocytosis, and intra- and extracellular cytotoxicity. Updated results relative to the clinical trials and to the modulation of mature myeloid cell function by colony-stimulating factors will be presented. The ability to regulate the number and activity of mature myeloid effector cells in vivo establishes unique therapeutic opportunities in the area of infectious disease, cancer treatment, bone marrow transplantation, and the augmentation of host defense in immunodeficient patients.

C 023 POTENTIAL ROLE OF BONE MARROW EFFECTOR CELLS IN THE THERAPY OF LEUKEMIA AND OTHER CANCERS, Ronald B. Herberman, Pittsburgh Cancer Institute and Departments of Medicine and Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213
Natural killer (NK) cells have been shown to have considerable cytotoxic activity against human as well as murine leukemia cells. NK cells are derived from bone marrow progenitor cells and differentiate into functionally active effector cells, mainly in response to exposure to interleukin 2 (IL2). Culture of functionally active NK cells with moderate to high doses of IL2 leads to higher levels of cytotoxic activity and also to proliferation of the effector cells. These observations provide the potential for at least two approaches to therapy of leukemia and other forms of cancer, and data have been obtained in animal tumor models to support the value of both approaches. One strategy has been to admix bone marrow cells with purified IL2-activated NK cells prior to transplantation. This has been shown to lead to reduction of at least four logs of leukemic or other tumor cells, without appreciable effect on stem cells or engraftment. An alternative approach has been to administer IL2 alone or in combination with other cytokines to the recipients of bone marrow transplants. Treatment shortly after transplantation with even very low doses of IL2 has been found to substantially accelerate the development of NK activity in the spleen of mice. The addition of IL1 or tumor necrosis factor plus interferon has led to even more rapid development of NK activity. When such treatments have been performed in tumor bearing mice, the acceleration of regeneration of NK activity has been paralleled by an anti-tumor effect and prolonged survival of the mice. These preclinical results offer encouragement for performing analogous clinical trials.

Graft-versus-Host Disease

C 024 GRAFT-VERSUS-HOST DISEASE (GvHD)-SECONDARY DISEASE OR ADJUVANT THERAPY? H.J.Deeg, University of B.C., Vancouver,B.C., Canada. With single agent methotrexate (MTX), cyclophosphamide, or Cyclosporine (CSP) 40-70% of patients given bone marrow transplants (BMT) from an HLA identical sibling develop acute GvHD. With combinations of MTX + CSP or CSP + Prednisone the incidence is 20-30% (40-80% with HLA non-identical transplants), and as low as 10% with the addition of immunoglobulins i.v. or i.v./p.o. With T lymphocyte depletion of donor marrow the incidence of acute GvHD with HLA identical sibling transplants is $\lesssim$10%. However, 5-40% of patients fail to achieve lasting engraftment, and with some diseases, e.g.CML, the incidence of leukemic relapse is increased. Leukemic relapse may also be more frequent in patients given MTX + CSP, indicating that effective GvHD prophylaxis reduces the anti-leukemic efficacy of BMT. A graft-versus-leukemia effect of BMT has clearly been shown in patients developing GvHD after single agent GvHD prophylaxis. In an attempt to prevent graft failure and recurrent leukemia, more intensive conditioning regimens are being used. The resulting toxicity may interfere with in vivo GvHD prophylaxis. Target cell damage and repair are associated with cytokine release, inflammatory changes, and increased expression of HLA antigens. These reactions involve not only normal but also malignant cells, although their sensitivity may differ. The epithelial tropism of T cells with various $\gamma\delta$TCR repertoirs may further influence clinical manifestations. This interplay is altered by T cell depletion and drugs. It is likely that stochastic and non-stochastic processes occur. Designing improved transplant regimens will require refinements at the levels of conditioning, marrow manipulations and post-grafting drug administration.

C 025 SUMMARY OF CLINICAL STUDIES OF ANTI CD-5 IMMUNOCONJUGATE (XOMAZYME®-H65) IN PROPHYLAXIS AND TREATMENT OF ACUTE GRAFT-VERSUS-HOST DISEASE (aGvHD), Pavel L. Lomen and the XOMA BMT Study Group, XOMA Corporation, 2910 Seventh Street, Berkeley, CA 94710

XomaZyme®-H65 is a murine monoclonal antibody conjugate composed of a non-complement-fixing IgG_1 immunoglobulin and ribosomal inhibiting protein ricin A chain (RTA). The antibody is reactive with a human leukocyte differentiation antigen CD-5 found on the surface of mature human T lymphocytes. In multicenter (27) clinical trials, XomaZyme®-H65 was administered to 106 patients with moderate to severe aGvHD (80% of patients with Grade III-IV overall and 20% of patients with Grade I-II overall) who failed the first line therapy with steroids. In addition, XomaZyme®-H65 was a component of aGvHD prophylactic regimens in 54 BMT patients. Additional therapeutic, prophylactic and graft rejection prevention trials are in progress. Preliminary results from therapeutic trials indicate that XomaZyme®-H65 is a safe and effective immunoconjugate capable of reversing or improving signs and symptoms of advanced aGvHD. Approximately 77% of patients with skin disease, 71% of patients with gut disease and 35% of patients with liver disease achieved partial or complete response. Overall (all organ stages graded), approximately 61% of patients achieved complete (25%) or partial (36%) response. Side effects were reported in about 50% of patients and included reversible hypoalbuminemia and mild to moderate weight gain. Elevations of serum creatinine were seen in some patients and are believed to be related to concomitant nephrotoxic drugs. In one prophylaxis trial (high risk patients with HLA matched donors) the time to graft function recovery was significantly shortened in patients receiving XomaZyme®-H65. In another prophylaxis trial, a group of patients with metabolic diseases receiving bone marrow grafts from haploidentical (1-3 antigen disparity) donors showed 88% engraftment and 88% survival from 30 to 672 days following transplantation and severe (Grade III-IV) aGvHD was seen in only one patient.

Detailed results on efficacy, survival and pharmacokinetics from completed trials will be presented.

C 026 ENHANCEMENT OF BONE MARROW ALLOGRAFTS FROM "NUDE" MICE INTO MISMATCHED RECIPIENTS BY T CELLS VOID OF GRAFT VS. HOST ACTIVITY, Yair Reisner, Tsvee Lapidot, Ido Lubin, Yifat Faktorowich, and Porath Erlich, Department of Biophysics, The Weizmann Institute of Science, Rehovot, Israel 76100

We previously presented in a murine model of allogeneic bone marrow transplantation, evidence suggesting that competition between donor and residual host stem cells may play an important role in controlling the establishment of durable donor type chimerism. Engraftment of donor type hematopoietic cells was found to be enhanced either by treatment of the host with myeloablative drugs in addition to conditioning with 8 Gy TBI, or by increasing the number of T cell-depleted bone marrow cells transplanted. Although the effect of the latter is likely to be due to the increased number of donor stem cells, it could be mediated in part by T cells remaining in the bone marrow after T cell depletion. In the present study, further investigations were carried out so as to distinguish between these two possibilities. C57BL/6-Nu/Nu ("nude") donors served as a source of T cell-depleted pluripotent stem cells, with minimal residual T cell activity. Furthermore, this bone marrow was subjected to one-step T cell depletion, as was a control bone marrow from normal C57BL/6 donors. Mature thymocytes separated by peanut agglutinin from C57BL/6 or from (C57BL/6 x C3H/HeJ)F_1 mice served as purified sources of competent T cells. Mixing experiments using different numbers of "nude" bone marrow cells, with or without mature thymocytes, revealed that engraftment of allogeneic T cell-depleted bone marrow is T cell dependent. To ensure engraftment, a large inoculum of "nude" bone marrow must be supplemented with a trace number of donor T cells, whereas a small bone marrow dose from "nude" donors requires a much larger number of T cells for engraftment. The use of F_1 thymocytes in this model strongly indicates that the enhancement of bone marrow engraftment by T cells is not only mediated by alloreactivity against residual host cells, but may rather be generated by growth factors, the release of which may require specific interactions between T cells and stem cells, or between T cells and bone marrow stroma cells.

C 027 **THALIDOMIDE THERAPY OF GRAFT-VERSUS-HOST DISEASE,**
G.B.Vogelsang, A.D.Hess, G.W.Santos, Bone Marrow Transplantation Unit, The Johns Hopkins University, Baltimore, MD 21205
Thalidomide has been shown to be an effective agent in prevention and treatment of Graft-versus-Host disease (GVHD) in experimental animal models. Rats receiving major histocompatibility mismatched marrow grafts after lethal total body irradiation could be prevented from developing GVHD (44/54 animals) and treated for acute GVHD (46/48 animals). Likewise, chronic GVHD responds to thalidomide (15/18 animals). Studies done using combination low dose thalidomide and CsA therapy suggest that they are at least additive, if not synergistic in both acute and chronic GVHD. Current animal studies are looking at thalidomide derivatives and the effects of thalidomide on the intact immune system. One of the major hindrances to the use of thalidomide has been the lack of an IV formulation. We have found that a chemically modified β-cyclodextrin will complex with thalidomide (CDT). A complexed concentration of 1.6 mg/ml as opposed to 0.06 mg/ml was obtained. Kinetic studies in the rat have given satisfactory peak plasma concentrations and sustained release. Pharmacokinetic studies done in normal volunteers with oral thalidomide showed a peak concentration 1.15 $\pm$ .2 ug/ml, absorption half life 1.7 $\pm$ 1.05 h, elimination half life 8.7 $\pm$ 4.1 h , volume of distribution 120.7 $\pm$ 45.4 L, and total body clearance 10.4 $\pm$ 2 L/h. Because of the variability of levels seen in normal and patients, plasma levels are necessary to assess how well the drug is being absorbed. Clinical trials at a number of different centers are currently underway. Results from these studies will be summarized. Our initial studies have shown a response rate of about 65% in all patients entered onto protocol with chronic GVHD.

Alternative Donors for BMT

C 028 MARROW GRAFTING FROM HLA MATCHED UNRELATED DONORS,
PG BEATTY, JA HANSEN, ED THOMAS for the Seattle Marrow Transplant Team, Fred Hutchinson Cancer Research Center, Seattle, WA.

Bone marrow transplantation has traditionally been limited to those 30% of patients fortunate enough to have an HLA genotypically identical sibling. In hopes of making marrow grafting more widely applicable, several transplant centers have begun exploring alternative donor sources. We report here on 52 patients who received HLA-matched unrelated transplants for the treatment of leukemia. Fourteen had acute leukemia in relapse, 12 CML in advanced stage, 3 lymphoma in relapse, 3 pre-leukemia, 17 CML-CP and 3 acute leukemia in remission. For each of these 52 patients, two controls were chosen who received HLA-matched sibling grafts and were of similar age, diagnosis, and stage of disease. Thirty-five percent of those patients receiving an HLA-matched related transplant developed grade II to IV Acute Graft Versus Host Disease (AGVHD) versus 73% who received unrelated donor transplants. Furthermore, the day of onset of Grade II or greater AGVHD was sooner in those receiving unrelated grafts (median day 13 vs day 17). The median day to achievement of 1000 granulocytes was 24 days for both groups. With a median follow-up of 15 months (range 4-52), there was no significant difference in relapse-free survival between the groups (p=.17), with both showing survival plateaus of 32%-36% at 36 months. It is too early to compare the incidence of chronic graft versus host disease and relapse.

We conclude that although the use of HLA-matched unrelated donors in bone marrow transplantation is still in its infancy, the initial data indicate that the survival of patients receiving such transplants is comparable to survival of patients receiving grafts from matched siblings, thus justifying efforts to increase the number of available unrelated donors.

C 029 BONE MARROW TRANSPLANTS WITH MATCHED UNRELATED DONOR PATIENTS WITH SEVERE APLASTIC ANEMIA, James L. Gajewski, Div. Hematology/Oncology, UCLA, Los Angeles, CA 90024. Bone marrow transplantation from an HLA-matched sibling donor is a curative therapy for young patients with aplastic anemia. For patients lacking an HLA-matched sibling the only alternative therapy has been antithymocyte globulin (ATG) which has a response rate of approximately 40%. Patients failing ATG had little alternative other than clinical trials with growth factors, androgens, cyclosporine, etc. Few patients have been complete responders with these agents. The first attempts of matched unrelated donor bone marrow transplants were done for patients with aplastic anemia. Because aplastic anemia patients have a higher rate of graft rejection with HLA-matched siblings, which is even higher when there is HLA disparity between donor and recipient, most centers have used intensive conditioning regimens (similar to those used for leukemia patients) for aplastic anemia patients receiving matched unrelated donor transplants. This has increased toxicity of the conditioning regimens, but possibly enabled engraftment in 80 to 90% of patients with 6 of 6 HLA, A, B, DR matches. Since only a few patients have had conditioning therapy with limited field irradiation and cyclophosphamide, little data is available on less intensive conditioning regimens. Patients receiving transplants from donors matched for less than 6 antigens have had poor engraftment rates, particularly with T-cell depletion techniques. Matched unrelated donor transplants have a grade II-IV AGvHD rate of 60 to 80%. This higher incidence of AGvHD has been associated with higher mortality in patients matched for less than 6 HLA, A, B, DR antigens. Improved AGvHD prophylaxis is needed in matched unrelated donor transplants. Despite these increased toxicities the survival rate with matched unrelated donor bone marrow transplants has ranged from 20 to 40% in otherwise incurable aplastic anemia patients. The poorer survival in these patients compared to matched sibling bone marrow transplants may be due to the fact that the time from diagnosis to transplant has often been longer than one year. If searches are begun shortly after diagnosis and larger registries of fully typed donors become available, survival should improve for aplastic anemia patients receiving matched unrelated donor transplants.

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2. Beatty PG et al: Clin Transplantation 1:117-124; 1987
3. Gordon-Smith EC et al: British Med Jnl; 285:835-87; 1982
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6. Bacigalopo A et al: Bone Marrow Transplantation 3:531-535; 1988
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C 030 HLA COMPATIBILITY IN MARROW TRANSPLANTATION: TRANSPLANTS FROM HLA PARTIALLY MATCHED FAMILY MEMBERS, John A. Hansen, Claudio Anasetti, Patrick G. Beatty, Paul J. Martin and Effie Petersdorf, Fred Hutchinson Cancer Research Center and Department of Medicine, University of Washington, Seattle, WA 98104

We have analyzed the effect of HLA incompatibility on GVHD, relapse and survival in 281 patients transplanted from a haploidentical HLA partially matched family member for the treatment of hematological malignancy. Results were compared to 967 patients transplanted for hematological malignancy from an HLA identical sibling donor during the same period of time. Transplants occurred from November 1975 to March 1986, and all patients received unmodified donor marrow. Conditioning regimen and GVHD prophylaxis varied over time, but was the same for both the study and control groups. The relationship of the donor to the recipient in the study group was parent (n = 137), sibling (n = 137), child (n = 12) or other (n = 13). Although all donors in the study group were haploidentical, donor-recipient pairs were either phenotypically identical for HLA-A, B and D (n = 29), incompatible for *one A, B or D locus* (n = 119), *two loci* (n = 164) or *three loci* (n = 29). The incidence and severity of acute GVHD was correlated with the degree of recipient HLA incompatibility, but GVHD also varied according to type of GVHD prophylaxis. Recipients incompatible for *one locus* had an incidence of severe grade III to IV GVHD of 72% when receiving standard methotrexate (MTX), but only 34% when receiving "short MTX" and cyclosporine (CSP). Similarly, the incidence of severe GVHD in recipients incompatible for *two or three loci* was 84% and 63%. In multivariate analysis, HLA incompatibility was the most significant GVHD risk factor (relative risk = 2.16 per incompatible HLA locus, $p < 0.0001$), and "MTX only" versus "short MTX+ CSP" was the second most significant GVHD risk factor (relative risk = 3.45, $p < 0.0001$). Patient age per decade was the third and last significant GVHD risk factor (relative risk = 1.17, $p = 0.0373$). Clinically significant acute GVHD grade II to IV was associated with a lower relapse in patients transplanted for ALL ($p = 0.04$), but not ANL or CML. Chronic GVHD was associated with a lower relapse in patients transplanted for ANL in relapse or CML in blast crisis ($p = 0.01$). In good risk patients (ANL in 117 remission, ALL in 1st or 2nd remission and CML in chronic phase) the rate of relapse in "one locus" incompatible subgroup (n = 61) compared to controls (n = 561) was 22% vs 37% ($p = 0.095$). Overall, survival was negatively associated with degree of HLA disparity ($p = 0.0001$), however, survival of *one locus* incompatible recipients was equivalent to controls. The favorable outcome for "one locus" incompatible recipients occurs because of a lower relapse rate, presumably secondary to a clinically significant "graft-vs-leukemia" (GVL) effect.

C 031 UNRELATED DONOR BONE MARROW TRANSPLANTS - AN UPDATE FROM EUROPE. J.M. Hows, Department of Haematology, Hammersmith Hospital, Royal Postgraduate Medical School, London, United Kingdom. Unrelated donor bone marrow transplants (UD-BMT) are increasingly employed for treating patients who lack a suitable family donor. In the UK during the first 4 months of 1989, two major donor registries, the Anthony Nolan Research Centre (ANRC) and the British Bone Marrow and Platelet Donor Panel (BBMPDP) performed 450 and 156 searches, cumulating in provision of 31 and 6 donors respectively. The total number of UD available in Europe in 1989 is 227,000: ANRC 150,000, BBMPDP 20,000, France Transplant 45,000, Leiden 3,500, Belgium 5,000, W. Germany 2,000, and Spain 1,500. Most European BMT centers consider patients with chronic myeloid leukemia (CML), poor risk acute leukemia (AL), myelodysplasia (MDS), severe aplastic anemia (SAA), Fanconi's anemia (FA), other constitutional bone marrow failures and inborn errors of metabolism as candidates for UD-BMT. A recent survey of UK UD search requests to ANRC and BBMPDP shows 40% are for CML patients. Retrospective analysis of results of UD-BMT in the UK[1] 1977-87 shows 32% one year survival for UD-BMT compared with 63% (p=0.0007) for identical sibling BMT, matched for year of BMT, diagnosis, disease risk, age and transplant center. Multivariate analysis showed patients with CML in chronic phase survived best after UD-BMT (p=0.03). At Hammersmith 15/28 patients with CML survive after UD-BMT, with 40% probability of survival at 4 years. In 24/28 cases, T cell depletion was used as GVH prophylaxis. Of the survivors, 3 have cytogenetic and one hematogic relapse. 5 patients with SAA, MDS or FA were transplanted at Hammersmith using intensive immunosuppressive protocols, 3/5 survive. To formally evaluate the efficiency of UD searches and the outcome of UD-BMT the 'International Marrow Unrelated Search and Transplant' (IMUST) Study[2] has been commenced. 72 transplants have been registered, and preliminary data will be presented.

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2. Bradley BA, Gore SM, Howard MR et al. Bone Marrow Transplant 1989 4: (suppl 2) 414.

C 032 THERAPY OF CHRONIC MYELOGENOUS LEUKEMIA WITH UNRELATED DONOR BONE MARROW TRANSPLANTATION: RESULTS IN 102 CASES, Philip B. McGlave, Patrick Beatty, Robert Ash, Jill M. Hows, Department of Medicine, University of Minnesota Hospital, Minneapolis, MN 55455 From April, 1985 to February, 1989 102 consecutive patients received unrelated donor bone marrow transplantation therapy for chronic myelogenous leukemia (CML) at four centers. Median age of the group was 31 years (range = 4.5 to 51 years). Fifty-four patients were in first chronic phase (CP) at time of transplantation and 48 had evidence of more advanced disease (AD) (accelerated phase = 32; blast crisis = 9; second chronic phase = 7). In 44 cases the donor and recipient were identical at the HLA A, B, and DR loci and were nonreactive in bidirectional mixed leukocyte culture (MLC) ("matched"). In 58 cases non-identity between donor and recipient could be determined at at least one HLA locus or in bidirectional MLC ("mismatched"). All patients were prepared for transplantation with a combination of cyclophosphamide and fractionated total body irradiation. In 44 cases recipients received additional preparative therapy. In 58 cases GVHD prophylaxis consisted of methotrexate alone or in combination with cyclosporine, prednisone or antithymocyte globulin (ATG). In 44 cases recipients received marrow depleted of mature T lymphocytes by ex vivo incubation with either anti-CD3 antibody plus complement (n = 24) or Campath-1 (n = 20). Primary engraftment defined by neutrophil count >0.5 x 109/L was demonstrated in 92 cases and occurred at a median of 21 days (range = 11 to 46 days). In 10 cases primary engraftment did not occur. Hematologic relapse was seen in 4 cases, and recurrence or persistence of the Ph1 without evidence of hematologic relapse in four additional cases. The incidence of grade II to IV acute GVHD is 65% (95% confidence interval [C.I.] = ± 10%). After adjustment for recipient age and donor matching status, recipients of T lymphocyte-depleted donor marrow had a significantly lower incidence of grade II-IV acute GVHD (p = < .01); however, T depletion was not significantly associated with improved survival (p = .34), disease free survival (p = .51) or increased incidence of relapse (p = .39). Forty-six of 102 patients are alive with a median survival of 12 months (range = 3 to 46 months) and the Kaplan-Meier estimate of survival is 35% (95% C.I. = ± 13%) at 2-1/2 years. A trend towards better survival in the 44 recipients of matched donor marrow (46%; 95 C.I. = ± 20%) when compared to 58 recipients of mismatched marrow (27%; 95% C.I. = ± 14%) (p = .10) was observed and could not be attributed to significant differences in recipient age, disease stage, incidence of GVHD or graft failure between the two groups. Unrelated donor bone marrow transplantation may provide an alternative form of curative therapy for patients with chronic myelogenous leukemia who do not have an HLA-matched sibling donor.

Immune Recovery and Genetic Diseases

C 033 ENGRAFTMENT OF NORMAL AND LEUKEMIC HUMAN HEMATOPOIETIC CELLS INTO IMMUNE-DEFICIENT SCID AND BNX MICE. John E. Dick, Suzanne Kamel-Reid, Christian Sirard. Dept of Genetics, Research Institute, Hospital for Sick Children; and Dept. of Medical Genetics, University of Toronto, Toronto, Ontario Canada M5G 1X8. Understanding the process of differentiation and development remains as a major challenge in biology. The blood-forming system of mice and humans consists of a heterogeneous array of cells, ranging from large numbers of differentiated cells with defined function to rare pluripotent stem cells with considerable developmental and proliferative potential. Our understanding of the biology of the human hematopoietic system has suffered relative to that in the mouse because of the lack of an in vivo assay system for human pluripotent stem cells. In addition, progress in understanding human leukemic transformation and progression has been hampered by the lack of experimental in vivo models. We have attempted to develop such in vivo models by transplanting immune-deficient scid and bnx mice with normal and leukemic bone marrow. We have found that human myeloid progenitors rapidly expand in the hematopoietic tissues of bnx mice transplanted with normal human bone marrow and persist there for up to 3 months. The fact that human in vitro progenitors have little if any self-renewal potential and the fact that progenitor cells were continuously produced for at least 3 months suggests that earlier progenitor or stem cells have engrafted these animals. Human bone marrow was infected with a neo vector using high efficiency retrovirus mediated gene transfer techniques and infused into mice; after one month a substantial proportion of the CFU-GM contained the neo gene. It should now be possible to use the retrovirus integration site to follow stem cell clones in the transplanted mice. In addition to normal bone marrow we have engrafted animals with a variety of human lymphoid and myeloid cell lines. In particular we have found that the growth of a human ALL cell line in mice is analogous to the spread of the disease in children. After initial growth in the bone marrow, cells gradually spread to other peripheral tissues and eventually many organs including the lungs, liver, kidney, and brain were massively infiltrated leading to the death of animals by 12 weeks. Bone marrow taken directly from some patients with ALL has also engrafted immune-deficient animals. The establishment of in vivo models for the growth of normal and leukemic cells presents a unique system in which to study numerous biological parameters governing their growth, as well as a system in which to test novel chemotherapeutic agents, biological response modifiers, and immunotherapy techniques for the treatment of human leukemia.

C 034 PREVENTION OF GRAFT REJECTION AFTER HLA NON-IDENTICAL BONE MARROW TRANSPLANTATION USING AN ANTI-LFA-1 (CD11a) ANTIBODY. A. Fischer, W. Friedrich, A. Fasth, S. Blanche D. Frappaz, P. Hervé, P. Bordigoni, J. Vossen, C. Griscelli, M. Hirn. Hôpital des Enfants-Malades, Paris, Ulm, Goteborg, St Etienne, Besançon, Nancy, Leiden (Immunotech). The in vivo infusion of a murine monoclonal antibody specific for the -chain of LFA-1 (.2 mg/kg daily from day -3 to +6) has been used in order to try to improve the rate of acceptance of HLA non identical, T-cell depleted bone marrow. Sixty seven children with either inborn errors (n=49, including partial immunodeficiency, osteopetrosis, Fanconi anemia and metabolic diseases) or leukemia (n=18, mainly ALL in 2nd CR) have been included in these trials. Two thirds of the patients received transplants from full haplotype-mismatched parents. The rate of sustained engraftment is the first group was 71 % (compared to 14 % in an historical group and 12.5 % in a group of similar patients who received anti-T cell antibodies and 80 % in the leukemia group. Acute graft versus host disease (GVHD) ≥ II occurred in 27 % of the patients in the first group (cGVHD 10 %) and 33 % in the second group. The survival rates are 47 % and 22 % with a mean follow of 22 (3-50) and 15 months respectively. The higher incidence of death in the leukemia group was caused by infections and relapse. We conclude that the in vivo use of an anti-LFA-1 antibody as additional immunosuppressive therapy in patients undergoing HLA non identical BMT may promote engraftment and survival for children with inborn errors such as immunodeficiency and some metabolic diseases. The combination of anti-LFA-1 Ab with aggressive antileukemic conditioning regimen appears to induce an high incidence of toxicity due to immunosuppression.

C 035 RATIONALE FOR BONE MARROW TRANSPLANTATION IN THE TREATMENT OF AUTOIMMUNE DISEASES. S. Ikehara, M. Inaba, R. Yasumizu, N. Nagata, N. Oyaizu, J. Toki, H. Hisha, H. Ogata, T. Ishida, F. Takao, N. Nishioka and R. A. Good. Department of Pathology, Kansai Medical University, Moriguchi, Osaka 570, Japan, and All Children's Hospital, University of South Florida, St. Petersburg, FL33701, U.S.A.

Using an animal model for type I diabetes mellitus (NOD mouse), we have demonstrated that allogeneic bone marrow transplantation (ABMT) has preventative effects on insulitis and diabetes, and that a combined transplantation of newborn pancreas and ABMT can be used to treat overt diabetes in NOD mice. In addition, we have shown using $(NZBxNZW)F_1$, BXSB, and MRL/lpr mice that ABMT has completely curative effects on autoimmune diseases (lupus nephritis, etc.) in these mice except for MRL/lpr mice; since MRL/lpr mice possess abnormal radioresistant stem cells, they regularly suffer a relapse approximately 5 months after ABMT.

We have recently succeeded in purifying murine hemopoietic stem cells (HSCs) in the G_0 phase, and found that MHC-matched stromal cells in the bone marrow are essential to growing HSCs in the G_0 phase. We therefore attempted to treat autoimmune diseases in MRL/lpr mice by transplantation of stromal cells with HSCs. Transplantation of HSCs with a stromal cell line (PA-6) or bone (without bone marrow) were indeed found to have curative effects on autoimmune diseases (lupus nephritis and arthritis) in MRL/lpr mice. These results indicate that ABMT will become a valuable strategy for the treatment of patients with autoimmune diseases, and that donor-derived stromal cells play a crucial role in the acceptance and growth of donor HSCs.

To prove that the etiopathogenesis of autoimmune diseases can be attributed to defects at the level of HSCs, we are in the process of transferring HSCs of autoimmune-prone mice into normal mice. Long-term observation in [NOD->C3H/HeN] chimeras revealed that the chimeric mice develop insulitis followed by overt diabetes. This finding suggests that the susceptibility to autoimmune diseases is programmed at the gene level of HSCs.

C 036 BONE MARROW TRANSPLANTATION FOR IMMUNE DEFICIENCY STATES, Robertson Parkman, Kenneth I. Weinberg, Donald B. Kohn, Leonard S. Sender and Carl Lenarsky, Division of Research Immunology/Bone Marrow Transplantation, Childrens Hospital of Los Angeles and Department of Pediatrics, University of Southern California School of Medicine, Los Angeles, California 90027.

Allogeneic bone marrow transplantation has an established role in the treatment of oncological, hematological and genetic diseases. In addition, bone marrow transplantation has been successfully used to treat primary immunodeficiency states involving either the lymphoid and/or hematopoietic stem cells. Allogeneic bone marrow transplantation is the treatment of choice for all forms of severe combined immune deficiency (SCID), although certain forms (IL-2 deficiency, bare lymphocyte syndrome) appear to be relatively resistant to the engraftment of T lymphocyte depleted haploidentical bone marrow. The successful transplantation of all forms of SCID results in donor T lymphocyte engraftment; B lymphocyte engraftment is variable depending upon the form of SCID. Overall transplantation results with histocompatible and T lymphocyte depleted haploidentical bone marrow are similar (70% survival). Bone marrow transplantation has also been used to successfully treat immunodeficiencies of the hematopoietic stem cell including Kostmann's syndrome and chronic granulomatous disease. The recent demonstration that the systemic administration of cytokines can also successfully treat these disorders means that the role of bone marrow transplantation in the treatment of hematological immunodeficiency states needs to be reassessed. Inadequate data are available to determine the role of transplantation in the treatment of the X-linked lymphoproliferative syndrome (XLP), Chediak-Higashi syndrome and the erythrophagocytic syndromes.

Acquired immunodeficiency due to infection with HIV-1 has been treated in the past by both bone marrow transplantation and leukocyte infusions without demonstrable benefit. The successful use of bone marrow transplantation for the treatment of HIV-1 infected patients may require combined therapy, including pre-transplant cytoreduction, anti-viral therapy, post-transplantation immunosuppression and, ultimately, the insertion of genetic elements that inhibit HIV replication into the transplanted bone marrow, either autologous or allogeneic.

C 037 THERAPEUTIC EFFECT OF BONE MARROW TRANSPLANTATION IN ARTHRITIC RATS, Dirk W. van Bekkum, Radiobiological Institute TNO, 2280 HV Rijswijk, The Netherlands. Total body irradiation followed by bone marrow transplantation has been found to cure rats suffering from progressive adjuvant arthritis. This treatment was most effective when applied shortly after the clinical manifestation of the disease, i.e. 4-7 weeks after the challenge with M. Tuberculosis. Permanent cures of arthritis were obtained equally well with allogeneic bone marrow grafts from a donor strain that is not susceptible to the induction of arthritis, with syngeneic bone marrow of healthy donors and with autologous bone marrow from arthritic rats. The effect of bone marrow replacement was not due to local irradiation effects on the affected joints, nor to a lack of M. Tuberculosis antigen. Further evidence is provided that bone marrow transplantation abolishes the autoimmune reaction that is causing chronic arthritis in our rat model.

C 038 THE CREATION OF HEMATOPOIETIC CHIMERISM IN NON-HUMAN PRIMATES BY IN UTERO TRANSPLANTATION OF HEMATOPOIETIC STEM CELLS, Esmail D. Zanjani and Michael R. Harrison, Departments of Medicine and Physiology, Veterans Affairs Medical Center, University of Nevada, Reno, NV 89520, and Department of Surgery, University of California, San Francisco, CA 94143
We transplanted hematopoietic cells (HC) obtained from livers of early gestational age normal fetal Rhesus monkeys (60 days-old) into unrelated normal Rhesus monkey fetuses (40-120 days-old; 10^8-10^9 cells/kg body weight) of opposite sex intraperitoneally under ultrasound guidance. Donor cell engraftment was confirmed by karyotype analysis of peripheral blood leukocytes and bone marrow cells of the recipients at birth and at monthly intervals thereafter. Four of five 60 day-old and 2 of three 80 day-old recipients exhibited significant donor cell engraftment. Fetuses significantly younger than 60 days aborted soon after donor cell infusion and fetuses 100 days or older failed to engraft. Engraftment involved lymphoid (2.9 - 8.0%), erythroid (5.3 - 12.5%), and myeloid (8.5 - 15.4%) donor cell lineages. The chimerism has persisted for about 2.2 years without evidence of graft-vs-host disease or graft loss. In utero transplantation of preimmune fetal HC into a similarly preimmune fetus without the use of cytoablative procedures may eliminate the problems that limit postnatal hematopoietic stem cell transplantation (e.g. rejection and graft-vs-host disease), and offer an effective therapy of genetic disorders of blood in utero.

Viral Infections

C 039 CORRELATION BETWEEN INFECTION WITH AND IMMUNITY TO HERPES VIRUSES AND GRAFT-VERSUS-HOST DISEASE. Gratama JW, Zwaan FE, Ringden O, Stijnen T, and Ernberg I. Departments of Immunohematology and Blood Bank, and Hematology, University Hospital, Leiden; Department of Medical Statistics, Erasmus University, Rotterdam, the Netherlands; Department of Clinical Immunology, Huddinge Sjukhus, Huddinge; and Department of Tumor Biology, Karolinska Institute, Stockholm, Sweden.

Results of experimental and clinical BMT have shown a positive correlation between the presence of intestinal bacteria and the severity of GVHD. These bacteria are thought to contribute to GVHD by providing antigenic stimuli to donor T lymphocytes. Viruses which remain latent in their hosts after initial infection may similarly contribute to GVHD, and immunity of the marrow donors against such viruses may further enhance this effect. The pretransplant antibody status against such viruses can be taken as a marker for carrier status and immunity in recipients and donors. The correlation between pretransplant herpes virus serology and GVHD in HLA-identical sibling transplants has been investigated in 2 single-center studies. In Leiden (126 patients), donor seropositivity for herpes simplex virus (HSV), recipient seropositivity for Epstein-Barr virus (EBV) and donor seronegativity for EBV were significant risk factors for Grades II-IV acute GVHD. In Huddinge (111 patients), recipient seropositivity for cytomegalovirus (CMV) was a significant risk factor for Grades II-IV acute GVHD, and donor HSV seropositivity reached borderline significance. Recipient carrier status of an increasing number of herpes virus types added significantly to the risk for GVHD in a retrospective survey of the European cooperative BMT group (379 patients). In addition, active HSV infection was a significant risk factor for Grades II-IV acute GVHD in the Leiden patients, whereas active CMV infection was not. Most cases of active HSV infection preceded GVHD in these patients. The role of active EBV infection is being evaluated in Leiden and Huddinge and these results will be reported. The combined results of these studies suggest that herpes virus carrier status, infection and immune reactivity associated with these conditions may contribute to the development of GVHD. Pretransplant herpes virus serology may be helpful in estimating the risk of GVHD in HLA-identical sibling transplantation; the exact role of individual virus types and the mechanism by which they contribute to GVHD remains to be established.

C 040 CLINICAL ASPECTS OF CYTOMEGALOVIRUS INFECTION,
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Although progress has been made both in prevention and treatment, cytomegalovirus (CMV) infection remains a hazard among patients (primarily those seropositive before transplant) undergoing marrow allografting. Autograft recipients also have a substantial risk of serious CMV infection, albeit lower than after allografting: CMV pneumonia occurred in 11 of 157 patients (7%) receiving autografts for hematologic malignancy between 1980 and 1987. In previous studies among allograft recipients, the probability of CMV disease (pneumonia or enteritis) by 100 days after transplant was .49 among seropositive patients and .13 among seronegative patients. CMV viremia or excretion of CMV in throat or urine had a 50% positive predictive value for subsequent disease. The probability of CMV pneumonia was .35 and .10, and of CMV enteritis .30 and .08, among seropositive and seronegative patients, respectively, within the first 100 days after transplant. Deep endoscopic biopsy is crucial for diagnosis of CMV enteritis, with viral cultures or immunohistology being positive more often than standard histology. Virtually all patients with CMV enteritis have nausea, vomiting, anorexia or diarrhea. Abdominal pain is less frequent and fever occurs in only one-third. Approximately 25% of patients with enteritis progress to CMV pneumonia within the succeeding 2-12 weeks. Ganciclovir alone was not effective therapy for either CMV pneumonia or enteritis, whereas the combination of ganciclovir and intravenous immunoglobulin (IVIG) was associated with greater than 50% response in several trials in patients with CMV pneumonia. Recent study showed that prophylactic CMV IVIG had no effect on CMV disease in seronegative patients with seropositive marrow donors. In another study high-dose (500 mg/kg) unscreened IVIG reduced CMV pneumonia (but not enteritis) apparently by modulating acute GVHD, but had no direct effect on CMV infection. Suppression of CMV reactivation with antiviral agents (e.g. acyclovir, ganciclovir or foscarnet) among seropositive allograft recipients remains of major interest. The efficacy and toxicity of ganciclovir is being tested both when given "prophylactically" and for "early treatment" among patients excreting CMV after transplant.

C 041 GANCICLOVIR AND INTRAVENOUS IMMUNOGLOBULIN IN BONE MARROW TRANSPLANTS
Drew J. Winston, Winston G. Ho, and Richard E. Champlin, UCLA Medical Center, Los Angeles, CA 90024. The indications and limitations of ganciclovir and intravenous immunoglobulin (IVIG) in bone marrow transplants (BMT) have been defined from several recent studies. In uncontrolled studies, neither ganciclovir alone (1,2) or given with corticosteroids (3) improved survival in BMT with CMV pneumonia. Ganciclovir given with IVIG containing CMV antibody, however, improved survival to 50-85% at some transplant centers (4-7) but not at others (survival in 0 of 9 patients treated at Utrecht and UCLA) (8,9). Ganciclovir appears more effective when given earlier in the course of CMV infection before the development of pneumonia (2). The efficacy and safety of prophylactic ganciclovir in allogeneic BMT is currently being evaluated in a double-blind, placebo-controlled trial at UCLA. CMV-seropositive BMT are being randomized to receive either placebo or ganciclovir at a dosage of 2.5 mg/kg IV q. 8hrs for one week before transplant. The ganciclovir is then stopped on day of infusion of the donor marrow. After transplant, when the granulocyte count reaches 1000 cells/mm^3, prophylactic ganciclovir is resumed at a dosage of 6mg/kg IV per day, Monday through Friday, until day +120. Sixty-three evaluable BMT have completed the study. The study code has not yet been broken but the overall incidence of CMV infection is 30% and of symptomatic CMV disease 11%. Neutropenia developed in 40% of BMT. In CMV-seronegative BMT, CMV infection can be prevented by using CMV-seronegative blood products when the marrow donor is CMV-seronegative (10). For other CMV-seronegative BMT, prophylactic IVIG can modify CMV infection and prevent CMV pneumonia (11). IVIG also has the additional benefits of preventing bacterial infections and modifying GVHD (11,12).

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C 042 THE PATHOGENESIS OF HUMAN CYTOMEGALOVIRUS-ASSOCIATED DISEASES: IMPLICATIONS FOR PREVENTION AND TREATMENT, John A. Zaia, Division of Pediatrics, City of Hope National Medical Center, Duarte, CA 91010. The relationship between human cytomegalovirus (HCMV) and its host remains incompletely understood, but recent clinical and laboratory evidence suggests mechanisms of pathogenesis. These mechanisms must explain disease occurrence during primary infection, during reinfection, and following reactivation of primary infection. In addition, the variability of disease presentation in different patient groups, including different immunodeficient population, should be explained. Evidence will be reviewed to support the hypothesis that the pathogenesis of HCMV-associated disease is due to an inbalance between three factors: persistent virus infection, immunopathologic mechanisms, and virus-induced expression or repression of cellular genes. HCMV retinitis and infection of the nervous system appears to be due to direct cytopathic effects of virus replication. HCMV-associated interstitial pneumonis (HCMV-IP), on the other hand, is more complex and appears to be a result of persistant infection and its effect(s) on cellular immune function. HCMV-associated alimentary disease in the transplant recipient is so incompletely understood that it cannot be explained in any present pathogenetic schema.

Since the initiation point for disease pathogenesis is HCMV infection and replication, prevention of these HCMV-syndromes must focus on prevention of virus infection or on inhibition of continuous virus replication. Treatment, on the other hand, is only effective using antiviral agents when the viral cytopathologic effects cause disease. Thus, retinitis improves on ganciclovir therapy, but HCMV-IP does not usually respond to antiviral treatment alone. Rather, the combination of an antiviral agent with biologic response modifiers should, in theory, be therapeutic for a more complex disease process such as HCMV-IP.

BMT for Lymphoma and Related Disorders

C 043 BONE MARROW TRANSPLANTATION IN LYMPHOMA, James O. Armitage, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68105. Bone marrow transplantation is an increasingly widely applied treatment for patients with relapsed, refractory, and poor prognosis non-Hodgkin lymphoma and Hodgkin disease. The number of transplants done annually has been increasing steadily. Approximately 800 autologous transplants alone will be done to treat patients with lymphoma in 1989. It is now clear that patients undergoing autologous or allogeneic bone marrow transplantation for lymphoma can be cured. The important and unanswered question is how many patients are cured with bone marrow transplantation that could not have been cured with another form of therapy. This question becomes more difficult to answer as transplants are done earlier in the course of the illness. Reported results today suggest that approximately 30% of patients with relapsed Hodgkin disease undergoing bone marrow transplantation can be cured with an associated 10% treatment related mortality. As is always true in transplant series, patients treated earlier in the course of the illness have better results. In aggressive non-Hodgkin lymphoma, 30-40% of patients transplanted for relapsed disease that is still chemotherapy sensitive achieve long-term, disease-free survival with 10-20% of patients suffering treatment related mortality. In both Hodgkin disease and non-Hodgkin lymphoma treatment related mortality seems to rise as patients are treated at more advanced stages of the disease. In indolent non-Hodgkin lymphoma most patients treated to date remain in complete remission with a treatment related mortality of about 10%. Unfortunately, the follow up in not nearly long enough to determine whether or not this disease can be cured with transplantation. Important unanswered questions include what is the best high dose treatment regimen, whether or not *in vitro* marrow treatment is necessary to remove potentially contaminating tumor cells, the relative merits of allogeneic versus autologous transplantation, the use of peripheral stem cells versus bone marrow derived stem cells, and the place of hematopoietic growth factors in bone marrow transplantation. The exact contribution bone marrow transplantation will eventually make to the treatment of patients with lymphoma is not yet clear, although data is rapidly accumulating.

C 044 **TRANSPLANTS IN CHRONIC LYMPHOCYTIC LEUKEMIA. Sante Tura, Giuseppe Bandini. Institute of Hematology "L. & A. Seràgnoli", St.Orsola University Hospital, Bologna, Italy.**

The classic treatment of chronic lymphocytic leukemia (CLL) aims at prolonging survival, without attempt at eradicating the disease .Howevere, more recently new approaches have been attempted, and one is allogeneic bone marrow transplantation(BMT).Very few patients(pts) have been transplanted and in a search of the literature, we found only 9 cases (1). They had a median age of 40 yrs. 8 were fully HLA identical and 1 was mismatched. 1 pt underwent BMT without previous therapy ,all the others had proved resistant to conventional or "intensive" therapy (CHOP or COP) and received BMT a median 36 mos after diagnosis. At the time of BMT 7 pts were RAI stage III or IV, and 2 stage I. Conditioning consisted of Cyclophosphamide and TBI, with the addition of Chlorambucil(1pt) or Etoposide (2pts). All pts had a disappearance of lymphnodes, splenomegaly, hepatomegaly and lymphocytosis. 3 pts died (2 of aGVHD and 1 of cerebral hemorrhage). 6 were alive, of whom 5 in continuos complete remission at 5 to 33 mos after BMT, while one relapsed at 54 mos. These results indicate that intensive chemoradiotherapy and BMT determine a prolonged disapperance of the leukemic clone in all patients. BMT deserves further exploration in selected pts with CLL.

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C 045 ALLOGENEIC BONE MARROW TRANSPLANTATION IN MULTIPLE MYELOMA,
G. Gahrton*, S. Tura, C. Belanger, M. Cavo, B. Chapvis, A. Fernant, M. Flesch, M. Gore, A. Gratwohl, P.J. Gravett, J.L. Harrouseau, A. Lindeberg, P. Ljungman, B. Löwenberg, G. Lucarelli, M. Michallet, J. Reiffers, O. Ringdén, M.T. Van Lint, J.P. Vernant, B. Sallerfors, B. Simonsson, A. Toivanen, X. Troussard, L.F. Verdonck, L. Volin and F.E. Zwaan, for the European Group for Bone Marrow Transplantation (EBMT), * Department of Medicine, Huddinge Hospital, S-141 86 Huddinge, Sweden.

50 patients (M/F=25/25, median age 41 y., range 29-54) who underwent allogeneic bone marrow transplantation for multiple myeloma have been reported to the EBMT registry at Huddinge Hospital. At BMT, 35 patients were on second-line treatment, and 15 one first-line treatment. 24 patients were considered refractory to previous treatment. 42 patients received marrow from HLA-matched sibling donors, 3 from identical twin donors, and 5 from unrelated or related non-sibling donors. The conditioning for BMT was TBI + cyclophosphamide in 24 patients, TBI + cyclophosphamide + melphalan in 10 patients, TBI + other chemotherapy in 13 patients, busulphan + cyclophosphamide in 2 patints and melphalan only in one patient. 21 patients entered complete remission, while 15 had persistant disease following repopulation of the marrow. 14 patients were not evaluable for remission status because of early, transplantation-related death. The overall median survival from BMT was 27 months, with a projected long-term survival of 34%. Patients who were 40 year of age or older had a survival that was not significantly different from that of patients between 29 and 40 years of age. The median disease-free survival of patients in complete remission post BMT was 41 months. These patients tended to have a longer survival than patients with persistant disease following repopulation of the bone marrow. Allogeneic bone marrow transplantation appears to be a promising method for selected patients of multiple myeloma.

C 046 USE OF HIGH DOSE ETOPOSIDE (VP-16) FOR CYTOREDUCTION WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION IN HIGH RISK NON-HODGKIN'S LYMPHOMA (NHL) AND HODGKIN'S DISEASE (HD).
SJ Horning, NJ Chao, RS Negrin, RT Hoppe, KG Blume, Stanford University Medical Center, Stanford, CA. 94305

High dose VP-16 (60 mg/kg) was used in combination with 1200 cGy fractionated total body irradiation (FTBI) and cyclophosphamide (CY 100 mg/kg) or, for those previously treated with thoracic irradiation, carmustine (BCNU 10-15 mg/kg) /CY as cytoreduction for 52 patients (pts) transplanted between December 1987 through August 1989. All pts engrafted but the first required backup allogeneic marrow. Thereafter, peripheral mononuclear cells were used to supplement bone marrow or exclusively (median nucleated cell dose 7.3 x 10^8/kg). In vitro purging with a panel of monoclonal antibodies and complement was employed in 12 pts. 32 pts also participated in a randomized, double-blind trial of GM-CSF (code not broken). 29 pts with HD and 23 pts with NHL have been transplanted. Of 3 (6%) transplanted-related deaths, all among pts treated with BCNU/VP-16/CY, one each was due to veno-occlusive disease, pulmonary failure and delayed cytopenia. A syndrome of dyspnea and pulmonary infiltration developed in 3 other pts receving BCNU; all completely responded to corticosteroids. Three additional deaths were due to recurrent disease (2 HD, 1 NHL). With a median time from transplant of 9 months, the projected one-year survial is 78%. 30 pts participated in protocols designed to transplant patients with less than 25% chance for cure with conventional therapy at an optimal time and in a minimal disease state. With a median followup of 8 months, all protocol pts are disease-free. We conclude that high dose VP-16 containing regimens are well-tolerated and preliminary data supports their efficacy in high risk NHL and HD.

Category	Pts	Alive, Disease-free	Recurrence	Transplant Deaths	Death d/t Disease
ALL	52	42	7	3	3
PROTOCOL	30	27	0	3	0
FTBI/VP-16/CY	19	16	3	0	1
BCNU/VP-16/CY	33	26	4	3	2
HD	29	21	5	3	2
Protocol	15	12	0	3	0
NHL	23	21	2	0	1
Protocol	15	15	0	0	0

C 047 BONE MARROW TRANSPLANTATION (BMT) FOR HODGKIN'S DISEASE (HD). Phillips, Gordon L, Reece, Donna E, Connors, Joseph M, Leukemia/Bone Marrow Transplantation Program of British Columbia, Divisions of Hematology and Medical Oncology. Cancer Control Agency of British Columbia, Vancouver General Hospital, and the University of British Columbia, Vancouver, B.C., Canada.

Over the last few years, the rate of autologous (Au) BMT has increased more rapidly for HD than for any other diagnosis. The reason for this recent increase is likely related both to the realization that patients who fail modern 7 or 8 drug regimens have a low rate of durable remission with conventional salvage therapy, and to the emergence of a favorable experience with AuBMT in such patients. In this regard, it is interesting that many series report durable remission in roughly 50% of all patients who failed primary chemotherapy -- an unusually favourable result in the BMT experience for advanced hematologic malignancy.

Currently, major issues in BMT for HD may be summarized as follows: 1) Patient selection; 2) Conditioning regimens; 3) Source of stem cells; 4) Use of growth factor augmentation. Of these issues, patient selection is likely the most critical, and it has profound influence on each of the others. For instance, if patients are transplanted after multiple relapses, the incidence and severity of toxicity from conditioning regimens, the availability of the different stem cell sources and the requirement for growth factors are unlikely to be identical to patients transplanted with less advanced HD. Accordingly, we believe that the earliest sign of failure of primary chemotherapeutic treatment regimens such as "MOPP/ABV(D)" is the optimal time to perform BMT. Conditioning regimens including total body irradiation are effective, but excessively toxic, and chemotherapy (only) regimens are preferred. The usual source of stem cells is autologous marrow, but peripheral blood stem cells and allogeneic marrow may be useful in some cases as well. Finally, growth factors may be useful in accelerating hematopoiesis in some patients.

In summary, the "final barrier" to tumor eradication in HD may be overcome using existing tools. Successive increases in the efficacy of primary chemotherapy over the last decade have reduced the number of incurable HD patients; since foreseeable improvements in conventional chemotherapy are unlikely to cure a very much larger proportion of patients, the development of effective BMT regimens is critical to further reductions in mortality in HD. As with most other hematological malignancies, it is imperative that the medical oncologist and the marrow transplant team work closely together to achieve this goal.

Biology of Bone Marrow Transplantation-III

C 048 STEM CELL INVOLVEMENT IN NORMAL AND MALIGNANT HEMATOPOIESIS. I. Bernstein, R. Andrews, J. Singer, P. Fialkow, R. Berenson, W. Bensinger, C. Buckner, Fred Hutchinson Cancer Research Center and University of Washington School of Medicine, Seattle, WA

The isolation and characterization of human hematopoietic stem cells has been elusive. We have identified primitive CD33- CD34+ cells that are incapable of forming hematopoietic colonies in vitro, but attain this capacity after culture in the presence of marrow stroma. Direct and indirect evidence indicates that these cells are responsible for hematopoietic engraftment after myeloablative therapy and are thus candidate human stem cells. In CML, an abnormality of pluripotent stem cells, CD33- CD34+ precursors are partly or mainly of clonal origin. In contrast, in some cases of AML where expression of the clonal abnormality was restricted to granulocyte/monocyte differentiation, CD33- CD34+ precursors were predominantly or completely of normal origin. Recent studies to establish a clonal in vitro assay for these putative human stem cells will be discussed.

C 049 MINIMAL RESIDUAL DISEASE IN ACUTE LEUKEMIA: PRECLINICAL STUDIES, Anton Hagenbeek, Ying-Lin Lu, Ger J.A. Arkesteyn, Yan-Ying and Anton C.M. Martens, Radiobiological Institute TNO, Rijswijk and The Dr. Daniel den Hoed Cancer Center, Rotterdam, The Netherlands.

Minimal residual disease (MRD) is defined as the relatively few leukemic cells ($1-10^{10}$) which have survived successful remission-induction chemotherapy. These residual cells finally determine the outcome of treatment in still the majority of patients: a relapse. Decreasing the detection level for MRD obviously offers various advantages, such as: quantifying the efficacy of a given treatment in terms of "log leukemic cell kill", deciding whether or not to go on with treatment, detecting arising drug-resistant leukemic cell clones, deriving prognostic factors indicating which patients will and which will not profit from marrow ablative chemo-radiotherapy followed by bone marrow transplantation, quantifying the number of leukemic cells present in autologous marrow grafts, studying the kinetics of regrowth of MRD, etc.

The possibilities to study MRD in human acute leukemia are limited. Based on studies in a relevant rat model for human acute myelocytic leukemia (BNML) a selection of studies will be presented, dealing with lowering the detection level of MRD. These studies include flow karyotyping and premature chromosome condensation (PCC); fluorescent in situ hybridization (FISH) with chromosome-specific DNA probes; detection of MRD after retroviral transfer of the lacZ-gene in leukemic cells. Extrapolations to man of these methodologies developed in preclinical models will be discussed.

C 050 SYNGENEIC GRAFT-VERSUS HOST DISEASE (SGVHD), Allan Hess, Anne Fischer, Georgia Vogelsang, Richard Jones, and George Santos. Bone Marrow Transplant Unit, The Johns Hopkins University, Baltimore, MD 21205

Tolerance to self major histocompatibility (MHC) antigens is one of the fundamental tenets of immunology. Acquisition of tolerance to self MHC antigens not only occurs in the neonate but must also occur after autologous or syngeneic bone marrow transplantation (BMT). Perturbation of the developing immune system leading to an imbalance in the mechanisms responsible for self:non-self discrimination could result in severe autoaggression. Recent studies have shown that treatment of rats or man with the immunosuppressive drug Cyclosporine (CsA) following lethal irradiation and autologous or syngeneic BMT, results in the development of autoaggression. The disease which occurs 10-14 days after cessation of CsA therapy (10mg/kg/d x 30 d) resembles GVHD developing after allogeneic BMT. There appears to be two fundamental immunobiological mechanisms accounting for the induction of syngeneic GVHD; (1) the development of autoreactive T cells in the thymus and their release into the periphery, and (2) elimination of a peripheral, autoregulatory system. Current studies in the rat indicate that associated with syngeneic GVHD is the development of $CD8^+$ cytotoxic T lymphocytes which recognize a public determinant on class II MHC antigens including self. Similar results have been obtained in man. Adoptive transfer studies suggest that the class II specific cytotoxic T cell is amplified *in vivo* by $CD4^+$ T helper cells also recognizing self class II antigens. CsA appears to inhibit the clonal deletion of these autoreactive cells in the thymus. On the other hand, an autoregulatory system present in unmodified recipients which regulates the amplification and/or action of these autoreactive cells must be eliminated to allow for the induction of syngeneic GVHD. This autoregulatory system requires the action of both $CD4^+$ and $CD8^+$ T cells and is sensitive to radiation and cyclophosphamide. CsA also appears to interfere with the development of this system post BMT. Taken together, these data suggest that CsA uncouples autoreactive and autogegulatory mechanisms which leads to autoaggression in the autologous/syngeneic BMT recipient. Furthermore, the induction of autologous GVHD is being applied to clinical transplantation to provide an anti-tumor effect on residual leukemia cells bearing class II MHC antigens, the target antigens recognized in syngeneic GVHD.

C 051 IS THERE A ROLE FOR LONG-TERM MARROW CULTURE AUTOTRANSPLANTS?

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For reasons that remain unclear, clonogenic leukemic cells can disappear in long-term bone marrow culture while normal hematopoietic progenitors are retained. This exciting observation described by the Eaves group has recently been exploited clinically in several centres including Manchester and Vancouver. Patients with acute myeloid leukemia in relapse, and in remission, have undergone intensive therapy and hematopoietic rescue with autologous long-term marrow culture cells. More recently, autotransplants using long-term culture cells have been performed in patients with chronic myeloid leukemia. However, results from these pilot studies suggest that a role for long-term marrow culture as a purging method is difficult to establish. Further laboratory and clinical investigations are required. Pertinent issues that need to be addressed include the time required to culture bone marrow such that sufficient clonogenic leukemic cells are lost but adequate normal stem cells capable of engraftment are retained. Results may depend in part on the type and extent of prior chemotherapy. The sensitivity of detecting leukemic clones in long-term culture by cytogenetics and other methods also requires scrutiny. The stage of disease at transplantation and the specific intensive therapy regimens need to be assessed. The clinical efficacy of this innovative approach will be evaluated in the context of the cell biology of long-term marrow cultures and clinical studies of autotransplants employing unpurged peripheral blood or marrow stem cells.

C 052 CHRONIC GRAFT-VERSUS-HOST DISEASE AND LONG-TERM FOLLOW-UP CARE.

Keith Sullivan, Jean Sanders and Robert Witherspoon, Fred Hutchinson Cancer Research Center and the University of Washington, Seattle, WA 98104

Late complications following allogeneic transplantation may result from the pretransplant preparative regimen, immunodeficiency and associated infection, recurrent or secondary malignancy or chronic graft-versus-host disease (GVHD). Chronic GVHD is observed in 25-50% of allograft survivors. Prognostic factors of adverse outcome include increasing patient age, prior acute GVHD, obliterative bronchiolitis, hyperbilirubinemia, and thrombocytopenia. Supportive care with antibiotic or immunoglobulin prophylaxis is currently under study. Without immunosuppressive treatment, < 20% of patients with extensive chronic GVHD survive with Karnofsky performance scores > 70%. Controlled trials have demonstrated that early therapy of standard-risk chronic GVHD with prednisone alone is more effective than prednisone and azathioprine, due to more frequent infections with cytotoxic treatment. Cyclosporine combined in an alternating-day regimen with prednisone has reduced morbidity and mortality in high-risk patients with thrombocytopenia or those failing primary treatment. A graft-versus-leukemia effect has been observed in patients developing acute or chronic GVHD. The relative risk of treatment failure (that is, mortality or relapse) was decreased in patients with ALL transplanted in relapse (RR = 0.70, P < 0.033) and CML in blast crisis (RR = 0.37, P < 0.009) compared to those patients who did not develop acute or chronic GVHD. This effect was independent of age, sex, preparative regimen, GVHD prophylaxis or length of follow-up. Chronic GVHD has not been associated with secondary neoplasms. Total body irradiation (TBI) used in the preparative regimen (RR, 3.9) or treatment of acute GVHD with antithymocyte globulin (RR, 4.2) or anti CD-3 antibody (RR, 13.6) has been associated with secondary cancers, particularly non-Hodgkin's lymphoma. Other late effects of TBI include cataracts, gonadal failure and neuroendocrine abnormalities. Among patients given 10.0 Gy single-dose TBI, growth hormone deficiency developed in 90% of children given cranial irradiation prior to TBI compared to 42% of children without prior irradiation (P = 0.009). For adult females, gonadal insufficiency can cause significant symptomatology. Among 44 post-pubertal women studied 0.7 - 12.7 years after transplant, atrophic gynecologic abnormalities were found in 33 of 36 recipients of TBI, compared to 2 of 8 women not given TBI (P = 0.02). Diagnosis and treatment of late complications can improve survival and the quality of life of the marrow graft recipient.

C 053 DETECTION OF MINIMAL RESIDUAL DISEASE (MRD): IMPLICATIONS FOR OUTCOME OF AUTOLOGOUS BONE MARROW TRANSPLANTATION (BMT) IN HIGH RISK REMISSION ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

Fatih M. Uckun, John H. Kersey, Robert Haake, Daniel Weisdorf, Daniel A. Vallera, Dorothea E. Myers, Kevin G. Waddick, and Norma K. C. Ramsay. The University of Minnesota Bone Marrow Transplant Program, Minneapolis, MN 55455.

We report our results on the prognostic value of a new MRD detection assay for outcome of high risk remission ALL patients after autologous BMT. 15 patients with high risk B-lineage ALL and 14 patients with high risk T-lineage ALL were transplanted in complete remission (REM) using ex vivo purged autografts. Patients were prepared for BMT with single dose TBI plus high dose Ara-C or hyperfractionated TBI plus high dose cyclophosphamide. Quantitative analysis of REM BM samples for the presence of residual leukemic blasts was performed using a novel detection method for minimal residual leukemia which combines fluorescence activated multi-color/multiparameter flow cytometry and cell sorting with LPC assays. Specifically, FACS sorted $CD19^+sIgM^-$ B-lineage lymphoid cells were cultured for 7 days in the presence of low molecular weight B-cell growth factor and assayed for B-lineage ALL blast colony formation, and FACS sorted $CD5,7^+CD3^-$ and $CD5,7^+CD3^+$ T-lineage lymphoid cells were assayed for T-lineage ALL blast colony formation in the presence of 10% PHA-LCM plus 100 U/ml rIL-2.

B-lineage ALL: Eleven of 15 patients (73.3%) relapsed at 2.4-24.4 months post BMT (median = 5.9 months) and 7 (46.7%) subsequently died of leukemia. Three patients are alive disease free at 19.9, 25.1, and 27.1 months post BMT. The mean number of blast colonies/10^5 FACS sorted cells ranged from 4-523 (median = 145). The calculated number of LPC/10^8 BM mononuclear cells (MNC) ranged from 1,560 (=0.002% of MNC)-3,460,000 (=3.5% of MNC) (median = 350,000=0.35% of MNC). There was a significant difference in the probability of remaining in remission between patients with >0.35% LPC vs patients who had <0.35% LPC among the MNC of their prepurge BM (0% vs 38%; P=0.004).

T-lineage ALL: Eight patients relapsed at 1.2-17 months (median = 2.2 months) post BMT and 6 have subsequently died of leukemia. Three patients are alive disease free at 2, 13, and 20 months post BMT. The number of T-lineage LPC/10^8 MNC ranged from <237 (=<0.0002% of MNC) to 214,047 (=0.2% of MNC) (median = 8,568 = 0.009% of MNC). This parameter inversely correlated with the probability of remaining in remission after BMT which was 53.3% for patients with <0.009% LPC as compared to 14.3% for patients with >0.009% LPC among the MNC of their BM samples (P=0.01).

Conclusion: Taken together, these findings suggest that in high risk remission ALL patients undergoing autologous BMT the number of LPC in pretransplant remission BM samples, as quantitated by this new MRD detection assay may help to predict the outcome.

Autologous BMT

C 054 DOSE INTENSIVE CHEMOTHERAPY IN BREAST CANCER

Karen Antman, M.D., Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115

While generally sensitive to the initial standard dose chemotherapy regimens, metastatic breast cancer virtually always recurs and is uniformly fatal with a median survival after the diagnosis of metastatic breast cancer of about two years. Women with estrogen receptor positive tumors, (median survival 2.3 years), and those who achieve a complete response with standard dose therapy (median 2.5 years) or who have only small amounts of local disease (median > 4 years) have a somewhat better prognosis. Lessons learned from the early leukemia and lymphoma transplantation experience are clearly applicable to the current studies of dose intensive therapy in solid tumors. Patients with sensitive tumors must be treated early in the disease course, before the development of resistance to available ablative chemotherapy regimens. Alkylating agents are ideal for use in dose intensive regimens in that they exhibit a steep dose-response curve, and non-hematologic toxicity varies among the different agents. Combinations of alkylating agents produce significant therapeutic synergy and subadditive toxicity in a variety of experimental tumor systems. There are 14 studies of high doses of single drugs followed by autotransplantation in 86 patients with advanced refractory breast cancer. Although all complete responses were obtained with alkylating agents (melphalan or thiotepa), partial responses were observed with all drugs evaluated. The response rates for alkylating agents was 41% compared to 26% for non-alkylating agents. Twenty one combinations of high dose chemotherapy with or without total body irradiation have been reported in 97 patients with failed or refractory breast cancer. Regimens of two or more alkylating agents seemed to produce the highest response rates. The response rate for radiation containing regimens (10 of 17, 59%) was not significantly different from regimens without radiation, (59 of 80, 73%). There are 2 studies of combination chemotherapy in 25 previously untreated patients with inflammatory or metastatic breast cancer. (Some patients had received prior adjuvant therapy.) Twelve regimens have been used in patients responding to induction therapy. A total of 70% of these later patients have achieved complete responses. Follow up remains short. More than 40% of patients with metastatic breast cancer and about 55% of those with a positive bone scan or metastases evident on bone x-rays will have bone marrow involvement detected by conventional studies of bone marrow biopsies, aspirations, or clot sections.The importance of either overt or occult bone marrow involvement in the setting of autologous bone marrow transplantation is unknown. Response rates of 30-50% in *refractory* breast cancer, (with complete responses in 10-20% of patients) provide evidence of a dose-response relationship. As in high dose therapy for leukemia and lymphoma, long-term disease-free survival of patients treated for refractory disease is rare. Transplant early in the course of the illness, after a good response to standard dose therapy yields a complete response rate much higher than the 10 to 30% reported with standard dose therapy. With follow-up intervals of 12 to 35 months from the time of transplant (16-40 months from the beginning of 2 to 4 cycles of induction therapy), these *unmaintained* responses appear to be relatively durable (35% in continuous complete response).

C 055 AUTOLOGOUS BMT (ABMT) FOR CANCER IN CHILDREN, John Graham Pole and Robert Marcus, Departments of Pediatrics and Radiation Oncology, University of Florida (UF) College of Medicine, Gainesville, FL 32610 and the Pediatric Oncology Group.

Myeloablative chemoradiotherapy supported by ABMT is used increasingly to treat children with refractory cancer. I will review our results in two diseases, neuroblastoma (NBL) and Ewing's sarcoma (ES).

We have treated 94 NBL patients (pts) in first or second remission (rem) on a Pediatric Oncology Group protocol with either melphalan 180 mg/m^2 or cytoxan (Cy) 3600 mg/m^2/etoposide (VP16) 1800 mg/m^2 + fractionated total body irradiation (FTBI) 9 or 12 GY followed by ABMT . Among 74 pts treated at least 18 months (m) ago, there have been 44 (59%) relapses (rel) and 7 (9%) toxic deaths. There is a significant association between event-free survival probability (EFS prob) and remission (rem) number at ABMT (rem 1 pts EFS 40% vs. rem 2 pts EFS prob 8%, p<.001). In rem 1 pts EFS prob was also significantly associated with diagnosis-to-BMT interval (EFS prob <9 m interval 25% vs. EFS prob >9 m 46%). Use of local irradiation (LI) and use of 12 GY TBI was also associated with better EFS prob than use of no LI and use of 9 GY TBI.

At UF we have treated 25 ES pts in first rem but at high risk of relapse (primaries >8 cm and/or metastases at diagnosis) with either vincristine (2 mg/m^2) + CY (1800 mg/m^2) + adriamycin (90 mg/m^2) + FTBI 8 GY, or CY 3600 mg/m^2 + VP16 1800 mg/m^2 + FTBI 12 GY, followed by ABMT. There was 1 toxic death. EFS prob is 63% at 36 m, compared with 21% in 33 similar ES pts receiving chemoradiotherapy at UF without ABMT and 0% in 9 pts receiving ABMT after rel. 6 of 11 (55%) with metastases at diagnosis and 10 of 14 (70%) with large primaries are event-free a median of 18 m since ABMT.

The results of these pilot studies of ABMT in pediatric solid tumors are promising. However, multicenter phase 3 studies are needed to compare end-intensification + ABMT with non-ABMT regimens in these diseases.

C 056 PHASE I/II TRIALS: ADULT SOLID TUMORS. Roger H. Herzig, James Graham Brown Cancer Center, University of Louisville, Louisville, Kentucky 40292.

Dose escalation studies in the treatment of refractory malignancies have been carried out over the past two decades. Single agent and multi-agent phase I trials have elucidated the nonmyeloid toxicities of mainly the alkylator drugs when autologous marrow is used to ameliorate the anticipated myelosuppression. These studies have demonstrated modest to substantial dose escalation compared to conventional dose. The increased dose has translated in improved responses in malignancies generally considered resistant to standard therapy (eg. melanoma, colon, lung). The beneficial effect on response to increased dose was also observed in patients with "sensitive" tumors which had become resistant to standard therapy (eg. lymphomas, neuroblastoma, ovarian, germ cell, breast, Ewing sarcoma). An overview of these phase I/II trials will be presented.

C 057 **CONCEPTS: BONE MARROW TRANSPLANTATION FOR SOLID TUMORS,** Geoffrey Herzig, Division of Hematology/Oncology, Washington University School of Medicine, St. Louis, MO 63110

In the absence of effective new agents for cancer therapy, increasing dose intensity may be the only way to improve outcome for patients who fail conventional treatment. When myelosuppression is the dose limiting toxicity, bone marrow transplantation (BMT) can be used to increase dose intensity. However, the maximum tolerated dose with BMT is rarely more than 50% greater than the maximum that can be given with good supportive care alone. The major exceptions are total body irradiation, busulfan, and thiotepa which demonstrate dose increments of approximately 2-3 fold over their MTD's without BMT. Combinations of myelosuppressive agents might produce additive toxicity and benefit more from BMT, but data from clinical trials is lacking. The decision to use hematopoietic stem cell support with dose intensive therapy should balance the estimated benefit against the risk of reinfusing tumor with autologous stem cells (marrow or peripheral blood), and the toxicity of graft-vs-host disease with allogeneic BMT. Regimens containing at least 2 active agents will probably be required for curative therapy. Alkylating agents (and radiation) are well-suited for use in dose-intensive combinations because, in general, they exhibit a steep, log-linear dose response relationship over an extended range, they lack cross-resistance, and do not readily induce high-grade resistance. Because of overlapping non-hematologic toxicities, high-dose combinations using more than 2 agents are possible only if significant (>20%) reductions from the single-agent MTD's are accepted. However, possible advantages of using multiple agents are synergism, and the increased probability of including the "best" agent(s). An alternative which might permit the use of more than two agents without dose reduction is administration of multiple courses of dose intensive therapy. Feasibility remains to be demonstrated, at least with maximally tolerated regimens, however, in the future the use of growth factors may further reduce toxicity and facilitate this approach.

C 058 HIGH DOSE CHEMOTHERAPY FOR TESTICULAR CANCER... FROM DESPERATION TREATMENT TO PRIMARY THERAPY, C. R. Nichols, Indiana University School of Medicine, Indianapolis, IN 46202. Testicular cancer is uniquely sensitive to chemotherapy and over 90% of patients with this disease are cured. However, patients with recurrence after primary therapy have a poor outcome. High dose chemotherapy with autologous bone marrow rescue in patients with recurrent testicular cancer is a subject of current investigations in Europe and the United States. At Indiana University, 29 patients were enrolled in a phase I/II trial of high dose carboplatin plus etoposide. All patients had failed at least two prior chemotherapy regimens for testicular cancer or had progressive disease on cisplatin. Sufficient bone marrow was harvested prior to chemotherapy for two courses of treatment. Carboplatin dose ranged from 900 mg/m^2 to 2000 mg/m^2 and was given with a fixed dose of etoposide at 1200 mg/m^2. Patients responding to the first course of treatment were given a second course of identical treatment with reinfusion of the remaining half of the cryopreserved marrow. Dose-limiting extramedullary toxicity of this regimen was hemorrhagic colitis and hepatic enzyme elevation. Ototoxicity, neurotoxicity, and renal toxicity were not dose-limiting. Medullary toxicity was severe with all patients developing granulocytopenic fevers and 5 patients developing thrombocytopenic hemorrhage. Four patients (14%) died during therapy. All therapy-related deaths were associated with uncontrolled infections. Fifteen patients (52%) exhibited response with 9 complete remissions (31%) and 6 partial remissions (21%). Response continues in 6 patients at 12+,14+,18+,27+,28+, and 30+ months.

These results, along with those obtained at other centers, are sufficiently encouraging to investigate the use of high-dose chemotherapy earlier in the course of treatment of testicular cancer. Trials through Indiana University and the Eastern Cooperative Oncology Group and at Memorial Sloan Kettering Hospital (New York) incorporate high dose chemotherapy plus etoposide into initial salvage chemotherapy or, in some settings, primary therapy of testicular cancer.

C 059 DOUBLE AUTOLOGOUS BONE MARROW TRANSPLANTATION IN SOLID TUMORS, Spitzer G., Dunphy F, Yau J, Dicke K, Huan S, LeMaistre C.F., Spinolo J.The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030. Most high dose therapies incorporate only a single cycle of therapy at the maximum tolerated dose. As a further strategy to enhance the response rate and progression-free survival of human tumors, we initiated eight years ago the principle of a double high dose therapy intensification using cyclophosphamide and etoposide as the initial important core of drugs. We have progressively escalated the dose of cyclophosphamide to the present level of 6mg/M^2, etoposide to 1500 mg/M^2 and introduced cisplatinum at doses presently between 165mg-180mg/M on each course. The second cycle has usually been repeated within 6 weeks of the initiation of the first cycle, to maintain the dose intensity over time. From these studies a number of important lessons have been learned. These drugs and intensities have been associated with rapidly reversible toxicities. Absence of any viral infection and rapid return in performance status allows the administration of the second high dose therapy within a short period upon hematological recovery from the first cycle. There has been no difference in morbidity or mortality between cycles of therapy and >80% of the patients have received the second cycle of therapy. Mortality (approximately 5%) has almost been purely infection related.

This approach of double high dose intensification has some theoretical advantages, including the potential to overcome some of the kinetic resistance of tumors with long cycling times, the administration of possibly more dose intensity for less toxicity and the introduction of noncross resistant combinations at high dose. To approach the last concept we have investigated mitoxantrone-thiotepa based combinations (which hopefully would be noncross resistant to CVP) in >60 patients with more resistant or advanced breast cancer. Because of the significant mucositis and the protracted hematopoietic recovery with the use of this regimen, patients receive a second transplant with this approach after a longer interval of 4-6 weeks. Impressive response rates have been obtained in resistant tumors with these drugs and potentially these combinations could form the second high dose intensification following CVP.

CONCLUSIONS:

With careful choice of drugs and dosages double autologous transplant can be regularly given to patients up to advanced years (60 years). Response rates have been impressive using double autologous transplant incorporating identical drugs in each course. Studies are needed to evaluate if two high dose possibly noncross resistant programs are superior to high dose therapies of identical drugs or a single high dose intensification. Many tumor models are suitable for testing, for instance, bulky disease metastatic breast cancer, bulky disease ovarian cancer, refractory relapses of lymphoma.

Late Additions

C 060 AUTOLOGOUS BONE MARROW TRANSPLANTATION (BMT) FOR MULTIPLE MYELOMA (MM). Bart Barlogie, Sundar Jagannath, Raymond Alexanian and Karel A. Dicke. From the University of Arkansas for Medical Sciences, Little Rock, Arkansas; the M.D. Anderson Cancer Center, Houston, Texas; and the University of Nebraska, Omaha, Nebraska.

MM is an incurable malignancy affecting primarily older patients (median age, 65 years) who survive a median of 2-3 years. Because of its predominantly terminal B cell phenotype, MM was selected as a model for unpurged autologous BMT in support of high-dose melphalan (HDM) and TBI, in order to evaluate the determinants of short and long-term prognosis. The median age of the 52 study patients was 55 years. Early mortality was almost 25% in the 21 patients with refractory MM contrasting with 3% among the 31 treated in first or later remission, usually induced with the VAD regimen. All patients with refractory disease and about half of those with sensitive MM experienced >75% tumor cytoreduction, although the frequency of true complete remission was 10% in the former and 25% in the latter group. The durations of relapse-free and overall survival after ABMT were only affected by pre-treatment serum beta 2-microglobulin (B2M) levels. Thus, the one-half of patients with low levels $\leq$ 2.5mg/L had a median relapse-free survival of 15 months and 85% two-year survivorship compared with median durations of 6 and 12 months for those with higher B2M levels ($p < .01$). Importantly, once B2M was accounted for, marrow-plasmacytosis (up to 30% in refractory MM), timing of ABMT for sensitive vs. refractory disease, and patient age were not significantly associated with prognosis. Management of post-BMT relapses included a variety of regimens including second BMT. Subsequent survival increased with the duration from initial BMT to post-BMT salvage therapy. We conclude that, if applied in remission, ABMT affords relatively safe administration of HDM/TBI even to older patients with MM, whose prognosis is mainly related to pre-B2M as a tumor burden-related parameter. Unless attributable to the re-establishment of benign monoclonal gammopathy, the relatively low incidence of complete remission calls for better pre-BMT cytoreduction with alternating VAD and HDM as 2 non-cross resistant regimens, possibly further intensification of the marrow-ablative regimen, and post-BMT interferon maintenance.

C 061 BONE MARROW TRANSPLANTATION FOR THALASSEMIA. THE EXPERIENCE OF PESARO.

Galimberti M., Lucarelli G, Polchi P., Angelucci E., Baronciani D., Durazzi SMT., Giardini C., Nicolini G.,

Divisione Ematologica e Centro Trapianto Midollo Osseo di Muraglia, Ospedale di Pesaro, Pesaro, Italy.
Today September 15, 1989.

We report here on up date of the study of consecutive of 222 pts with beta homozygous thalassemia ages 1-15 years (mean age 7.8 yrs) that received bone marrow transplant (BMT) from 212 HLA identical siblings and 10 identical parents from January 1983 and August 1988. All 222 patients received unmodified bone marrow. Initially six patients were prepared for the transplant with 16 mg/Kg of Busulfan (BU) followed by 200 mg/Kg of Cyclophosphamide (CY) while all the following 216 consecutive patients received BU 14 mg/Kg and CY 200 mg/Kg. One hundred and eighty-six patients (84%) are alive, 169 (78%) are event-free and 17 (8%) are alive with return of thalassemia, one to more than six year after the transplant. Twentyfour patients died of transplant related complications within 100 days and twelve died between 100 and 290 days post-transplant of infectious complications mainly associated with GVHD. In total there was failure of engraftment or rejection in 26 pts (10%). In this group of patients the Kaplan-Meier calculation of the probability of survival after the transplant is 82% and the probability of disease free survival is 74% at six and half years post-transplant.

Leukemia; Aplastic Anemia; Graft vs. Leukemia

C 100 MARROW TRANSPLANTATION (BMT) FOR HODGKIN'S DISEASE (HD) USING SINGLE OR SEQUENTIAL MYELOABLATIVE (MYAB) REGIMENS T. Ahmed, J. Ascensao, E. Feldman, J. Szyalyga, D. Ciavarella, D. Wuest, L. Helson, S. Biguzzi, P. McDonald, J. Perchick, J. Ayello, A. Mittelman, J. Nelson, S. Gulati, M. Coleman and Z. Arlin. New York Medical College, Valhalla, NY 10595. 45 pts with advanced HD participated in a trial of potentially MYAB chemotherapy. Therapy consisted of VP-16 1800 mg/m^2, BCNU 400 mg/m^2 and Cytoxan 5 G/m^2 (BEC-2) followed by BMT. Pts with resistant relapse or persistent disease were offered a second course of therapy with ThioTEPA 900 mg/m^2, Velban 0.4-0.6 mg/kg_2 Ara-C 3-6 G/m^2 (TAVE) or ThioTEPA 750 mg/m^2, mitoxantrone 40 mg/m^2 and carboplatinum (JM-8) 900 mg/m^2 (TMJ) and a second BMT (BMT2). There were 24 males, median KPS 80, and prior regimens pre BMT was 3. 10 pts with sensitive relapse had 1 BMT only following BEC-2, 7 are alive in CR. 7 pts are too early for BMT2, 2 died prior to BMT2, 3 refused BMT2, 4 were judged unfit and 2 progressed prior to BMT2. 11 of 17 pts who underwent BMT2 are alive and free of disease with no relapses after BMT2. Toxicity was tolerable with the BEC-2 regimen; there was 1 toxic death. Of 12 pts who had BMT2 after TAVE, 6 experienced toxic death. Toxicity with TMJ was much less and no toxic deaths were encounteredin the 5 pts so treated. Sequential BMT is tolerable and is associated with a high probability of disease free survival in pts with refractory relapse of HD, as do pts with sensitive relapses undergoing a single BMT with BEC-2.

C 101 INTENSIVE POST REMISSION CHEMOTHERAPY VERSUS UNPURGED AUTOLOGOUS BONE MARROW TRANSPLANTATION IN ADULT ANLL IN FIRST COMPLETE REMISSION, Emilio P. Alessandrino, Paolo Bernasconi, Mario Lazzarino, Daniela Caldera, Maurizio Bonfichi, Carlo Bernasconi. Division of Hematology, Policlinico S.Matteo, IRCCS, Pavia, Italy. In an attempt to compare two different modalities of intensive post remission therapy, 44 patients (pts) studied in our Institution affected with ANLL in first C.R. were submitted to different protocols within 6 months from the reached C.R.: 23 pts (group A) were treated by 4 courses of Cytarabine at the dosage of 3 g/m2 every 12 h. for 3 consecutive days by 3 h. i.v. infusion every 21 days, while in 21 pts (group B) intensification of remission was performed by a chemotherapeutic association (BCV: BCNU 800 mg/m2, Cytarabine 900 mg/m2 c.i. over 3 days, VP 16213 900 mg/m2 in 3 days) rescued by unpurged ABMT harvested within 8 weeks from C.R. We have now a significant follow up for both groups. The median remission duration was 21 months (group A) and 37 months (group B). DFS was 35 % at 46 months for the cytarabine group and 49.6 for the transplanted pts at 54 months. The median time to complete the treatment was 120 days (89-140 range) and 36 days (17-60 range) respectively. None of these pts died during the procedure; both regimens were associated with mild toxicity and comparable support requirement. In this study the BCV protocol induced a lower incidence of relapse and the median duration of treatment was significantly shorter.

C 102 INTERFERON THERAPY FOR PH1-POSITIVE CML PATIENTS RELAPSED AFTER T-CELL DEPLETED ALLOGENEIC BONE MARROW TRANSPLANTATION, William Arcese, Francesca R. Mauro, Giuliana Alimena, Maria Screnci, Maria R. De Cuia, Anna P. Iori, Francesco Lo Coco, Paola Fazi, Michele Cedrone and F. Mandelli, Department of Hematology, University of Rome, Italy. Eighteen CML pts with hematological (4 pts) or only cytogenetic (14 pts) relapse occurring after T-cell depleted allogeneic BMT have been treated with alpha 2b IFN (Schering, Essex, Italy) at a starting dose of $5x10^6$ IU/m^2, subcutaneously, three times a week. All 4 pts with hematological relapse achieved a long lasting hematological remission without reduction of BM Ph1+ cells. At time of starting IFN the median percentage of BM Ph1+ metaphases was 50% (range 9%-100%) for the 14 pts with cytogenetic relapse. Twelve (85.7%) out of these pts are alive with a median follow-up of 25 mos (range 20-37 mos) from the cytogenetic relapse and 33 mos (range 27-49 mos) from BMT. Six (43%) out of the 14 pts have progressed to hematological relapse and 8 pts (57%) are still in hematological remission with 2 pts achieving complete cytogenetic remission confirmed at molecular level by disappearance of the bcr rearranged band. IFN therapy can be considered a good alternative to conventional chemotherapy for transplanted CML pts with hematological relapse and the treatment of choice for pts with a persistent cytogenetic relapse occurring after T-cell depleted BMT. CNR-PFO CONTRACT No. 88.00793.44

C 103 EVALUATION OF REMISSION STATE AFTER BMT IN PATIENTS WITH CML, R. Arnold, C.R. Bartram, B. Heinze, D. Bunjes, T. Hoffmann, M. Wiesneth, B. Kubanek and H. Heimpel, Departments of Internal Medicine III, Paediatrics II, Occupational Medicine, and Transfusion Medicine, University of Ulm, D-7900 Ulm, FR Germany

17 patients were studied by standard cytogenetics and molecular genetic approaches (Southern blots and PCR analysis) after allogeneic bone marrow transplantation for detection of residual leukemia. The time of follow up ranged from 2 to 78 months after bmt. Eight out of 17 patients showed no evidence of residual disease by cytogenetic or Southern blot analysis of the breakpoint cluster region (BCR) gene. Further analysis by PCR technique revealed residual BCR/ABL mRNA in three of these eight patients. All three had received a T-cell depleted transplant. In five patients with a cytogenetic relapse and in four patients with a hematological relapse after bmt cytogenetic, Southern blots as well as PCR revealed congruent results. In conclusion, it remains to be seen if minimal residual leukemia demonstrated by PCR is associated with the later development of a leukemic relapse.

C 104 LECTIN-SEPARATED BMT FOR LEUKEMIA PATIENTS AFTER CONDITIONIG REGIMEN THAT INCLUDES THIOTEPA (TT). Franco Aversa, Yair Reisner*, Adelmo Terenzi, Alessandra Carotti, Pier G. Pelicci, Paolo Latini and Massimo F. Martelli. BMT Unit-Dpt. Hematology University of Perugia 06100, Italy,* Dpt. Biophysics Weizmann Institute Rehovot, Israel.

Our preliminary experience in a serie of 44 leukemia pts who received a lectin-separated BMT confirmed that a more aggressive conditioning regimen (15 Gy HFTBI, 120 mg/kg Cy and ATG) overcomes rejection but increases cardio-pulmonary system toxicity and so has a negative impact on overall survival. Reisner et al. reported that mice treated with 8Gy TBI and Cy manifested less donor-type chimeras than those who received TBI + BU or DMM or TT. Since it would, therefore, seem that the addition of these myeloablatives to the conditioning regimens should help overcome both rejection and leukemia relapse after a T-depleted BMT, we included 10 mg/kg TT in our cytoreductive protocol of 15Gy HFTBI, 120mg/kg Cy and ATG, in an effort to enhance the myeloablative effect without significantly increasing extramedullary toxicity. So far, 10 evaluable pts (5CML,5ALL,1AML-M6) have achieved primary engraftment, as proved by DNA sequence polymorphism analysis. The presence of residual disease was evaluated in the bone marrow of 5CML pts by enzymatic amplification (PCR) of the bcr-abl mRNA. As in none of the pts the bcr-abl mRNA was detectable, our conditioning regimen would seem to lead to a both more rapid engraftment and total eradication of the disease. However large series with adeguate follow-up would be required to evaluate leukemia relapse.

C 105 A RANDOMIZED STUDY OF CY-TBI vs BUS-CY + ALLO BMT FOR ANL IN FIRST CR. D. Blaise, J. Reiffers, D. Guyotat, J.L. Harousseau, N. Ifrah, M. Michallet, P. Bordigoni, E. Vilmer, M. Attal, A. Jouet and D. Maraninchi for the GEGMO - Marseille, FRANCE.

Allogeneic BMT is a highly efficient consolidation for patients with ANL in first CR with a 20 to 30% relapse rate (1). However long term survival probability in retrospective studies is not superior to 40 to 60%, mainly affected by transplantation toxicities and GVHD. GVHD prophylaxis is under intensive investigations, but BMT related mortality should also be decreased with performing BMT sooner, in less heavly treated patients, with less toxic conditioning regimens. Busulfan and Cytoxan association has been shown to have a good antileukemic activity with a good tolerance (2). We initiated a prospective randomized multicentric study, comparing effects of Bus-Cy vs Cy-TBI in ANL within 3 months after first CR. A two years interim analysis will be presented. On june 1989, 45 patients are already randomized (Bus-Cy : 24, Cy-TBI : 21). No significant differences existed among the 2 groups in terms of age (mean : 32), sex ratio (M/F : 27/18) ; FAB classification (M1-M3 : 65%, M4-M5 : 35%) and time to reach CR (median : 2 months (1-5)). No difference was seen in terms of toxicity, infection, GVHD and 6 months survival. Currently with a median follow-up of 11 months (2-18), the relapse rate does not differ between each arm.

(1) Appelbaum et al. Ann of Int. Med., 1984, 101, 581
(2) Tutschka P. et al. Blood, 1987, 70, 1382.

C 106 SEROPOSITIVITY TO A FEW HERPES VIRUSES INCREASES THE RISK OF LEUKEMIC RELAPSE. [1]Lennart Boström, [1]Olle Ringdén, [2]Niels Jacobsen, [3]Ferry Zwaan and [1]Bo Nilsson for the Leukaemia Working Party of the European Group for Bone marrow transplantation. The Bone Marrow Transplantation teams at [1]Huddinge Hospital, Stockholm, Sweden; [2]Copenhagen, Denmark; and [3]Leiden, the Netherlands.
Two hundred and ninety-four leukaemic recipients of HLA-identical bone marrow, surviving at least 90 days after bone marrow transplantation (BMT), were reported from 17 European BMT teams. Using a Cox bivariate regression analysis the following factors were associated with an increased risk of relapse: seropositivity for 0-2 different herpes viruses (compared to 3-4) in the recipient prior to BMT (p=0.002), cytogenetic abnormalities present at diagnosis (p=0.036), patients with leukaemia in later stages than 1st remission or 1st chronic phase (p<0.001), and patients without chronic myeloid leukaemia (CML) (p=0.026). In a Cox multivariate analysis seropositivity for 0-2 different herpes viruses prior to BMT (compared to 3-4) among the recipients (p=0.003), and patients with leukaemia transplanted beyond 1st remission or 1st chronic phase (p=0.003) were associated with relapse of leukaemia. In a Cox bivariate regression analysis the following factors were associated with poor patient survival: recipient seropositivity for CMV (p=0.01), high recipient age (p=0.03), serpositivity for 3-4 different herpes viruses (compared to 0-2) among the donors (p=0.04), low bone marrow cell dose (p=0.005), a previous grade II-IV acute graft-versus-host disease (GVHD) (p=0.001) and patients with leukaemia in later stages than 1st remission or 1st chronic phase (p=0.01). In multivariate analysis the following factors were associated with poor patient survival: recipient CMV seropositivity (p=0.0008), high recipient age (p=0.03), low bone marrow cell dose (p=0.007), grade II-IV acute GVHD (p=0.004) and patients with leukaemia beyond 1st remission or 1st chronic phase (p=0.004).
Conclusion: 1) Patients seropositive to 0-2 different herpes viruses and those beyond 1st remission or 1st chronic phase had an increased risk of relapse. 2) Poor patient survival was associated with CMV seropositive recipients, high recipient age, low bone marrow cell dose, acute GVHD and leukaemia beyond 1st remission or 1st chronic phase.

C 107 MARROW TRANSPLANTATION IN THE CHRONIC PHASE OF CHRONIC MYELOGENOUS LEUKEMIA
Buckner CD, Clift RA, Storb R, Hansen J, Thomas ED for the Seattle Marrow Transplant Team, Fred Hutchinson Cancer Research Center, Seattle, Washington, U. S. A.
Recent results of marrow transplantation were analysed to determine the optimal timing for patients in the chronic phase of chronic myelogenous leukemia (CML).
From August 1983 through October 1988, 78 patients were treated with a regimen of cyclophosphamide (120 mg/kg), fractionated total body irradiation (12.0 Gy) followed by methotrexate and cyclosporine. The 3-year probabilities of survival (S), leukemia-free survival (LFS) and relapse (R) were .78, .66 and .21. When the patients were categorized on the basis of the interval from diagnosis to transplant (DXTX) these statistics were as follows:

DXTX	N	S	LFS	R
≤ 1 year	49	.92	.77	.21
> 1 year	29	.48	.51	.22
p-value		.0001	.002	.64

Among the 49 patients transplanted within 1 year there were 2 deaths (one from cytomegalovirus interstitial pneumonia (IP) on day 880). Among the 29 patients transplanted after a longer delay there were 11 deaths (4 from IP, 1 from veno-occlusive disease of the liver and 2 each from other infections, leukemia, and complications of chronic graft-versus-host disease). The 3-year post-transplant survival probability for patients who relapse after transplantation is .44. The survival expectations for newly diagnosed patients are not reduced by immediate transplantation, even in the short term. Patients who have suitable donors should be transplanted as soon as possible after diagnosis.

C 108 ALTERNATIVE BONE MARROW DONORS FOR CHILDREN WITH APLASTIC ANEMIA, B. Camitta, J.T. Casper, J. Hunter, J. Menitove, 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). Fourteen children (ages 1-13, median 6 years) with SAA recieved a marrow transplant at our center using alternative donors (sibling 5, parent 5, unrelated 4). Conditioning for the first three patients included cyclophosphamide (CYCLO) ± irradiation, no T-depletion of the marrow, and methotrexate for GvHD prophylaxis. Subsequent patients received: CYCLO + cytosine arabinoside + TBI, 2 log monoclonal antibody T-cell depletion of the marrow, and methylprednisolone + cyclosporine for GVHD prophylaxis. Three patients failed to engraft and died; all had been heavily pretransfused; two had received T-depleted marrow. Eleven patients engrafted. Acute GvHD was > grade 2 in only one patient (non T depleted); this patient is the only one with severe chronic GvHD. Three engrafted patients died (pneumocystis pneumonia, day 127; systemic parainfluenza, day 116; severe venocclusive disease, day 56). The remaining eight children are alive 43+ to 2872+ days (median 752+). Donors for the survivors were: siblings 4, parents 1, unrelated 3. 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.

C 109 T-CELL DEPLETED BMT FOR SEVERE APLASTIC ANEMIA IN ADULTS. H Castro-Malaspina, B Childs, I Cunningham, N Flomenberg, J Young, N Kernan, L Mandell, RJ O'Reilly. Memorial Sloan-Kettering Cancer Center, New York, New York 10021.

Acute and chronic graft-versus host disease (GvHD) are important factors limiting the utility of unmodified bone marrow transplantation (BMT) in the treatment of aplastic anemia in adults. Nine patients have been entered in this pilot trial of allogeneic BMT using HLA-matched sibling marrow grafts depleted of T-cells by the soybean lectin agglutination and E-rosette depletion method. Pretransplant conditioning included TBI (1375-1500 cGy) with lung shielding +/- rib boost (600 cGy), followed by cyclophosphamide (60 mg/kg x 2). No GVHD prophylaxis was given. Rejection prophylaxis consisting of anti-thymocyte globulin (ATG) and methyl prednisolone was given in the early post-transplant period to recipients of male grafts. There were 6 male and 3 female patients with a median age of 25 years (range 16-44). Previous treatments included ATG in 2 patients, steroids in one, and transfusions alone in 6. One patient experienced graft rejection and died of an intracranial bleed 2 months post-transplant. Eight patients engrafted. One developed late graft failure of unclear etiology and died 3 months post transplant shortly after a marrow boost. Another developed late graft failure secondary to CMV, which resolved following therapy with gancyclovir and Ig G. These patients who developed graft failure had not received rib boost irradiation and had eye shields during TBI. No patient developed acute GvHD and none of the 6 evaluable for chronic GvHD has developed this complication. One patient, still fully engrafted, died 7 months post-transplant of complications of leukoencephalopathy likely secondary to large amounts of Amphoterecin B required in the post-ATG period for lung aspergillosis. Six patients are alive 1 to 52 months postransplant, with a median of 10 months. T-cell depleted allogeneic BMT appears to be an effective therapy for adults with severe aplastic anemia. It obviates acute and chronic GvHD. Graft failure may be prevented by preparation with a full dosage antileukemic regimen.

C 110 BONE MARROW TRANSPLANTATION FOR CHRONIC MYELOID LEUKEMIA IN FRANCE. FACTORS ASSOCIATED WITH RELAPSE. Agnès Devergie, Eliane Gluckman and the members of GEGMO. BMT Unit, St Louis Hospital, Paris, France.

281 patients with chronic myelogenous leukemia have been treated with allogeneic bone marrow transplantation. The median follow up time was 40 months. 170 patients were in 1st chronic phase, 14 were in 2nd chronic phase, 73 were in accelerated phase and 24 were in blastic crisis. The overall actuarial survival is 50 % at 5 years. In multivariate analyses, the probability of relapse was correlated with the phase of the disease, the method of total body irradiation, the T cell depletion of the marrow and the occurrence of a chronic GVH D. The probability of survival was better for patients with grade 0-1 GVH D than for patients with grade 2-4 GVH D. In contrast, the probability of disease free survival was significantly better for the patients who received a non T cell depleted marrow than for recipients of T cell depleted marrow. Interval between diagnosis and transplant, splenectomy before transplant, patient age and donor recipient sex match were not significantly associated with outcome. Bone marrow transplantation in first chronic phase with an HLA identical non T cell depleted marrow offers the better chance of prolonged leukemia free survival.

C 111 GENERATION AND EXPANSION OF LYMPHOKINE-ACTIVATED KILLER (LAK) CELLS FROM T-CELL DEPLETED HUMAN BONE MARROW, Drobyski W, Piaskowski V, Ash RC, Truitt RL, Departments of Pediatrics and Medicine, The Medical College of Wisconsin, Milwaukee, WI 53226.

Allogeneic bone marrow (BM) transplantation is thought to cure leukemia in part because of a graft-versus-leukemia (GVL) effect which is typically coexpressed with graft-versus-host disease (GVHD). T-cell depletion of the donor graft decreases the incidence and severity of GVHD, but often at the expense of GVL reactivity. Lymphokine activated killer (LAK) cells might be a potential therapeutic approach to augment the GVL reactivity of T-cell depleted (TCD) donor grafts. To study whether LAK activity in BM is compromised by T-depletion, we examined the effect of the T-cell specific MoAbs against CD3 and CD6 alone, or in combination, on the generation and expansion of LAK cells in recombinant interleukin-2 (rIL-2). TCD BM generated LAK activity equivalent to non-TCD BM after long term culture in rIL-2. Expansion of TCD BM cultures for four weeks yielded a predominant population of $CD3^-$ $NKH\text{-}1^+$ natural killer cells. Limiting dilution analysis revealed that depletion with CD3 and/or CD6 antibodies did not significantly alter the frequency of LAK cell precursors able to expand and mediate LAK activity. These results suggest that selective T-cell depletion can eliminate GVH reactive T-cells without complete loss of effector cells with potential to contribute to an antileukemia effect in vivo. The data also show that TCD BM is a potential source of LAK cells for adoptive immunotherapy.

C 112 DETECTION OF BCR/ABL MRNA USING AMPLIFICATION OF CDNA FOLLOWING BMT FOR CHRONIC MYELOGENOUS LEUKEMIA, Pamela Ely and Wesley J. Miller, Division of Hematology, University of Minnesota, Minneapolis, MN 55455

We have utilized the polymerase chain reaction (PCR) to sensitively detect persistence of the chronic myelogenous leukemia (CML) malignant clone and to study BCR/ABL mRNA splicing patterns following bone marrow transplantation (BMT). Thirteen of sixteen patients displayed persistent malignant cells during post-BMT clinical remission. Eleven patients with detectable malignant cells post-BMT remain in clinical remission while two patients had BCR/ABL mRNA detected 4 and 9 months prior to clinical relapse. Two patients have lost all evidence of BCR/ABL transcript at 1 year and 2 years respectively, while nine have shown persistence of the BCR/ABL transcript from 28 days to 3 years post-BMT. Intriguingly, mRNA splicing patterns changed in 5 patients following BMT with complete loss of mRNA containing BCR exon 3 (n=2) or new appearance of mRNA not containing BCR exon 3 (n=2). A single patient transiently lost evidence of BCR exon 3 expression while persistently expressing the BCR exon 2/ABL exon 2 splice. Our data suggest that the majority of patients harbor small numbers of malignant cells following transplantation and that such persistence may not inevitably predict clinical relapse. Complete elimination of the malignant CML clone post-BMT may rely on immunological mechanisms (e.g. graft-vs-leukemia.).

C 113 MARROW TRANSPLANTATION FOR ACUTE NON-LYMPHOCYTIC LEUKEMIA (ANLL); A PRELIMINARY REPORT FROM THE CHILDRENS CANCER STUDY GROUP (CCSG). Stephen A. Feig, Jonathan Buckley, Beatrice Lampkin, Mark Nesbit, Mark Krailo, and Denman Hammond. CCSG, Pasadena, CA 91101.

One hundred forty four children with ANLL in first remission were treated with bone marrow transplantation on two consecutive CCSG studies. All donors were HLA-identical siblings or relatives, matched for at least 5 of the A,B, and DR loci. Patients were conditioned with cyclophosphamide and total body irradiation. In the first study, CCG-251, graft-versus-host disease (GVHD) prophylaxis consisted of standard methotrexate for 100 days after transplantation. In an attempt to diminish the risk of GVHD and its sequelae, the subsequent study (CCG-213) used GVHD prophylaxis with short course methotrexate and 6 months of cyclosporine-A. The risk of GVHD and its fatal complications was successfully reduced by this regimen but an equivalent increase in relapse risk was observed.

	CCG-251	CCG-213
Follow-up after transplant	53m (24-72m)*	21m (3-44m)*
Actuarial 2-Year Survival	56% (45-67%)**	62% (45-77%)**
Actuarial 2-Year Event Free Survival	54% (43-65%)**	58% (44-72%)**
Actuarial 2-Year Relapse Risk	19% (9-29%)**	34% (19-49%)**
Actuarial 2-Year Other Risk	34% (22-46%)**	13% (4-22%)**

* median (range) ** (95% confidence interval)

C 114 LONG TERM REMISSION FOLLOWING SECOND MARROW TRANSPLANT FOR RELAPSED LEUKEMIA IN DONOR CELLS, Steven M. Fruchtman, Vesna Najfeld, Eileen Scigliano, Marsha Simon, Department of Hematology, Mount Sinai Medical Center, New York, NY 10029 and Collaborative Research, Inc., Waltham, MA.

The vast majority of relapses following marrow transplantation for acute leukemia occur in host cells. Rarely, relapsed leukemia has been reported in donor cells (Exp Hem 1986; 14:178), suggesting a transfer of leukemogenic material to these cells. Optimal management of these patients is unknown. The patient presented at age 5 with non B, non T ALL; WBC >100,000 cells/ul. Following 3rd remission and CNS leukemia, a BMT was performed from his HLA identical brother. The preparative regimen consisted of Busulfan 16mg/kg and Cyclophosphamide (Cy) 120mg/kg. Marrow relapse occurred six months later. He was reinduced with chemotherapy and underwent a second BMT from the same donor using hyperfractionated total body irradiation 1,225 rads and Cy 120mg/kg. The leukemic clone had no prior exposure to radiation. GVHD prophylaxis consisted of cyclosporine A and methotrexate. High molecular weight DNA was isolated from skin fibroblasts and bone marrow pre and post second BMT. Polymorphic probes were used to distinguish the genotypes of the patient and sibling donor. Genomic DNA prior to, and following, second BMT is of donor origin. Three years following second BMT there is no evidence of disease. Thus, second BMT can be done successfully with long term remissions by using innovative preparative regimens.

C 115 AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION (ABSCT) AFTER G-CSF COMBINED CONDITIONING FOR TREATMENT OF ACUTE MYELOGENOUS LEUKEMIA

Mine Harada, Shuichi Taniguchi, Shigeyoshi Makino, Koichi Akashi, Takanori Teshima, Yasushi Takamatsu, Tsunefumi Shibuya and Yoshiyuki Niho,
First Department of Internal Medicine, Kyusyu University, Fukuoka 812, Japan.

Three patients with AML were treated with high-dose busulfan (4mg/kg, days-7 to -4) and cytosine arabinoside ($6g/m^2$, days-3 and -2) followed by the infusion of autologous peripheral blood stem cells collected during hematologic recovery after remission induction or consolidation chemotherapy. Two days before the conditioning chemotherapy, intravenous infusion of 5mcg/kg of recombinant human G-CSF (Chugai Pharm., Tokyo) was started and continued until day-1. Patient 1 (29yo/F) received ABSCT at 1st relapse and is now surviving in remission for 5 mo. But she requires platelet support because of delayed recovery. Patient 2 received ABSCT at 3rd relapse and engraftment (PMN>500) has not yet obtained 3 weeks after ABSCT. ABSCT after G-CSF combined chemotherapy may be used as an alternative to remission reinduction therapy in relapse or early intensification in remission. More experience will be requiered to assess its efficacy.

C 116 ALPHA INTERFERON (IF) INDUCES CYTOGENETIC REMISSIONS IN PATIENTS WHO RELAPSE WITH CHRONIC MYELOGENOUS LEUKEMIA (CML) AFTER ALLO-GENEIC BONE MARROW TRANSPLANTATION (BMT). CS Higano, W Raskind, D Durnam, JW Singer, VA Medical Center, Fred Hutchinson Cancer Center, Univ of Wash., Seattle.

A significant number of patients (pts) develop clinical relapse of CML after allogeneic BMT. Second (2nd) BMT is an option for some pts but the results have not been encouraging. In this study, IF (Roferon) was administered to pts who relapsed with CML chronic phase after allogeneic BMT. Eleven pts were registered: 2 are ineleigible (blast crisis), 1 pt relapsed after 2nd BMT, 2 pts were transplanted in accelerated phase, 1 in BC, and 1 pt received T-depleted marrow. IF treatment began 0-36 mos after relapse at a dose of $3mU/M^2/d$ which was escalated by $1.5mU/M^2/d$ each month as tolerated to a maximum dose of $6mU/M^2/d$. After 1 year responding patients are maintained with IF $1.5mU/M^2$ 3x/wk. Pts have been treated for 3+,4,6+,8+,9,10, 11,19+,26+ mos. Two pts lost 5-10% body weight. Four pts maintain plt cts of 60-100K. Other toxicities include: mild-mod depression 3 pts, mental clouding 2 pts, paresthesias 3 pts, TIA-like episodes 1 pt. Five of 9 pts remain on study; 1 pt is too early to evaluate; 4 pts have had major sustained cytogenetic responses determined by analyses of generally at least 25 marrow metaphases for Ph every 3 mos: 1 pt had a decrease from 100% to 20% Ph+ metaphases (Ph+m) after 3 months of IF, 1 pt who relapsed after a 2nd BMT decreased from 100% to 30% Ph+m for 2 consecutive studies, 1 pt decreased from 94% to 2% Ph+m (<10% Ph+ for 21 mos), and 1 pt decreased from 100% to 0% Ph+m after developing pancytopenia following 9 days of IF. In 2 of 3 responders in whom there was a donor sex difference, Ph- cells were donor while in the 3rd pt they were host. Four pts did not respond after 9-12 mos of IF. After BMT, pts are less tolerant of standard CML treatment doses of IF, yet 4 of 8 response evaluable pts achieved durable cytogenetic and hematologic remissions. Further studies should address whether the addition of prophylactic IF to BMT in pts with high risk CML might decrease the relapse rate.

C 117 THE DRUG SENSITIVITY OF BONE MARROW GRAFT OCCULT CLONOGENIC LEUKEMIA (CFU-L) PREDICTS RELAPSE AFTER AUTOLOGOUS MARROW TRANSPLANTATION (ABMT). R.J. Jones, S.D. Rowley, C.B. Miller. The Johns Hopkins Oncology Center, Baltimore, MD 21205

We prospectively studied 30 consecutive patients (pts) with AML in complete remission (CR) who underwent 4-hydroperoxycyclophosphamide (4HC)-purged ABMT after preparation with busulfan and cytoxan, between 1/8/88 and 2/28/89. We attempted to detect and determine the biologic significance of occult CFU-L in the pts. Pts had cells removed from the harvested marrow grafts prior to clinical purging. T-cell depleted marrow mononuclear cells were incubated with graded concentrations of 4HC and cultured in methylcellulose. CFU-L sensitivity to 4HC was determined by the slope of the dose-response curve obtained by plotting the fractional survival (log) of CFU-L versus 4HC concentration. Individual pt CFU-GM sensitivity to 4HC was determined as a control. CFU-L were cultured from 26 pts. CFU-L sensitivity to 4HC was ≥ 0.1 in 17 pts (sensitive) and < 0.1 in 9 pts (resistant). The median CFU-L sensitivity to 4HC was 0.12 in the pts with sensitive AML versus 0.06 in resistant pts ($p<0.0001$), while there was no difference in the median CFU-GM sensitivity to 4HC between the 2 groups (0.039 vs 0.037, $p=0.8$). Actuarial disease-free survival was 82% in the sensitive group versus 17% in the resistant group at 18 months ($p=0.009$). In the sensitive pts, the actuarial relapse rate was 0% compared to 82% in the resistant group ($p=0.0001$). CFU-L can be detected in the majority of pts with AML in CR at the time of ABMT, and the in vitro drug sensitivity of the CFU-L predicts the pts' relapse-free survival after ABMT. The correlation is independent of CR number. It is likely that the sensitivity of occult CFU-L predicts the effectiveness of both the purging and the cytoxan-containing preparative regimen.

C 118 TUMORICIDAL ACTIVITY OF MONOCYTES AFTER BONE MARROW TRANSPLANTATION, Hans-G. Klingemann, Fred Kohn and Gordon L. Phillips, Leukemia/Bone Marrow Transplant Program of B.C. and Terry Fox Laboratory, Vancouver, B.C. Canada.

Our laboratory is interested in developing new strategies to eliminate residual malignant disease after bone marrow transplantation (BMT). One aspect focusses on the ex vivo activation of monocytes (MO) to become cytotoxic towards malignant cells. We tested purified MO (Percoll separation) from 20 patients transplanted for acute and chronic leukemia at various times after BMT to determine if they could develop tumoricidal activity against tumor necrosis factor (TNF) insensitive (T 24 human bladder carcinoma) and TNF sensitive cell lines (WEHI 164 mouse fibrosarcoma) after incubation with different stimuli i.e. LPS, and recombinant IL-2, α-IFN, γ-IFN, M-CSF, and GM-CSF. The results were compared with those obtained from 6 normal donors. Using a standard ^{51}Cr release assay, killing of T24 cells by patient MO was normal after stimulation with IL-2. No other cytokine induced MO killing of T24 cells. WEHI cells were lysed only after stimulation of MO with LPS or a combination of γ-IFN and GM-CSF. This lysis could be blocked by an antibody directed against TNF-α. As with T24, MO from BMT patients reacted normally. Northern analysis showed that only LPS or the combination of γ-IFN/GM-CSF induced gene expression of TNF-α in normal MO. These results indicate that MO from BMT patients are fully functional with regard to tumor cell killing, even at a time (<4 months post BMT) when lymphocyte function is highly impaired. Attempts to develop strategies to use cytokine activated MO to overcome residual disease after BMT are ongoing.

C 119 A COMPARISON OF THERAPEUTIC EFFICACY BETWEEN AUTOLOGOUS BLOOD STEM CELL (ABSCT) AND BONE MARROW TRANSPLANTATION (ABMT) IN 43 PATIENTS WITH FIRST REMISSION AML, Martin Körbling, Bernd Dörken, Rainer Haas, Anthony D. Ho, Rolf Holle, Werner Hunstein, Department Internal Medicine V and Inst. Med. Biometrics, Heidelberg University, 6900 Heidelberg, FRG. We undertook this study to compare autologous transpl. of blood derived from marrow derived stem cells in the treatment of AML pts in first CR. We treated 43 pts with TBI (12.1-16.7 Gy) and CY (200 mg/kg) and monitored them for up to 29 mo (ABSCT) and 55 mo (ABMT). 20 pts received their own blood stem cells, 23 pts received a marrow autograft purged of residual leukemic cells by means of Mafosfamide. Except for median age (41, ABSCT, versus 33, ABMT, $p<0.05$), both patient subgroups showed no significant differences regarding sex, FAB subclassification, WBC at diagnosis and duration of CR preceding transplant (3.5 mo, ABSCT, versus 4 mo, ABMT). The median number of MNC/kg transfused was 80.0×10^7 (ABSCT) versus 4.7×10^7 (ABMT) or 2.35×10^4 CFU-GM/kg versus 0.14×10^4. WBC engraftment occurred earlier in patients who received blood stem cells (median time to reach 1000 WBC/ul 10 days, ABSCT, versus 28 days, ABMT, $p<0.001$). There was no significant difference in platelet reconstitution. Recipients of ABSCT had shorter hospital stays (median time 45 days, ABSCT, versus 73 days, ABMT, $p<0.001$). Whereas one ABMT patient died of transplant related complications, there was not one mortality amongst the ABSCT patients. The probability of disease free survival (DFS) was estimated to be 36% in the ABSCT group (median follow-up 14.3 mo) versus 59% in the ABMT group (median follow-up 31 mo), although statistically not different. We evaluated the following potential prognostic factors like sex, age, WBC count, duration of CR preceding transplant for their influence on DFS. Except for FAB subclassification M5, none of these factors was found to be associated with a poor prognosis.

C 120 MINIMAL RESIDUAL LEUKEMIA IN CHRONIC MYLOID LEUKEMIA PATIENTS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION :COMPARISON BETWEEN CY+TBI,HIGH DOSE ARA-C+CY+TBI, BUSULFAN+CY CONDITIONING. Kosei Matsue, Shuki Mizutani, Yoshinori Takemoto, Kouichi Miyamura, Mine Harada, and Hirofumi Teshima, Department of Hematology, Kameda General Hospital, Department of Viology, National Children's Medical Center, 2nd Department of Internal Medicine, Hyogo Medical College, 1st Department of Internal Medicine, Nagoya University, 1st Department of Internal Medicine, Kyushu University, and Center for Adult Disease of Ohsaka, JAPAN

Since enzymatic amplification genomic or cDNA target sequence by polymerase chain reaction (PCR) allows identification of neoplastic cells at frequencies 1 in 10^6, PCR has been used in the detection of minimal residual disease. Philadelphia chromosome (Phl) in chronic myeloid leukemia (CML) results in fusion of the bcr geneand c-abl oncogene, this new bcr/abl fusion gene could be a characteristic marker of leukemicclone. In this study, we investigated the remission state of 17 Phl positive CML patients in complete remission after allogeneic bone marrow transplantation (BMT) using PCR technique. All patients showed clinical and hematological remission following BMT and also showed cytogenetic remission that indicated by the absence of Phl. After the generation of cDNA from RNA samples of peripheral blood of BMT recipients, cDNA was amplified with oligomers detecting bcr/abl fragment and analysed by Southern blotting using bcr probe. All cases had a positive internal abl control. Residual bcr/abl mRNA was detected in 9 patients studied after marrow transplantation. In patients who received CY+TBI as a conditioning regimen, 3 out of 3 patient (100%) had positive bcr/abl mRNA by PCR. On the other hand, patients who received high dose Ara-C+CY+TBI and busulfan+CY as a conditioning regimen, bcr/abl mRNA was detected 2 out of 8 patients (25%) and 3 out of 5 patients (60%), respectively. There was no association between the presence or absence of GVHD and the detection of bcr/abl mRNA. These data provide us a important information for designing a new therapeutic stategies.

C 121 CYCLOSPORIN AND RELAPSE RATE IN PATIENTS TRANSPLANTED FOR CHRONIC MYELOID LEUKAEMIA (CML), H.A. Messner,J.M. Meharchand, M.D. Minden, G.M. Fyles, J.E. Curtis, Ontario Cancer Institute and University of Toronto.58 patients with chronic myeloid leukaemia were transplanted between 1/1/83 and 15/3/89. All patients were prepared with 100/mg/m2/dx5 ARA-C, 60mg/kg/day x2 of CTX and TBI at 500 cGy delivered as a single fraction with a dose rate of 42-91 cGy/min. As outlined in the table 3 consecutive protocols for GVHD prophylaxis were used. in the Table.

Group	n	GVHD Prophylaxis	GVHD Treatment	Relapse
1	24	MTX, PRED	CyA	2
2	17	MTX, CyA x 6 months	PRED	10
3	11	MTX, CyA x 2 months	PRED	0

The groups differed significantly in their relapse rate. Patients receiving a six month course of cyclosporin (CyA) had a significantly higher relapse rate than patients without CyA or patients that received CyA for two months. Four of the ten patients in the second group lost their Philadelphia chromosome after discontinuation of CyA. Survival of the first group at five years is 46%, the survival of the second group at three years is 80%, the survival of the third group at eighteen months is 75%.

C 122 GVHD IN CHILDREN:A REPORT FROM THE AIEOP BMT GROUP,F.Porta,L.Nespoli,C.Uderzo,V.Con ter,R.Miniero,W.Arcese,G.Dini,P.Colleselli,S.Bagnulo,M.Andolina,P.Paolucci,A.Pession, R.Rondelli,Italy.
Out of 363 children registered by the BMT Group of the Italian Association of Pediatric Hema tology Oncology (AIEOP) who underwent BMT,95(38F,52M)received allogeneic BMT from HLA id. do nors:78 pts with malignant hematological disorders(40 ALL,26 AnLL,10 CML,2 NHL),5 NB and 12 by other diseases.All but 7 pts received Cy A.Acute GVHD was present in 63%(34 g.I,18 II,7 III,4 IV);chronic GVHD in 18% (76% over 80% Karnofsky score).Donor recipient sex pairs and recipient age did not influence the overall incidence of acute and chronic GVHD,while older donors were associated with a higher frequency of aGVHD and cGVHD.Overall survival at 2 yrs post BMT was 55%.SUR at 2 yrs post BMT of pts with aGVHD was 53%,as compared to 54% of those who did not present aGVHD.SUR at 2 yrs post BMT of pts presenting cGVHD was 83%,as opposed to 47% of those who did not.When data were analyzed in terms of DFS at 2 yrs post BMT,figures were similar to those relative to SUR.Our data point out that children receiving Cy A present:1)an incidence of aGVHD comparable to adults,but remarkably less severe;2)a very low incidence and severity of cGVHD.Therefore,low incidence and severity of GVHD may not exert a negative impact on SUR and DFS.

C 123 TREATMENT OF AQUIRED APLASTIC ANEMIA IN CHILDREN:BONE MARROW TRANSPLANTATION OR IMMUNOSUPPRESSIVE THERAPY? E. Fred Saunders, Daniel S. Halperin and Melvin H. Freedman. Division of Hematology-Oncology, Hospital for Sick Children, Toronto, Ontario. Canada.
Two equally effective therapies are available in adults with severe aquired aplastic anemia (SAA), either immunosuppressive therapy (IST) or BMT. To compare these treatments in children we reviewed 36 patients (age 0.9 to 17.5 years, median 7.5 years) diagnosed between 1977 and 1987. BMT was performed in 16 patients (14 HLA-MLR matched, 2 partially mismatched) using conditioning of cyclophosphamide ± horse antithymocyte globulin (ATG) or rabbit antithymocyte serum (RATS) ± procarbazine ± TBI. Twelve patients were given 10 to 14 day courses of ATG or RATS ± high dose methylprednisolone. Eight patients diagnosed prior to introduction of IST received supportive care (SC) only. Patient characteristics, clinical manifestations, and time to initiation of therapy were similar in each group. Both mismatched BMT failed. Normal hematopoiesis was achieved in 11 patients (79%) with matched BMT, 3 patients (25%) with IST, and 1 patient (12%) with SC. Partial remission (absence of transfusion requirements) was achieved additionally in 1 patient with IST and 1 with SC. Survival was 79% for matched BMT (median follow-up 52 months), 42% for IST (median 15 mo) and 25% for SC (median 1.5 mo). Time to hematologic recovery (Hb 100 g/L, ANC 0.5×10^9/L, platelets 50×10^9/L) was significantly shorter in BMT than IST (median 38 vs 129 days, $P < 0.005$). These data support the superiority of BMT over IST in children with SAA. IST should be reserved for children without a matched donor.

C 124 REDUCING THE RISK OF RELAPSE AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) FOR HEMATOLOGIC MALIGNANCY-BuCyVP, William P. Vaughan, David Dennison, Lynell Klassen, Peter Coccia, Sarah Strandjord, James Armitage, Departments of Internal Medicine and Pediatrics, University of Nebraska Medical Center, Omaha, NE 68105

BMT currently offers the best chance of cure for patients (pts) who fail conventional chemotherapy for hematologic malignancy. Regimens using total body irradiation (TBI), have been most widely used but dose escalation is limited by extramedullary toxicity, and relapses are frequent except in very good risk pts. Between 8/86 and 10/88 we treated 12 pts with hematologic malignancy at high risk for relapse after BMT with an intensified chemotherapy only preparative regimen (Bu4mg/kg/d x4,Cy60mg/kg/d x2,VP16 60mg/kgx1). Twelve pts with high risk of relapse disease had previously undergone BMT using a TBI based regimen in this institution and served as controls.

REGIMEN	AGE(MEDIAN)yrs	DISEASE	ALIVE IN CR	TOXIC DEATHS	RELAPSES
BuCyVP	1-46(27.5)	AML=5,LYMPHOMA=4,ALL=2,CML=1	3(25%) 74,94,156wks	5(42%)	4(33%)
TBI/Ara/Cy	2-40(14)	AML=5,LYMPHOMA=3,ALL=3,CML=1	2(17%) 178,193wks	4(33%)	6(50%)

Three BuCyVP pts are alive in CR at 74,94 and 154 wks. They had refractory AML (2pts) and refractory NHL (1pt).Non-hematologic toxicity of BuCyVP consisted of skin rash and mucositis. The 5 toxic deaths were due to graft failure (1) and aspergillosis and interstitial pneumonia (2 each). Three pts relapsed and 1 had persistent disease after BuCyVP as compared to 4 relapses and 2 persistent disease after TBI. These data suggest that chemotherapy-only regimens produce results as good or better than TBI based regimens for high risk patients. Further dose escalation may be more easily accomplished with chemotherapy-only protocols.

C 125 CYTOLITIC FUNCTION OF CLONABLE T CELLS AFTER HUMAN BONE MARROW TRANSPLANTATION. Andrea Velardi, *Paola Varese, *Carlo E.Grossi, Nicola Albi, Chiara Dembech, *Maria C.Mingari, *Lorenzo Moretta, Franco Aversa and Massimo F.Martelli. BMT Unit-Dpt. Hematology University of Perugia, 06100 Italy. *Cancer Institute, Genova, Italy.

Aim of this study has been to evaluate the efficiency of non-specific T-cell mediated cytotoxicity against tumor-cell targets (LAK effect) during the late (>5 months) reconstitution phase after T cell-depleted allogeneic BMT. These functions may contribute to the control of leukemia relapse in the post-grafting phase (GvL effect). Since cytotoxic functions against tumor cells are sustained by IL2, we also investigated the ability of post-BMT T cells to produce IL2. Cytotoxic functions and IL2 production have been investigated at the clonal level and phenotypic analysis have been conducted on both precursor cells and clonal cultures. More than 200 T-cell clones from six long-term BMT recipients were generated in presence of exogenous IL2 and compared to 60 T-cell clones derived from 2 normal controls. Almost the totality of CD8+ clonal cultures from BMT recipients expressed cytolytic activity in a LDCC assay. A higher proportion of BMT-recipient-derived cytolytic clones were able to mediate LAK activity in comparison to control clones (28% vs 4%, $p<0.05$). However, T-cell clones from BMT-recipients, as opposed to control clones, were largely incapable of producing IL2. Given the high proportions of post-BMT circulating CD8+ T cells, it appears that, in long-term BMT-recipients, precursors of LAK effectors are present at above normal levels.

C 126 TOTAL BODY RADIATION FOR BONE MARROW TRANSPLANTATION. H.M. Vriesendorp, Johns Hopkins Oncology Center, Baltimore, MD 21205

Total body irradiation of human patients requires a large field and extended treatment distance. The tissue inhomogeneities and the irregular contour of the human body, as well as off-axis-beam factors will cause dose inhomogeneities. At best, moderately homogenous TBI can be obtained in man with a ratio between highest and lowest absorbed dose of between 1.1 and 1.2. Many permutations of TBI exist in dose, dose rate, fraction size, treatment time, patient orientation, partial body shields and others. Optimalization of TBI for bone marrow transplantation is best performed on the required immunosuppression, as an endpoint. Models predicting prospectively in vivo side effects of TBI remain to be formulated and tested. At the low dose rates of TBI (± 10 cGy/min), the following radiation survival curve parameters were estimated from previously published patient or large animal TBI studies. The single hit, multiple target model was used as it was more revealing than alpha/beta ratios from the linear quadratic model. The estimates of extrapolation numbers (n) and D_o for various tissues were:

	n	(Gy)
Bone marrow	1.1	0.70
Lung	6	1.35
Intestinal tract	10	1.35
Immune system	1.25	1.50

These parameters appeared to be predictive in dog and miniature swine studies and were compatible with retrospective analyses in human patients. The current recommendation for a generic TBI schedule in man, is a schedule that lasts for 3-4 days, with one fraction a day, lung shielding on one day and, a total dose of 12 Gy and the highest possible dose rate. Modifications to obtain an optimal TBI schedule, for a given patient will depend on type of bone marrow donor available, type of bone marrow purification and GVH prophylaxis and type of recipient disorder.

C 127 COMPARISON OF INTENSIVE CONSOLIDATION CHEMOTHERAPY, AUTOLOGOUS AND ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) IN ADULT ACUTE MYELOID LEUKEMIA (AML). THE LEIDEN EXPERIENCE.

R. Willemze, W.E. Fibbe, D.J. Richel, J.C. Kluin-Nelemans, and F.E. Zwaan. Department of Hematology, Leiden University Medical Center, Leiden, The Netherlands.

The median duration of first CR in patients with AML between 15 and 65 years of age is 12 months with only 20-25% disease-free survivors at 3-4 years (LAM-5,LAM-6 EORTC trials). Further intensification of consolidation regimens has been advocated in order to increase CR duration and DFS. High dose cytosine arabinoside (HD Ara-C) containing protocols and autologous bone marrow transplantation (A-BMT) has been introduced recently at the Leiden University Hospital for those patients who lack an HLA identical family donor. From 1985 till 1989 95 patients with primary and secondary AML in first remission received either an allo-BMT (30), or one or two courses of HD Ara-C/m-Amsa alone (44) or followed by HD cyclophosphamide/TBI and A-BMT (21). The median age of the whole group was 41 years (range 16-62 years), for A-BMT: 40 yrs (25-62 yrs) and for allo-BMT 32 yrs (16-49 yrs). Two patients died due to overwhelming infection and ARDS during consolidation chemotherapy. The median interval between the last consolidation course and BMT was 10 weeks (range 6-20 weeks). One patient relapsed just before A-BMT, and another patient just before allo-BMT. One patient died after A-BMT due to non-engraftment. At present the median DFS-duration for the HD Ara-C consolidation group as well as for the HD Ara-C/m-AMSA + A-BMT group is 18 months and for the allo-BMT group 8 months. Twenty-eight patients (67%) who received only HD Ara-C relapsed, compared to 9 of 21 patients who received A-BMT (44%) and only 6 of 30 patients who received an allo-BMT (20%). With a median follow-up of 15 months the actuarial DFS rate at 3 yrs is 25% of the HD Ara-C group, 40% of the HD Ara-C + A-BMT group and 38 % of the allo-BMT group. Conclusion: DFS curves for HD Ara-C/m-AMSA and HD Ara-C/m-AMSA + ABMT overlap up to 24 months. At present it remains to be seen whether intensive consolidation chemotherapy and ABMT or allo-BMT, will have a different impact on the long term DFS.

C 128 INTRODUCTION OF THE BACTERIAL β-GALACTOSIDASE (lac-Z) GENE AND THE NEOMYCINE RESISTANCE (neoR) GENE IN THE CELL LINE LT12 FROM THE BNML RAT LEUKEMIA MODEL.

Ying Yan* Anton C.M. Martens, P. Jan Hendrikx, Dinko Valerio, Anton Hagenbeek and Dirk W. van Bekkum. Radiobiological Institute TNO, Rijswijk, The Netherlands. *: Hua Shan Hospital, Shanghai Medical University, Shanghai, P.R. China.

A subline (LT12) from the BN acute myelocytic leukemia (BNML) was infected with a retroviral vector (BAG) carrying both the neoR gene and the lac-Z gene (Price et al., 1987). Several LT12-neo-lac-Z (LT12nl) clones were produced in an agar colony system containing G418 (200 μg/ml). Individual colonies were picked and expanded in liquid cultures containing G418 (600 μg/ml). The expression of lac-Z was detected by FACS-FDG measurements (Nolan et al., 1988) and by cytochemical X-gal staining. The fraction of lac-Z expressing cells was shown to be stable in individual cell lines, however, lac-Z expression varried between different LT12 cell lines. LT12 cell lines with the highest level of lac-Z expression were FACS-FDG sorted and cultured to generate a marked cell line from the BNML model. Results will be presented on the in vivo growth characteristics of this cell line and on the sensitivity of this technique for determining the mechanism of relapse from minimal residual disease (MRD).

J. Price et al., Proc Natl Acad Sci USA 84:156-160 (1987)
G.P. Nolan et al., Proc Natl Acad Sci USA 85:2603-2607 (1988

C 129 ALLOGENEIC BONE MARROW TRANSPLANTATION FOR LEUKEMIA IN EUROPE: REGIONAL DIFFERENCES

F.E. Zwaan, A. Gratwohl, J. Hermans, A.J. Barrett, P. Ernst, F. Frassoni, G. Gahrton, A. Granena, H.J. Kolb, H.P. Prentice, J.P. Vernant. Report from the Working Party Leukemia of the European Group for Bone Marrow Transplantation.

1'904 allogeneic HLA-identical sibling donor bone marrow transplants performed in 52 European centers between 1979 and 1986 and reported to the EBMT leukemia registry were analyzed depending on the geographical place of the transplant. Patients were grouped in six regions: United Kingdom, Scandinavia, Benelux, France, Central Europe, Southern Europe. There are significant differences within and between regions. They concern the patient population and outcome. The relative proportion of the three major disease categories, stage and subtype of the diseases, GvHD prevention methods, donor recipient sex combinations, age of the patient, year of the transplant and the time intervals from diagnosis to transplant, from diagnosis to first complete remission for acute leukemia and the time from first complete remission to the transplant vary from region to region. The analysis shows that leukemia free survival differs from region to region. This difference in leukemia free survival is due to a significant difference in relapse incidence. This influence of region is confirmed in a multivariate analysis and is independent of the other factors known to affect outcome. Transplant related mortality is not different from region to region. The reasons for these differences cannot be explained by the data in the registry. We conclude from these data, that regional factors have to be considered when BMT data are compared and we postulate that pretransplant factors probably affect outcome more than previously thought.

Engraftment; GvHD; Histocompatibility

C 200 INCUBATION OF BONE MARROW WITH VINCRISTINE AND METHYLPREDNISOLONE AND GVH PROFILAXIS WITH CY-A AND I.V. IMMUNOGLOBULINS: A MODEL FOR MISMATCHED BMT, Marino Andolina, Ebriberto Agosti, Andrea de Manzini, John L. Millar, R.L. Powles, Istituto per l'Infanzia Trieste, Italy and the Royal Marsden Hospital, Sutton, U.K.

Since 1986, 17 children have undergone a mismatched (2 or 3 loci) BMT for end stage leukemia (resistant relapses, relapses after a 1st BMT), CML in chronic phase (2), SAA (1), RAEB (1) CGD (1), Lesch Nyhan syndrome (1). The donors were haploidentical parents and siblings, and their marrows were incubated with vincristine (1 mcg/ml) and methylprednisolone (3 mg/ml)for 30'. The patients were treated with CyA and intravenous Ig, 100 mg/kg every day for 15 days and then every other day for 1-12 months. The patients with acute leukemia engrafted rapidly The aGVHD was absent or mild in all but two patients. Three patients still survive at 34, 21 and 7 months from the BMT. The CML patients survive after an autologous engraftment, in one case Ph negative for 30 months. The RAEB engrafted only after a second BMT, with another donor; the GVHD was severe (death of candida pneumonia). The patients with SAA and Lesch Nyhan died before the take (VOD and cardiac failure). The patient with CGD had a trouble_ some take of the donor marrow and eventually died of cardiac failure.

As a conclusion a mismatched BMT resulted rather easy to perform in heavily treated patients and very hard in patients not immunecompromised.

C 201 **T CELL DEPLETION OF BONE MARROW: COMPARISON OF TWO ANTI-CD5 MONOCLONAL ANTIBODIES AND TWO INTENSIVE CONDITIONING REGIMENS. J Antin, B Smith, B Bierer, E Guinan, J Ferrara, N Tarbell, R Macklis, M Provost, J Rappeport, S Burakoff, H Weinstein. Harvard Medical School, Boston, MA. T cell depletion is an effective means of preventing GvHD, but the best way to minimize GvHD, graft failure, and leukemic relapse is uncertain. We treated 68 leukemic or myelodysplastic patients at high risk of acute GvHD with one of two protocols using anti-CD5 MoAb's: 1) 20 HLA/MLC matched and 10 mismatched patients received ara-c 500 mg/m^2/d x 7 days, cytoxan 1800 mg/m^2/d x 2, TBI 12-14 Gy (5-10 cGy/min) in 8 fractions followed by marrow treated with anti-Leu1 (Becton-Dickinson) plus complement and in some patients 4 doses of MTX; 2) 24 matched and 14 mismatched patients received ara-c 3 g/m^2 x 6, cytoxan 1800 mg/m^2 x 2, and TBI 14 Gy (10cGy/min) in 8 fractions followed by ST1-immunotoxin (anti-CD5 F(ab')$_2$ conjugated to ricin A chain; Sanofi) treated marrow and no additional GvHD prophylaxis.**

TREATMENT	CD3+ CELLS INFUSED (x10^5/kg) *mean ± sd*	AGvHD (number with Grade 0-1/2/3/4) match	mismatch	GRAFT FAILURE match	mismatch	DAY TO PMN ≥500/μL *median (range)* match	mismatch
anti-Leu1 + C'	3.7 ± 4.2	13/2/2/0	3/2/3/1	1/19	2/10	19(15-36)	22(17-27)
ST1-IT	0.9 ± 0.8	18/4/2/0	8/1/2/3	2/24	1/14	19(15-33)	18(12-67)

Neither protocol depleted the marrow of CD5$^-$ T cells or NK cells. Similar degrees of acute GvHD control and graft failure were observed in both groups, but graft failure occurred primarily in patients with myelodysplasia and marrow fibrosis (p=0.0008). We conclude that selective CD5+ T cell depletion in association with an intensive conditioning regimen results in good control of acute GvHD and a small risk of graft failure in most patients. Because of the heterogeneous diagnoses and stages, risk of leukemic relapse could not be assessed, but it is noteworthy that 8/11 patients with ANLL in CR1 are alive and well and only 1 relapsed. Further studies are required to determine whether this type of enhanced conditioning regimen and selective CD5+ T cell depletion increase survival.

C 202 A DECREASED INCIDENCE OF ACUTE GRAFT-VERSUS-HOST DISEASE IN HLA MISMATCHED BONE MARROW RECIPIENTS USING A COMBINATION OF METHOTREXATE + CYCLOSPORINE. Johan Aschan, Olle Ringdén, Ulla Persson, Per Ljungman, Erna Möller, Thomas Paulin & Jan Tollemar. Departments of Transplantation Surgery, Clinical Immunology and Medicine, Karolinska Institute, Huddinge Hospital, Stockholm, Sweden.

Since 1975 we have performed bone marrow transplantation (BMT) with 33 mismatched related donors and 4 unrelated donors. Between 1975-85, monotherapy was used as graft-versus-host disease (GVHD) prophylaxis with 5 patients receiving methotrexate (MTX), and 10 receiving cyclosporine A (CSA). Since 1985, combination therapy with MTX+CSA has been used in 22 patients. There was one antigen mismatch from 27 related donors (18 siblings, 8 parents and 1 son); two antigens mismatch from 3 donors (2 siblings and 1 uncle) and three antigens mismatch from 1 parent. Two donors were HLA identical parents. Three of the unrelated donors were HLA identical and 1 had a one antigen mismatch. HLA matching was similar in patients with monotherapy or MTX+CSA. However, there were more low-risk patients (non-malignant diseases, acute leukemia in 1st remission or chronic myeloid leukemia in chronic phase) in the combination therapy group (57%) than in the monotherapy group (33%).

Results. With MTX+CSA the incidence of grade II-IV acute GVHD decreased to 38% from 73% (p=0.004); deaths due to GVHD decreased to 18% from 47% and the 3-year survival increased to 37% from 20%. Two of the 4 unrelated bone marrow recipients died due to GVHD, and 2 are alive and well one year and 6 months after BMT respectively without experiencing any GVHD.

Conclusion: The incidence of acute GVHD and death due to GVHD has been reduced with the combination of MTX + CSA as compared to monotherapy with either drug.

C 203 SELECTIVE INHIBITION OF ALLOREACTIVE RESPONSES OF HUMAN BONE MARROW T LYMPHOCYTES BY AN IMMUNO-A-TOXIN CONJUGATED WITH A ANTIBODY DIRECTED AGAINST THE IL2 RECEPTOR.
M. Cavazzana, C. Fromont, J.M. Derocq, J. Gerota, F. Le Deist, C. Griscelli, A. Fischer.
Graft-versus-host disease (GVHD) and graft rejection remain the two principal causes of morbidity and mortality after MHC-mismatched bone marrow transplantation. Since the human mixed lymphocyte culture (MLC) and the HLA-restricted host directed cytotoxicity (CTL) may reflect cellular interactions occurring during GVHD and graft rejection, inhibition of these responses may be useful for screening functional T cell depletion in experimental bone marrow transplantation studies. For this purpose, we have tested the possibility of removing host-specific allogeneic T cells present in the graft. After a two day MLC, the specifically activated host-alloreactive bone marrow T cells were incubated with the ricin A-chain toxin conjugated with the antibody 33B3.1 directed against the human receptor of IL2. An inhibition of a primary MLC and of cytotoxic activities as demonstrated by CTL assay was observed. The specificity of this depletion was demonstrated by the relative preservation of reactivity (80 % of control) against third party cells. Limiting dilution analysis of residual host specific alloreactive T cells indicated a magnitude of 1.3 to 1.6 log of depletion. Growth of myeloid marrow progenitors (CFU-GM) was not inhibited. Similar results were obtained using murine cells. In vivo evaluation of the procedure is presently underway in mice both for GVHD and graft rejection prevention.

C 204 DIFFERENTIAL SENSITIVITY OF HUMAN T HELPER CELL PATHWAYS TO CYCLOSPORIN A, Mario Clerici and Gene M. Shearer, Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892
We recently demonstrated that human T cell (Th) recognition of HLA allogeneic peripheral blood leukocytes in vitro is mediated by three distinct pathways that involve $CD4^+$ or $CD8^+$ Th and self(s) or allogeneic (a) antigen presenting cells (sAPC or aAPC): 1) $CD4^+$ Th-sAPC; 2) $CD4^+$ Th-aAPC; and 3) $CD8^+$ Th-aAPC. In the present study we tested whether these Th-APC pathways were differentially susceptible to the immudomodulating effect of cyclosporin A (CsA). We identified a dose dependent hierarchy of sensitivity of these pathways to CsA (added to the 7-day in vitro cultures) such that the most sensitive pathways was $CD4^+$ Th-sAPC, followed by $CD8^+$ Th-aAPC, and finally by $CD4^+$ Th-aAPC. Analysis of these dose-dependent effects of CsA on different Th-APC pathways should be useful determining optimal drug doses in transplantation immunology and for elucidation of the pathways responsible graft rejection.

C 205 DEPLETION OF T LYMPHOCYTES FROM SOYBEAN LECTIN AGGLUTININ TREATED BONE MARROW WITH THE AIS T-CELLECTOR, Nancy H. Collins, Jane Lebkowski, Thomas Okarma, David Okrongly, Vibeke Strand, Richard J. O'Reilly, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, Applied ImmuneSciences, Inc., Menlo Park, CA 94025-1109.
Soybean lectin agglutination and sheep red blood cell rosetting have been used to prevent GVHD in over 400 allogeneic bone marrow transplants. This combined procedure reduces total clonable T cells by 3 $\log_{10}$. To improve efficiency and speed, we replaced the rosetting step with the AIS T25 or T624 T Cellector, which are solid phase polystyrene devices to which mAbs to the CD5 and CD8 T cell antigens are covalently bound. The non-adherent $SBA^-5^-/8^-$ cells had similar T cell frequencies ($\bar{X}$ 1/530 vs 1/534) to the standard SBA^-SRBC^-cells, but greater cell recovery ($\bar{X}$ 97.7% vs 48.7%), which resulted in a lower (50.9%) total $\log_{10}$ T cell depletion. The $SBA^-5^-/8^-$ cells had similar or greater hematopoietic precursor recovery: CFU-GM 71.1% vs 57.5%; BFU-E 81.7% vs 95.5% and CFU-GEMM 69.7% vs 60.2%. The T Cellector had significant advantages in ease of operation and manipulation in a closed system. This technology has the potential to increase the total number of progenitor cells administered. Future clinical trials will define to what degree improvement in this feature of the transplants will affect the frequency of durable engrafment.

C 206 BONE MARROW CHIMERISM DOES NOT GUARANTEE TOLERANCE TO VASCULARIZED ALLOGRAFTS. Donald V. Cramer*, Allen L. Hoffman, Leonard Makowka* and Thomas E. Starzl. Departments of Surgery*, Cedars-Sinai Medical Center, Los Angeles, CA 90048 and the University of Pittsburgh School of Medicine, Pittsburgh, PA, 15261.

The establishment of mixed syngeneic and allogeneic bone marrow chimeras between strains of mice that are congenic for the major histocompatibility complex has been associated with the establishment of specific tolerance to allogeneic skin grafts. We have created similar bone marrow chimeras in rats to establish whether this type of tolerance induction may extend to vascularized organ grafts of clinical importance. Irradiated LEW rat recipients were transplanted with 50 x 10^6 syngeneic and allogeneic T-lymphocyte depleted ($OX19^+$) bone marrow cells. The presence of long-term stable chimerism was established by the detection of donor class I antigens on RBCs by hemagglutination and DNA hybridization of recipient splenic DNA with a synthetic oligonucleotide specific for the ACI donor AFP genes. LEW rats that were stable mixed BM chimeras were transplanted with ACI skin, heart, and small intestine. Despite the presence of stable, long-term hematopoietic and lymphoid chimerism, the survival of the allogeneic grafts was not significantly different from that seen for control animals. These results suggest that the induction of tolerance with bone marrow chimerism may depend upon donor/host immunoregulatory events that may be more complex than early experiments have suggested.

C 207 VIRAL ENHANCEMENT OF ANTI-HOST ALLOREACTIVITY IN MICE UNDERGOING CONCURRENT GRAFT-VERSUS-HOST REACTION AND MCMV OR HSV-1 INFECTION. **Carolyn Cray and Robert B. Levy, Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, Florida 33101.** Although the DNA viruses CMV and HSV are known to be associated with an increased incidence of GVHD in humans, the specific nature of this relationship remains to be determined. Using the murine P -> F1 model of graft-versus-host reaction (GvHR) with concurrent MCMV or HSV-1 infection, we have previously reported viral infection can clearly enhance GvHR associated alterations when a class I or class I/II MHC disparity is present between donor and host. Subsequently, experiments were performed to determine whether, a) anti-pathogen specific immune reactivity is demonstrable in animals undergoing GvHR, and b) a specific donor anti-host (i.e. allogeneic) response is augmented by the presence of the pathogen. The results have shown that mice undergoing concurrent MCMV infection and GvHR produce anti-MCMV antibody of the IgM class (but not IgG) during the course of infection. Additionally, anti-host allogeneic CTL activity (CD8+) was clearly enhanced as early as day 7 post injection in mice undergoing GvHR together with virus infection in comparison to that observed in non-virally infected GvHR animals. These findings thus demonstrate that immune responses against the pathogen can be induced early during the GvH reaction. In total, the present observations are consistent with a hypothesis whereby virus infection can enhance alloreactivity (i.e. donor anti-host response) and contribute to exacerbating graft-versus-host reactions.

C 208 BONE MARROW TRANSPLANTATION FOR ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) USING GENOTYPICALLY HLA-IDENTICAL DONORS. RESULTS OF A MULTIVARIATE ANALYSIS OF FACTORS AFFECTING GRAFT-VERSUS-HOST DISEASE (GVHD), SURVIVAL, AND RELAPSE, Kris Doney, C. Dean Buckner, Jean Sanders, Rainer Storb, and E.D. Thomas for the Seattle Bone Marrow Transplant Team, Division of Clinical Research, Hutchinson Cancer Research Center, Seattle, WA 98104
Between Feb. 1972 and Nov. 1987, 192 adults (>17 yrs old) with ALL were transplanted in Seattle using genotypically HLA-identical marrow donors. Median patient (pt) age was 23 yrs. Eighty-nine pts were in marrow remission (REM)(41=1st REM, 48=>1st REM) and 103 pts were in marrow relapse (REL). Conditioning regimens included chemotherapy alone (3 pts) or in combination with 9.2-17 Gy total body irradiation (189 pts). GVHD prophylaxis consisted of methotrexate and/or cyclosporine. 79 pts (41%) developed grades II-IV acute GVHD and 28/122 pts (23%) developed chronic GVHD. Actuarial survival at 8.5 yrs was 26% for pts in 1st REM, 10% for pts in >1st REM, and 15% for pts in REL. Corresponding relapse rates were 50%, 59%, and 71%. A multivariate analysis evaluated both prognostic variables associated with conventional chemotherapy and transplant-related pt and donor characteristics for their potential effect on development of acute GVHD, survival, relapse, and disease-free survival (DFS). Increased risk of developing acute GVHD was associated only with increasing donor age. Variables significantly associated with both increased survival and DFS included transplantation in 1st REM, younger pt age, and younger donor age. A decreased probability of relapse was associated with transplantation in 1st REM, male pt sex, grade II-IV acute GVHD, and the presence of both acute and chronic GVHD. These data suggest that for adults with ALL, transplant-related factors rather than disease characteristics at diagnosis predict long-term DFS after marrow grafting.

C 209 ASSOCIATION BETWEEN HCMV INFECTION AND ACUTE GVHD, H. Einsele, M. Steidle, A. Vallbracht, H. Schmidt, G. Ehninger, C. A. Müller
Medizinische Klinik und Poliklinik, Virologie, Hygieneinstitut der Universität Tübingen, 7400 Tübingen, West Germany

Patients undergoing bone marrow transplantation were followed up weekly for HCMV infection by different techniques including slot-blot hybridization, conventional and rapid virus culture techniques as well as polymerase chain reaction using primer pairs specific for the immediate early region of the HCMV genome. Blood, urine and throat washings were analysed. PCR technique proved to be the most sensitive method and allowed virus detection at a very early stage posttranplant. All of the seropositive patients or negative bone marrow transplant recipients with seropositive donors were found to develop viremia after BMT, although only in a minority of these patients symptomatic HCMV infection was observed. In some patients HCMV infection preceded acute GvHD. Immunological phenomena associated with the development of symptomatic infection will be analysed and presented. Additionally viral presence in lung, liver and skin detected by different techniques was correlated with immunohistological changes like abnormal expression of HLA-class II antigens or T-cell infiltrates known to be chararcteristic features of GvHD-mediated tissue alterations after BMT.

C 210 REGULATION OF MURINE GRAFT-VERSUS-HOST DISEASE BY NON-MHC GENES AND AGING, Loren D. Fast, Department of Medicine, Rhode Island Hospital and Brown University, Providence, RI, Nicola M. Kouttab, Department of Pathology, Roger Williams General Hospital and Brown University, Providence, RI, Mahnaz Badamchian and Allan Goldstein, Department of Biochemistry and Molecular Biology, The George Washington University School of Medicine, Washington, D.C. A murine model of GVHD that is often studied is one in which an acute form of GVHD is generated following injection of C57BL/6 spleen cells into unirradiated (C57BL/6 x DBA/2)F_1[B6D2F_1] mice. In contrast, injection of DBA/2 spleen cells into the same recipients results in a chronic form of GVHD. The distinguishing characteristics of these two forms of GVHD is the presence of CTL in the acute form and the hyperproduction of immunoglobulin and autoantibodies in the chronic form. Genetic analysis using BXD recombinant inbred mice and (B10.D2 x DBA/2) x DBA/2 backcross mice indicated that a single locus, Gvh, located 35±5 map units from Hbb on chromosome 7 controlled the GVHD responses of the parental spleen cells. During these studies it was also discovered that spleen cells from 30-40-week-old DBA/2J mice induced the acute form of GVHD instead of the chronic form induced by cells from 6-10-week-old DBA/2J mice. Further studies have shown that administration of thymosin fraction 5 (TF5), a preparation of thymic hormones, to the aged DBA/2J mice for 4 days prior to obtaining the spleen cells to test for GVHD response resulted in the chronic form of GVHD. Thus, the administration of thymic hormones modulated the effects of age on GVHD responses in DBA/2J mice. Because these experiments also showed that thymosin α_1, a purified peptide present in TF5, did not alter GVHD responses in older mice, purification of the active peptides present in TF5 was started. Partial purification of at least two active peptides in TF5 has been achieved and additional purification steps are ongoing. In addition, genetic analysis to determine if the changes in GVHD response caused by aging and thymic hormone administration are the result of altered expression of the Gvh gene have been initiated.

C 211 INCREASED BIOAVAILABILITY OF CYCLOSPORINE (CSA) BY KETOCONAZOLE IN PATIENTS AFTER ALLOGENIC MARROW TRANSPLANTATION.
A. A. Fauser, J.Schmid, G.W. Löhr. Bone Marrow Transplant Unit, Department of Hematology, Med.Univ.-Klinik, Freiburg, FRG. Acute graft versus host disease (aGVHD) is a major cause of morbidity and mortality during the first few months after allogenic marrow transplantation. Since the treatment of established acute GVHD is unsatisfactory, most patients receive immunosuppressive therapy as prophylaxis. However, despite prophylaxis with a combination of cyclosporine and methotrexate or steroids, acute GVHD of grades II to IV develops in 20 to 40 percent of the recipients of nonmanipulated HLA-identical marrow grafts. Since the immunosuppressive effect of cyclosporine appears to be reversible and since the concentration can vary considerably from patient to patient given the same dose of drug one explanation is that cyclosporine concentrations might be low and subtherapeutic. Co-administration of ketoconazole with CSA profoundly elevates cyclosporine blood levels. The exact mechanism for this interaction remains unclear. All patients at high risk to develop aGVHD received cyclosporine 2.5 mg/kg i.v. twice daily, infused over a period of 4 hrs, starting at day-3, prednisone 0,5 mg/kg i.v., starting at day + 7, and ketoconazole 400 mg, p.o. every day, starting at day-7. Blood samples for the measurement of trough cyclosporine concentrations were obtained at least three times a week, 8-10 hours after the administration of cyclosporine. The trough cyclosporine concentrations ranged between 500 to 950 ng/ml during the first week of transplantation, and 350 to 680ng/ml during the second week. All patients tolerated the immunosuppressive therapy, however most patients complained about nausea, and headaches. The results of our study confirm that ketoconazole profoundly increases the bioavailability of cyclosporine. The interaction is safely manipulated as evidenced by no dramatic changes in renal or hepatic function. All patients (n=7), who have been at high risk, did not develop aGVHD; however it is not known yet whether high serum concentrations during the first two weeks of treatment do correlate with a lower incidence of clinical GVHD of grade II or higher.

C 213 EPITOPIC ARCHITECTURE OF HLA CLASS I ALLOANTIGENS DERIVED USING HUMAN ANTIBODIES (aAb), Thomas C. Fuller, Anne Ahern-Fuller, Glenn E. Rodey and Peter Parham, Massachusetts General Hospital, Boston MA, Emory University, Atlanta GA and Stanford University, Stanford CA.
The human HLA Class I antigens have been probed with murine monoclonal antibodies (mAb) and shown to be composed of a diverse array of epitopes. Our objectives were to provide similar information using human aAb reagents. HLA aAb were purified on HLA antigen-affinity columns. Competitive binding inhibition assays with aAb and mAb probes in conjunction with indirect immunofluorescence (FACS) were used for comparative analysis of antigenic structure; both the HLA-A2 and HLA-B7 cross-reactive families were studied. Our data demonstrate that four (4) structurally distinct determinants can be identified by aAb on each HLA molecule, including a unique private epitope and at least 3 public determinants. Specific amino acid substitutions on the α-helices and the β-loops of the two distal heavy chain domains are likely responsible for the epitopes recognized by the Ab panel. From our studies, the concept of HLA serologic cross-reactivity can be established to arise mainly from the sharing of similar if not identical public epitopes between members of each CREG. We conclude that the role of HLA compatibility in allotransplantation must be reassessed from the molecular view (viz, epitope matching). This perspective could offer additional promise in the successful search for HLA disparate donors of bone marrow by lessening the antigenic load and the severity of GvH disease.

C 214 TREATMENT OF ACUTE GRAFT VERSUS HOST DISEASE (GVHD) WITH 2'-DEOXYCOFORMYCIN (DCF), D.S. Gordon, L.T. Heffner, W.R. Vogler, and E.F. Winton. Division of Hematology/Oncology, Emory University School of Medicine, Atlanta, GA.
DCF is known to be a potent inhibitor of T-lymphocytes and to bind to the purine salvage pathway enzyme adenosine deaminase with extremely high affinity. Seven allogeneic bone marrow transplant patients with untreated GVHD have received 8 courses of DCF given weekly at a dose of 4 mg/m-2 followed by a taper. Toxicities have been limited to moderate nausea and vomiting. Three patients had disease initially limited to the skin (>50% involvement) and responded by the end of the second week of therapy with significant clearing of the disease. One of these patients then developed biopsy proven GVHD involving the liver and responded to an additional course of DCF. Of these three, one is alive early after transplant, one died of recurrent disease and the third died of multiple thromboemboli and Legionella infection without evidence of GVHD. Four patients had multi-system GVHD (3 skin/liver/gut and 1 liver/gut) that did not respond to DCF therapy and were subsequently treated with corticosteroids. All died of complications of the GVHD and/or steroid therapy. We conclude while DCF is well tolerated, its efficacy in GVHD appears to be modest. The addition of deoxyadenosine to DCF for therapy, a combination known to be significantly more immunosuppressive than either alone, may be more efficacious.

C 215 IL-2 ENHANCEMENT OF VETO SUPPRESSOR CELL FUNCTION IN T CELL DEPLETED BONE MARROW IN VITRO AND IN VIVO. R. Gress and H. Nakamura. Experimental Immunology Branch, National Cancer Institute, Bethesda, MD 20892. In allogeneic bone marrow transplantation, graft-versus-host disease can be prevented by the removal of T cells from the donor bone marrow. The risk of marrow graft rejection is, however, greater for T cell depleted marrow than non-depleted marrow. Cells with a specific type of suppressor activity, termed veto cells, which might be able to suppress the host rejection response, have been reported to be present in murine marrow. We have studied veto activity of T cell depleted marrow activated with IL-2. T cell depletion was carried out with antibody and complement with no detectable T cells by FACS analysis following depletion. FACS analysis of marrow activated with IL-2 (ABM) demonstrated two major cell populations, Thy1.2^{+}NK1.1^{+}Mac-1^{-} and Thy1.2^{-}NK1.1^{-}Mac-1^{+}. In mixed lymphocyte cultures, the generation of H-2^{b} anti H-2^{d} (but not anti-H-2^{k}) effectors was suppressed in the presence of H-2^{d} or H-2$^{b/d}$ ABM. Cold target inhibition was ruled out by the elimination of residual H-2^{d} cells at the end of the culture period. To examine ABM veto activity in vivo, H-2^{b} mice were sublethally irradiated 700-750 cGy and given allogeneic H-2^{d} bone marrow alone, marrow plus H-2$^{d/b}$ ABM, marrow plus ABM with IL-2 (to sustain ABM in vivo), or marrow plus IL-2. Chimerism was examined by FACS six weeks later. Chimerism was reproducibly present only in the group of animals receiving marrow plus ABM and IL-2. Inoculation of H-2$^{b/k}$ ABM was not effective in enhancing engraftment of H-2^{d} marrow. These results demonstrate that ABM has veto activity in vitro and is able to specifically suppress allogeneic responses in vivo with enhanced engraftment of MHC-mismatched, T cell depleted marrow.

C 216 REQUIREMENT FOR DONOR BONE MARROW COMPONENTS FOR INDUCTION OF SPECIFIC TRANSPLANTATION TOLERANCE, Suzanne T. Ildstad,M.D.,David H. Sachs,M.D.,Department of Surgery,University of Pittsburgh,Pittsburgh,PA,and Immunology Branch,NIH,Bethesda,MD.
Transplantation of a mixture of T-cell depleted donor + recipient bone marrow results in donor-specific transplantation tolerance. Theoretically, bone marrow stem cells are "re-educated" to recognize donor antigens as self during reconstitution. To determine whether this same tolerance can be achieved without the use of donor bone marrow, transplantation of syngeneic bone marrow plus an MHC-incompatible skin graft was evaluated. Three groups of B10 recipient mice were studied: Group I:syngeneic + allogeneic bone marrow transplant (B10+B10.D2->B10); Group II:fully allogeneic bone marrow inocula (B10.D2->B10); Group III:syngeneic bone marrow (B10+>B10). A simultaneous allogeneic (B10.D2) skin graft was placed. Donor-specific B10.D2 skin grafts were prolonged on all animals. However, rejection occurred rapidly after full reconstitution in those animals which had not received donor bone marrow inocula (Group III). A second B10.D2 skin graft placed on those animals at day 50 was rejected rapidly (MST-8 days) and probably represents a second set rejection. These data suggest that some component of donor bone marrow is required for the induction of specific transplantation tolerance in this model. Studies are in progress to identify the required allogeneic donor cell phenotype.

Group	Reconstitution	Skin Graft	#	Median Survival Time Skin Graft
I	B10+B10.D2->B10	B10.D2	5	> 90 days
II	B10.D2->B10	B10.D2	5	> 90 days
III	B10->B10	B10.D2	8	36 days
	Normal B10 (no BMT)	B10.D2	5	10 days

C 217 THE INCIDENCE OF GRAFT VERSUS HOST DISEASE IN ALLOGENEIC BONE MARROW TRANSPLANT PATIENTS USING SURGICAL PROCUREMENT OF BONE MARROW AS COMPARED TO ASPIRATION, Joseph Kapelushnik, Melanie Kirby, E.F. Saunders and Hassan Solh, Division of Haematology/Oncology, The Hospital for Sick Children, Toronto, Ontario, M5G 1X8.
We evaluated the incidence of graft versus host disease (GVHD) in allogeneic bone marrow transplant patients (pts.) using marrow obtained either surgically in one group (Group I) or by marrow aspiration in another (Group II) from HLA-MLR matched sibling donors. There were 32 pts. in Group I: 8 SAA, 1 thalassemia major, 13 ANLL in CR 1 or 2, 10 ALL in CR 2 or 3 and 34 pts. in Group II: 9 SAA, 1 Blackfan-Diamond, 7 ANLL in CR 1 or 2, 6 ALL in CR 2 or 3, and 1 CML. In the surgical group, spongy bone was removed from 1 iliac wing via window in the cortical bone. A marrow cell suspension was produced by washing the crushed bone particles in TC 199 and heparin. After filtering the final volume was 100-300 ml. Red cell contamination was negligible (mean hematocrit 0.01). All pts. in both groups received similar conditioning regimens and same GVHD prophylaxis with Cyclosporin and a short course of IV methotrexate. No statistical significance was found between the two groups comparing age, sex, number of cells received per kg (although the cell number required using the surgical procedure was lower), time to engraftment (WBC: 0.5, granulocyte: 0.5, platelet count: 50K). Using student t-test the incidence of GVHD (Grade I-III) was statistically higher in Group II when compared to Group I ($p < 0.05$). In addition to the other advantages of the surgical procedure including ability to cross the ABO barrier without marrow manipulation and lack of donor transfusion, the lower incidence of GVHD may also be an important factor. Marrow obtained by aspiration is usually diluted with peripheral blood and consequently one would assume that more T cells are present when this technique is used. We are conducting a prospective study evaluating the number and subtypes of T cells in marrows obtained surgically compared to aspiration and the incidence of GVHD in both groups.

C 218 USE OF ALLOGENEIC LAK CELLS TO PREVENT LETHAL GRAFT-VERSUS-HOST DISEASE, Joseph Kaplan and Hatsumi Yamamoto, Departments of Pediatrics, Medicine, and Immunology-Microbiology, Wayne State University School of Medicine, Detroit, MI 48201.
We have previously shown (J. Immunol. 143:1524) that LAK cell preparations produced by culturing lymphocytes with high dose IL-2 for 4 days prevent lethal graft-versus-host disease (GVHD) when injected into sublethally irradiated syngeneic mice together with allogeneic spleen cells, but themselves cause lethal GVHD when injected into sublethally irradiated allogeneic mice. Reasoning that more prolonged culture of lymphocytes with IL-2 might selectively deplete LAK cell preparations of contaminating alloreactive T cells, we tested 14 day-cultured LAK cells for i) ability to produce lethal GVHD, and ii) ability to prevent lethal GVHD. Sublethally irradiated A/J mice were injected with 4 million allogeneic B6 spleen cells and/or 10 million "4 day" or "14 day" B6 LAK cells. As expected, lethal GVHD occurred in 7/8 injected with B6 spleen cells alone and in 4/5 injected with "4 day" B6 LAK cells alone. By contrast, lethal GVHD occurred in only 1/8 injected with "14 day" B6 LAK cells alone, and in only 2/9 injected with B6 spleen cells together with "14 day" B6 LAK cells. Therefore, by prolonging the culture of lyphocytes with IL-2 from 4 to 14 days LAK cell preparations are generated which can prevent lethal GVHD in allogeneic recipients. These findings imply that it may be possible to use donor and 3rd party as well as recipient-type LAK cells to prevent GVHD in human bone marrow transplant recipients.

C 219 STABLE ENGRAFTMENT OF ALLOGENEIC T CELLS IN A PATIENT WITH SEVERE COMBINED IMMUNODEFICIENCY: *RECOGNITION OF HOST HISTOCOMPATIBILITY* ANTIGENS AND ENGRAFTMENT FAILURE OF A BONE MARROW TRANSPLANT HLA-IDENTICAL TO THE HOST, Christian Knobloch, Shraga F. Goldmann, Herbert Lattke, and Wilhelm Friedrich, Departments of Pediatrics II, Human Genetics, and Transfusion Medicine, University Ulm, and Red Cross Blood Bank Ulm, D-7900 Ulm, FRG.

We have analyzed the immunocompetence of blood donor derived, allogeneic T cells that had engrafted in a patient with severe combined immunodeficiency following a transfusion with unirradiated blood. The blood donor T cells multiply and persist in the patient's bone marrow and peripheral blood over several months without leading to a graft-versus-host disease. Yet, following expansion in vitro, the allogeneic T cells can be specifically restimulated by cells expressing host HLA-DR antigens. Moreover, engraftment of a bone marrow transplant from an HLA-identical sibling was not successful until the blood donor derived T cells had been eliminated in vivo. We conclude that allogeneic T cells sensitized against host histocompatibility antigens may play a role in the fate of a bone marrow graft that shares HLA-antigens with the host even if the sensitization does not lead to a clinically apparent graft-versus-host-disease. Our results may also be relevant with regard to conditioning regimens and parental marrow donor selection in severe combined immunodeficiency patients with engrafted maternal T cells.

C 220 EFFECT OF CYCLOSPORIN A ON LYMPHOPOIESIS : AUGMENTATION OF NK ACTIVITY IN BONE MARROW TRANSPLANTED MICE TREATED WITH CYCLOSPORIN A. Atsushi Kosugi and Gene M. Shearer, Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892. Recently, we and others have demonstrated that cyclosporin A (CsA) induces a maturational arrest of T cell in the thymus. In the present study, we investigated the effect of CsA on the development of lymphocyte other than T cells. In the spleen of syngeneic bone marrow transplanted mice subsequently treated with CsA, marked elevation of NK activity was observed, whereas T cells were greatlty reduced in such mice. This elevation of NK activity was correlated with the increase of $NK1.1^+$ cells, and appeared to be unrelated to the CsA-induced effect on T cell maturation in the thymus. These results indicate that CsA not only downregulates the development of T cells, but also upregulates the generation of NK cells, a finding that may be important in clinical bone marrow transplantation.

C 221 PRE-EMPTIVE THERAPY OF GRAFT VS. HOST DISEASE (GVHD) WITH A PAN-T-CELL IMMUNOTOXIN (IT) IS ASSOCIATED WITH ACCELERATED ENGRAFTMENT. C.F. LeMaistre, C. Meneghetti*, J.C. Yau*, J. Reuben,* M.Duvic,* D. Moloney,* P. Lomen,* and A.B. Deisseroth. The University of *Texas M.D. Anderson Cancer Center. Houston, TX, and Xoma Corporation, Berkeley, CA.*

An IT created by linking the A-chain or ricin to a murine monoclonal antibody which binds the pan-T-cell antigen CD_5 (XomaZyme®-H65) is effective therapy for steroid resistant GVHD. Because GVHD remains a major problem associated with bone marrow transplantation (BMT) in adults, this study was designed to establish the feasibility of using an IT to prevent GVHD in adults who undergo BMT from matched sibling donors. Six patients (pts) (2 male, 4 female, ages 21, 30, 30, 33, 40, 40) have been enrolled in this trial with the following diagnoses: AML (3), ALL (2), CML (1). The first cohort received 0.1 mg/kg/day (d) IT from d+7 to d+16 after BMT (4 pts); the present cohort received 0.1 mg/kg/d from d+3 to d+16 (2 pts). All patients are evaluable for toxicity and engraftment. Toxicities associated with IT were mild and included myalgia (3 pts), arthralgia (1 pt), proteinuria >1 gram/24 hrs (5 pts) and weight gain >5% (5 pts). Human anti-mouse antibody levels are available in the first 4 pts. Only one pt developed a significant IgM or IgG response, suggesting pts can be retreated. Notably, the median time to 100 absolute neutrophil count (ANC)/μl was d+10 and was significantly shorter by log-rank analysis than for historical controls who had received cyclosporin (CSA) alone ($p=0.012$) and for concomitant patients who received CSA and methotrexate ($p=0.002$). Engraftment was extremely rapid, with all pts having d+10-d+14 bone marrow examination demonstrating trilineage engfaftment and $\geq$15% cellularity. Additionally, 4 pts had 1000 ANC/μl by d+12. Early after transplantation patients remain lymphopenic with normal CD_4: CD_8 ratios. By day 100, there is a perferental recovery in CD_8 populations, but the majority of these are not cytotoxic T-cells. Approximately half of lymphoytis are CD_8 positive by this time. Three pts developed Stage II GVHD of skin without other documented organ involvement, but two pts also developed a cytomegalovirus colitis. Thus patients remain immunosuppressal with this approach early after BMT. In conclusion, a pan-T-cell IT may be used to prevent GVHD in adults undergoing matched allogeneic BMT and is associated with accelerated engraftment. Immune recovery following the use of this IT requires further examination.

C 222 CYCLOSPORINE ADMINISTRATION BY CONTINUOUS INFUSION (CI) AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT).
K. B. Miller, C.D. Hillyer, T.A. Fogaren, D.E. Wazer and J.F. Desforges. Division of Hematology and Radiation Oncology, New England Medical Center, Boston, Massachusetts 02111.
Patients (ages 18 - 43) undergoing HLA matched allogeneic BMT for acute leukemia or aplastic anemia (n=5) were given continuous I.V. cyclosporine and I.V. methotrexate (10 mg/m^2 days 1, 3, and 6) for graft versus host disease (GVHD) prophylaxis. CI cyclosporine was started one day prior to the marrow infusion at a dose of 2.5 mg/kg/24 hours and continued for 20 days. On day 21 post BMT oral cyclosporine was begun. Cyclosporine was mixed in 1000 ml normal saline and administered via peripheral vein. Patients tolerated the infusion well. Cyclosporine levels ranged from 170-431 ng/ml. All patients received concommitant nephrotoxic drugs including amphotericin during their course. All patients developed hypertension with a mean increase in systolic blood pressure of 46 mg Hg and diastolic blood pressure (DBP) of 36 mm Hg. All patients had DBP greater than 100 mg Hg (range 100 -110 mm Hg). No patient's creatinine rose to greater than 2.0 mg/dl and none required dialysis. Serum creatinine increased by a mean of 0.8 mg/dl, total bilirubin rose by a mean of 2.4 mg/dl. There were no neurologic toxicities or serious GVHD. One patient had mild (grade I) GVHD skin disease. These findings suggest that CI cyclosporine is effective in preventing GVHD with acceptable toxicity for patients undergoing allogeneic BMT.

C 223 INFECTION WITH MURINE CYTOMEGALOVIRUS CAN LEAD TO INCREASED HYBRID RESISTANCE AGAINST PARENTAL BONE MARROW GRAFTS, Satish C. Muluk, Fran Hakim, and Gene M. Shearer, Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892. Lethally irradiated (C57BL/6 x C3H)F1 (B6C3F1) and (C57BL/6 x DBA/2)F1 (B6D2F1) mice are known to exhibit resistance to engraftment by parental C57BL/6 (B6) bone marrow. This hybrid resistance was found to be markedly enhanced by injection of the hosts with murine cytomegalovirus (CMV) 3 days prior to irradiation and bone marrow injection. In contrast, engraftment of syngeneic marrow, or bone marrow from the non-B6 parent (C3H for B6C3F1, DBA/2 for the B6D2F1) was not affected by CMV infection. To test whether the CMV-induced enhancement of resistance was mediated by NK cell, splenic NK activity (YAC-1 killing) and frequency (NK 1.1 staining) were assessed. Both parameters were found to be elevated at 3 days after CMV infection but to return to normal levels by 9 days. B6 bone marrow engraftment was in fact found to be normal when the marrow was administered to F1 mice 9 days after CMV infection. Thus, the NK enhancement resulting from CMV infection appears to play a major role in enhanced hybrid resistance. This effect may be of importance in clinical bone marrow transplantation, a situation in which patients are susceptible to viral infection.

C 224 CLASS III TYPING AS A STRATEGY FOR IDENTIFYING POTENTIAL BONE MARROW TRANSPLANT (BMT) DONORS. S. Neudorf, L.S. Beischel, N. Bishof, K. Balakrishnan, L. Whitacre, M. Schroeder, N. Kobrinsky, and T.R. Welch, Children's Hospital Research Foundation, Cincinnati, Ohio and University of Manitoba, Winnipeg, Manitoba
An HLA identical sibling is often the preferred donor for BMT. Screening for donors using HLA typing may be an expensive part of the pretransplant work-up and may be complicated by lymphopenia, abnormal HLA expression, or the requirement for significant volumes of blood from small subjects. The genes encoding the complement proteins Bf, C4A, and C4B ("Class III") are within the MHC, between HLA-B and -DR loci. Electrophoresis of a fraction of a milliliter of plasma permits assignment of phenotypes (the complotype) for these proteins. The complotype is as polymorphic as any individual class I or II locus. To evaluate the utility of complotyping as a means to screen for HLA matched donors, 27 families were prospectively complotyped as part of pre-BMT HLA typing. Of 10 sibs in whom identity to the recipient was suspected from the complotype, HLA identity was confirmed in 9 cases and 1 recipient was shown to have a -B/-DR crossover. Complotyping was used to screen for a donor for a 4 year old patient with severe aplastic anemia in a large inbred family with no HLA identical sibs. Of 98 family members studied, 77 (79%) were excluded by the complotype. Complete HLA typing of the 21 individuals not excluded by the complotype led to identification of a donor who was identical to the recipient at all class I and class II loci and was nonreactive in MLC. Complotyping may be used to exclude obviously nonidentical sibs and, thus, permit a more focused approach to complete HLA typing for donor identification.

C 225 HIGH DOSE METHYLPREDNISOLONE (MP): EFFECTIVE THERAPY FOR ACUTE GRAFT VERSUS HOST DISEASE (aGVHD), David Oblon, Gerald Elfenbein, Roy Weiner, John Graham-Pole, University of Florida and the Gainesville VA Medical Center, Gainesville Florida 32610.
We have treated 46 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 a male:female ratio of 2:1. The incidence of aGVHD in 121 consecutive allogeneic BMT was 48% (58/121) and for grade II-IV aGVHD was 38% (46/121%). 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% (38/42) with confidence limits of 80-100%. 76% (32/42) of pts responded to the initial dose level; 60% (4/10) of escalated pts responded to MP at 10 mg/kg/d. Only 2 of 5 patients responded to MP at 20 mg/kg/d. Four pts were unevaluable for response. 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 followup 6 mo.) for grade 0-1 is 49% (36/74); for grade II-IV it is 33% (15/46). The rate of relapse of leukemia was significantly higher in pts without aGVHD (8/62) versus pts with aGVHD (2/35) at $p<.05$ level. We conclude that high dose MP is effective therapy for aGVHD. A survival benefit is not apparent due to multiple competing causes of death in BMT pts and/or the toxicity of high dose MP.

C 226 MODIFIED T-CELL COLONY-FORMER TECHNIQUE AS A POSSIBLE ASSAY FOR DONOR ALLOSENSITIZATION IN MHC-MATCHED UNRELATED-DONOR TRANSPLANTATION: PRELIMINARY FINDINGS, James P. OKunewick, Deborah L. Kociban, Mary J. Buffo, Allegheny-Singer Research Institute, Allegheny General Hospital, Pittsburgh, PA 15212.
Utilizing an allogeneic mouse model involving donors and recipients which are H-2 identical, but differ in their minor histocompatibility determinants, we have been attempting to devise an in vitro assay of potential predictive value for GvHD in MHC-matched unrelated donor transplantation. For this we have developed a modified T-cell colony former assay. Briefly, donor lymphoid cells are exposed to irradiated recipient marrow cells in vitro in liquid co-culture for 48 hours and then transferred to petri dishes in a soft agar medium to which IL-2 is added. Colony growth is allowed to proceed for 5 days, at the end of which the number of colonies is counted. The resultant data are then compared with control data obtained from cultures in which the allogeneic recipient cells used as stimulators are replaced by syngeneic donor marrow. The initial results obtained with this model suggest that it may be a sensitive detector of prior allosensitization of the potential donors to recipient antigens. Specifically, in using this technique to assay for the effect of transfusion-induced prior allosensitization of the donors, we have observed increases in the in vitro colony former response of up to 10-15 times above the level seen when the donors were not previously allosensitized. Two types of colonies appeared to be formed in response to this allosensitization: one being IL-2 dependent and specific to recipient antigens in the culture medium, and the other being non-specific and IL-2 independent, with the former colonies being twice as numerous as the latter.

C 227 IN VIVO ANTI IL2 RECEPTOR MAB IN THE PREVENTION OF GVHD PRELIMINARY RESULTS OF A RANDOMIZED MULTICENTRIC PROSPECTIVE STUDY OF GEGMC. D. Olive, D. Blaise, D. Guyotat, J. Reiffers, M. Michallet, M. Attal, JP. Vernant, N. Ifrah, E. Vilmer, V. Leblond, P. Paret, M. Hirn, C. Mawas and D. Maraninchi for the GEGMO - Marseille, FRANCE.
AGVHD is mediated by cytotoxic T lymphocytes activated by IL2 through a specific receptor ; 33B31 is a rat IgG2 Mab, highly efficient to block this receptor (1). In animal models such Mab have been shown to prevent GVHD (2). We studied the feasability of 33B31 administration after allogeneic BMT in humans (3). To assess the impact of such approach on GVHD prophylaxis, we designed a randomised trial comparing 33B31 + MTX-CSA versus MTX-CSA after allogeneic BMT for acute or chronic leukemia in first CR or first CP. 33B31 Mab was given from day 1 to day 28, as a single daily bolus. On june 89, 26 patients received 33B31 Mab and 24 were entered in CTRL group. No significant differences appeared between the 2 groups in term of age (mean = 31), sex ratio (M/F = 33/17), diagnosis (CML : 21, ALL : 16, ANL : 13) and conditioning regimen (Cy-TBI : 35, Bus-Cy : 16). No toxicity was reported. Probability of grade $\geq$2 AGVHD was 25% vs 38% in CTRL group ($p = 0.07$), with no grade 4 GVHD versus 8% in CTRL group (p = NS). There was no difference in relapse rate, 33B31 monitoring showed good trough levels and no immunisation. 50 patients are scheduled to be entered in each arm prior december 89 and the overall results will be presented.
(1) Olive et al. Eur. J. Immunol. 16, 1986, 611
(2) Ferrara et al. J. Immunol. 137, 1986, 1874
(3) Blaise et al. Lancet, 1989, june 10, 1333

C 228 THE KERATINOCYTE-REACTIVE T-LYMPHOCYTES FROM ALLOGENEIC BONE MARROW TRANSPLANTATION RECIPIENTS WITH SKIN GRAFT-VERSUS-HOST DISEASE, C. Lucy Park, Yasuhiko Ikeda, and F. Leonard Johnson, Dpt. of Pediatrics, University of Chicago, Chicago, IL 60615. The skin is one of major target organs of graft-vs-host disease(GVHD). Skin biopsy from patients with acute skin GVHD show T cell infiltration. Previously it was reported that freshly prepared epidermal cells(EC) stimulated proliferation of allogeneic lymphocytes and stimulating element was Langerhan's cell expressing DR antigens. Since acute GVHD of skin occurs in patients receiving HLA-matched/MLC-unreactive donor, we speculated that T cells infiltrating skin were reacting to non-DR antigen expressed on skin tissue. Skin EC culture was established from allogeneic BMT recipient who recovered from acute skin GVHD(RM) and normal voluneteer(YI). Following 3 weeks of EC culture with epidermal cell growth factor, these cultured EC were 100%keratinocytes(KC) and DR(-), and they became DR(+) only after exposure to interferon- for 2 days. Mixed lymphocyte-keratinocyte culture(MLKC) was performed using peripheral blood mononuclear cells(PBMC) as responders and irradiated KC as stimulators for 6 days. RM-PBMC showed proliferative response to RM-KC(stimulation index; SI=6.8), but not to YI-KC(SI=1.02). YI-PBMC did not proliferated to either YI-KC(SI=0.35) or RM-KC(SI=1.36). YI and other normal PBMC did not proliferate to allogeneic DR(-) KC even after in vitro sensitization for 6-10 days before testing(primed MLKC). These findings suggest that KC express antigen that is weakly stimulatory to T cells from minor-antigen incompatible donor, and in vitro proliferative response can be seen only after in vivo sensitization.

C 229 CD8 ANTIGEN LEVELS IN ALLOGENEIC AND AUTOLOGOUS BONE MARROW TRANSPLANTATION, Christopher H Poynton, Steven J Cousins and Susan John, Department of Haematology, University of Wales College of Medicine, Cardiff UK.

Soluble CD8 antigen is present in the serum as a 52kD homodimer and as a 66kD membrane associated antigen. The soluble form may be a marker of total suppressor cell activity, although its physiologic role is currently unknown.

We have measured CD8 antigen by enzyme linked immunoassay (Biological Industries - UK) in the sera of 35 bone marrow transplant patients. Measurements were done at up to daily intervals at various stages of the procedure. CD8 antigen levels rose up to 7 fold in the presence of graft versus host disease. The peak CD8 levels correlated well with the peak of clinical severity but the initial rise did not predate the clinical signs. Where a second surge of acute GvHD recurred after initialization of therapy, the CD8 antigen remained elevated, whereas if the GvHD settled completely, CD8 rapidly returned to normal. In chronic GvHD, T8 levels were elevated up to 3 fold. In proven CMV pnemonitis in allogeneic and autologous BMT, CD8 levels were elevated up to 4 fold. Episodes of neutropenic fever with bacterial isolates were not associated with a significant rise in CD8 levels.

In conclusion, serum CD8 antigen levels may be useful in monitoring the effectiveness of therapy for GvHD, but will not readily distinguish GvHD and CMV infection. Further studies with CD4 and CD25 antigens may reveal other useful patterns of activity.

C 230 SIX YEAR FOLLOW UP OF T CELL DEPLETED (TCD) MARROW TRANSPLANTATION FOR ACUTE MYELOBLASTIC LEUKAEMIA IN FIRST CR. H.G. Prentice, M.K. Brenner, A. V. Hoffbrand, L. Gandhi, B. Ward, M. Aldouri, M. Hamon, M. Gilmore, J. Burger. Department of Haematology, Royal Free Hospital School of Medicine, London, U.K.

From 5/83 38 patients (age 7-48 median 26) in first CR of AML received an HLA identical BMT using our standard conditioning regimen and marrow purged of T lymphocytes by murine monoclonal antibodies (MBG6 or RFT12/CD6 and RFT8/CD8 ± RFT2/CD5) plus rabbit complement (n=28) or Campath and autologous serum (n=3) or an anti CD5 Ricin A chain conjugate (n=7). Conditioning was: cytoxan 60mg/kg x 2 and 7.5Gy single fraction TBI at a received mid-plane dose rate of 15cGy ± 2 per min. Four patients died from infection (complicating GvHD in one) one after BM rejection and one from cardiac toxicity. At a median follow-up of 33 months 3 have had a leukaemic relapse. Actuarial leukaemia-free survival is 73% at 6 yrs (and 87% for 28 patients less than 37 yrs old). Leukaemia relapse occurred in 2 of 7 having αCD5/IT treated marrow which may remove more T cells than other methods we have used. The low relapse risk (9% actuarial) may be due to the combination of the host immunosuppressive properties of fast rate TBI allowing a donor immune advantage and the avoidance of post-BMT IS drugs which enables the effectors of graft versus leukaemia (GvL) to operate. But with more effective methods of TCD better immunosuppressive conditioning is needed and the protocol has therefore been amended to include busulphan 5mg/kg and the cyclophosphamide reduced to 50mg/kg x 2.

C 231 REPRODUCIBLE CONTROL OF T-CELL CONTENT IN PARTIALLY MATCHED ALLOGENEIC BMT, R. Quinones, C. Carter, R. Gutierrez, C. Bare, P. Lucas, G. Reaman, R. Gress, Children's National Medical Center, The George Washington University, Washington, D.C. and National Cancer Institute and Clinical Center, Bethesda, Maryland. The ability to reproducibly manipulate the T cell content of infused bone marrow (BM) is desirable to prevent GVHD and to investigate the role of T cells in engraftment and GVL. To this end, HLA-disparate, MLC-reactive BM, depleted extensively of mature T cells by counter centripetal elutriation (CCE) and to which controlled numbers of T cells were added, was used to reconstitute five patients with refractory leukemia. Approximately 10^9 BM nucleated cells/kg (recipient) were harvested from related donors, RBC's were removed using an apheresis device (Fenwal CS3000) and then elutriated using a Beckman elutriator (JE-10X or 5.0 rotor) in a semiclosed system. High flow rate fractions (>140 ml/min) were used to optimize separation of lymphoid cells and capture larger sized cells. Fractions were assessed by FACS analysis and, based on previous studies, fractions with no mature T cells detected, were pooled for infusion. All CCE separation cell fractions were sterile and viable, thereby allowing the add-back of mature T cells (5-10 x 10^4/kg) from the first fraction containing T cells. T cell content of the fraction was subsequently confirmed by quantitation using limiting dilution analysis. Engraftment (ANC > 500) occurred in 4/4 evaluable patients. GVHD occurred in the one patient who had been given $>10^5$ T cells/kg, therefore, subsequent patients received 10^4 T cells/kg. Elutriation offers a practical and reproducible method for T cell depletion which allows for quantitative T cell add-backs and would be adaptable to a closed semi-automated system.

C 232 PRELIMINARY REPORT OF A RANDOMIZED TRIAL COMPARING T CELL DEPLETION WITH CYCLOSPORIN COMBINED WITH METHOTREXATE IN ADULT LEUKEMIC MARROW-RECIPIENTS. [1]Olle Ringdén, Lola Markling, Berit Sundberg, Peter Pihlstedt, Ingvar Båryd, Johan Aschan, Per Ljungman, Berit Lönnqvist, Jan Tollemar and George Janossy. Departments of Clinical Immunology, Transplantation Surgery, Medicine, Huddinge Hospital and Blood Bank, Radiumhemmet, Karolinska Hospital, Sweden; Department of Immunology, Royal Free Hospital, London, UK.

Twenty-three adult leukemics were randomized to receive T-cell depleted marrow, and 23 were randomized to receive methotrexate (MTX) combined with cyclosporin (CSA). Of those, 18 and 16 respectively were in 1st remission or 1st chronic phase. The median age was 36 and 33 years respectively. For T cell depletion the marrow was treated with the monoclonal antibodies RFT8 and RFT12 plus complement, depleting a mean ±SEM of 95±6% of the T cells. The median marrow cell dose given to the T cell depleted recipients was $0.3x10^8$/kg compared to $2.1x10^8$/kg in patients treated with MTX+CSA. Engraftment ($>0.2x10^9$ WBC/L) occurred earlier in the T cell depleted recipients: 15±1 days compared to 18±1 ($p<0.001$), but the T depleted marrow recipients required more erythrocyte transfusions ($p=0.01$). Platelet and granulocyte transfusions, and infections were the same in both groups. There were no differences in acute or chronic graft-versus-host disease (GVHD). Only a few patients developed grade II (6) or grade III (1) acute GVHD. At 30 months the probability of relapse was 41% among the T depleted marrow recipients compared to 50% in those treated with MTX+CSA. The 30-month probability of survival was 62% and 65% in both groups respectively and after 30 months the leukemia-free survival was 44% in both groups.

Conclusion: Among adult leukemic marrow recipients, those who were treated with T cell depleted marrow had earlier engraftment, but required more erythrocyte transfusions compared to those given MTX+CSA. Infections, GVHD, relapse and survival figures were the same in both groups.

C 233 ANALYSIS OF HEMOPOIETIC GRAFT FAILURE IN UNIRRADIATED RECIPIENTS: FAILURE TO SEED THE HOST MARROW vs FAILURE TO PROLIFERATE, Michel W.J. Sadelain, The Whitehead Institute for Biomedical Research, Cambridge, MA 02142. It is generally believed that early graft failure may result from immune rejection or lack of "space" in the host marrow cavity. Either mechanism results in preventing proper seeding and subsequent activity of transplanted hemopoietic stem cells. To understand the mechanisms controlling hemopoietic engraftment, the fate of parental hemopoietic stem cells was investigated in unconditioned F1 hybrid recipient mice. By administering an anti-host H-2K monoclonal antibody as a conditioning regimen, thereby targetting host cells whilst sparing the donor, we found that chimerism could be induced by delayed conditioning in animals with apparent graft failure. Engraftment kinetics in the host were followed by typing individual CFU-GM colonies for their origin and showed that parental cells, which were otherwise virtually absent, became promptly detectable within the marrow cavity following antibody administration. Marrow transfers to secondary hosts suggested that parental stem cells were present in the marrow of the untreated recipients. These findings establish that the elimination of all parental cells cannot account for the absence of peripheral blood chimerism in the unconditioned F1 hybrid recipient. Thus, viable and functional donor stem cells, which remain quiescent in the host marrow, can be activated by a selective conditioning regimen and can rescue an apparent graft failure.

C 234 GRAFT VERSUS HOST DISEASE IN SCID MICE, David Spaner and Robert A. Phillips, Dept. of Hematology/Oncology, Hospital for Sick Children, Toronto, Ontario M5G 1X8 Graft versus host disease (GVHD) was studied in the SCID mouse. The advantages of using this immunodeficient mouse as a model system include: 1. No conditioning regimen is required. 2. Host lymphocytes cannot confound the analysis. 3. Requirements for pathogen free conditions imply infection cannot confound results.

5x10**6 C57/B6 lymph node cells (LNC) kill unirradiated SCID mice in about 60 days. As few as 5X10**4 B6 LNC kill sublethally irradiated animals in about 14 days. Unirradiated animals have profound liver damage but only minor skin and gastrointestinal involvement. They also develop pancytopenia but their actual cause of death is presently unclear. Sublethally irradiated animals die of bone marrow failure whereas control animals without LNC injections survive. This implies that host lymphocytes need play no role in some aspects of GVHD.

This model allows the role of host lymphocytes as a target for GVHD to be assessed. Injection of 6X10**7 F1 (C57/B6 x Balb/c) spleen cells before induction of GVHD delays mortality by up to a month. Effects of loading other cells as targets for GVHD are currently under investigation.

C 235 IL-2 PLUS TCD SYNGENEIC MARROW PREVENT GVHD MORTALITY WHILE PERMITTING ALLOENGRAFTMENT. Megan Sykes, Molly L. Romick, Kim A. Hoyles, and David H. Sachs. Immunology Branch, NCI, NIH, 9000 Rockville Pike, Bethesda, MD 20892. Previous work from this laboratory has demonstrated that T cell depleted (TCD) syngeneic marrow can delay, but not prevent, the mortality from acute GVHD caused by allogeneic lymphocytes administered to lethally irradiated mice. We now demonstrate that this protective effect can be markedly potentiated by in vivo treatment with IL-2 in the early post-transplant period. In lethally irradiated recipients of TCD syngeneic marrow plus non-TCD fully MHC-disparate allogeneic bone marrow and spleen cells, administration of 50,000 U of IL-2 twice daily for the first 5 days after bone marrow transplantation reduced acute GVHD mortality from 80 to 100% in control animals to 0 to 20% in experimental animals. Chronic GVHD mortality was also attenuated by IL-2. Complete allogeneic reconstruction was observed in all long-term survivors. While either IL-2 or TCD syngeneic marrow administered alone was protective in some experiments, the maximal protective effect was observed following administration of both components. The timing of IL-2 administration may be critical, since preliminary results indicate that a delay of 7 days in commencing IL-2 treatment led to accelerated GVHD mortality. Preliminary results in an EL-4 leukemia model indicate that the anti-GVHD effect of IL-2 did not abolish the anti-leukemic effect of allogeneic lymphocytes. This new approach to the prevention of GVHD therefore retains the alloengraftment-promoting and anti-leukemic effects of T cells in allogeneic marrow.

C 236 ASSOCIATION OF SERUM TNF-ALPHA WITH DEVELOPMENT OF ACUTE GVHD, Frank W. Symington, Margaret S. Pepe, and Anna Deliganis. Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98104. Antibody prophylaxis data suggest that tumor necrosis factor alpha (TNF, cachectin) mediates murine GVHD. In addition, TNF inhibits human basal keratinocyte growth and mainly localizes in skin and gut of mice given i.v. TNF. These findings led us to ask whether serum TNF co-associates with acute GVHD in humans. An enzyme-linked, double antibody sandwich assay was used to detect TNF in serum samples from allogeneic marrow recipients sampled once between 4 and 52 days posttransplant. A strong association between the presence of immunoreactive TNF in serum and development of grades II-IV acute GVHD was noted for these patients ($p=0.06$, Fisher's exact test). More than 90% of patients with positive TNF levels developed acute GVHD compared with 59% of those without detectable serum TNF. The association was not explained by bacterial, viral, or fungal infections, and was in fact stronger for those patients with no known infections within a week of sampling ($p=0.04$). As only one sample from each patient was evaluated, and as the dates of sampling versus GVHD onset were not considered here, the reported association is a conservative one. These results are compatible with a role for TNF in mediating acute GVHD in humans.

C 237 *IN-VIVO T CELL DEPLETION WITH CAMPATH-1G* MONOCLONAL ANTIBODY AS COMPARED TO RADIOTHERAPY AND COMBINATION CHEMOTHERAPY USING LIMITING DILUTION ANALYSIS, Matthias Theobald, Till Hoffmann, Donald Bunjes, Renate Arnold and Wolfgang Heit, Department of Internal Medicine III, BMT-Unit, University Hospital Ulm, D-7900 Ulm, FRG. Host T cells surviving preconditioning radio- and chemotherapy have been identified to mediate graft rejection after ex vivo T-depleted BMT. Attempts to eliminate these cells with chemo- and/or radiotherapy have only been moderately successful. An alternative is the in-vivo application of immunosuppressive mAbs to the marrow recipient. We here report on the efficiency of the mAb Campath-1G to deplete T lymphocytes (Tl) in-vivo as compared to standard conditioning regimens as TLI, TBI and a combination chemotherapy (CT) with Bus and Ctx. Using limiting dilution analysis the frequencies of proliferating (PTL), cytotoxic (CTL-p) and IL-2-secreting helper (HTL-p) Tl precursors as well as mature nonproliferating (radioresistant) IL-2-secreting Tl (HTL) were determined prior to and following treatment. Both TBI and CT were highly efficient at depleting PTL, CTL-p and HTL-p (> 97,6% to > 99,9%) but spared HTL to a variable extent. In eight patients treated with Campath-1G mAb a similar degree of PTL, CTL-p and HTL-p depletion was achieved in PB and bone marrow (> 85,5% to > 99,9%), and, in addition, HTL were effectively removed. Consequently Campath-1G in-vivo could be successfully employed in depleting radio- and CT-resistant host Tl prior to ex-vivo T-depleted BMT.

C 238 ANTIBODY CONCENTRATION ON T CELLS LEADING TO T CELL DEPLETION, Stefan Thierfelder, Udo Kummer and Josef Mysliwietz, Institut für Immunologie, GSF, Hämatologikum, Marchioninistr.25, 8000 Munich, FRG. High antigen density expressed on T cells is the prerequisite for coating T cells with antibodies sufficient to suppress gvh (and/or -residual hvg). In this regard Thy-1 is sufficiently densily expressed even for suppressing gvh in fully mismatched BMT. Since all other known murine and human T antigens are considerably less densily expressed so that sufficient antibody coating appears only attainable by antibody cocktails, it would be desirable to know the threshold of effective antibody coating. We therefore set up a mouse model which allows to study 5 different Thy-1 antigen densities above or below the threshold affecting antibody coating and suppression of gvh. It exploits a) the polymorphic expression of Thy-1 and b) our observation that Thy-1.1 is considerably less densily expressed on T lymphocytes than Thy-1.2. This allows us to define a scale of 5 phenotypes of C57BL/6 mouse bone marrow donor strains with decreasing Thy-1 antigen densities: 1.) Thy-1.2^+/Thy-1.2^+, 2.) Thy-1.2^+/Thy-1.1^+, 3.) Thy-1.1^+/Thy-1.1^+, 4.) Thy-1.2^+/Thy-1.1^-, 5.) Thy-1.2^-/Thy-1.1^+. The threshold i.e. the antibody concentration on T cells below which prevention of gvh in fully mismatched mice is no longer possible (even with the most suppressive anti-Thy-1 antibody isotypes) can be expressed in antibody molecules per T lymphocytes. It lies between 2.) and 3.).

C 239 THE ROLE OF NATURAL KILLER (NK) CELLS IN THE PATHOGENESIS OF aGVHD. JS Thompson, PJ Henslee, CD Jennings, ML Cibull, MJ Messino and JS Macdonald, Departments of Pathology and Medicine, University of Kentucky College of Medicine and Veterans Administration Hospital, Kexington, KY.

Acute GVHD (aGVHD) is characterized in man and experimental animals by mild to severe damage to the thymus, lymph nodes, skin, liver, bowel and occasionally other organs. Associated with these changes is a profound immunological incompetence. Although donor T-lymphocytes are essential for the recognition and initiation of aGVHD, a striking feature of aGVHD is the comparative absence of mature T-cells in the peripheral blood. In this study we report the results of a randomized trial which revealed that a reciprocal increase in $CD56^+/CD16^+/CD8\ dim^+/Ia^+$ NK cells and decrease in $CD3^+/\alpha/\beta\ TCR^+$ cells is associated with GrI-II aGVHD and is more marked in GRIII-IV aGVHD. Immunohistology of the skin revealed a general paucity of all inflammatory cells as compared to solid organ rejection. As severity of the lesion increased there were, however, progressively more NK cells in the lesions, as contracted to virtually no $CD8^+$ T-lymphocytes. Lymphocyte functional assays including PHA, Con A and Poke Weed Mitogen stimulation were performed serially post transplantation on 40 patients receiving histocompatible marrow grafts. Patients developing GVHD had significantly depressed responses to all mitogens as compared to allogeneically transplanted patients without GVHD or autologous and syngeneically transplanted patients. Similar to the alteration in peripheral blood T and NK cells, the depression of lymphocyte response was most marked in patients with GRIII-IV aGVHD. This evidence strongly implicates the role of activated $CD56^+/CD16^+/CD8\ dim^+/Ia^+$ cells in the pathogenesis of aGHVD in man.

C 240 IN VIVO ANTI-IL-2 RECEPTOR MONOCLONAL ANTIBODY; A NOVEL THERAPEUTIC STRATEGY FOR STEROID-RESISTANT ACUTE GvHD: P Tiberghien, P Herve, J Widjenes, E Racadot, JY Cahn, P Bordigoni*, N Milpied~, JP Bergerat^. Besancon, Nancy*, Nantes~ and Strasbourg^ BMT centers, France.
45 patients (pts) with steroid-resistant acute GvHD were treated by in vivo administration of an anti-IL-2R MoAb (B-B10) in a multicenter pilot study. 33 pts had received a marrow from matched related donors (MRD), 6 from matched unrelated donors (MUD) and 6 from partly MRD. GvHD was grade II in 22 pts, III in 17 pts and IV in 6 patients. B-B10 was very well tolerated with the exception of transient leucopenia in 6 pts. GvHD resolved in 29 pts (64.5%)(16 grade II, 11 grade III and 2 grade IV), improved in 7 pts (15.5%) (4 grade II, 2 grade III and 1 grade IV) and was resistant in 9 pts (7 grade III or IV). The actuarial probability of survival at one year was of 42%. GvHD recurred in 13 pts (36% of the evaluable responders) after completion of B-B10 treatment. The delay between the onset of GvHD and B-B10 treatment correlated with GvHD response (p=.03). Sequential analysis of serum IL-2R, CD-8 and TNF in 19 pts showed that high levels of these parameters correlated with grade III or IV GvHD and remained high in partial or non-responders to B-B10 (except for sIL-2R for which evolution did not correlate with B-B10 response). A double blind randomized trial of B-B10 as 1st line grade >2-GvHD therapy is underway. Lastly, a pilot study with B-B10 as part of GvHD prophylaxis in MUD or partly MRD BMT is generating interesting preliminary results.

C 241 ACUTE UPPER GI GRAFT-VERSUS HOST DISEASE (GVHD): CLINICAL PRESENTATION AND RESPONSE TO IMMUNOSUPPRESSIVE THERAPY. Daniel J. Weisdorf, Dale C. Snover, Robert Haake, Wesley J. Miller, Philip McGlave, Bruce Blazar, Norma K.C. Ramsay, John H. Kersey, Alexandra Filipovich, University of Minnesota Bone Marrow Transplantion Program, Minneapolis MN 55455.
Recognized manifestations of gastrointestinal acute GVHD include secretory diarrhea, abdominal pain, and bleeding. We have recognized a syndrome of upper GI GVHD presenting as anorexia, dyspepsia, food intolerance, nausea and vomiting. It was recognized and confirmed histologically in 64 of 469 patients undergoing matched sibling donor BMT (15% by Kaplan-Meier projection at the initiation of systemic GVHD therapy), a subset of 197 with grade II - IV GVHD. These 64 patients with upper GI GVHD were older than the overall BMT population & than those with grade II - IV GVHD. They frequently had skin GVHD (39/64), but less often lower GI disease with diarrhea (12/64) or liver involvement (8/64). Twenty-five had upper GI GVHD accompanied only by limited skin GVHD while 14 patients who initially had upper GI GVHD & skin involvement later progressed to multiorgan GVHD. Twenty-five others presented with upper GI & multiorgan GVHD. We have recognized this upper GI GVHD syndrome only since 1982 and it has been observed more frequently than lower GI GVHD. The upper GI GVHD syndrome is more responsive to immunosuppressive therapy than grade II GVHD defined by conventional clinical criteria with complete and continuing responses to treatment observed in 69±18% (95% confidence interval) of those with the upper GI GVHD syndrome compared to only 37±10% complete responses in other patients with grade II GVHD (p = .002). Upper GI GVHD appears part of the acute GVHD syndrome in that it is frequently followed by chronic GVHD (75% of patients). Patients failing immunosuppressive therapy for upper GI GVHD often progress to lower GI involvement with frank diarrhea, suggesting that this syndrome may be an earlier, more treatable manifestation of this unique intestinal immunopathology. Though the diagnosis of upper GI GVHD requires histologic confirmation, we believe this syndrome has been underreported following BMT & deserves recognition within the GVHD scoring system.

C 242 USE OF A GRAFT-VERSUS-HOST DISEASE ASSESSMENT GUIDE FOR PATIENTS UNDERGOING ALLOGENEIC BONE MARROW TRANSPLANTATION, Terry Wikle, Mary Beth Neff, Jan Luzins, Marla Gagnon, Lesley Myers, and Jerry Janiec, Bone Marrow Transplant Unit, University of Florida, Gainesville, FL 32610. Acute Graft versus Host Disease (AGVHD) remains a cause of morbidity and mortality in 30-60% of patients (pts) who undergo allogeneic bone marrow transplantation (BMT), despite the use of T-cell depletion and/or prophylactic immunosuppressive drugs. We reviewed our experience in treating AGVHD (grade 2B or greater) and found a very high response rate (35/39 or 90%) to methylprednisolone. Analysis of all variables indicated that early and intensive nursing assessment through the use of our GVHD Assessment Guide was the major factor. Quantitative AGVHD assessment was carried out from Day -1 through Day +28 and continued for longer if AGVHD develops. Parameters monitored are: rash description, stool volume, hyperbilirubinuria, and elevation of liver enzymes. This tool allows nursing and medical staff to plot out the patient's AGVHD status and quickly determine if the pt fits the pre-established criteria for the use of high-dose steroids.

C 243 EXPERIMENTAL EVIDENCES FAVORING PHYTOHEMAGGLUTININ (PHA) AS AN EFFECTIVE IMMUNOSUPPRESSIVE AGENT IN ALLOGRAFT TRANSPLANTATION, Bruce M. Wimer, Department of Medicine, Texas Tech University School of Medicine, Lubbock, TX 79430

The potential value of mitogenic PHA as an immunosuppressive agent in allograft transplantation has gone largely overlooked. Various experimental studies have shown that PHA can • suppress primary humoral responses in mice or rats to sheep or chicken erythrocytes and to typhoid antigen • blunt or block reaction to full-strength PPD in guinea pigs previously immunized against tubercle bacillus • prolong renal allograft survival in dogs, skin allograft as well as rat xenograft survival in mice, and heart allograft survival in rats • substantially inhibit GVH reactions in F_1 hybrid and newborn mouse models. The mitogenic lectin also has the capacity to accentuate inhibition of rejection responses by other immunosuppressive agents, substantial amplification of the suppression of renal allograft rejection by azathioprine and of skin allograft rejection by azathioprine and prednisone having been documented in dogs. PHA offers the advantage of low toxicity in providing concomitant myeloproliferative stimulation, antineoplastic effects, and immunostimulation, the discordance of both stimulative and suppressive actions on immunities apparently having to do with dosage, scheduling, and timing with respect to antigen exposures. The majority of these studies were flawed by using potently erythroagglutinating PHA preparations that are highly toxic in small animals, the weight of evidences indicating that subleukoagglutinating doses of the purely mitogenic nonerythroagglutinating L4 isolectin of PHA would have been more efficacious and comparatively nontoxic. Thus, the most striking results were achieved by Marcus, et al. (1968) using appropriately low doses of purely mitogenic PHA to inhibit GVH reactions markedly in newborn mice by co-culturing incompatible donor lymphocytes with PHA for 60-90 minutes before injection, or by systemic administration to the donors prior to harvesting and giving their spleen cells.

C 244 AUTOGRAFTING IN CHRONIC MYELOID LEUKEMIA (CML) AFTER MAINTENANCE OF MARROW IN CULTURE, Michael J. Barnett, Connie J. Eaves, Gordon L. Phillips, R. Keith Humphries, Dagmar K. Kalousek, Hans-G. Klingemann, Peter M. Lansdorp, Donna E. Reece, Gloria J. Shaw, John D. Shepherd, Ali G. Turhan, Allen C. Eaves, Leukemia/BMT Program of BC, Division of Hematology, Cancer Control Agency of BC, Vancouver General Hospital, and the University of BC, Vancouver, British Columbia, Canada.

We have previously shown that Philadelphia chromosome (Ph^1)-positive cells rapidly disappear when CML marrow is set up in long-term culture whereas hematopoiesis from co-existing normal stem cells is maintained. Five patients aged 22-53 (median 41) years with CML in chronic phase (2 in 1st, 2 in 2nd, 1 in 3rd) have now been treated with intensive therapy and transplantation of cultured autologous marrow. From each patient ~ 2 x 10^{10} nucleated cells were set up in culture and 10 days later ~ 1.3 - 2.1 x 10^8/kg cells were collected and infused. In the interval, patients received etoposide 1.8 g/m^2 x 1, cyclophosphamide 2.0 g/m^2 x 3 and total body irradiation 200 cGy x 5 or 6 (3 patients) or busulfan 1 mg/kg x 16, cyclophosphamide 60 mg/kg x 2 and melphalan 90 mg/m^2 x 1 (2 patients). After transplantation, recovery of the neutrophil count to >1.0 x 10^9/L was achieved by Day 31 in 4 patients and by Day 72 in 1 patient. Recovery of the platelet count to >20 x 10^9/L was achieved by Day 16, 35 and 70 in 3 of 4 patients surviving > Day 28. Regenerating hematopoietic cells were 100% Ph^1-negative in 4 patients and predominantly so in 1 patient. One patient, in whom a minor population (~5-10%) of Ph^1-positive cells reappeared, remains well on α-interferon therapy 22 months post-transplant without evidence of Ph^1-positive cells by cytogenetic or molecular studies. Two patients continue well and in cytogenetic and molecular remission off treatment 3 and 6 months post-transplant. One patient died of therapy-related toxicity, and one had a fatal recurrence of blast phase disease 3 months post-transplant. These data suggest that in some patients with CML normal stem cells with repopulating potential can be maintained in culture for 10 days. Thus, the feasibility of this approach has been established.

C 245 ROLE OF THE MICROFLORA OF THE GASTROINTESTINAL TRACT IN THE DEVELOPMENT OF GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION. Peter J. Heidt and Dirk W. van Bekkum, Radiobiological Institute TNO, P.O. Box 5815, Rijswijk, The Netherlands.

An important factor which influences graft-versus host disease (GvHD) after allogeneic bone marrow transplantation (BMT) is the recipient's gastrointestinal microflora. In experiments using mice, the mechanism responsible for the influence of the microflora on GvHD after allogeneic BMT was investigated. Recipient mice (C3H/Law) carrying a defined microflora (which we named Houston Flora [HF] after its place of origin) were housed under gnotobiotic circumstances. After total body irradiation (9 Gy, X-rays) they received a bone marrow graft from C57Bl/Rij donors, which had either the same (Houston) flora, or another defined flora (SPF-flora). The results showed, that only severe lethal graft-versus-host disease (GvHD) was observed, when the recipients harboured microorganisms which were not present in the donor, as was the case in the HF recipients of SPF bone marrow (SPF→HF) When there was identity in microflora between the donor and the recipient, GvHD was mitigated in the recipients of an allogeneic (H-2 mismatched) BMT. Complete gastrointestinal decontamination of the HF recipients of SPF bone marrow mitigated GvHD, in contrast to selective gastrointestinal decontamination, which leaves the anaerobic microflora unaffected.

These studies show that GvHD can be induced by activated T-lymphocytes from donor origin reacting against bacterial antigens which might be cross-reactive with the recipients epithelial tissue antigens. Activation of these T-lymphocytes is confined to antigens of certain bacteria of the recipient which are not present in the indigenous microflora of the donor mice. These bacteria most likely belong to the anaerobic flora of the recipient. The latter hypothesis is strongly supported by the observation in human pediatric patients that, in contrast to complete GID, selective decontamination of the gastrointestinal tract did not exert any benificial effect on moderately severe to severe GvHD after transplantation with MHC-matched sibling donor bone marow grafts.

Immunodeficiency; Genetic Diseases; Lymphoma; Infection

C 300 MURINE THYMIC RECONSTITUTION AND FUNCTION AFTER TOTAL BODY IRRADIATION (TBI): EFFECTS OF GROWTH HORMONE (GH) ADMINISTRATION, Carroll A. Brennan, Susan Brief, Daniel A. Vallera, Bruce R. Blazar, Departments of Pediatrics and Therapeutic Radiology, University of Minnesota, Minneapolis, MN 55455.
GH has been shown to influence thymic development and cell-mediated immunity (CMI) in animals and humans in whom GH-deficiency and immunodeficiency co-exist. In our institution, approximately 25% of bone marrow transplantation (BMT) recipients have GH deficiency. Most of these BMT recipients have CMI defects in the early post-BMT period. We sought to assess the effect of GH in irradiated murine recipients that do or do not receive a bone marrow rescue. C57BL/6 mice were conditioned with 600 rads TBI followed by bovine (b) GH injections (10 μg subcutaneously twice daily). A biologic effect of bGH was demonstrated by significantly greater weight gain in the treated mice (n = 35) versus saline injected controls (n = 35) at numerous intervals over the study period. On days 7, 10, and 12 following TBI, 5-7 mice per group were sacrificed to assess total thymocyte numbers, cell surface expression of CD4 and/or CD8 antigens, and thymocyte functional responses. Total cell yield from thymuses of bGH-treated mice was significantly ($p < 0.05$) greater than controls at each time period studied. Results from cell surface antigen phenotyping and functional responses are currently being analyzed and will be presented. In separate experiments, C57BL/6 (Thy 1.2^+) mice received 550 rads TBI followed by infusion of C57PL.6 (Thy 1.1^+) congenic bone marrow which had been T-cell depleted with anti-Thy 1.1 plus complement in vitro. Mice were untreated or received bGH injections (10 or 100 μg subcutaneously once daily). All mice had autologous thymocyte recovery. On days 8 and 11 post-TBI, 3-5 mice per group were sacrificed. Thymocyte proliferative responses (using PMA as a co-mitogen and recombinant IL-1,IL-2,IL-4 and/or IL-6 with or without soluble anti-CD3) in both of the bGH-treated groups of mice were significantly ($p < 0.05$) greater than untreated controls. We conclude that GH may have an important role in promoting thymic reconstitution and immune recovery following TBI.

C 301 BUSULFAN CONTAINING REGIMENS AND AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) FOR RELAPSED LYMPHOMA. S. Bulova, P. Crilley, D. Topolsky, I. Brodsky, Department of Neoplastic Diseases, Hahnemann University, Philadelphia, PA 19102.
Busulfan (Bu) 16 mg/kg and Cytoxan 120 mg/kg (BuCy2) with bone marrow transplantation has shown efficacy in the treatment of acute myelogenous leukemia. Since Busulfan is not used in conventional treatment regimens for lymphoma we undertook a trial of this regimen with unpurged ABMT in 7 patients with heavily pretreated relapsed lymphoma. 4 patients had nonHodgkin's lymphoma (NHL) and 3 patients had Hodgkin's disease (HD). The average age was 35 (18-62). The average number of courses of chemotherapy (CT) previously received was 2.4 and radiation (XRT) was 0.9. All patients had active disease at the time of transplant. All patients tolerated the procedure well and 1 patient had venooclussive disease of the liver (VOD). The average time to engraftment as measured by >500 granulocytes was 29 days. 3 patients relapsed and 4 of the 7 patients have expired. The median survival is 418 days. Since the regimen was well tolerated and resulted in some disease response we added VP-16, 40 mg/kg (BuVPCy) and treated an additional 6 patients, 4 with NHL and 2 with HD with an average age of 32 (21-50). They all had relapsed active disease and had received an average of 2.5 courses of CT and 0.5 courses of XRT. 2 patients had nonfatal VOD and 1 had severe skin toxicity. The average engraftment time, >500 granulocyte, was 20 days. 5 of the 6 patients are alive and disease free with an average followup of 200 days. The Bu containing regimen BuCy2 followed by ABMT showed limited efficacy. The addition of VP-16 gave a regimen (BuVPCy) was adequately tolerated by heavily pretreated patietns with active relapsed lymphoma and appears to show efficacy in controlling the disease. The results suggest that this Busulfan containing regimen with ABMT be considered for patients with relapsed lymphoma who have received many of the drugs conventionally used for this disease.

C 302 SUCCESSFUL BONE MARROW TRANSPLANTATION (BMT) FOR CORRECTION OF HURLER SYNDROME UTILIZING HAPLOIDENTICAL MARROW GRAFTS. Ciocci G, Henslee-Downey PJ, Romond E, Messino M, Macdonald J, Harder E, Marciniak E, and Thompson J; Department of Medicine and Pediatrics, University of Kentucky, Lexington, KY 40536. Since November of 1987, six patients (pts) between 11 and 43 months of age have received allogeneic BMT for correction of Hurler Syndrome, an autosomal recessive disorder characterized by the absence of alpha-L-iduronidase resulting in accumulation of glycosaminoglycan substrates in body organs and tissues leading to deterioration and death within the first decade of life. Therefore, despite the lack of a matched sibling donor, marrow grafting is being explored for enzyme replacement using alternative donors including haploidentical family members who are partially mismatched at HLA antigens(ag). In our series, pts were conditioned for BMT with total body irradiation, Cytosar, Cytoxan and Methylprednisolone. A partial *ex-vivo* T-lymphocyte depletion of the marrow graft with an anti-CD3 MoAb, T10B9, is followed by an *in vivo* depletion with daily administration of a lymphocyte targeted immunotoxin, Xomazyme H-65, given on either day+10 for 7[1],+7 for 10[2], or+5 for 12 days[3]. In the GVHD direction, the pt was mismatched for either one[3] or two[3] ag involving Class I ags[3], Class II[2], or both[1], the majority demonstrating bidirectional reactivity in mixed lymphocyte cultures. Three donors were parents with partial production of enzyme and 3 donors were family members with normal enzyme levels. All pts engrafted promptly with a mean day to a peripheral white count >500 occurring at +12 and >1000 at +16 days post BMT. The highest overall acute GVHD Grade observed was 0 in 2, I in 2, II in 1 & IV in 1 pt. Five pts(83%) are surviving between 19-661 days following BMT. All evaluable pts have demonstrated persistent enzyme production appropriate to donor levels. Diverse clinical manifestations of storage material reversal, as well as, progression in both physical and mental development has been observed; impressive in children <two years of age. These encouraging results support the continued application of allogeneic BMT including mismatched grafts for the correction of Hurler Syndrome; the results may be improved with earlier transplantation.

C 303 PROPHYLAXIS AGAINST CYTOMEGALOVIRUS (CMV) INTERSTITIAL PNEUMONIA (IP) IN CMV TITER POSITIVE RECIPIENTS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT). Elfenbein G, Krischer J, Rand K, Graham-Pole J, Hong R, Jansen J, Lazarus H, Winton E., Dept. of Int. Med., University of South Florida, Tampa, FL, USA. CMV IP is a major cause of death after allogeneic BMT especially in CMV titer positive (POS) recipients. We have evaluated the role of intravenous immunoglobulin (IVIG) as prophylaxis against CMV IP in 181 consecutive patients (pts) receiving total body irradiation (TBI) in a 5 institution balanced trial. All pts received 500 mg/kg unscreened Sandoglobulin(R) weekly from day-8 to day +96. Median age was 27.5 yrs with range of 1 to 53 yrs. Forty pts had acute lymphoblastic leukemia, 56 acute myelogenous leukemia, 56 chronic myelogenous leukemia, 12 severe aplastic anemia, 7 lymphoma, 5 myelodysplasia and 5 miscellaneous diagnoses. There were 17 pts whose donors were not HLA identical and 154 who were. The median dose of TBI was 1200 cGy with range of 450-1375 cGy. 102 (56%) pts were CMV POS, 84 (46%) donors were CMV POS and only 72 (42%) pts received CMV titer negative blood products (NEG BP). Acute graft versus host disease (GVHD) prophylaxis was methotrexate (MTX) for 17 pts, MTX and prednisone for 11, cyclosporine (CSA) for 6, MTX and CSA for 92, T-cell depletion for 35, cyclophosphamide ± MTX for 2 and nothing for 17. Within 150 days after BMT, IP was seen in 30 (17%) pts, CMV IP was seen in 13 (7%) pts and 8 pts died from CMV IP. Acute GVHD occurred in 87 (49%) pts. CMV IP occurred in 3/50 (6%) pts who were CMV NEG, had CMV NEG donors and received CMV NEG BP. CMV IP occured in 9/101 (9%) pts who were CMV POS and/or whose donor was CMV POS and/or received CMV unscreened BP. Finally, CMV IP occurred in 5/51 (10%) pts who were CMV POS, whose donors were CMV POS and who received CMV unscreened BP. The most remarkable finding of this study is the low rate of CMV IP, especially in the highest risk category, when IVIG was used to prevent CMV IP. In this setting, significant risk factors for CMV IP after IVIG were age 40 and over, total dose of TBI (1320-1375), and T-cell depletion to prevent acute GVHD.

C 304 CMV PNEUMONITIS FOLLOWING BMT: NATURAL HISTORY AND RESULTS OF TREATMENT. Helen Enright, Dennis Confer, Robert Haake, John Kersey, Philip McGlave, Norma Ramsay, Daniel Weisdorf, and Wesley Miller. Departments of Medicine, Pediatrics, and Laboratory Medicine, Bone Marrow Transplantation Program, University of Minnesota, Minneapolis MN 55455.

Cytomegalovirus (CMV) pneumonitis (IP) is a devastating and frequent complication following BMT. CMVIP was documented in 95 among 1105 patients undergoing BMT between August 1975 and July 1989. Although most cases (86) developed in allogeneic BMT recipients, CMVIP occurred in 8 autologous recipients. Patients ranged in age from 4 months to 59 years (median 28 years) and 75 patients were aged 17 years or greater. Sixty-eight of 86 allogeneic BMT patients with CMVIP also had acute GVHD. CMVIP was temporally associated with relapse of previous malignancy in 7 patients. The median time from BMT to diagnosis of IP was 68 days in all patients (70 days in allogeneic and 36 days in autologous recipients). Median survival from diagnosis of IP was 20 days, 16 days in allogeneic and 34 days in autologous recipients. Patients were treated according to available agents:

Treatment Group	n	Median Survival	Number Surviving	Initial Improvement	Relapse CMVIP
None	40	7.5 days	1	1	0
Acyclovir	3	17 days	0	1	0
Acyclovir + Ig	13	22 days	2	4	1
Ganciclovir	13	47 days	1	5	2
Ganciclovir + Ig	26	42 days	8	14	5

Of 48 patients who were ventilated to provide respiratory support, only 3 (6%) recovered sufficiently to allow discontinuation of support. Despite encouraging result with Ganciclovir and Ig, earlier recognition and better therapy for CMVIP is urgently needed. Prolonged antiviral therapy may decrease CMVIP relapse.

C 305 UNRELATED DONOR (URD) BONE MARROW TRANSPLANTATION (BMT) FOR CONGENITAL IMMUNODEFICIENCIES (ID) A.H. Filipovich, R.S. Shapiro, P. McGlave, T Kim, J.H. Kersey, N.K.C. Ramsay. University of Minnesota, Minneapolis, MN.

Fewer than 10% of infants with lethal ID have immunocompetent histocompatible sibling donors for BMT. In recent years T-depleted haploidentical parental BMT has been investigated as curative treatment for severe combined immunodeficiency (SCID), and to a lesser extent for other lethal ID; this approach is frequently associated with delayed, partial immunoreconstitution and a high incidence of posttransplant EBV-associated lymphoproliferative complications. For these reasons we have initiated a pilot study of URD BMT for ID. Acceptable URD, defined as HLA A, B, DR, Dw identical or 1 HLA locus (A or B) mismatched but DR, Dw identical, have been identified for 15 of 18 children with ID. To date 13 patients (6 mos. to 9 yrs.) received BMT for: SCID (6), Chediak Higashi (CHS) (2), Wiskott Aldrich (WAS) (1), Ataxia Telangiectasia (1) and other combined ID (3). 12/13 patients received pretransplant conditioning; 10 patients received GvHD prophylaxis with methotrexate, cyclosporin and prednisone and 3 with methotrexate, ATG and prednisone. 8/13 patients are alive. Ten (6 SCID, 1 WAS, 2 CHS, 1 combined ID) achieved prompt engraftment. Seven (5 SCID, 1 WAS, 1 CHS) have achieved immunoreconstitution and are currently well without GvHD median 12 mos. (range: 3-22 mos.) post BMT. One patient (CHS) is still on therapy for grade 3 acute GvHD. Five pts. have died: 1 pt. with SCID on d.20 of preexisting infection, and the remaining 4 pts. of complications related to poor engraftment (3) and pulmonary hemorrhage (1). We conclude that suitable URD can be found for a majority of ID pts. and that URD BMT offers a promising alternative to T depleted haploidentical BMT for children with several lethal ID.

C 306 LONG TERM EVALUATION OF HLA-MISMATCHED BMT IN CONGENITAL IMMUNODEFICIENCIES
Wilhelm Friedrich, Christian Knobloch and Sharga F. Goldmann, Department of Pediatrics and of Transfusion Medicine, University of Ulm, D-7900 Ulm, FRG
In order to further define the potential and current limitation of HLA-mismatched, T-cell depleted BMT in the treatment of Severe Combined Immuodeficiency Syndromes, we restudied a group of 16 pts., transplanted between 1982 and 1986 and surviving between 3 and 7 yrs. after BMT. Pertinent findings include: 1) When transplanted without conditioning (12/16 pts.) engraftment of donor cells is confined to T cells. 2) Once normalized (during the first year after BMT), the number and function of these T cells remain regular (14/16 pts.), while in 2/16 pts. initially subnormal functions of donor T cell have remained so until now. 3) In the subgroup of pts., where circulating non functional B cells are present at diagnosis (B+ variants of SCID), these B cells undergo variable functional maturation after BMT. This may develop as late as during the third year after BMT. However, we have never observed complete normalization of humoral immunity, as reflected by persistance of reduced serum levels of one or more classes of immunoglobulins < 2SD as well as by persistantly subnormal antibody responsiveness. 4) In B^- variants of SCID, humoral immunity does not develop. 5) Prior cytoreductive conditioning favours development of humoral immunity. 6) In none of these patients there is clinical evidence of late GvHD.
This analysis has been useful to identify subgroups of patients where modifications of the transplant approach are necessary in order to improve long term results after BMT.

C 307 CMV HYPERIMMUNOGLOBULIN AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION, Andrew P. Grigg[1], Gordon L. Phillips[1], Michael J. Barnett[1], Noel A. Buskard[2], Donna E. Reece[1], John D. Shepherd[1], Hans-G. Klingemann[1], Leukemia/Bone Marrow Transplant Program of B.C.[1] & Canadian Red Cross Society[2], Vancouver, B.C., Canada.
There is evidence that prophylactic use of immunoglobulin (IG) preparations containing high titers of antibody to CMV is effective in reducing the incidence of CMV disease after bone marrow transplantation (BMT). As the half-life of such preparations may be as short as 3-5 days, and CMV disease occurs most frequently between 4 and 12 weeks after BMT, frequently coinciding with GVHD, we tried to rationalize use of this expensive product. CMV-IG (Immuno, Vienna) at 150 mg/kg was administered every week for a total of 6 doses beginning on day 28 post BMT to patients seropositive for CMV and/or with a seropositive donor, undergoing allogeneic BMT. Fifty-four were eligible for the protocol. Six patients died early and were ineligible for analysis; none had evidence of CMV disease. The remaining 48 were stratified according to no acyclovir (n=3), low dose (250 mg/m^2 q 8h from day -1 to +30)(n=19) and high dose acyclovir (500 mg/m^2 q 8H)(n=26). In the first 120 days, CMV infection (positive culture) occurred in 1, 10 and 6 patients and CMV disease occurred in 1, 4 and 2 patients respectively. Two patients had CMV pneumonia diagnosed on day +52 and +79 after BMT and died shortly thereafter; another patient died of multiorgan failure and grew CMV from a bronchoalveolar lavage. No patients receiving CMV-IG and high dose acyclovir developed CMV pneumonia. Four patients, two of whom received high dose acyclovir, developed CMV enteritis at day 54, 70, 76 and 114. In conclusion, CMV-HIG started 4 weeks post BMT, in combination with high dose acyclovir, appears to prevent CMV pneumonia. However, since some patients developed CMV disease after the completion of CMV-IG at day +65, an extension of prophylaxis should be considered.

C 308 LONGTERM $CD4^+$ T HELPER DEFICIT INDUCED BY GRAFT-VS-HOST REACTION.
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Most splenic immune functions recovered from a period of acute graft-vs-host (GVH) associated immune deficiency to near normal levels within 4-5 months after the induction of GVH by injection of B10 splenocytes into unirradiated (B10xB10.BR)F1 hosts. This restoration of function, which occurred concurrent with repopulation of the host lymphohematopoietic system by donor cells, included Con A and LPS stimulated proliferation and cytotoxic T lymphocyte (CTL) responses to allogeneic stimulators. Yet even after 12 to 18 months, a deficit in L3T4 (CD4) T helper function remained. L3T4 dependent responses were reduced or absent, including CTL generation against TNP-modified syngeneic stimulators, IL-2 generation in autologous mixed lymphocyte reaction and anti-CD3 stimulated proliferation in Lyt-2 (CD8) depleted cultures. FACS analysis demonstrated near normal frequencies of L3T4 cells, all of which were $CD4^+$ $CD8^-CD3^+$ T cells of donor origin. No suppression of CTL responses was found in coculture, suggesting that L3T4 function was deficient, not suppressed as in early GVH. Induction of GVH with a combination of B6 Thy 1.1 T-depleted bone marrow and B6 Thy 1.2 lymph node cells (lacking pre-T cells) demonstrated that mature donor T cells (Thy 1.2) predominated in the host spleens at 8 weeks of GVH, but that donor pre-T cells (Thy 1.1) maturing in the GVH host predominated in long term GVH (9 months). Thus a defect in the maturation of T cells in the GVH host could produce a long standing T helper functional deficit.

C 309 DEFECTIVE ACTIVATION OF T CELLS FROM MICE WITH MINOR ANTIGEN GvHR, Brian L. Hamilton, Department of Pediatrics, University of Miami, Miami, FL 33136. Profound immunodeficiency is a major manifestation of the graft-vs-host reaction to minor H antigens in mice. Previous work from this lab demonstrated normal B cell function and a defect in T helper cell function in vivo. The present study was done to further define the T helper cell defect in vitro. A mixture of T-depleted bone marrow plus spleen cells from B10.D2 mice was injected i.v. into lethally irradiated BALB/c (GvHR) or B10.D2 (transplant control) mice. Spleen cells were recovered from transplanted and normal control B10.D2 mice from 4-8 weeks after transplant. Cells were stimulated in vitro with Con A or anti-CD3 antibody. IL-2 production and IL-2 receptor (IL-2R) expression were assayed at 24 hours and proliferation measured at 72 hours. The number of spleen cells recovered from mice with GvHR was significantly decreased compared to controls, but the proportions of CD4 and CD8 T cells were relatively normal. Stimulation of T cells from the normal and transplant controls resulted in IL-2 production, an increase in IL-2R expression, and proliferation. T cells from mice with GvHR also produced IL-2, but failed to express IL-2R, and did not proliferate. These results demonstrate that T helper cells from mice with GvHR can be induced to produce IL-2 by Con A and via antibody to the Ti-CD3 complex. In contrast to controls, T cells from mice fail to express IL-2R in response to these signals. The deficiency in T helper function may result from a failure of clonal expansion of T helper cells due to the inability of these cells to express IL-2R upon activation.

C 310 **A RANDOMIZED STUDY OF PROPHYLACTIC INTRAVENOUS GAMMAGLOBULIN (GAMMAGARD) IN PEDIATRIC MARROW TRANSPLANTATION, Richard E. Harris, Steven Neudorf, James Sambrano, Daniel Pietryga, Christopher Morris, Cynthia DeLaat, Robin Mueller, and Peggy Kaiser, Bone Marrow Transplant Section, Children's Hospital Medical Center, University of Cincinnati, Cincinnati, OH 45229.** To date, 59 pediatric marrow transplant patients have been entered into a randomized controlled trial of weekly intravenous gammaglobulin prophylaxis utilizing Gammagard (Baxter) 500 mg/kg on days -8, -1, +6, then weekly until day +97. Pharmacokinetic studies were performed on days -8, +13, and +56 to determine the rate of clearance of the IVGG at various stages of the transplant. Cytomegalovirus titers by CFT, ELISA, and neutralization were determined pre-BMT and at set intervals post transplant. Serial IgG levels and antibody titers to HSV, VZV, EBV, adenovirus, RSV, parainfluenza and influenza were obtained. Patients were monitored for the number of febrile days, days of hospitalization, number and types of pneumonias, bacteremias and other infectious episodes, usage of antimicrobial agents, and the number of days of oxygen therapy, and ventilatory and presser support. In the event of any pneumonic process, definitive diagnosis was attempted. Of the 59 patients, 61% were CMV seronegative pre-transplant and thus received CMV negative blood products exclusively. The two groups were comparable with respect to diagnosis, type of transplant, intensity of preparative therapy, receipt of total body irradiation, recipient CMV status, and age. Preliminary analysis reveals a trend for improved survival for the first six months after transplant among the patients receiving IVGG, but with no evidence for improvement in ultimate survival. Most of the adverse events among the patients randomized to not receive IVGG were in those patients receiving the most intensive preparative therapies and those who were CMV seropositive pre-transplant. The data will be updated and reanalyzed just prior to the UCLA meeting for presentation.

C 311 NATURAL KILLER FUNCTION FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION. M. Hokland, B. Nielsen and P. Hokland, Institute of Medical Microbiology and Department of Haematology, University of Aarhus, Denmark.

Natural killer (NK) cell function was followed sequentially after allogeneic bone marrow transplantation using three approaches: (1) chromium-release assay with purified mononuclear effector cells, (2) chromium-release assay with whole blood effectors, and (3) enumeration of lymphocytes bearing the NK-associated antigen NKH-1. The two latter methods enabled us to demonstrate a very early reappearance (at day 4 posttransplant) of pre-NK cells, which after interferon-α enhancement effectively lysed K562 cells and carried the NKH-1 antigen. During the first month NK function steadily increased, and at day 28 activated NK cells, which lysed the otherwise resistant P815 cell line, could be demonstrated concomittant with a substantial over-shoot in the proportion of NKH-1 positive cells. Furthermore, the increase in NK lysis was more pronounced in patients with cytomegalovirus (CMV) infections (primary or reactivated). After the first month of increase, NK declined reaching levels near those observed in their respective bone marrow donors at day 90. These data demonstrate a surprisingly early recovery after allogeneic bone marrow transplantation, which can largely be related to external factors among which CMV seems to be a prime candidate.

C 312 HERPES ZOSTER (HZ) INFECTION FOLLOWING AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) FOR HODGKIN'S DISEASE (HD), Neal P. Christiansen and David D. Hurd, Department of Medicine and The Bone Marrow Transplant Program, University of Minnesota, Minneapolis, MN 55455
HZ infections are seen in as many as one-third of patients who have undergone standard treatment for HD. Various factors such as age, level of complement fixation antibody, intensity of therapy and stage and subtype of HD have been reported to predict which patients are at an increased risk for developing HZ. Although HZ infections have been reported after ABMT, there is little information on factors which might be useful in predicting risk for developing HZ following ABMT for HD. Between June, 1985 and May, 1988, 30 sequential patients with HD undergoing ABMT with a uniform chemotherapy regimen (cyclophosphamide 1500 mg/m^2 x 4d, etoposide 150 mg/m^2 bid x 3d and BCNU 300 mg/m^2 x 1d), with (n=6) or without (n=24) involved field irradiation (IFR) (2000 cGy in 10 fractions), were evaluated for the development of HZ post ABMT. 8 patients developed HZ in the first 150 days post ABMT. Neither pretransplant antibody titer to HZ, age, gender, extent of prior therapy, nor the use of IFR in the preparative regimen were predictive. However, among the 10 patients with a history of HZ infection subsequent to the diagnosis of HD and prior to ABMT, 6 (60%) developed HZ in the first 150 days compared to 2 cases of HZ among the 20 patients without a prior history of HZ ($p<0.01$, Fisher's Exact). Among the 4 patients who were at risk due to a prior history of HZ and who did not develop HZ in the early post transplant period, 3 had been given acyclovir as prophylaxis for H. simplex. We conclude that a history of HZ subsequent to the development of HD is a significant risk factor for the development of HZ post ABMT. Although acyclovir may have been beneficial in the our patients with a prior history of HZ who did not develope recurrent HZ, a randomized study would need to be done to evaluate its role in prophylaxis for HZ post ABMT.

C 313 MARROW METASTASES AT THE TIME OF AUTOLOGOUS PERIPHERAL STEM CELL HARVESTING AND RESPONSE OF REFRACTORY HODGKIN'S DISEASE TO HIGH DOSE CYCLOPHOSPHAMIDE, CARMUSTINE AND ETOPOSIDE (CBV), Kessinger A, Bierman PJ, and Armitage JO, Section of Oncology/Hematology, University of Nebraska Medical Center, Omaha, NE 68105
Autologous peripheral stem cell transplantation was used for patients with refractory Hodgkin's disease whose bone marrows were inadequate for transplantation to restore marrow function following high dose chemotherapy. Autologous marrow was considered inadequate if there was histopathologic evidence of marrow metastases, skeletal metastases or hypocellularity in conventional harvest sites, or a history of prior marrow metastases. To determine if patients with marrow metastases at the time of peripheral stem cell collection were as likely to have a response to high dose therapy as those who had no evidence of marrow metastases, we reviewed the records of all patients with refractory Hodgkin's disease treated with CBV and autologous peripheral stem cell transplantation at the University of Nebraska Medical Center who had bone marrow examined for evidence of malignancy at the time of peripheral stem cell harvesting. Seventeen of 19 patients with marrow involvement and 19 of 21 patients without marrow involvement were evaluated for response. For marrow + patients, there were 10 complete responses and 6 partial responses. For marrow - patients there were 12 complete responses and 5 partial responses. Patients with marrow metastases at the time of peripheral stem cell harvesting are not at decreased risk to achieve a complete response to CBV therapy (p=NS). Four of 17 responding patients with negative marrow have died or relapsed, and 10 of 16 patients with positive marrow have died or relapsed (p=NS, Fisher's exact test).

C 314 75 PATIENTS(PTS)WITH METABOLIC DISORDERS TREATED IN THE UNITED STATES WITH BONE MARROW TRANSPLANTATION AT EIGHTEEN INSTITUTIONS,(U.OF MINNESOTA et al) Krivit, W., Whitley,C.B., Ramsay, N.K.C., Kersey, J.H., Henslee-Downey,J.,Yeager,A., Klemperer M., Bayever,E.,Kirkpatrick,D.,Parkman,R.,Coccia,P.,Malatack,J., Harris, R.,Sanders,J. Feig,S., Grabowski,G.,Cowan.,M.,Trigg,M.E., Rappeport,J.,Casper,J.,Schorin,M.:A recent survey of Bone Marrow Transplant (BMT)Programs in the U.S. has been conducted to determine the strategies employed and outcome following BMT for metabolic disorders. 71 patients with lysosomal storage diseses have been identified in 18 institutions. The number of pts treated for specific entities are Hurler(32)MetachromaticLeukodystrophy(12),Gaucher(7),Sanfilippo(6),Hunter(4),Krabbe3, Nieman-Pick(2),Maroteaux-Lamy(2),Morquio(1),Pompe(1)and Wolman (1). In addition, adrenoleukodystrophy(3) a peroxisomal disorder and Lesch-Nyhan(1) a metabolic disease have had bone marrow transplantation. Diverse methods of choice of donor, preparative regimen, method of prevention and treatment of graft versus host disease have been utilized. For pts with Hurler disease comprising the largest group, 15/32 received histocompatible sibling grafts,12 of whom are currently surviving. 17/32 received alternative grafts, 10 from family members with 4 surviving, and 7 from unrelated phenotypically similar volunteer donors with 6 surviving. These preliminary results suggest that marrow transplantation using alternative donors can be successful in pts with genetic disorders where the availability of matched siblings is limited. A complete summary of all patients with be presented. The experience to date supports a continued effort in transplantation of these pts. Furthermore, the need for establishing a working study group to formulate objectives and common protocols will be explored.

C 315 THE RECONSTITUTION OF IMMUNITY AFTER HLA HAPLOIDENTICAL BMT IN A SCID WITH ADA DEFI CIENCY,A.Lanfranchi,L.Nespoli,F.Porta,M.F.Martelli,F.Aversa,F.Bonetti,F.Locatelli, M.Martinetti,G.R.Burgio.Dep.Pediatrics,IRCCS Policlinico S.Matteo,Pavia;Dep.Hematology,Peru gia;HLA Lab.AVIS,Pavia,Italy.

ADA converts adenosine and deoxyadenosine to inosine and deoxyinosine and its deficiency is associated with a combined immunodeficiency(CID).The mode of inheritance of this defect ap pears to be autosomal recessive and the carrier state can be easily shown.Since BMT is a sug gested treatment for SCID,and only a minority of pts has a id. donor,to avoid GVHD in haplo identical BMT T cell depletion has been attempted, but this procedure has been associated wi th failure of engraftment.E.F. was born after uneventful pregnancy (weight 3.500 gr.).Imme diately after discharge from hospital she presented diarroea,upper and lower respiratory tract infections and failure to thrive.Absence of thymic shadow,leucopenia,absence of IgM and IgA low levels of IgG,impairment of T cell functions were detected.ADA was absent in E.F. RBC,re duced of 50% in parents.She received BMT from the father after T cell depletion with soybean lectins agglutination and E rosetting.14 months after BMT E.F.is a full chimera (100% donor) and her cellular immunity shows an almost normal response to PHA and NK a ctivity.T cell sub population are in the normal range and titers of natural antibodies,IgM,are present.

C 316 INVOLVED FIELD RADIOTHERAPY (IFRT), HIGH-DOSE BCNU, CISPLATIN, VP-16, AND AUTOLOGOUS BONE MARROW TRANSPLANT (AuBMT) FOR REFRACTORY LYMPHOMA. H. Lazarus, P. Crilley, N. Ciobanu, R. Creger, R. Fox, D. Shina, S. Bulova, R. Gucalp, I. Brodsky, Ireland Cancer Center, Case Western Reserve University, Cleveland, OH 44106; Hahnemann University, Philadelphia, PA 19102; Albert Einstein Cancer Center, New York, NY 10467.

40 relapsed or refractory lymphoma patients (pts) received IV BCNU 200-350 mg/m²/d x 3, Cisplatin 40 mg/m²/d x 5, & VP-16 800-1000 mg/m²/d x 3 followed by AuBMT. 22 pts had Hodgkin's Disease (HD) & 18 had non-Hodgkin's lymphoma (NHL). Pts did not receive additional salvage chemotherapy to decrease tumor burden prior to beginning the AuBMT regimen. 29 pts received IFRT 1200-2000 cGy to sites of active bulky disease. Marrow was either treated in vitro with VP-16 [50-75 μM] for 60 min at 37°C (n=17) or frozen unpurged (n=23). Toxicities included fever, bacteremia, fungemia, severe nausea & vomiting, high-tone hearing loss, stomatitis, esophagitis, diarrhea & transient cardiac conduction defects. For 39 evaluable pts, median duration of neutropenia (<500/μl) was 16 days (range: 8-35) and thrombocytopenia (<20,000/μl, untransfused), was 15 days (range: 0-52). No statistical differences in recovery of peripheral blood counts were noted between VP-16 purged and unpurged bone marrow. 1 pt died early (fungemia), partial responses occurred in 12 pts and two pts did not respond. Complete responses (CR) were noted in 25 pts (12 HD pts and 13 NHL pts) & 23 pts remain in CR. CR duration ranged 1.5+ - 28+ mo (median:8.5+ mo). This regimen is effective salvage treatment in relapsed HD and NHL, and VP-16 purging of marrow may be a useful adjunct.

Days:	Neutropenia median	range	Thrombocytopenia median	range
VP-16 purging	17	11-35	16	7-52
No purging	14	8-33	14	0-49

C 317 **SELECTIVE DEFECT IN CD4+CD8- BUT NOT CD4-CD8+ SPLENIC T-CELLS IN MICE UNDERGOING GRAFT-VERSUS-HOST REACTIONS. Robert B. Levy, Monica Jones, and Carolyn Cray, Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, Florida 33101.** The nonspecific nature of depressed T-cell reactivity occurring during GvHR is reflected in the loss of proliferative responses against mitogens, help for antibody production, and the ability to generate cytotoxic T-cell activity. The present studies were designed to precisely determine the nature of the defect in host T-cell responsiveness during GvHR. Highly purified CD4+CD8-and CD4-CD8+ populations from spleens of mice undergoing GvHR induced by the P -> F1 model were examined for responses induced by anti-CD3 mAb and ConA in the presence of irradiated syngeneic accessory cells. Following 3 weeks of GvHR, low levels of anti-CD3 and ConA induced proliferation were detected in the CD4+ population. In contrast, CD8+ cells from these GvHR mice responded as well as normal CD8+ to these agents. Notably, GvHR CD4+ cells could proliferate in response to anti-CD3, anti-Ly-6A/E, and calcium ionophore when incubated in the presence of PMA. Furthermore, the ability of anti-CD4 mAb to inhibit anti-CD3/PMA stimulation GvHR CD4+ cells indicated the CD4 molecule on this GvHR population apparently possessed some functional capacity. Nylon adherent spleen cells were found to contain a population capable of suppressing normal CD4+ T-cell responses. Thus, in total, these findings suggest at least two distinct abnormalities may contribute to T-cell unresponsiveness in GvHR induced immune deficiency: one involving a defect in the peripheral CD4+ T-cell population and the second involving an active suppression mechanism.

C 318 CYTOTOXIC ACTIVITY AGAINST VARICELLA-ZOSTER VIRUS-INFECTED TARGET CELLS AFTER MARROW TRANSPLANTATION. Per Ljungman, Raleigh A. Bowden and Joel D Meyers, Fred Hutchinson Cancer Research Center, Seattle, Wa 98104 and Department of Medicine, Huddinge Hospital, Huddinge, Sweden.

Patients and methods: 17 patients who underwent allogeneic marrow transplantation were repeatedly studied during the first 100 days after transplantation. Autologous and HLA mis-matched fibroblasts were infected with varicella-zoster virus (VZV). Cytotoxic activity by peripheral blood lymphocytes against VZV infected fibroblasts and K562 targets was analyzed with a ^{51}Cr-release assay.

Results: No HLA-restricted lysis was detected. Lysis of HLA mis-matched VZV infected target cells by patient's lymphocytes was significantly reduced after marrow transplant compared to healthy normals ($p<0.05$). In contrast, lysis of K562 targets by peripheral blood lymphocytes from patients was similar or increased compared to controls. Three patients developed herpes zoster during the study. No significant difference was found in the lysis of VZV infected target cells between patients who developed herpes zoster compared to those patients who did not.

Conclusion: Depressed natural killer cell activity against VZV infected targets might be important for the development and outcome of VZV infection but studies in more patients and with longer follow-up time are needed.

C 319 AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) FOR RELAPSED HODGKIN'S DISEASE (HD) AND LARGE CELL NON-HODGKIN'S LYMPHOMAS (NHL) EMPLOYING HIGH DOSE CYCLOPHOSPHAMIDE, ETOPOSIDE AND CISPLATIN (CEP). A PHASE I-II STUDY. Mangan K.F., Glenn L.D., Cropper, T. and Mullaney, M. Temple University School of Medicine, Bone Marrow Transplant Program, Philadelphia, PA 19140. Intensive doses of cyclophosphamide (CTX), etoposide (VP-16) and cisplatin were administered to exploit the anti-lymphoma synergism of etoposide and cisplatin and to provide a non-cross resistant and non-pulmonary toxic option for patients failing conventional treatments with Stage III-IV HD or NHL. CTX (6 gm./m^2 as 1.5 gm./m^2 IV QDX4D) and cisplatin (150 mg./m^2 as 72 hr. continuous infusion) was administered to all patients. VP-16 was escalated from 900 mg./m^2 to 2400 mg./m^2 (Q12H IV in 6 divided doses). Local field irradiation (LFI, 2000-4000 cGy) was only given to patients with bulky disease (BD $\geq$ 5cm.) pre or post ABMT. All patients were rescued with 1.8 - 2.6 x 10^{10} autologous, unpurged marrow containing 10^4 CFU_{GM}/kg. body weight. As of 9/89, 13 patients (HD, NHL) who relapsed following standard MOPP, ABVD, CHOP, MACOP-B and M-BACOD regimens have been entered at 900 (N=1), 1500 (N=3), 1800 (N=4) and 2100 (N=4) and 2400 (N=1) mg./m^2 VP-16 levels. Bone marrow aplasia with severe neutropenia (nadir 25 WBC/μl) occurred in all patients. All patients engrafted: 500 neutrophils/μl (mean 19d, range 12-29 d); 20,000 platelets/μl. (mean 24d, range 18-29 d), 1% retics (mean 21d, range 14-27d). There was 1 toxic death (hepatic veno-occlusive disease) and 1 septic death (fungal). Mild to moderate dermatitis, mucositis, diarrhea, hepatitis, ototoxicity and nephrotoxicity were common but reversible in all others. Serious cardiac or pulmonary toxicity has not been observed. Of 11 evaluable patients, 6 achieved complete remission (CR). Three (without LFI) remain disease free at 368+, 136+ and 119+ days. Two with bulky disease achieved CR post ABMT with LFI and remain disease free at 427+, 187+ days. Five others achieved partial remissions with $\geq$ 80% tumor cell reductions. These preliminary results suggest that high dose CEP with ABMT has acceptable toxicity and excellent antilymphoma activity in heavily pretreated relapsed HD or NHL. Marrow support is probably required in this protocol for VP-16 doses 1800 mg./m^2.

C 320 SEROLOGICAL AND CLINICAL STUDIES OF EPSTEIN-BARR VIRUS INFECTION IN ALLOGENEIC MARROW GRAFT RECIPIENTS, M.A.P. Oosterveer, J.W. Gratama, J.M.M. Lepoutre, J.J. van Rood, F.E. Zwaan, J.M. Vossen, G. Klein and I. Ernberg. Dept. of Tumor Biology, Karolinska Institute, Stockholm, Sweden; Depts. of Immunohematology and Blood Bank, Hematology, and Pediatrics, University Hospital, Leiden; and Laboratory for Virology, National Institute for Public Health and Environmental Protection, Bilthoven, the Netherlands.
A serological study of Epstein-Barr virus (EBV) infection was conducted in 153 allogeneic BMT recipients and their donors to estimate the frequency of virus eradication as reported earlier for 2 patients (Gratama et al, PNAS 1988; 85: 8693). We took a decrease in IgG antibodies against EBV viral capsid antigen (VCA) to 1/100 of the pretransplant concentration as a marker of virus eradication. This contention was confirmed by virus isolation studies which were consistently negative in such patients. There were 94 patients who were EBV seropositive prior to BMT and who had serological follow-up times exceeding 100 days post BMT. Twenty patients showed a decrease in VCA antibody titers as specified above; 10 of them even became completely seronegative which lasted for more than 1 year in 4 patients. This serological pattern was significantly more frequent among patients with chronic GVHD than among those without: 12 of 20 patients with decreasing VCA titers developed chronic GVHD vs. 22 of 73 without chronic GVHD ($p=0.02$). Among patients transplanted for acute leukemia in remission or chronic myelogenous leukemia in chronic phase, the incidence of relapse was lower in those with decreasing VCA titers than in those without (1 of 17 vs. 18 of 62 patients; $p=0.04$). These results suggest that GVH reactivity may contribute to eradication of EBV by eliminating EBV-carrying cells of recipient origin.

C 321 PROGNOSTIC SIGNIFICANCE OF OCCULT TUMOR CELLS IN BONE MARROW OF PATIENTS WITH INTERMEDIATE AND HIGH GRADE NON-HODGKIN'S LYMPHOMA, J.G. Sharp, S.S. Joshi, J.O. Armitage, P.J. Bierman, A. Kessinger, P.F. Coccia, D.A. Crouse, D.S. Harrington, S.L. Mann and D.D. Weisenburger, Depts. of Anatomy, Radiology, Internal Medicine, Pediatrics and Pathology & Microbiology, University of Nebraska Medical Center, Omaha, NE 68105
When histologically-negative bone marrow harvests from patients with intermediate or high grade non-Hodgkin's lymphoma are placed into liquid culture with phytohemagglutinin stimulated human spleen cell medium (1%), monoclonal T or B cell populations with gene rearrangements matching those of the original tumor grow out in approximately one-third of the cases. We have recently analyzed the clinical outcomes of 70 consecutive patients whose harvests were placed into this culture system. Of the 70 patients, 17 were not transplanted and 53 underwent high dose therapy and autologous marrow transplantation. Six of the 17 non-transplanted patients were culture positive and had an actuarial survival at three years of 50% versus 73% for the culture negative patients. Of the 53 patients who were transplanted, 24 achieved a completed response and were evaluable for long term survival. Eleven of these 24 patients were culture positive and their actuarial survival at three years was 9% versus 69% for the culture negative patients. We conclude that the detection of occult lymphoma cells in marrow harvests is associated with a poorer prognosis especially in patients who undergo autologous transplantation. (Supported by Imogene Jacobs Memorial grant from the American Cancer Society.)

C 322 HIGH DOSE THERAPY WITH MITOXANTRONE, VP-16 AND THIOTEPA (MVT) PLUS AUTOLOGOUS BONE MARROW TRANSPLANT (ABMT) FOR HODGKIN'S DISEASE (HD) IN RESISTANT RELAPSE (RR). J.A. Spinolo, S. Jagannath, J.C. Yau, F.C. LeMaistre, F.R. Dunphy, F. Cabanillas, R.O. Wallerstein, G. Spitzer. The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030.
High dose therapy with ABMT is an established therapy for relapsed HD. The CBV combination (cyclophosphamide, BCNU and VP-16), frequently achieves durable remissions in patients (pts) still responsive to conventional dose salvage therapy. However, many of these responsive pts relapse, and only few pts with RR have achieved long term survival with CBV; different regimens might achieve better results. We gave a new combination of mitoxantrone 30 mg/m^2 on day 1, VP-16 400 mg/m^2/day x 3 and TT 250 mg/m^2/day x 3 to 13 pts with HD in RR. Three pts had received CBV 2-3 years before MVT; one had not responded to CBV. ABMT was given on day 6-9. Median age was 36 years (24-61), and median number of prior failures was 3 (2-5). Ten patients are evaluable, and three are too early. Response: Two pts achieved CR (9+ and 6+ months), including the pt who had failed to respond to CBV; 3 had PRs, 4 did not respond, and 1 died early. Four of the 10 evaluable pts are still alive at 9, 6, 4, and 2 months. Toxicity: Hemopoietic recovery showed median times (ranges) to 0.5 x10^9 granulocytes/l of 28 days (18-45), and to 50 x10^9 platelets/l of 45 days (31->100). Three pts had delayed platelet recovery (<100 days), due to myelodysplasia (1 case), or bone marrow hypoplasia (2 pts). The two latter pts received ABMT on day 6 and 7; subsequent cases have been transplanted on day 9. All pts had mucositis: 2 grade 2, 7 grade 3 and 1 grade 4. One patient developed congestive heart failure. One patient died early (day +6) of sepsis. Discussion: This group of very poor prognosis pts shows that MVT is active in some pts with HD in RR. MVT is potentially non-cross reactive with CBV as shown by the patient resistant to CBV who had a CR with MVT. Timing of the bone marrow infusion is important to avoid delayed platelet recovery. MVT could be used as a second high dose non-cross resistant therapy in patients with prognostic factors suggestive of poor or short response to CBV or in good prognosis pts who fail CBV.

C 323 AUTOLOGOUS BONE-MARROW TRANSPLANTATION (ABMT) IN LYMPHOPROLIFERATIVE DISEASES. PRELIMINARY RESULTS WITH ETOPOSIDE, THIOTEPA AND CYCLOPHOSPHAMIDE (ETC) CONDITIONING REGIMEN, Tabilio A, Aversa F, Carotti A, Falzetti F, Tesoro S, Gambelunghe C, Pasqualucci V, Martelli M.F., Bone Marrow Transplantation Unit, Perugia, Italy. ABMT after ETC cytoreductive program was performed in 12 patients, 5 with conventional-and salvage-therapy resistant Hodgkin's disease (HD), 6 high-grade non-Hodgkin's lymphoma (NHL), 3 in partial remission and 3 in relapse, 1 traditional-therapy resistant multiple myeloma (MM). The conditioning design was 1gr/m^2 etoposide in 4 days, 900mg/m^2 thiotepa in 3 days, 200mg/kg cyclophosphamide in 4 days for the first 5 patients, the other 7 received 120mg/kg in 2 days. Four of the six NHL patients were given radiotherapy at the site of the bulky disease 2 months after bone marrow reconstitution. Two patients died early, one from cerebral haemorrhage, the other from haemorrhagic alveolitis; two relapsed at 4 and 6 months post-transplant; 7 are in complete remission (CR), median 8.5 months (range 2-13). The MM patient has not yet obtained CR. The main complications were: a)haemorrhagic cystitis (WHO grade IV) in the first 5 cases. This side effect was not seen when the dose was reduced to 120mg/kg in 2 days; b)mucositis (WHO grade II-III). These preliminary data indicate that the ETC schedule is reasonably well tolereted and that it achieves a high incidence of CR, even in patients previously treated with various combined therapy regimens.

C 324 LIPOSOMAL AMPHOTERICIN B (AmBisome) TREATMENT IN BONE MARROW AND SOLID ORGAN TRANSPLANT RECIPIENTS. J. Tollemar, O. Ringdén, Gunnar Tydén. Depts of Transplantation Surgery and Clinical Immunology, Karolinska Institute, Huddinge Hospital, Stockholm, Sweden.
Amphotericin B (Amp-B) is the drug of choice for severe fungal infections. However, nephrotoxicity, especially when given with cyclosporine, and other side-effects often complicate a therapeutic course of treatment. In order to reduce the toxicity and improve efficacy, Amp-B can be incorporated into liposomes. We have used liposomal Amp-B (AmBisome, Vestar Inc. USA) in 8 transplant patients: 4 bone marrow transplants (BMT), 1 combined kidney and pancreas (KP), 1 single pancreas (P), 1 kidney (K) and 1 liver transplant recipient (L). No side-effects occurred. During treatment renal function was unchanged or improved in 5/7 patients. In 2 cases the creatinine rose due to cyclosporine* and aminoglycosides**, and one patient was already on long-term dialysis therapy.

Pat	Dose max,mg/kg	Dose total,g	S-crea % change	Type of Candida infection	Diagnostic tools Culture	Diagnostic tools Free C.ag	Outcome Free of fungi	Outcome Status	Follow-up months
K+P	2.5	6.5	- 38	Peritonitis	Abdomen	+	+	Alive & well	>5
K	2.1	3.5	-	Pneumonia	BAL	+	+	Alive on dialysis	>5
P	1.1	0.5	+ 50**	Candidemia		+	+	Alive & well	>4
BMT	1.1	0.4	- 48	Candidemia		+	+	Alive & well	5
BMT	1.4	1.0	- 1	Candidemia		+	+	Died (autopsy)	
BMT	2.3	0.9	- 2	Disseminated	BAL	+	?	Died (no autopsy)	
BMT	0.9	0.6	- 15	Candidemia		+	+	Alive & well	>2
L	2.2	2.1	+ 74*	Liver	Biopsy	-	+	Alive & well	>2

Conclusion: Six out of 8 patients with documented or suspected fungal infections were successfully treated with AmBisome. The drug was well tolerated.

C 325 IMMUNOSUPPRESSIVE POTENCY OF PREPARATORY AGENTS FOR ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT): COMPARISON OF CYCLOPHOSPHAMIDE (CY), ACNU, BCNU, IFOSPHAMIDE (IFOS), MELPHALAN (MELPH), AND CARBOPLATIN (CARB), Lutz Uharek , Winfried Gassmann, Andreas Erbersdobler, Helmut Loeffler and Wolfgang Mueller-Ruchholtz, Departments of Immunology and Internal Medicine II, University of D-2300 Kiel, FRG.
We have compared different cytostatic agents for their effectiveness to prevent graft rejection following allogeneic BMT. LEW rats received a lethal dose (35 mg/kg) of busulfan followed by injection of 1 x 10^8 F1(CAPxLEW) marrow cells which are unable to induce a GvHR in LEW recipients. Rejection of the marrow graft was assessed by monitoring hematocrit, granulocyte and platelet count either until death of the animal or until day 80. Surviving animals received a donor type skin graft to confirm persistence of allogeneic hematopoiesis. Due to its weak immunosuppressive properties, busulfan by itself is unable to allow engraftment of allogeneic marrow. Therefore, additionally administered agents could be tested for their capacity to prevent marrow graft rejection. The following rejection rates were observed: CY (i.p., day -2): 30 mg/kg 100%, 60 mg/kg 60%, 90 mg/kg 20%, 120 and 180 mg/kg 0%; ACNU (i.v., day -2): 3, 5, 7, and 10 mg/kg 100%, 15 mg/kg 50%, 20 and 30 mg/kg 0%; BCNU (i.p., day -2): 5 mg/kg 100%, 10 mg/kg 71%, 20 mg/kg 56%, 30 and 40 mg/kg 0%; IFOS (i.p., day -2): 60 mg/kg 100%, 90 mg/kg 75%, 120 mg/kg 60 %, 180 mg/kg 80%, 240 and 360 mg/kg 0%; MELPH (i.p., day -2): 1, 2, 3, and 5 mg/kg 100%, 7 mg/kg 67%; CARB (i.p., day -2): 10 to 80 mg/kg 100%. Thus, the following doses are nearly equivalent to the standard dose (120 mg/kg) of CY: 20 mg/kg ACNU, 30 mg/kg BCNU, 240 mg/kg IFOS. In the dose range tested, MELPH and CARB were not able to reach the immunosuppressive potency of 120 mg/kg CY.

C 326 EXPANSION OF CIRCULATING LYMPHOCYTES BEARING $\tau : \delta$ RECEPTOR, WITH A RESTRICTED DIVERSITY, AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION. VILMER E. and BENSUSSAN A. Unité d'hémato-immunologie, Hôpital Robert Debré 75 019 PARIS - Unité INSERM 93, Hôpital Saint Louis 75 010 PARIS.

The generation of T lymphocytes diversity after allogeneic bone marrow transplantation (BMT) is likely a crucial issue which has not yet defined. Two out of 27 patients (pts) who received a BMT for a leukemia presented a predominant expansion of circulating TCR $\tau : \delta$ cells over a several months period (1). Molecular studies showed a restricted diversity of variable (V) repertoire : TCR $\tau : \delta$ cells from pt1 express $V\tau 5$ and $V\delta 4$ which is a member of $V\alpha 6$ gene family (2), these V usage being rare in TCR $\tau : \delta$ cells from normal PBL. Circulating TCR $\tau : \delta$ cells from pt2 express $V\tau 9$ and preferentially $V\delta 2$, this V usage being comparable to that of normal PBL. In pt1 a sustained and durable immunodeficient state may be explained by TCR $\tau : \delta$ cells which seems to be responsible for the unresponsiveness of PBL as demonstrated by cell sorting experiments (3). In addition, these TCR $\tau : \delta$ cells were unable to proliferate when the CD2 or the CD3 molecules were trigerring. Some TCR $\tau : \delta$ clones from both pts as well as TCR $\tau : \delta$ clones derived from ungrafted pts with acute lymphoblastic leukemia, displayed a cytotoxicity against autologous or allogeneic leukemic cells (4). These observations might suggest that this TCR $\tau : \delta$ subset may be implicated in the graft versus leukemia effect.

(1) Blood 1988;72:841 (2) Proc Acad Sci USA 1988;85:5634 (3) J Clin Invest 1988;92:155 (4) Blood 1989;73:2077

C 327 **TREATMENT WITH GABEXATE MESILATE FOR INTERSTITIAL PNEUMONIA AFTER BONE MARROW TRANSPLANTATION.**

Hisashi Wakita, Takayoshi Asai, Akira Hirasawa, Satoko Morio, Nobuyuki Aotsuka, Kiyoshi Hiruma, Hirotoshi Nakamura, Nobuyuki Endoh, Tadahiko Igarashi, Hakumei Oh, Kuniaki Itoh and Sho Yoshida.
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The incidence of interstitial pneumonia (IP) after bone marrow transplantation (BMT) were decreased by a variety of prophylactic procedures. However, the establishment of much better prophylactic procedures and treatments for IP after BMT is essential when considering the mortality rate. On the other hand, it has been reported that superoxide and some proteases released by neutrophils may be related to the progression of interstitial pneumonia.

Gabexate mesilate (GM), a protease inhibitor, was used in two cases which were clinically diagnosed to be IP after BMT. Effects of GM were recognized by the improvement of clinical findings and by recovery from IP. The inhibitory effect of GM on superoxide production by neutrophils was examined in vitro and in vivo. The suppression of neutrophil superoxide production was not observed in vitro, but was observed in vivo. These results suggest that GM improved the clinical findings of patients with IP after BMT by the indirect suppression of the superoxide production by the neutrophils.

C 328 CULTURE OF BER-H2 (CD30) POSITIVE REED-STERNBERG-LIKE CELLS FROM PERIPHERAL BLOOD STEM CELL AND BONE MARROW HARVESTS OF PATIENTS WITH HODGKIN'S DISEASE,

Dennis D. Weisenburger, James O. Armitage, Ann Kessinger, Sally Mann, Joanne M. DeBoer, and J. Graham Sharp, Departments of Pathology and Microbiology, Internal Medicine, and Anatomy, University of Nebraska Medical Center, Omaha, NE 68105.

The origin and characteristics of the Reed-Sternberg cell remain uncertain. In order to better detect and evaluate the properties of this cell, a reliable *in vitro* culture system for growing such cells would be useful. Although culture methods for Reed-Sternberg cells have been reported and a number of cell lines described, their characteristics are inconsistent and controversial (Leukemia 1:629, 1987). We have cultured harvested bone marrow in systems which support the growth of breast cancer cells (12 patients) and non-Hodgkin's lymphoma cells (21 patients) metastatic to bone marrow. In no instance were Reed-Sternberg-like cells detected. However, when bone marrow harvests (n=45) and peripheral blood stem cell harvests (n=23) from patients with Hodgkin's disease were cultured by the method of Douay et al. (Bone Marrow Transpl. 2:67, 1987), 3 of 32 (9%) evaluable bone marrows and 6 of 23 (26%) peripheral harvests grew large, morphologically-abnormal cells with the features of Reed-Sternberg cells. In sequential immunocytochemical studies in 5 patients, the first indication of abnormal cells was the appearance of small Ber-H2 positive mononuclear cells after about 2 weeks in culture. The frequency and size of these cells increased until they predominated after 5 weeks in culture. At 5 weeks, similar cultures established from harvests of normal donors and patients with non-Hodgkin's lymphoma show a predominance of benign-appearing, Ber-H2 negative histiocytes. Currently, the Ber-H2 positive cells have not grown in nude mice (10 week period); therefore, their malignant nature remains to be established. However, this culture system may be a useful method for growing Ber-H2 positive cells in patients with Hodgkin's disease. The clinical significance of these findings with regard to transplantation is currently being evaluated.

C 329 TREATMENT OF NEUROPATHIC MUCOPOLYSACCHARIDOSIS BY BONE MARROW TRANSPLANTATION, Chester B. Whitley, William Krivit, Norma K.C. Ramsay, Kumar G. Belani, Pi-Nian Chang, C. Gail Summers, and John H. Kersey. Bone Marrow Transplantation Program, University of Minnesota, Minneapolis, MN 55455. Bone marrow transplantation (BMT) is being evaluated for the treatment of inborn errors of lysosomal metabolism, and we have focused on mucopolysaccharidosis (MPS) diseases as a prototypic model for clinical trials. Nineteen patients with neuropathic MPS disease (15 Hurler syndrome; 1 Hunter syndrome; 3 Sanfilippo syndrome) were prepared with busulfan (from 12 to 24 mg/kg total dose) and cytoxan (240 mg/kg total dose), without T-cell depletion. After administration of 5 x 10^8 nucleated cells/kg, the rate of survival-with-donor-engraftment was acceptable in each of three donor-recipient groups: HLA-identical sibling donor (13/15), related phenotypically-matched donor (1/2), and unrelated donor (2/2). Systemic metabolic correction was observed with respect to donor-type enzyme levels in peripheral leukocytes and bone marrow aspirate, and reduction of glycosaminoglycan content in urine and liver. Patients with increased lumbar spinal pressure prior to engraftment showed reduction to normal within 18 months post-BMT. Electroretinographic measurements showed cessation of retinal deterioration post-transplantation, and returned to normal, or toward normal, in every case. In those children evaluated at least 1 to 6 years post-transplant, current behavior assessment scores (IQ) were inversely related to age-at-engraftment, and were strongly correlated (r=0.898) to pre-transplantation IQ score. For children with neuropathic MPS diseases, successful donor-type engraftment can be achieved without irradiation. The procedure results in systemic metabolic correction with stabilization of the central nervous system against the usual progressive deterioration. These observations provide the mandate for early, presymptomatic diagnosis and treatment of neuropathic MPS disease by means of BMT.

C 330 **INABILITY TO INCREASE INTRACELLULAR IONIZED CALCIUM CONCENTRATION ($[Ca^{2+}]i$) IN T-CELLS FROM ALLOGENEIC BONE MARROW TRANSPLANT RECIPIENTS, Masahiko Yamagami, Lawrence Lum, Voravit Ratanatharathorn, and Lyle L. Sensenbrenner, Departments of Medicine and Pediatrics, Wayne State University (WSU), Detroit, MI 48201.** This study addressed the question of whether the binding of CD3 molecules to T cell receptor complex (CD3-TCR) of T cells from marrow recipients with anti-CD3 Abs could induce normal increases in $[Ca^{2+}]i$. PBL from 25 T-depleted marrow graft recipients (Medical College of Wisconsin) and 4 T-repleted recipients (WSU) were studied. After loading indo-1, cells were assessed by flow cytometry. 38.1 (IgM isotyped mouse anti-CD3 monoclonal Ab) was used to activate T cells. PBL from 10 of 22 short-term recipients (< 1 yr after BMT) responded poorly (< 35% of control) to anti-CD3 stimulation and PBL from 8 of 22 had blunted calcium responses (35 - 70% of control). The magnitude of responses was a function of the percentage of responding cells and not a function of the mean increase of $[Ca^{2+}]i$ in responding cells. PBL from several short-term recipients had high endogenous $[Ca^{2+}]i$ in the absence of stimulation. In contrast, PBL from 5 of 7 long-term recipients (> 1 yr after BMT) responded normally, and 2 of 7 had blunted calcium flux responses to anti-CD3 stimulation. These results suggest that the TCR of T cells from recipients may be structurally or functionally immature.

C 331 PROPHYLAXIS OF CYTOMEGALOVIRUS (CMV) INFECTION AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION. J.C. Yau, J.J. Tarrand, P.E. Oefinger, H.E. Fischer, K.A. Dicke, L.A. Williams, J.A. Spinolo, F.R. Dunphy, G. Spitzer, C.M. Meneghetti, R.O. Wallerstein, S.D. Huan, A.B. Deisseroth, and C.F. LeMaistre. UT M. D. Anderson Cancer Center and University of Texas Medical School, Houston, TX 77030
CMV infection is one of the most common causes of morbidity and mortality after allogeneic bone marrow transplantation. The response of established CMV pneumonitis to treatment is low despite ganciclovir and intravenous immunoglobulin. We studied a prophylactic regimen of immunoglobulin 500 mg/Kg IV every 2 weeks starting on day -2, acyclovir 250 mg/M^2 IV Q8H starting on Day 1 for 3 weeks, and CMV negative blood products for transfusion. Consecutive samples of 46 patients with leukemia, myeloma, and aplastic anemia undergoing allogeneic marrow transplantation were studied. Leukocytes from patients were cultured for CMV at weekly intervals for nine weeks after transplantation. Viremia correlated with detection of CMV recovery by early antigen expression or development of cytopathic effect are shown in the following table:

CMV serology (Recipient/Donor)	+ / +	+ / -	- / +	- / -
Total number of patients	22	10	4	10
CMV viremia	7	4	0	0
CMV pneumonitis	5	2	0	0
Total interstitial pneumonitis	8	2	0	1

The result shows that primary CMV infection can be effectively prevented in seronegative patients undergoing allogeneic bone marrow transplantation. For the seropositive patients more aggressive prophylaxis such as intravenous ganciclovir should be considered.

Autologous BMT

C 400 SIMPLIFIED METHOD FOR CRYOPRESERVATION OF HEMOPOIETIC STEM CELLS USING DIMETHYLSULFOXIDE AND HYDROXYETHYL STARCH

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Peripheral blood mononuclear cells (PBMC) and bone marrow mononuclear cells (BMMC) were cryopreserved using cryoprotective solution containing both 5% dimethlysulfoxide (DMSO) and 6% hydroxyethyl starch (HES) at -80°C without rate-controlled freezing. Compared to prefreezing values of PBMC, the mean nucleated cell recovery was 95 ± 4% and trypan-blue viability was 90 ± 5% 3 months after cryopreservation; the mean CFU-C and BFU-E recoveries were 75 ± 13% and 96 ± 7%. Frozen-thawed BMMC gave similar CFU-C recoveries (80 ± 20%). When large-scale samples of PBMC in 100ml aliquots in freezing bags were cryopreserved for the clinical application, CFU-C recoveries in two cases were 72% and 63% respectively. Thus the DMSO/HES mixture proves to be a simplified method for cryopreservation of hemopoietic stem cells from PBMC and BMMC. This method has advantage over the conventional one since rate-controlled freezing and storage in liquid nitrogen are not required.

C 401 MONOCLONAL ANTIBODIES ELIMINATE BREAST CANCER FROM BONE MARROW.
Edward D. Ball, James J. Vredenburgh, and Gregg W. Crabtree Department of Medicine, Dartmouth Medical School, Hanover, N.H. 03756

The majority of stage III and IV breast cancer patients have bone marrow involvement , as detected by monoclonal antibodies (mAb) and immunofluorescence. We determined the reactivity of a panel of 15 tumor-reactive mAb with three breast cancer cell lines and normal bone marrow by cytofluorography. We selected the 4 mAb , PM-81, TFS-2, 113-F1, and 251.03, that showed the highest reactivity. PM-81 reacts with CD-15, TFS-2 reacts with a 39 kD antigen found on many carcinomas, 113-F1 reacts with a 73 kD protein found on breast and ovarian carcinomas, and 251.03 reacts with the Her-2/neu oncogene product, a 185 kD protein. PM-81, TFS-2, and 113-F1 were found to react with over 90% of breast cancer tumors, and 251.03 with 30%, as determined by immunohistochemistry. Various combinations of the mAb and immunomagnetic beads (IMB) selectively eliminated the contaminating tumor cells from a mixture of 10% breast cancer cells and 90% marrow cells, as determined in a clonogenic assay or limiting dilutions and demonstrated in the table:

Exp. No.	mAb	Tumor Cell Line	Log Tumor Cell Separation
10	PM-81,TFS-2,113-F1	SK-BR-3	5 log
3	PM-81,TFS-2,113-F1	MCF-7	5 log
3	TFS-2,113-F1,251.03	BT-20	4 log

The effect of each mAb combination and IMB on hematopoietic progenitor cells was determined, and there was an average of a 40% reduction in CFU-GMs and a 30% reduction in BFU-Es. The excellent tumor cell separation with the mAb and IMB may obviate the need for in vitro cytotoxic drugs, which have more adverse effects on the hematopoietic progenitor cells, and avoid the potential leukemogenic effects of the cytotoxic drugs. These experiments will provide the background to begin using the mAb for purging of bone marrow for ABMT.

C 402 **ELIMINATION OF LYMPHOID TUMOR FROM NORMAL MARROW IN LONG TERM BONE MARROW CULTURE,** **Steven K. Bergstrom, Department of Pediatrics,University of Connecticut School of Medicine, Farmington CT 06032.** **The elimination of tumor from long term bone marrow culture, with maintenance of normal hematopoietic function has been demonstrated in Acute and Chronic Myelogenous Leukemia. In these experiments, bone marrow taken from patients with acute lymphoblastic leukemia, either at the time of diagnosis, relapse, or at various times during remission, has been placed in long term cultures. At intervals, samples have been taken from these cultures. Myeloid/macrophage cells have been removed using immunomagnetic beads, and the DNA from the remaining cells extracted. Following digestion with restriction endonucleases, the DNA was analyzed for rearrangement of the immunoglobulin gene or T-cell receptor molecule on standard southern blotting. Using this method, residual disease of 0.1% of total nucleated cells present can be detected. Our experiments show the rapid disappearance of lymphoid tumor in long term marrow culture. Further experiments will be necessary to determine the suitability of using autologous marrow , purged with this method, for transplantation.**

C 403 VERAPAMIL MODULATION OF VINCRISTINE AND VP-16 CYTOTOXICITY DURING PURGING OF MDR (L-100) AND ATYPICAL MDR (Nalm-6-500) ALL FOR AUTO BMT. Cairo M.S., Toy C., Knoppel E, van de Ven C, Childrens Hosp of Orange County, Univ CA Irvine, Orange, CA

Multiple drug resistance (MDR) has recently been observed during relapse in some patients with ALL (Rothenberg; Blood:74;1388, 1989). ABMT for ALL is characterized by ineffective methods of bone marrow purging and a high reoccurance rate. Vincristine (V) and VP-16 are active agents in the treatment of ALL and additionally, Verapamil (VPL) has been demonstrated to enhance the cytotoxicity (C) of V and VP-16 in pleotropic resistance ALL cells. L-O, a T-lineage and Nalm-6, a B-linage ALL cell lines were passed repeatedly in the presence of sublethal concentrations of V and VP-16, respectively, to develop L-100 and Nalm 6-500 resistant lines. L-100 was determined to be a typical MDR line and Nalm 6-500, an atypical MDR line by Northern and Western blot analysis. Additionally, L-100 was characterized by an increase in CD_5, CD_7 and CD_{10} expression compared to L-O (37x,6x15x) by FACS immunophenotyping. VPL (10uM) had no effect on the V IC_{50} of L-O (0.13$\pm$.02uM) but enhanced the IC_{50} of L-100 to (79$\pm$10uM). Likewise, VPL had no effect on the VP-16 IC_{50} of Nalm 6 (3.5$\pm$2.0uM) but enhanced the VP-16 IC_{50} of Nalm 6-500 to 22.3$\pm$12.3uM. VPL, additionally, had no effect on bone marrow CFU-GM growth. A remission marrow was simulated by adding 5% Nalm 6-500 to BM. VPL increased VP-16 inhibition of CFU-GM from 85.1$\pm$2.0% to 94$\pm$3.4% ($p<.02$), an increase of 9.4$\pm$2.0%. Previously, VPL potentiated the VP-16 IC_{50} of Nalm 6-500 by 78.7$\pm$9.0%. Therefore, VPL potentiated VP-16 cytotoxicity of Nalm-6-500 vs CFU-GM inhibition by seven fold ($p<.0001$). These studies suggest that VPL may modulate and enhance chemopurging of drug resistant ALL for future ABMT without increasing BM toxicity.

C 404 **THREE YEARS ACTIVITY DATA OF ITALIAN CHILDREN BMT REGISTRY.** Paolo Colleselli, Marino Andolina, Serenella Bagnullo, Giorgio Dini, William Arcese, Roberto Miniero, Paolo Paolucci, Fulvio Porta, Franco Schettini, Cornelio Uderzo, for the AIEOP BMT Group. The italian cooperative group for pediatric bone marrow transplantation (AIEOP BMT Group) collects in a registry the experience of 10 centers of pediatric hematooncology in which BMT is currently performed. We have summarized here the data of 3 years of registry activity. A total of 442 tranplants were registered up to June 1989. 257 autologous 163 allogeneic, 16 mismatch, 4 singeneic and 2 fetal liver transplants were enrolled during these 3 years. The majority of patients were affected by lymphoblastic leukemia (39%), the others by non lymphoblastic leukemia (25%), neuroblastoma (16%), chronic mielogenous leukemia (3%), aplastic anemia (3%), non H. lymphoma (3%). The remaining part of transplanted patients includes solid tumors, immunodeficiency diseases and storage diseases. The crude survival without any correlation to disease and follow up period results in 142/257 (58%) for autologous transplants and 102/163 (63%) for allogenic transplants. We think that the number of pediatric bone marrow transplantation collected and to be collected in our registry, constitutes an important matter for prospective and retrospective studies in this field.

C 405 MARROW ABLATIVE THERAPY (MAT) AND AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) FOR NEUROBLASTOMA (NB), Giorgio Dini, Alberto Garaventa, Edoardo Lanino, Sandro Dallorso, Claudio Viscoli, Graziana Manno, Paola Franzone. Department of Pediatric Hematology-Oncology, Bone Marrow Transplantation Unit, Istituto "G. Gaslini", Genova, Italy. From October '84 to November '87, 38 children aged from 1 year 1 month to 12 years 4 months with resistant or relapsed NB (Group 1, 14 patients), unselected disseminated NB (Group 2, 14 patients) or selected disseminated NB (Group 3, 10 patients) were treated with an *intensive protocol, including chemotherapy, surgery to the primary tumor and MAT followed by* unpurged ABMT. The induction protocol included: 1) High-dose peptichemio, one course; 2) OC-HDP regimen (vincristine, cyclophosphamide, high-dose cisplatin), two courses; 3) Surgery on the primary; 4) Teniposide and doxorubicin one or two courses. Subsequently patients received MAT followed by unpurged ABMT, within 5 months from diagnosis (or within 5 months from last relapse in the relapsed patients). The MAT regimen included vincristine, fractionated total body irradiation, and melphalan. Twenty-three patients were grafted in complete remission (CR), 6 in very good partial remission (VGPR), 5 in partial remission (PR), 4 in progressive disease (PD). The acute toxic death rate was 6.1%; the relapse and progressive disease rate was 65.7%. The median progression-free survival was 17 months for the 23 patients grafted in CR, 5 months for the 6 patients grafted in VGPR, 5 months for the 5 patients grafted in PR, 2 months for the 4 patients grafted in PD. We conclude that: 1) Progression free survival (24% at 45 months for all the 38 grafted patients) is almost double when compared to our previous studies (<10%). 2) Apparently the results obtained utilizing unpurged ABMT are not worse than those obtained utilizing electively purged ABMT.

C 406 AUTOLOGOUS BONE MARROW TRANSPLANTATION IN A 5 MONTH OLD BABY
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A 5 month old boy with a congenital megacaryoblastic leukemia(FAB-M7) was treated with conventional chemotherapy and came into 1st complete remission. He suffered from an atypical liver- and spleen-fibrosis and had a severe bone-marrow fibrosis due to his leukemia. To intensify and shorten chemotherapy regimen the baby underwent an autologous bone marrow transplantation. 200 ml bone marrow was collected and after incubation with ASTA Z cryopreserved. Conditioning regimen included 12 mg/kg Busulfan, 40 mg/kg VP 16, 2 x 60 mg/kg Cyclophosphamid and 2 x intrathecally MTX 3 mg, ARA-C 8 mg, Prednisone 2 mg. The supportive care consisted of gut decontamination, CMV - negative blood products, immunoglobuline-infusion, CMV-hyperimmunglobulins and continous nursing in laminar-airflow-room. The patient was in excellent condition, no toxic side-effects were observed, the haematopoietic reconstitution was very rapid, and we discharged the baby only 20 days after bone-marrow infusion. Details of treatment and haematopoietic recovery will be presented. 50 days after ABMT the boy is in 1st CCR. Conclusion: high dose chemotherapy with bone-marrow rescue might shorten treatment of high risk leukemia and improve life-qualitiy even in very young children.

C 407 MEROCYANINE 540-SENSITIZED PHOTOINACTIVATION OF L1210 LEUKEMIA CELLS: INHIBITION OF TRANSPORT SYSTEMS, David K. Gaffney, Matthew S. Anderson, Donald L. Traul, and Fritz Sieber, Departments of Biochemistry and Pediatrics, Medical College of Wisconsin, Milwaukee, WI 53226.

Merocyanine 540 (MC540) is presently used in a phase I clinical trial to purge autologous bone marrow grafts of residual tumor cells in the treatment of leukemias and lymphomas. A combination of MC540 and white light which achieves a >5 log reduction in clonogeneic L1210 leukemia cells reduces pluripotent hematopoietic stem cells by <1 log. Transport of radiolabelled substrates was inhibited without a lag phase. The illumination times required to inhibit cycloleucine (active transport), leucine (active transport), thymidine (facilitated diffusion), uridine (facilitated diffusion), and ribose (facilitated diffusion) transport by 50% were 25, 36, 38, 54, and 65 minutes, respectively. Recovery of transport did not occur after mild photosensitization (15 or 30 min of illumination). There was no change in the Km for cycloleucine after MC540 treatment, suggesting that the affinity for the substrate was not altered. However, there was a significant decrease in Vmax ($p < 0.05$), which is consistent with a loss of functional transport sites. These data implicate the plasma membrane of tumor cells as a likely target of MC540-sensitized photoinactivation. Supported by CA 42734, the MACC Fund, and a Leukemia Society of America Scholarship to F.S.

C 408 ALLO OR AUTO BMT FOR METASTATIC NEUROBLASTOMA: A WORLDWIDE SURVEY, John Graham Pole for 28 Participating Investigators, Department of Pediatric Hematology/ Oncology, University of Florida, Gainesville, FL 32610. We have examined the event-free survival (EFS) and causes of failure of 124 neuroblastoma (NBL) patients (pts) receiving allogeneic (allo) marrow transplants (BMT) and compared this with Autologous (auto) Bone Marrow Transplant Registry data for 304 pts receiving auto BMT. 81% of allo and 66% of auto BMT pts were in first remission (rem). Myeloablation consisted of 1-4 drugs, including at least 1 alkylating agent in all pts and total body irradiation (TBI) in >90%.

Auto BMT was associated with a significantly higher EFS than allo BMT ($p = .036$). 38 (31%) allo BMT pts died of toxicity, which was significantly associated with number of myeloblative drugs and TBI dose. Rem status at BMT was significantly associated with EFS probability (prob) at 2 years post-BMT ($p = .0002$).

	2 year EFS Prob (95% CI)	
Status at BMT	Allo	Auto
Relapse	.00 (-,-)	.11 (.00,.23)
Partial Rem (PR)	.20 (.08,.32)	.28 (.20,.36)
Very good PR	.25 (.00,.55)	.34 (.14,.54)
Complete Rem	.40 (.21, .59)	.33 (.23,.43)

We conclude that the dominant factor in predicting EFS prob after BMT for NBL is rem status, that overall EFS prob is better after auto than allo BMT, and that the high toxic death rate after allo BMT may mask any difference in relapse prob between allo and auto BMT pts.

C 409 PROTEIN C AND ANTITHROMBIN III DEFFICIENCIES OCCUR DURING AUTOLOGOUS MARROW TRANSPLANTATION, William D. Haire, Anne Kessinger and James O. Armitage,University of Nebraska Medical Center, Omaha, NE., 68105. Subclavian vein thrombosis after Hickman catheter placement occurs with an inordinate frequency during autologous marrow transplantation, exceeding 13% by 28 days after chemotherapy. To evaluate for the development of a hypercoagulable state during transplantation we measured protein C (PC) antigen and activity, free and total protein S (PS), antithrombin III activity (ATIII), fibrinogen, tissue plasminogen activator (TPA) antigen, plasminogen activator inhibitor activity (PAI) and liver function studies weekly during the course of 10 patients undergoing autologous marrow transplantation for lymphoma or Hodgkin's disease. All patients received total parenteral nutrition with 10 mg vitamin K weekly. By day 14 there had been a significant drop in PC (from a mean of 90% of normal to 45%, $p=0.008$) and ATII activity (from a mean of 103% to 82%, $p=0.02$) and rise in fibrinogen level (400 to 695 mg%, $p=0.02$). There was no change in levels of TPA, PAI or either free or total protein S. The drop in PC antigen correlated strongly with the concomitant drop in albumin levels ($r=0.91$, $p=0.001$), suggesting impaired hepatic synthesis. Because vitamin K deficiency may have impaired PC production, 10 subsequent patients were studied while receiving 10 mg vitamin K daily in their parenteral nutrition solution. No differences were seen in the coagulation or fibrinolytic parameters between patients receiving daily or weekly vitamin K. The low levels of ATIII and PC and the rise in fibrinogen without an improvement in fibrinolytic variables create a potentially hypercoagulable state that may contribute to the thrombotic complications of autologous marrow transplantation.

C 410 PHASE I-II TRIAL OF BCNU,ETOPOSIDE,CARBOPLATIN,THIOTEPA(BECaT) & ADJUVANT AUTOLOGOUS BUFFY COAT(AABC) FOR NEURAL TUMORS, L.Helson, A.Mittleman, T.Ahmed, E.Feldman, R.R.Wolff,S.Kasoff D.Wuest,A.Donfrancisco G.Dep ,J.Allen+,New York Medical College,N.Y. Oepedale Bambino Jesu,Rome Italy, +NYU MEDICAL CENTER,N.Y.N.Y. The safety & efficacy of(BECaT)in 14 patients(pts) with systemic neuroblastoma(NB),brain or spinal cord glioma(GL) ependymoma(EP),& primitive neuroectodermal tumor(PNET) was studied.Myelo-suppressive doses of BECaT(in mg/sqM)(100,200,1000,100) respectively were given to 12 pts & myeloablative doses of BECaT (100,400,1500,750,)to 2 pts. Objective responses after 1-3 myelosuppressive courses were complete in 1 EP, & Partial in 2 NB,& 2 GL.Five pts were to early to evaluate(tete):1 PNET,& 1 GL did not respond.Two myeloablated pts are tete. We tested the ameliorating effect of cryo-preserved marrow or peripheral nucleated blood cells (>0.6x10 cells/kg) on drug toxicity.10/11 myelosuppressive courses without AABC resulted in grade IV hematologic toxicity including WBC <1000/c.mm/course; two with sepsis.In 6/6 myelosuppressive low dose courses plus AABC,hematologic toxicity was reduced to grade III without fevers or infections.Grade III gastrointestinal toxicity occured only in 2 pts with myeloablative doses.These data suggest BECaT to be an effective regimen for patients with neural tumors. We propose that AABC after BECaT or other myelosuppressive drug combinations will safely permit increased dose intensity & enhance tumor cytoreduction. Supported by the Neuroblastoma Foundation of New York.

C 411 THE THRESHOLD EFFECT IN PERIPHERAL BLOOD STEM CELL AUTOGRAFTING - DIFFERENCES BETWEEN ACUTE MYELOID LEUKAEMIA AND NON "STEM CELL DISEASES", James Q.K. Ho, Christopher A. Juttner, Luen B. To, David N. Haylock, Geoffrey W. Dart, Noemi Horvath, Pamela G. Dyson and Robert E. Sage, Division of Haematology, Institute of Medical and Veterinary Science and The Queen Elizabeth Hospital, Adelaide, South Australia 5000, Australia.

Haematological reconstitution (HR) after peripheral blood stem cell (PBSC) autografting was compared in 14 patients (pts) with acute myeloid leukaemia (AML) and 11 pts with non-Hodgkin's lymphoma (7), Ca ovary (1), multiple myeloma (2) or Ca breast (1). In AML pts PBSC were collected after DAT chemotherapy, in the others after cyclophosphamide 3-4gm/m IV. The mean CFU-GM dose was 119x10 /kg in the AML pts and 40x10 /kg in the others. Rapid HR occurred in all pts with no difference between AML and non-AML pts and no delay in pts receiving low CFU-GM doses (<50x 10 /kg). In succeeding weeks some AML pts experienced neutrophil falls to <1.0x10 /l (3/14) and/or platelets to <25x10 /l (7/14). AML pts receiving more than the threshold dose of 50x10 /kg were significantly less likely to have a neutrophil trough <1.0x10 /l. Non-AML pts did not experience neutrophil and platelet falls despite lower CFU-GM doses suggesting that the safe threshold CFU-GM dose for AML and non-AML is different, implying a lower ratio of totipotent stem cells to CFU-GM in AML (a stem cell disorder) than in non-AML. Further clinical experience in non-AML will probably show that PBSC autografting is even safer than in AML.

C 412 DELAYED PLATELET RECOVERY FOLLOWING SEQUENTIAL HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS BONE MARROW. Susan D. Huan, Frank R. Dunphy and Gary Spitzer, Department of Hematology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030.

The pattern of hematopoietic recovery following high dose chemotherapy has been extensively studied; however. hematopoietic reconstitution after sequential high dose chemotherapy has not been clearly defined. We treated 33 patients with solid tumors using myelotoxic doses of cyclophosphamide (C), etoposide (V), and cisplatin (P) for 2 cycles 4-6 weeks apart. Autologous bone marrow using equal cell numbers collected before the first cycle was infused during each cycle. Three increasing dose levels (L) of chemotherapeutic agents were given to evaluate the MTD (Table 1). The median days to absolute granulocyte count (AGC) >500/μl was not significantly different between the first and the second course on all 3 levels. Neither dose escalation nor repeated cycles seemed to affect granulocyte recovery. However, the impact of sequential high dose chemotherapy on platelet (PLT) recovery was evident at all levels. The median days to PLT count >50,000/μl were significantly delayed after the second course for L1 and L3 (p=.016 and p=.001 respectively) (Table 2). The effect of the second cycle on PLT recovery became more pronounced with subsequent dose escalation. Platelet reconstitution is a complex process. It is unclear whether PLT recovery from the second cycle originates in the infused marrow or remaining stem cells. Platelet recovery is delayed but not granulocytes by two courses of high dose chemotherapy with ABMT. Perhaps dose dependent damage to the host marrow stromal elements results in an unfavorable environment for thrombopoiesis, which cannot be overcome by autologous transplantation.

Table 1: *Dose Escalation Scheme*

	C	V	P
L1(mg/m²)	4500	750	120
L2	4500	900	150
L3	5250	1200	180

Table 2: *Median Days to Recovery*

	AGC >500			PLT >50,000		
	L1	L2	L3	L1	L2	L3
Course 1	23	26	22	20	21	21
Course 2	20	23	22	23	27	29

C 413 DIFFERENCES IN BCNU AND CYCLOPHOSPHAMIDE (CPA) PHARMACOKINETICS ASSOCIATED WITH INCREASED RISK OF HEPATIC VENO-OCCLUSIVE DISEASE (VOD) IN PATIENTS RECEIVING HIGH-DOSE CPA, CISPLATIN (cDDP), AND BCNU WITH AUTOLOGOUS BONE MARROW SUPPORT (ABMS). RB Jones, S Matthes, E Shpall, M Ross, J Wormley, and WP Peters, Duke University Autologous Bone Marrow Transplant Program, Durham, NC 27710.

VOD is a leading cause of mortality following marrow transplantation. An extreme elevation in serum bilirubin 2-4 weeks after treatment suggests this diagnosis. We have performed pharmacokinetic (PK) analyses for CPA and BCNU in 47 pts receiving CPA/cDDP/BCNU. All pts had normal pretreatment hepatic and renal function. The CPA/cDDP/BCNU regimen is: CPA 1875 mg/M^2/day for 3 consecutive days as a one hour infusion, cDDP 55 mg/M^2/day over the same 3 days by continuous infusion, and BCNU 600 mg/M^2 as a two hour infusion immediately after cDDP. CPA and BCNU PK were modeled by two and one compartment schemes, respectively. Mean (+/-sd) CPA AUC's (79,300+/-86,200, 57,400+/-98,300, and 38,800+/-21,900 ug-min/ml) and elimination half-lives (390+/-505, 243+/-255, and 195+/-54 min) decreased from the first to third days of treatment. BCNU AUC was 1,780+/-2,350 ug-min/ml and mean elimination half-life was 26+/-19 min. Regression analysis employing each maximal concentration, elimination half-life, and AUC demonstrated significant correlations between the maximal serum bilirubin concentration (mean 7.1+/-12.1, range 0.4-68 mg/dl) and BCNU AUC ($p=0.002$), BCNU elimination half-life ($p=0.02$), and the first day CPA AUC ($p=0.04$, $r^2=0.41$) only. We conclude that large interpatient differences in CPA and BCNU AUC exist despite uniform dosing by body surface area. These data confirm an association between BCNU and CPA AUC and risk of VOD. The relative magnitude of the associations supports previous data suggesting greater hepatic toxicity for BCNU than CPA. PK-directed dosing with this regimen should be studied.

C 414 HIGH DOSE CHEMOTHERAPY (CMT) AND BONE MARROW TRANSPLANTATION (BMT) IN THE TREATMENT OF METASTATIC BREAST CANCER, H. Kaizer, R. Ghalie, S.S. Adler, A.D. Korenblit, and C.M. Richman. The Thomas Hazen Thorne Bone Marrow Transplant Center, Rush Medical Center, Chicago, Il 60612.

Between June 1986 and May 1989, we have entered 15 patients with metastatic or recurrent breast cancer in a BMT study using a preparative regimen consisting of cyclophosphamide (60 mg/kg/day on days -3 and -2) and escalating doses of thiotepa (225 or 300 mg/m^2 x3 on days -11 to -9), and cisplatinum (50 or 100 mg/m^2 on days -11 and -3). This regimen was devised to maximize the dose of individual agents by not giving more than two at a time and separating the pairs of drugs by an interval of 5 days. The median age of patients was 37 years (range 21-44 years). All had measurable disease at transplantation. All patients except one were hormone receptor negative or had failed hormonal therapy. Two patients had received no prior CMT. Among the remaining patients, three received adjuvant CMT alone, 5 received salvage CMT alone, and 5 received both adjuvant and salvage CMT. Patients were transplanted with autologous marrow except for one patient with marrow metastases who received an allogeneic BMT. The median time to reach a granulocyte count of 500 was 18 days (range 11-22 days), and to become platelet transfusion independent was 27 days (range 12-36 days). Dose limiting toxicity was renal and gastrointestinal. Patients were retrospectively classified into 2 groups: group A includes 9 patients who were refractory to conventional salvage CMT; group B includes 6 patients who were transplanted at the time of initial primary treatment failure. Overall, 3 patients died too early to evaluate tumor response and the remaining 12 patients all exhibited an objective response. One patient in group A and 5 in group B achieved a complete response (CR). Three of these, all from group B, are in unmaintained CR, 8, 26, and 32 months after BMT. In conclusion, this preparative regimen is effective in patients who are not demonstrably refractory to conventional salvage CMT. Patients who fail conventional salvage CMT require alternate approaches. Because of toxicity at the highest dose level, our regimen currently does not exceed 750 mg/m^2 of thiotepa and 150 mg/m^2 of CDDP.

C 415 AUTOLOGOUS BONE MARROW TRANSPLANTATION IN HIGH-RISK EWING'S SARCOMA, Robert B. Marcus, Jr. and John Graham-Pole. Radiation Oncology and Pediatric Oncology, University of Florida College of Medicine, Gainesville, FL 32610

From January 1985 through August 1989, 25 patients with high-risk Ewing's sarcoma (metastases at diagnosis or primary lesion greater than 8 cm in maximum diameter) have completed treatment on two successive protocols employing autologous bone marrow transplantation (ABMT) as end-intensification. The first protocol (HR-3) ran from 1/85 through 12/87 and consisted of five monthly cycles of VAC chemotherapy (vincristine, 2 mg/m^2 on day 1; Adriamycin, 45 mg/m^2 on days 1, 2; cyclophosphamide, 900 mg/m^2 on days 1, 2), radiation therapy (RT) to all sites of initial disease, followed by an ABMT with VAC/total body irradiation (TBI) (400 cGy X 2) as a conditioning regimen. The present protocol (HR-4) started in 1/88 and consists of alternating courses of VAC and etoposide (600 mg/m^2 in a 3-day continuous infusion)/cyclophosphamide (1800 mg/m^2 in six divided doses over 3 days), RT to all sites of disease, followed by an ABMT with a conditioning regimen of VP-16 (1800 mg/m^2 in a 6-day continuous infusion), cyclophosphamide (3600 mg/m^2 in 12 divided doses over 6 days), and TBI (200 cGy twice a day X 3 days). Twenty patients were treated on HR-3 and five have completed treatment to date on HR-4. At present, 16 of 25 remain free of disease with a median follow-up of 18 months. At 3 years, the absolute survival rate is 78% and the event-free survival rate is 63% (Kaplan-Meier product limit method). Of the 11 patients with metastases at diagnosis, six remain free of disease, while 10/14 patients with large primary tumors are relapse-free. The results warrant further exploration of end-intensification using myeloablative therapy and ABMT.

C 416 HEMOLYTIC-UREMIC SYNDROME IN PATIENTS WITH ADVANCED NEUROBLASTOMA AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION: DECREASED TOLERANCE OF THE KIDNEY TO RADIATION AND CISPLATINUM? Hideo Mugishima[1], Akihiko Endo[1], Motoaki Chin[1], Takashi Suzuki[1], Hideo Takahashi[1], Masataka Ichikawa[1], Takahito Fujisawa[1], Masahiko Okuni[1], Kouhei Komatsu[2], Kunio Okano[2] and Kazunari Iidaka[3], Department of Pediatrics[1] & Department of Pathology[2], School of Medicine, Tokyo, 173 Japan. Department of Pathology[3], Dokkyo University, School of Medicine, Tochigi, 321-02. It has been noted that patients developed hemolytic-uremic syndrome (HUS) after bone marrow transplantation (BMT) have worse prognosis. We report two cases of HUS following autologous BMT in patients with neuroblastoma. Both cases are stage IV (Patient 1:2yr/F Patient 2:4yr/F). The induction regimen included cyclophosphamide, vincristine, THP-adriamycin, VP-16 and cis-platinum(CDDP). After six or seven courses of the induction therapy, primary tumors were removed and irradiated with 2500cGy (Pat.1) and 1500cGy (Pat.2) in a single fraction. Patient 1 and Patient 2 had received CDDP 630mg/m², 645mg/m² respectively as the induction therapy. They received modified VAMP and total body irradiation (TBI) (1000cGy/3f/3d, at 10cGy/min) as the conditioning regimen. Patient 1 died of renal failure on day 102 after transplantation and renal histology showed the appearance of HUS. Patient 2 developed hemolytic anemia from day 80, hypertension and imparied renal function from day 100. We successfuly controlled HUS in this patient by plasma exchange with fresh frozen plasma and administration of dipyridamole. Both patients had not recieved cyclosporin and no evidence of cytomegalovirus infection. It is postulated that TBI potentiated by CDDP may be the most important factor developing HUS. Possible pathogenetic mechanisms are discussed.

C 417 HIGH DOSE VP 16 IN CONDITIONING REGIMEN FOR BONE MARROW TRANSPLANTATION

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In the last 2 years 24 children with advanced malignant disease were treated with high-dose VP 16 before bone-marrow transplantation and/or peripheral blood stemm-cell infusion. Diagnosis: 9 ALL-(7 2nd rem., 1 1st rel., 1 2nd. rel.), 1 AML- 3rd rel., 1 congenital megacarioblastic leukaemia, 2 NHL- (2nd rem.), 4 Ewing-sarcoma, 4 Peripheral Neuroectodermal Tumors, 2 Neuroblastoma IV, 1 Embryonal Germ Cell Carcinoma. Methods: All patients underwent FTBI (8x1,5 Gy) or received 16 mg/kg VP 16 + 120 mg Cyclophosphamide, 60 mg/kg VP 16 + 120 - 140 mg/m2 Melphalan, 60 mg/kg VP 16 + 120 mg/m2 Melphalan + 150 mg/m2 Carboplatin. Results: 14 pts. are in complete remission (2-30 months, med. 14 months), 7 pts. relapsed (2-7 months after BMT, med. 5 months), 1 PR (6 months after BMT), 2 infection (day +10 after BMT: Pseudomonas; day +14 after BMT: Aspergillus). Toxicity: severe bone marrow aplasia (8-21 d, med. 14 d); severe oropharyngeal mucositis (10 pts. WHO III-IV); 1 anaphylactoid reaction. Conclusion: Conditioning regimen including VP 16 might offer advantages compared to other regimens used previously.Toxicities seem to be tolerable.Follow up is not long enough to evaluate relapse rates in these regimens.

C 418 HIGH-DOSE CHEMOTHERAPY(HDC) AND AUTOLOGOUS BONE MARROW TRANSPLANTATION(ABMT) IN REFRACORY GERM CELL TUMORS(GCT):A PILOT STUDY. G.Rosti°,M.Leoni°,R.Salvioni°°,S.Crispino+,F.Rasi*,F.Valzania*,and M.Marangolo°. Medical Oncology Unit-Ravenna°,INT-MIlano°°,Medical Oncology-Monza+,Neurophysiology Dept.-Cesena*. Italy.
The cure rate of GCT has been improved by the development of CDDP containing regimens.Nevertheless the prognosis of refractory GCT is still dismal.This pilot study has been designed to investigate the usefulness of HDC as a salvage treatment,and to evaluate the hematological & neurological toxicity of HD Carboplatin(JM8) and VP16. Twelve evaluable pts bearing different GCTs refractory to previous chemotherapy regimens,have been treated with JM8 450-600 mg/sqm + VP16 600-800 mg/sqm on days -7,-5,-3;ABMT an day 0.Eight pts received 1,3 pts 2 and 1 pt3 cycles of HDC.All pts were monitored for hematological toxicity;and neurological studies were performed before and after HDC,these included the speed of transport of sensorial and motorial stimuli,the visual and auditory evoked potentials,and the computerized analysis of the postural system. In 5 pts the serum markers turned negative;in 3/5 a CR and in 2/5 a PR have been documented;in 3 pts the serum markers were reduced to <10%,the corresponding figures were SD in 2 and NE in 1. In 3 pts the serum markers rose and all clinically progressed.The median duration of response and survival were 12 and 12.5 weeks(4-110+).All pts had grade 4 hematological toxicity:polys <500/µl on day 7.6 for 15.2 days;PLT <50K/µl on day 8.4 for 16.4 days.No severe neurotoxicity have been documented.HD JM8+VP16 may have curative potential.

C 419 CRYOPRESERVATION OF BONE MARROW STEM CELLS AT -150°C.

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High dose chemotherapy with autologous bone marrow rescue is now part of the therapeutic approach for several leukemias, lymphomas and cancers. Cryopreservation of the bone marrow is an important element in the success of this approach. Until recently, the bone marrow, once frozen, was stored in liquid nitrogen (-196°C). However, it is expensive and hazardous. Storage at -80°C has been proposed as an alternative but it has been shown to be less than optimal for long term preservation.

The aim of our study was to evaluate the performance of a halogen compressed freezer at -150°C to store bone marrows for periods of up to 1 year. 23 bone marrows were studied. Using the same protocol, bone marrows were harvested in 3 hospitals and sent within 1 hour to the Blood Services for processing. The bone marrows were processed using a Cobe 2991 cell washer and frozen in DMSO in a stepwise manner using a Cryomed cryopreservation system. Marrow cell count and morphology examination on the buffy coats were performed. Viability and cell recuperation were assessed by colony-forming capacity one day post freezing and monthly for 12 months. Our results indicate that after 12 months, no significant difference was observed in the number of BFU-E, CFU-E, and CFU-C.

We conclude that cryopreservation at -150°C for 12 months maintains excellent viability of human stem cells as assessed by their capacity to form colonies *in vitro*. Freezing at -150°C can thus become an economical and safe alternative to liquid nitrogen for long term preservation.

C 420 A PHASE I TRIAL OF HIGH DOSE CARBOPLATINUM (CBDCA) AND ETOPOSIDE (VP-16) FOLLOWED BY AUTOLOGOUS TRANSPLANTATION IN RECURRENT CHILDHOOD TUMORS. V.M. Santana, R. Williams, E. Thompson, D. Kalwinsky, J. Rodman, C. Patrick, and J. Mirro, St. Jude Children's Research Hospital, Memphis, TN, 38101. We treated 13 patients with relapse/refractory childhood tumors (neuroblastoma = 5, lymphoma = 3, brain tumor = 3, rhabdomyosarcoma = 1, germ cell tumor = 1) with escalating doses of CBDCA and VP-16 followed by autologous marrow rescue. The median age was 10 years (range: 4-24 years). All patients were extensively pretreated and had failed phase II relapse therapy; 11 had received prior cisplatin (total dose: 180-1340 mg/m^2) and/or VP-16 (total dosages: 1200-3000 mg/m^2). The first 4 patients received VP-16 320 mg/m^2 q.o.d. x 3 alternating with CBDCA 400 mg/m^2 q.o.d. x 3 . All subsequent patients received VP-16 400 mg/m^2 q.o.d x 3 with escalating CBCDA given q.o.d. x 3: 3 patients received 400 mg/m^2, 4 patients received 500 mg/m^2 and 2 patients received 600 mg/m^2. Five patients received a second course at the same dose level. A median of 2.1 x 10^8/kg (range; 1.2-4.5 x 10^8/kg) marrow cells were infused. The major toxicity was hematologic; median time to recovery of .5 x 10^9 granulocytes/L was 30 days (range 16-39 days) and 50 x 10^9 platelets/L was 29 days (range 16-38 days). Other toxicities included mild nausea and vomiting, diarrhea, high-tone hearing loss, and mucositis. One patient died of sepsis and another of tumor progression within three weeks of transplant. Among the 12 evaluable patients in this extensively pretreated group there was 1 CR and 8 PRs and we have not defined the maximum tolerated dose. The tolerable doses of CBDCA and VP-16 in children are equal to, or greater than, those reported for adults. This phase I study also demonstrated significant anti-tumor activity and indicates this drug combination warrants further phase II study.

C 421 HIGH-DOSE ALKYLATING AGENT CHEMOTHERAPY WITH AUTOLOGOUS BONE MARROW (ABM) SUPPORT IN PATIENTS (PTS) WITH STAGE III/IV EPITHELIAL OVARIAN CANCER (OCA), Elizabeth J. Shpall, D. Clarke-Pearson, J. Soper, A. Berchuck, R. Jones, R. Bast, Y. Lidor, K. Vanacek, T. Tyler, W. Peters, Division of Hematology-Oncology, Department of Medicine, Duke Univ. Med. Ctr., Durham, NC 27710 Previous studies in OCA demonstrate a steep dose response relationship for the alkylating agents (AA) Cyclophosphamide (CPA), Thiotepa (TT), and Cisplatin (CDDP). There is relative lack of cross-resistance between these agents, and additive or synergistic anti-tumor effects when they are used in combination. Intraperitoneal (IP) CDDP has been shown to have a pharmacologic advantage in the peritoneal cavity compared to IV use, without compromising systemic chemotherapy exposure. A Phase I trial was instituted with IV TT (300 mg/m^2 IV x1), CPA (1,875 mg/m^2 IV daily x3), and CDDP (30-60 mg/m^2 IP daily x 3). Four days following cessation of chemotherapy, ABM is administered. The dose of CDDP will be escalated by 10 mg/m^2/day in cohorts of 3 PTS until the maximally tolerated dose is reached. Thus far, 11 PTS with extensive disease refractory to standard therapy (mean: 3 prior regimens) have been entered. 5 of 7 evaluable PTS have had partial responses (71%) for a median duration of 6 months (range 2-8). One PT is too early to evaluate, and 3 PTS (27%) developed fatal toxicity (2-renal failure, 1-sepsis). The median time to marrow reconstitution following high dose chemotherapy was 23 days (range 12-28). Serial CA-125 measurements showed greater than a 1 log decrease in all patients with pre-treatment values greater than 200 u/ml. If the pre-treatment CA-125 value was normal, an acute four-fold rise with therapy, followed by return to normal was noted. These results and AA pharmacokinetic data will be presented. Once the regimen completes Phase I/II testing, and if toxicity is acceptable, we propose its use in an earlier high-risk disease setting where it should afford more important benefit.

C 422 A COMPARISON OF MYELOID ENGRAFTMENT RATES USING THREE METHODS OF PROCESSING PERIPHERAL BLOOD STEM CELLS (PBSC). Smith D.M., Weisenburger, D.D, Kessinger, A. University of Nebraska Medical Center, Omaha, NE 68105.
Early PBSC collections at our institution used a modified plt/gran protocol on a Heamonetics V50 apheresis machine. To decrease toxicity we have studied three methods, using the Heamonetics V50 to remove RBC and concentrate the PBSC products. The first two methods used a density gradient, or counterflow centrifugation (surge) to isolate mononuclear cells (MNC). The third method repeated the plt/gran protocol on the entire product during the final pass to collect both granulocytes and MNC. All three methods yielded greater than 90% of the MNC, CFU-gm and BFU-e, but both procedures which involved isolation of MNC resulted in prolonged aplasia. Patients processed using the "surge" method who received > 8 x 10^3 CFU-gm/kg had normal periods of aplasia while those receiving < 8 x 10^3 had prolonged aplasia. Patients processed using the repeated plt/gran method engrafted normally regardless of the CFU-gm dose. We conclude that MNC isolation resulted in prolonged aplasia and we hypothesize that the plt/gran method collects more hematopoietic progenitors our auxillary cells although they are not detected by our in vitro assays.

METHOD	Days to Neutrophyils > 500/mm3			
	N	Mean ± SEM	Median	Death
Plt/Gran	27	24.9 ± 2.6	22	6
Ficoll	7	37.4 ± 9.0	37	2
Surge	42	35.8 ± 2.7	31	3
Repeat	12	23.2 ± 2.3	21	0

METHOD	CFU-gm	Days to Neutrophils > 500mm3			
		N	Mean ± SEM	Median	Death
Lymph	< 8 x 103	16	40.1 ± 2.6	40	1
Surge	> 8 x 103	14	25.6 ± 3.5	20.5	0
Repeated	< 8 x 103	7	22.3 ± 2.6	21	0
Plt/Gran	> 8 x 103	5	23.6 ± 3.5	28	0

C 423 CUTANEOUS MASTOCYTOSIS AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION, Achiel Van Hoof, Arnold Criel, Melany Hidayat and Andries Louwagie, Department of Hematology, Algemeen Ziekenhuis St. Jan, B-8000 Brugge, Belgium.
A 40-year-old male patient underwent an autologous bone marrow transplant (ABMT) as part of intensive consolidation chemotherapy for treatment of a high-grade malignant non-Hodgkin lymphoma (immunoblastic). The chemotherapy regimen consisted of BCNU, VP-16, Ara-C and Cyclophosphamide (BEAC). The clinical course after ABMT was uneventful, with PMN > 0.5.10^9/L at day 15, and platelets > 30.10^9/L at day 17. 3 Months after ABMT, patient presented with pruritus and joint tenderness. A maculopapular skin eruption on scalp and forearms was noted. Biopsy showed infiltration (65%) by mast cells. There was no evidence for systemic mastocytosis : bone marrow biopsy, X-rays of bones and histamine levels were normal. Lymphocyte subsets were normal. Recurrence of the lymphoma could not be shown. Mast cell proliferation frequently coexists with dysplastic and neoplastic disorders of myeloid and, more rarely, of lymphoid cells. Mast cells are interleukin 3 responsive and may be myeloid in origin. After ABMT, hematological reconstitution may in rare cases lead to unbalanced proliferation of mast cells.

Unrelated Donors; Complications of BMT; Stem Cells; Hematopoietic Growth Factors

C 500 ADHESION OF CD34+ MYELOID PRECURSORS TO MARROW STROMA; ROLE IN STEM CELL HOMING. Camille N. Abboud, Jane L. Liesveld, Daniel H. Ryan, Maureen C. Kempski, and James K. Brennan, University of Rochester Medical School, Rochester, NY 14642.
Hematopoietic progenitor cells lodge, proliferate and differentiate in selective myeloid or erythroid microenvenronmental niches. We and others have shown that a subset of human CD34 antigen positive cells bind to marrow stroma and fibroblasts (Blood 73:1794, 1989). We now report on the binding properties of early myeloid leukemia cell lines which are CD34 positive (KG-1 and KG1-a) and mechanism of binding by studies of binding inhibition using chromium labelled cells. CD34+ myeloid progenitor adhesion was quantitated using agar CFU-GM assays. Both KG1 and KG1a cells bound to stromal layers and passaged fibroblasts. Binding of a CD34+ lymphoid precursor cell lines, RPMI 840, did not occur to the same extent as that of the myeloid precursor but was significantly greater than to plastic. The KG1a cell line did not demonstrate significant binding to single glycoprotein matrix component (e.g. fibronectin, laminin, or collagen), but did bind triton-X extracted matrix, but to a lesser degree than to intent stromal layers. Binding of normal CD34+ precursor was not inhibited by polyclonal anti-fibronectin, anti-laminin, or by RGDS peptides. These normal precursor cells were found to possess the beta chain (CD18) of the LFA-1 family of adhesion reception, but blocking antibody to this antigen did not inhibit precursor binding to stroma. Likewise, with the KG1a cell line, binding was not inhibited with RGDS peptide, anti-CD18, anti-ICAM, anti-fibronectin, anti-laminin antibodies or in the presence of 10^{-2}M galacto- and mannosyl pyranosides. Further definition of these adhesive receptor(s) is needed to fully understand myeloid stem cell homing.

C 501 EXERCISE ASSESSMENT OF CARDIAC FUNCTION FOLLOWING BONE MARROW TRANSPLANTATION. Charles S. August, MD; Ranae L. Larsen, MD; Charles T. Heise, Bonnie F. Auble, PA-C; Gerald Barber, MD. *The Children's Hospital of Philadelphia, Philadelphia, PA.*

Cardiac dysfunction is a potential complication of bone marrow transplantation (BMT), especially in patients (pts) with oncologic disorders. We assessed cardiac function by exercise (Ex) testing in 13 pts before BMT and 28 survivors of BMT (median survival: 3 years; range: 0.9 - 12.1 years) and compared the results with 17 normal subjects. Indications for BMT were leukemias (19), solid tumors (13) and aplastic anemia (6). Mean age at testing was 16.6 years.

Cycle ergometry using a standard protocol, with determinations of oxygen consumption (VO_2), cardiac index (CI), and stroke volume index (SVI), was performed in all pts. Left ventricular shortening fraction at rest by echocardiography was also measured, and was normal (Pre-BMT: 0.33±0.04; Post-BMT: 0.32±0.04).

Results:	Ex Time (% predicted) u±SD (p<.001)	Max VO_2 (% predicted) u±SD (p<.001)	Rest CI (l/min/M^2) u±SD (p=NS)	Ex CI (l/min/M^2) u±SD (p=NS)	Rest SVI (ml/M^2) u±SD (p=NS)	Ex SVI (ml/M^2) u±SD (p<0.05)
Pre-BMT	47.9±19.9	54.5±12.2	3.8±1.3	7.2±1.9	38.5±13.8	43.5±12.3
Post-BMT	59.2±20.1	59.1±12.5	4.0±1.1	7.4±1.7	43.2±13.4	43.6±09.8
Normals	99.7±28.0	88.0±13.1	3.9±0.5	8.3±2.1	43.8±05.7	50.0±06.2

Before and after BMT, pts had significantly reduced Ex time, maximum VO_2, and Ex stroke volume, indicative of cardiac dysfunction. The abnormalities were most severe in pts who had received anthracyclines and/or mediastinal irradiation. Resting indices of CI, SVI, and SF were not sensitive enough to detect these abnormalities. Exercise testing is a useful tool for the assessment of cardiac function in BMT pts.

C 502 ENGRAFTMENT OF AUTOLOGOUS CD34+ MARROW CELLS IN PATIENTS WITH ADVANCED BREAST CANCER AND NEUROBLASTOMA, Ronald J. Berenson, William I. Bensinger, Robert G. Andrews, Roger S. Hill, Dale F. Kalamasz, Beverly J. Still, C. Dean Buckner, Irwin D. Bernstein, E. Donnall Thomas, Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA. The CD34 antigen is expressed by 1-4% of human and baboon marrow cells, including virtually all hematopoietic progenitors detectable by in vitro assays. We have previously shown that CD34+ marrow cells can engraft lethally irradiated baboons. Since the CD34 antigen has not been detected on most solid tumors, positive selection of CD34+ cells may be used to provide engrafting marrow cells depleted of tumor cells. In fourteen patients with Stage IV breast cancer(n=12) or neuroblastoma(n=2), 2.5-21x10^9 marrow cells were separated by immunoadsorption with the anti-CD34 antibody 12-8 and 50-260x10^6 positively selected cells were recovered that were 60-90% CD34+. Six patients have been transplanted thus far with 1.0-3.8x10^6 CD34-enriched cells/kg after receiving marrow ablative therapy. Four patients engrafted, achieving granulocyte counts of >500/mm^3 at days 21-47 and platelet counts of >20,000/mm^3 at days 35-55 posttransplant. All of these patients showed sustained engraftment until the time of death 82-306 days posttransplant. Two additional patients died at days 14 and 17 posttransplant with granulocyte counts of 360/mm^3 and 120/mm^3, respectively. These studies suggest that CD34+ marrow cells are capable of reconstituting hematopoiesis in humans.

C 503 THE USE OF UNRELATED BONE MARROW DONORS FOR THE TREATMENT OF FATAL HEMATOLOGICAL DISEASES - A NATIONAL EXPERIENCE, N.A. Buskard, H.N. Messner. For the Canadian Unrelated Bone Marrow Donor Registry and the Canadian Cooperative Bone Marrow Study Group. During the past 20 months, 29 unrelated bone marrow transplants have been carried out in Canada with 3 more to be done shortly. The age range of the recipients was 4 to 50 yr. (av. 33 yr.). The diagnosis of those transplanted was; CML - 15, AML - 5, ALL - 4, MDS - 2, SAA - 2 and LBL - 1. At the present time 14 patients are alive and 15 dead. The most common cause of morbidity and mortality has been graft-vs-host disease and infection. There were 4 deaths associated with VOD. Uniform chemotherapy plus FTBI (1000 cGy) was used. Of the 29 transplants performed, 2 were 1 antigen mismatches and the MLR was reactive in some cases. The country of origin of the unrelated bone marrow donors has been Canada - 13, USA - 8, Great Britain - 7, Netherlands - 1. 20 of the donors have been male and 9 have been female. The age range of the donors has been 18-47 yr. with (av. 34 yr). For logistical reasons, it has been necessary for 3 Canadian donors to travel to a collection centre for the bone marrow harvest. In all other cases the bone marrow was harvested in the community where the donor resided. No complications have been experienced and all donors have indicated that they would be prepared to donate marrow again. Currently, there are 8,000 donors in Canadian files with a projected target of 50,000 over the next 2.5 yr. In Canada, all unrelated bone marrow donors must be part of another blood program so that there is a constant update of their health care questionnaire and infectious marker status. Two bone marrows have been supplied to the United States. This experience indicates that international collaboration is essential to be able to search optimally for an unrelated bone marrow donor.

C 504 CANINE MODEL FOR AUTOLOGOUS MARROW TRANSPLANTATION (AuBMT) WITH LONG-TERM CULTURE (LTMC) CELLS, RF Carter, A Ogg, SA Kruth, VE Valli, J Dick, B Bosch and ID Dube, Dept. of Pathology, U. of Toronto, Toronto, Ontario M5G 1L5. Singly inoculated LTMCs support the maintenance of CFU-GM for over 4 wks when the media contains 25% horse sera and 0.2 mg/ml catalase. LTMCs from 26 dogs with untreated lymphoma or leukemia were cytogenetically analyzed over 3 wks of culture. In 6/6 dogs with lymphoma and 1 of 2 dogs with ANLL, there was loss of cytogenetically marked tumor clones and retention of normal CFU-GM. Cytogenetic markers were uninformative for 15 dogs with lymphoma but normal-appearing CFU-GM were recovered. Failure of purging for1 dog with ANLL and total culture failure for 3 dogs with CLL suggest that this method may not be feasible for some tumors. To investigate engrafting potential in normal dogs, marrow was harvested from pelvis, femur, or humerus, and LTMCs were established in T-150 flasks (1.2 x 108 cells/flask). After 3 wks, LTMC cells were harvested for infusion immediately after the dogs received 7 or 8 Gy TBI delivered in a single fraction at 75 cGy/min. Pelvic harvests resulted in the lowest dilution of marrow by blood and the highest recovery of CFU-GM from cultures. One dog fully engrafted by 4.5 wks, 2 dogs died due to failure of support at 18 days, and studies of 1 dog are in progress. The engrafting dog had an initial harvest of 2 x 108 cells/kg BW, a final harvest of 1.5 x 107 cells/kg BW, and 9 x 103 CFU-GM/kg BW infused. We used a multiple supernatant exposure, non-selective retroviral method of transfection to mark the LTMC cells of 2 dogs with neor (achieving labelling of ~100% of infused CFU-GM in 1 dog), but we have not yet documented engraftment of labelled cells. Our findings suggest that this is a feasible method of purging for lymphomas in particular, and that singly inoculated cultures maintained for 3 wks can support engraftment. Our clinical trial with this model will help evaluate the potential of this approach for the treatment of lymphoma and leukemia.

C 505 UNRELATED DONORS FOR PEDIATRIC BONE MARROW TRANSPLANTATION.J. Casper, N.Bunin J. Hunter, L.A. Baxter-Lowe, K. Murray, C. Lawton, J. Menitove, R. Truitt, B. Camitta, R. Ash. Medical College of Wisconsin, Children's Hospital and The Blood Center of Southeastern Wisconsin, Milwuakee, WI 53226

Only one-third of children in need of a bone marrow transplant have an HLA-matched sibling donor. Concerns regarding non-engraftment and/or severe graft versus host disease (GVHD) have tempered the use of unrelated donors. Since 1986 we have performed 29 transplants utilizing matched and mismatched unrelated donors. Patient characteristics were: ages 1-15 (median 5); 22 male; 7 female; severe AA-3, SCID-2, WAS-1, JCML-2, preleukemia-3 ALL-10, AML-2, CML-5 and MLD-1. Donor and recipient were serologically identical for HLA A, B, DR (8), A mismatched (9), B mismatched (6), or D-region mismatched (6). Relative responses were > 10% in the graft rejection and/or GVHD direction in 50% of the MLC assays. The conditioning regimen included: busulfan (BU) 4mg/kg/dayx2; cytosine arabinoside 3 gm/m^2 q12hr x 6; cyclophosphamide (CY) 45mg/kg/dayx2; methylprednisolone 1gm/m^2 q12hr x 4 and total body irradiation (14 GY/9 fractions/3 days with lung, liver and kidney attenuation). AA patients did not receive BU; SCID patients received either CY, or CY/BU. IV cyclosporine 3 mg/kg/day was started on day-2. Marrows were T-cell depleted using a monoclonal CD3 antibody and normal rabbit serum. All patients engrafted. Median days to PMN >500 mm^3 and platelets >25,000/mm^3 were 16 and 16 respectively. GVHD $\geq$ II occurred in 10 patients (8 deaths). Mild chronic GVHD occurred in 18. Eighteen patients are alive and disease-free 3-35 months post-transplant (median 16 mos.). The 11 deaths were due to CMV (d69,76,97), lymphoma (d77, 127), pancreatitis (d509), P.Carinii (d127), aspergillus (d124), interstitial pneumonia (d221), recurrent ALL (d101) and GVHD(d74).

C 506 **MODULATION OF STEM AND PROGENITOR CELL NUMBER IN THE MARROW, SPLEEN AND PERIPHERAL BLOOD OF TUMOR BEARING MICE,** David A. Crouse, Changnian Liu, Anne Kessinger, Fred Ogren and J. Graham Sharp, Departments of Anatomy, Medicine and Otolaryngology/Maxillo-Facial Surgery, University of Nebraska Medical Center, Omaha NE 68105

Therapy for some cancer patients involves collection of bone marrow (BM) by aspiration, escalation of the cytotoxic treatment and rescue by transplantation of the autologous stored stem cell (HSC) product. In some patients, circulating cells collected by leukapheresis have been used as a source of HSC. However, the presence of non-hematological cancer is frequently associated with peripheral blood (PB) changes as well as alterations in BM hematopoiesis. The primary objective of this study was to determine what changes in the PB-HSC compartment may accompany the presence of a tumor in the donor. BDF$_1$ mice transplanted subcutaneously with the KLN205 squamous cell carcinoma cell line were used as donors for the determination of CFU-s and GM-CFC content in the BM, spleen (SPL) and PB at 30 days after tumor transplantation. Presence of small (<1 cm) KLN tumors was associated with a significant leukocytosis and granulocytosis beginning at about day 14 post-tumor grafting. The 8 & 12 day CFUs and GM-CFC content of BM, SPL and PB were determined using the Till & McCulloch spleen colony and semi-solid culture methods, respectively. Only spleen colonies greater than 0.5 mm and agar colonies of greater than 50 cells were scored. Both BM and PB day 8 CFUs were significantly elevated in tumor bearing mice compared to controls (3,800$\pm$200 vs 1,800$\pm$300/femur & 98$\pm$8 vs 21$\pm$1/ml PB). Day 12 colonies, studied only in the BM samples, were also significantly elevated in the tumor bearing mice (2,700$\pm$200 vs 1,700$\pm$200/femur). BM and SPL GM-CFC content in the tumor bearing mice were significantly greater than in the controls (30,500$\pm$5,300 vs 19,900$\pm$2000/femur & 15,503$\pm$1,629 vs 3,904$\pm$1,151/SPL). These data suggest that the presence of a solid tumor may alter the frequency of HSC in not only the BM and SPL but also the PB compartment. (Supported by NIH grant CA46686)

C 507 CHEMOTHERAPY - RELATED HEMOLYTIC UREMIC SYNDROME AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION, Cynthia DeLaat, James Sambrano, Richard Harris, Steven Neudorf, Daniel Pietryga, and Christopher Morris. Hematology/Oncology Division, Children's Hospital Medical Center, Cincinnati, OH 45229
A syndrome of renal dysfunction consistent with the hemolytic uremic syndrome (HUS) is being increasingly identified following bone marrow transplantation. Etiologic factors have included total body irradiation, cyclosporin A, graft-versus-host disease and cytomegalovirus infection. We describe a child who developed HUS following autologous transplant with high-dose chemotherapy only. The clinical and laboratory findings of this patient's HUS are similar to what is described with the chemotherapy related HUS following mitomycin C therapy. Our patient responded to treatment with plasmapheresis, Vincristine, steroids and aspirin; however she was chronic, but stable renal insufficiency. The early recognition of and more uniform treatment approach to this entity of post transplant HUS is necessary as its frequency may increase as more patients are undergoing bone marrow transplants.

C 508 ENDOCRINOLOGICAL FOLLOW-UP OF CHILDREN AFTER ALLOGENEIC AND AUTOLOGOUS BONE MARROW TRANSPLANTATION, R. Dopfer, M. Ranke, Th. Klingebiel, W. Blum, D. Niethammer, University Children's Hospital, Department for hematology and oncology, Tübingen, FRG
The endocrinological regulatory system of 30 patients who had been grafted for different malignancies (ALL, AML, CML, solid tumors) has been investigated before and at various times after bone marrow transplantation. A combined pituatary stimulation test was performed and hgH, TSH, LH, FSH and prolactin were measured. In addition basal levels for T3, T4, IGF-I, IGF-II, SMBP, testosterone and estradiol were determined. For children the most striking side effect of the treatment is impairment of growth leading to a persisting disability of the survivors. For this reason we focussed our interest on the influence of bone marrow transplantation on the growth of children. Spontaneous growth hormone secretion by night was measured in 10 patients and correlated to the stimulation test and growth. In patients with liver GVHD IGF-levels after short time growth hormone treatment were determined. The most frequent abnormality was primary hypogonadism, as exspected, followed by growth impairment. Primary hypothyroidism was a very rare finding. The endocrinological abnormalities are correlated to the irradiation dose and the presence or absence of graft versus host disease.

C 509 RAPID ENGRAFTMENT AND CONTROL OF GRAFT VS HOST DISEASE (GVHD) FOLLOWING MATCHED UNRELATED DONOR (MUD) BONE MARROW TRANSPLANT (BMT). Drosick R, Henslee-Downey PJ, Marciniak E, Romond E, Messino M, Macdonald J, Harder E, and Thompson J; Department of Medicine, University of Kentucky, Lexington, Ky 40536. Between November 1987 and July 1989, five of 76 referred patients(pts) (6.8%) with acute leukemia and without genotypic matched donors underwent BMT utilizing grafts from a MUD phenotypically identical for HLA A, B, DR and DRW loci and bi-directional non-reactive in mixed lymphocyte culture. The age of the recipients ranged from 14 to 37 and the donors from 26 to 39 years. They were of opposite sex in 2 cases. When possible, CMV seronegative donors were chosen for CMV- recipients. The mean time from initiating a donor search and receipt of marrow graft was 91.4 days, range 29-158. All pts received marrow and immunoablative therapy consisting of total body irradiation prior to high dose Etoposide, Cytosar, Cytoxan and Methylprednisolone. A two stage approach for GVHD prophylaxis was designed in an effort to enhance the engraftment process. Following a partial T-cell depletion of the graft with a complement mediated monoclonal antibody, T10B9, achieving approximately 1.5 log reduction in clonogenic lymphocytes, daily intravenous therapy with a T-lymphocyte targeted immunotoxin, Xomazyme H-65, was begun on day +10 for 7 days in 2, day +7 for 10 days in 2, or day +5 for 12 days in 1 pt. All pts engrafted promptly attaining a peripheral white count of >500 by a mean day of +13.6 and >1000 by +16.4 days post transplant, all of which demonstrated 100% donor origin by either analysis of cytogenetics or restricted fragment length polymorphism. Post transplant requirements for transfusion support was minimal with a median number of 9 red cell and 10 pheresied platelet units. The highest overall acute GVHD stage observed was 0 in 2, I in 2, and III in 1 pt with minimal skin or no GVHD in all pts receiving the immunotoxin by or before day +7. One pt has developed mild, limited chronic GVHD and no pts have relapsed with the underlying malignancy. These promising preliminary results support the feasibility of utilizing MUD grafts. For pts with acute leukemia it may be important to shorten the time required to obtain such grafts.

C 510 MEDIAN DAILY INCREMENT OF LEUCOCYTES DETERMINES THE STEM CELL YIELD OF TIMED LEUCAPHERESES IN CHILDREN, Wolfgang Emminger, Waltraud Emminger-Schmidmeier, Paul Höcker, Chris Peters and Helmut Gadner. Bone Marrow Transplantation Unit of St.Anna Children's Hospital and Intensive Blood Bank, Vienna, AUSTRIA.
22 consecutive patients with a median age of 11 years (range 3-23) underwent 103 leucaphereses for collection of peripheral stem cells. Our aim was to collect at least $2.5x10^4$ myeloid committed stem cells (CFU-GM) per kg BW. This stem cell number had resulted in rapid and sustained hematopoietic reconstitution after autografting in our institution. Stem cell collections during the 8 days following the first platelet rise after a chemotherapy induced aplasia contained median $1.02x10^4$ CFU-GM/kg BW/apheresis (range 0.2 -13.3), whereas the CFU-GM yield at any other time was always unsatisfactory - median $0.27x10^4$ CFU-GM/kg BW/apheresis (range 0.01-1.11).
The stem cell yield of "well timed" leucaphereses depended on the absolute number of circulating leucocytes ($p=.002$) and mononuclear cells ($p<.001$). The median daily increment of leucocytes predicted the stem cell yield of individual leucaphereses ($p=.002$). High stem cell numbers could be collected in various malignancies without the use of exogenously administered growth factors by using careful timing alone and resulted in rapid hematopoietic reconstitution. Our findings will help to economize the use of the peripheral stem cell source for hemapopoietic reconstitution after myeloablative therapy.

C 511 PSYCHOLOGICAL RESPONSES TO BONE MARROW TRANSPLANTATION. HILDE FUNAKI. PSYCHOLOGICAL MEDICINE DEPARTMENT, The Royal Marsden Hospital, Downs Road, SUTTON, SURREY.SM2 5PT
Bone Marrow Transplantation (BMT) is a traumatic treatment with great physical discomfort and great uncertainty regarding the patient's long term survival. Psychological responses to BMT tend to run parallel to the transplant process; problems are frequently experienced. Nonetheless the psychological impact of BMT on the patient has not yet been thoroughly examined. Existing research focuses on specific episodes such as isolation or the decision for transplant. This leaves the involved physician without guidelines as to when and to what degree to expect psychological problems, and how to proceed to ease these problems.This presentation describes a prospective, longitudinal study aimed at examining psychological morbidity, quality of life and individual differences in coping, as well as perceived support throughout the different stages (10) of BMT and then up to one year post-transplant. Subjects are a consecutive series of adult patients evaluated for BMT treatment. Patients are assessed using standardized questionnaires, and a structured interview aimed at gathering data on the specific components of BMT which contribute to reported psychological morbidity. These include measures of psychological responses such as anxiety and depression, "fighting spirit", emotional control and perceptions of locus of control. Data analysis involve within subject comparisons across the stages of BMT. To date 55 patients have been recruited and have passed the 3 months post transplant assessment stage. Preliminary results indicate high emotional morbidity during stages conditioning, isolation and 3 months. Physical and psychological distress are highest during isolation, "fighting spirit" at its lowest at stage 3 months.

C 512 LITHIUM ENHANCES RECOVERY OF MYELOPOIESIS AND LENGTHENS SURVIVAL IN AN ALLOGENEIC MURINE TRANSPLANT MODEL, Vincent S. Gallicchio, Michael J. Messino, Ben C. Hulette, Mark K. Bieschke, Nedda K. Hughes, and P. Jean Henslee, Bone Marrow Transplant Program, University of Kentucky Medical Center, Lexington, Kentucky 40536
Lithium (Li) has been shown to modulate many aspects of hematopoiesis, in particular the increased recruitment of various classes of hematopoietic progenitors. We report here the ability of Li to increase the rate of myelopoiesis and lengthen the survival of transplanted recipients in a murine allogeneic transplant model. HeN mice were pre-treated daily with 25 ug/kg/bw ultra pure Li_2CO_3 i.p. for 5 days. Following a 4 day rest period, lethally irradiated AKR recipients were transplanted with either BM alone or BM + spleen cells harvested from either Li or PBS treated donors. Myelopoiesis was monitored in transplanted recipients by quantitating CFU-GM from BM and spleen. On all days examined (0-35) increased BM derived CFU-GM was demonstrated (115%-249% of control) from the Li-BM transplanted recipients. Following 600 days after transplant, 48% survival was observed in the Li-BM/spleen recipients compared to 28% in the PBS controls. These results indicate Li pre-treatment may be an efficacious agent to modulate survival in an allogeneic transplant setting.

C 513 STRATEGIES FOR SELECTION OF UNRELATED BONE MARROW DONORS, S.F. Goldmann, M. Ballas, T. Eiermann, A. Wölpl and B. Kubanek, Department Transplantation Immunology, Red Cross Blood Bank Ulm, and Department Transfusion Medicine, University of Ulm, D-7900 Ulm, FR Germany

Use of HLA-A,B,DR and MLC identical unrelated marrow donors (UMDS) is essential for 70 % of patients needing allogeneic transplants but not having identical family donors. Current size of international UMDS pool and lack of HLA-DR as well as HLA-Dw(MLC) data of the potential donors limits donor finding for only 10 - 15 % of the patients. It has been postulated that correlation between restriction length polymorphism (RFLP) pattern and HLA-Dw typing as defined by HTCs exists. In order to simplify donor selection and, in addition, to enable one to find donors also for leukemic patients not reacting in the MLC due to disease and/or chemotherapy, we investigated the correlation between serologically defined HLA-class II antigens, RFLP patterns and MLC reactivity in unrelated HLA-A,B,DR phenotypically identical donor/recipient combinations. The genomic DNA of the individuals tested was digested with Pst1 and Taq1 restriction enzymes and hybridized with DRβ, DQα and DQβ probes. One to three different RFLP patterns were found in all 12 HLA-DR identical but MLC different pairs. Identical RFLP patterns were found in 9 of 13 HLA-DR and MLC identical pairs; one to two DQ restricted different RFLP patterns were found in 4 of 13 HLA-DR and MLC identical pairs. Our preliminary data seem to indicate that identical RFLP patterns with the described enzymes and probes parallele HLA-DR and MLC identity and that different RFLP patterns are highly associated with MLC disparity in HLA-DR identical unrelated pairs.

C 514 INTELLECTUAL AND SOCIAL QUOTIENTS IN SEVERE COMBINED IMMUNODEFIECIENCY (SCID) CHILDREN UNDERGOING BONE MARROW TRANSPLANT (BMT) WITH AND WITHOUT TOTAL BODY IRRADIATION (TBI), FE Halberg[1], JH Kramer[2], MR Cuttenden[2], WM Wara[1], DW Wara[2], KK Matthay[2], MJ Cowan[2], Depts of Radiation Oncology[1] and Pediatrics[2], Univ. of CA, San Francisco,94143. Pre and post BMT intellectual and social function were assessed in 11 children transplanted for SCID, ages 1 to 15 months. 6 children received minimal conditioning, generally antithymocite globulin (ATG). 5 children received TBI, as part of intensive immunosuppression for a mismatched T-cell depleted BMT, after nonengraftment of their first BMT, or mounting an intense in vitro response to donor cells. They received ATG, cyclophosphamide and TBI, 7.0 Gy, single dose, 15 cGy/min, with half-value blocks shielding brain, eyes and lungs to ~3.5 Gy. Bayley IQ and Vineland SQ tests were performed prior to and at 1 year post BMT.

PATIENTS:	BASELINE: IQ	SQ	1 YR. POST: IQ	SQ
Entire group	95	95	94	93
Unirradiated children	91	93	88	93
Irradiated children	101	97	100	93

Multivariate analysis of variance revealed no differences between the irradiated and unirradiated patients. This is remarkable in light of irradiation at such a young age. Follow-up testing is planned.

C 515 ASSESSMENT OF CHIMERISM FOLLOWING BONE MARROW TRANSPLANTATION USING THE POLYMERASE CHAIN REACTION (PCR), Mark Lawler, Shaun R.McCann and Peter Humphries, Department of Genetics and Haematology,St. James Hospital and Trinity College, Dublin , Ireland.

Mixed chimerism following allogeneic BMT may have implications for marrow rejection or leukemic relapse. We have described a rapid highly sensitive assay to detect mixed chimerism following BMT in sex mismatched transplants based on PCR (Lawler et al, Br.J. Haematol. in press.). As greater than 50% of BMTs will show no sex difference between donor and host, a sensitive technique to detect mixed chimerism in this situation is required. The recent discovery that the simple sequence block $(dC\text{-}dA)_n$: $(dG\text{-}dT)_n$ shows a high degree of polymorphism between individuals has prompted us to examine these sequences as highly informative markers of engraftment. Oligonucleotide primers were designed to span these simple sequence repeats (microsatellites) at the following loci - APOA2, RHO, IGF1 and APOC2. These loci were amplified by PCR using radioactive dCTP and the products analysed on denaturing polyacrylamide gels. Several blocks can be amplified simultaneously, allowing unambiguous typing of donor/host pairs. We have typed 18 donor/host pairs using APOA2/RHO primers and have found informative polymorphisms in 13 cases. Conditions have been optimised for PCR directly from peripheral blood, bone marrow aspirates and stored bone marrow slides (fixed and unfixed). The construction of a highly informative panel of additional microsatellites will allow the rapid and precise detection of mixed chimerism in BMTs where donor and recipient are sex matched. Multiple analyses can be performed on microlitre samples in 8-12 hours.

C 516 POSITIVE SELECTION OF HUMAN HEMATOPOIETIC STEM CELLS USING THE AIS STEM STEM CELLECTOR. Jane S. Lebkowski, Lisa Schain, Vibeke Strand, David Warren+, Roland Levinsky*, and Thomas Okarma. Applied ImmuneSciences, Inc. Menlo Park, CA; +Memorial Sloan Kettering Cancer Center, New York, NY; *The Institute for Child Health, London, UK.

We have developed a simple procedure to positively select for human hematopoietic stem cells using a well characterized CD34 monoclonal antibody and the AIS Stem CELLector device. The Stem CELLector device is a sterile polystyrene flask which contains the CD34 monoclonal antibody covalently bound to the internal surface. In this procedure, unfractionated human bone marrow nucleated cells are incubated in the device for one hour at room temperature. After incubation, the adherent stem cells are recovered by a simple physical process and are available for further use. The enriched CD34+ stem cells are greater than 85-90% viable and retain their ability to proliferate and differentiate. As measured by a variety of in vitro colony forming assays, the purified CD34+ cells are 10-50 fold enriched in both myeloid and erythroid progenitors. The long term hematopoietic activity of these cells is currently being defined in preparation for their use in bone marrow transplantation.

C 517 EFFECTS OF RECOMBINANT HUMAN HEMATOPOIETIC GROWTH FACTORS *IN VITRO*. Richard C. Meagher, Roger H. Herzig, Brown Cancer Center, Division of Medical Oncology/Hematology, University of Louisville, Louisville, KY 40202.

The availability of adequate supplies of recombinant human hematopoietic growth factors (rHGFs) permits study of the regulatory function(s) these cytokines exert on normal hematopoietic cell proliferation and differentation. Thus, it is possible to evaluate the potential role of rHGFs in the development of new therapies for cancer patients. The purpose of this study was to examine whether direct addition of rHGFs was capable of maintaining proliferation of normal hematopoietic precursors and whether rHGFs induce normal hematopoietic differentation *in vitro*. Long-term human bone marrow cultures (LTMC) established with either normal human marrow or with light-activated merocyanine-540 phototreatment (LAMP) purged marrow were exposed to continuous presence of rHGFs (GM-CSF, Ep, IL 1, or IL 3; 5-50 U/ml) during weekly feedings. LTMC were analyzed for total nucleated cell counts and for the presence of committed (BFU-E, CFU-GM) and multipotential (CFU-GEMM) progenitors. GM-CSF addition to LTMC caused enhanced myeloid proliferation with a doubling of recoverable nucleated cells. CFU-GM progenitors were increased 3 fold (2.2×10^2 - 1.7×10^3 vs. 8×10^1 - 5×10^2, respectively). Weekly replenishing of GM-CSF resulted in failure to sustain cell proliferation after 3 weeks; LTMC resembled mature macrophage cultures containing large vacoules. EP stimulated a mild increase in nucleated cell proliferation and BFU-E colony formation increased 2 fold during the first 2 weeks (3.0×10^2 - 0.8×10^4 vs. 1.2×10^1 - 5×10^3, respectively) followed by quiescence of LTMC. IL-1 and IL-3 both increased nucleated cell counts and hematopoietic precursors (BFU-E-1 fold, CFU-GM-4 fold, CFU-GEMM-2 fold). Unexpectedly, HGF addition to LTMC effected adherent stoma cell function. Similar results were obtained when LAMP purged marrow was used in LTMC. Thus, timing the addition of HGFs may be an important consideration when using hematopoietic cytokines in a clinical setting.

C 518 ABILITY OF IL-1 AND/OR TNFα TO PROTECT ENGRAFTABLE PLURIPOTENT STEM CELLS FROM 4-HYDROPEROXYCYCLOPHOSPHAMIDE (4-HC). J. Moreb, R. Neta, J.R. Zucali, G.D. Ledney and R.S. Weiner. University of Florida, Gainesville, FL 32610 and Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

Preincubation of bone marrow cells in vitro with IL-1 or TNFα can protect early hematopoietic progenitor cells from 4-HC. In this study, we determined whether IL-1 can protect pluripotent stem cells responsible for engraftment from 4-HC toxicity. B6D2F1 bone marrow cells (BMC) were incubated with or without 50 - 200 ng/ml IL-1 for 20 hours prior to treatment for 30 minutes with varying doses of 4-HC. After washing, 2×10^6 BMC were injected intravenously into groups of lethally irradiated mice. All mice receiving BMC treated with 40 or 60 μg/ml 4-HC in the presence or absence of IL-1 died by day 13 following transplantation. However, the survival at day 10 in mice receiving 40 μg/ml 4-HC with IL-1 was significantly better in comparison to the corresponding control group without IL-1 (91% versus 25%, respectively). Day 30 survival of mice receiving BMC treated with IL-1 and 20 μg/ml 4-HC was 82% versus 30% in the corresponding control group without IL-1. Similarly, all of the mice receiving BMC treated with IL-1 and 10 μg/ml 4-HC survived; whereas, 2 out 12 mice in the corresponding control group died. These results suggest that IL-1 preincubation offers a survival advantage for irradiated mice receiving 4-HC treated bone marrow. Recent in vitro studies in our laboratory suggest the involvement of TNFα in IL-1 protection. Thus, similar animal studies using TNFα may have great clinical significance.

C 519 DOPPLER ULTRASONOGRAPHY (DU) FOR EVALUATION OF VENO-OCCLUSIVE DISEASE OF THE LIVER (VOD). C.L. Morris, D.S. Babcock, D.W. Pietryga, S.M. Neudorf. Children's Hospital, Cincinnati, Ohio.

VOD is a major contributor to morbidity and mortality following BMT. Findings associated with a high likelihood of VOD include: jaundice, hepatomegaly with right upper quadrant pain, and unexplained weight gain or ascites (McDonald et al, Transplantation 39:603). However, the clinical status of symptomatic patients frequently precludes diagnostic liver biopsy. For this reason we evaluated the ability of DU to detect abnormalities in hepato-portal blood flow which confirm the clinical diagnosis of VOD. Three pediatric patients who fulfilled the clinical criteria for VOD were studied with DU. The first patient developed abrupt onset of symptoms 17 days after autologous BMT. Reversal of hepato-portal flow and gall bladder wall thickening was noted on day 14, and resolved by day 20 post BMT. The second patient developed symptoms 18 days after allogeneic BMT. Serial DU studies showed normal findings on day 14, gall bladder wall edema on day 20, and reversed hepato-portal flow on day 22 which persisted until death on day 30 after BMT. At autopsy, liver histology confirmed the presence of VOD and GVHD. The third patient developed acute GVHD on day 28 (Grade IV skin & gut, grade III liver). Four DU studies performed through the period of maximal liver function abnormalities showed no evidence for gall bladder wall edema or reversed hepato-portal flow. In summary, reversed hepato-portal blood flow and gall bladder wall edema were identified in 2 patients with VOD including a patient with histologically proven VOD and GVHD, and a patient who received an autologous BMT. A third patient with documented severe GVHD did not demonstrate abnormalities by DU. DU may provide an objective assessment of hepatic hemodynamics with findings specific for VOD.

C 520 CARDIAC MORBIDITY OF BONE MARROW TRANSPLANTATION (BMT) IN ELDERLY PATIENTS (PTS). S. Nair, P. Jetty, K. Musselman and D. Przepiorka, Pittsburgh Cancer Institute, Cadiology Unit, Montefiore Hospital, University of Pittsburgh, Pittsburgh, PA 15213.

Cardiac data were reviewed retrospectively for 7 pts age 55-67 yr who underwent autologous (5), syngeneic (1) or allogeneic (1 twice) BMT for hematologic disorders. The conditioning regimens consisted of cyclophosphamide (CYC) (50 mg/kg/day X 4 or 60 mg/kg/day X 2) with busulfan (6), TBI (1) or ATG plus TLI (1). Two pts had a history of pulmonary edema during remission induction, 1 had documented coronary artery disease (CAD) and stable angina 6 mos after having had an MI, and 4 had no prior cardiac problems. On pretransplant evaluation, 6 pts had left ventricular ejection fractions (LVEF) of 51-68% by ECHO or MUGA, and 3/6 had segmental or global hypokinesis. These 6 patients had 16 episodes of acute pulmonary edema with (4) or without (12) atrial fibrillation 0-86 (median 15) days after BMT. Only 1 pt, the 67 Y/O syngeneic transplant recipient with a history of CAD, had a documented transmural infarct. Cardiac events were usually coincident with hematocrit $\leq$ 30 (15), weight gain $\geq$ 2 kg (13), fever (9) or transfusion (7). The cardiothoracic ratio (CTR) increased $\geq$ 10% in all pts, and the sum of the voltage of the limb leads on follow-up EKG decreased $\geq$ 25% in 4 pts. In the absence of complicating noncardiac factors, all pts responded to therapy with diuretics $\pm$ digoxin. One pt did not develop pulmonary edema. He had an LVEF of 34% and segmental hypokinesis on ECHO prior to BMT, and his weight gain never exceeded 2 kg. Five pts died of BMT-related causes, but a myocardial event was never the primary cause of death. We conclude that cardiac morbidity is high in elderly BMT pts even with no history of cardiac disease, and a weight gain > 2 kg with anemia may predispose pts to pulmonary edema.

C 521 PRELIMINARY RESULTS OF A RANDOMIZED TRIAL OF rhGM-CSF AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN PATIENTS WITH LYMPHOID MALIGNANCY. J Nemunaitis, JW Singer, CD Buckner, CD Epstein, F Oldham, ED Thomas, FR Appelbaum. Fred Hutchinson Cancer Research Ctr and Immunex Corp., Seattle, WA.

To evaluate the benefits of rhGM-CSF in ABMT recipients, 30 patients (pts) were entered into a double blinded, randomized placebo-controlled trial. All pts received cyclophosphamide and TBI. The mean age of the 17 GM-CSF treated pts was 33 years compared to 34 years in the 13 placebo-treated pts. Eleven of the GM-CSF treated pts had NHL and 6 had ALL, whereas 7 of the placebo treated pts had NHL, 5 had ALL and 1 had Hodgkin's disease. Five of the 17 GM-CSF treated pts were in remission compared to 7 of the 13 control pts. RhGM-CSF (250μg/m2/day) or placebo was given by 2 hour infusion on days 0 to 20 after ABMT. There was 1 early death due to interstitial pneumonia in the control group and no early deaths in the GM-CSF group. The mean values of some parameters used to compare GM-CSF to placebo treated pts are shown below.

	GM-CSF	Placebo	P-value (Wilcoxon)
Day ANC >500	17	16	.55
Day last Plt Tx	22	4	.01
# units plts	69	109	.01
Max. Creatinine	1.1	1.8	.01
Max. Bilirubin	2.5	6.2	.07
Days Temp. >38	10	15	.02
Discharge Day	28	43	.02
Cost$x10^3	79	112	.02

Despite a minimal effect on neutrophil recovery, GM-CSF treated pts had significantly earlier platelet engraftment, fewer transplant related complications, shorter hospital stays and lower in-hospital costs.

C 522 DNA POLYMORPHISM ANALYSIS OF MATCHED FULL GRAFTS AND MISMATCH MIXED CHIMERISM IN T-DEPLETED BMT. Pier-Giuseppe Pelicci, Franco Aversa, Adelmo Terenzi, Amedea Mencarelli, Yair Reisner*, Massimo F.Martelli. BMT Unit University of Perugia, 06100 Italy,*Dpt.Biophisic Inst. Rehovot, Israel. The peripheral blood (PB) and bone marrow (BM) chimerism was evaluated in 10 BM transplanted leukemia patients (pts) by DNA sequence polymorphism analysis. All pts received allogeneic T-depleted BMT after a TBI,thiotepa and Cy conditioning regimen, 5 from an HLA-identical MLC-, 4 an haploidentical MLC+ donor and 1 from a phenotypically identical mother. DNA was extracted from donors' and patients' PB and the patients' PB and BM 1 to 10 months post-transplantation. Engraftment was quantitated by hybridizing DNAs to a minisatellite DNA probe which simultaneously recognizes many highly polymorphic loci. The 5 matched BMT pts achieved full PB and BM engraftment. Although BM engraftments was documented in the 6 mismatched BMT pts, they had varying degree of PB chimerism. Analysis of the PB of 1 mismatched BMT pt,who is still alive and disease free 10 months after BMT,showed that most T-cells were host derived,whereas B cells,neutrophils and macrophages were donor derived.The lack of mixed-chimerisms in the HLA-identical recipients suggests that our conditioning regimen is more ablative than conventional TBI and Cy; however, the mixed-chimerism in the PB of HLA-nonidentical recipients suggests that the regimen is not totally ablative. We are retrospectivly studying previously transplanted pts.

C 523 **IDENTIFICATION OF HOST CELLS IN MARROW TRANSPLANT RECIPIENTS BY IN SITU HYBRIDIZATION FOR THE Y CHROMOSOME (Y-ISH),** **Donna Przepiorka, E. Donnall Thomas, and Diane M. Durnam, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.**
The origin of peripheral blood mononuclear cells (PBMC) or granulocytes (GRAN) was studied prospectively by Y-ISH Day 7 to Day 84 in 51 recipients of sex-mismatched marrow not depleted of T cells. The conditioning regimens consisted of cyclophosphamide (CTC) and total body irradiation (TBI) with or without total lymphoid irradiation. HLA-matching with the donor was complete for 36 patients (pts) and partial for 15. Median follow-up is 27 mos (range 21-30 mos). The mean number of cells evaluated varied from 126 ± 23 PBMC and 172 ± 30 GRAN on Day 7 to more than 500 PBMC or GRAN on Day 21 and thereafter. Host PBMC could be detected in the majority of pts through Day 84, and in the absence of rejection (1 pt), the mean percentage of host PBMC plateaued at 1.0 ± 0.2% by Day 28. The mean percentage of host GRAN fell more rapidly, was less than 0.5% by Day 14, and thereafter was at background levels. In contrast, the mean absolute number of host cells did not vary substantially with time after Day 7. The mean absolute host PBMC was 5 ± 2 to 13 ± 3 per μL, and the mean absolute host GRAN was 0 ± 15 to 7 ± 7 per μL. Neither the percentage of host cells nor the absolute number of host cells on any day correlated with dose-intensity of TBI, HLA-matching, development of acute or chronic GVHD, or relapse after transplantation. We conclude that in the first 3 mos posttransplant, the presence of small numbers of host cells in pts conditioned with CTC and TBI is of no clinical significance in the absence of overt graft failure or relapse.

C 524 HEMORRHAGIC CYSTITIS (HC) DURING BONE MARROW TRANSPLANTATION (BMT): RISK FACTORS AND COMPLICATIONS. S.Sencer, R.Haake and D.Weisdorf, University of Minnesota Bone Marrow Transplantation Program, Minneapolis, MN 55455

Hemorrhagic cystitis is a major cause of morbidity following BMT; we have analyzed its incidence, risk factors and complications in 977 patients undergoing BMT between 1974 and 1988. Despite vigorous hydration and frequent voiding in all patients receiving Cytoxan (Cy), 135/977 (15% by Kaplan-Meier projection) developed HC (micro or gross hematuria, dysuria, bladder pain) between -7 and +100 days (median +22) following BMT. Of these, 60 had severe HC, including major urinary obstruction (4/60), renal failure (13/60) or need for surgical or chemical bladder cauterization (16/60). Allogeneic BMT recipients had more frequent HC than autologous patients (17% vs 9%, p=.02). Age was correlated with HC risks with children <10 at least risk (10%) and those 10-19 years with the highest rate of disease (20%, p<.01). The incidence of HC trended upward with higher dose Cy (none: 8%; 120mg/kg: 16%; ≥150mg/kg: 17%; p=.07), but was not increased in those receiving Busulfan, as has been previously suggested, even with high dose Cy (p=.47). Cy and radiation were prospectively assigned by diagnosis, therefore, independent assessment of HC risks related to diagnosis were not separable from risks imposed by Cy and radiation schedules. Patients with aplastic anemia conditioned with high dose Cy and Total Lymphoid Irradiation, had the highest rate of HC (22%) vs those with hematologic malignancies (15%, p=.03). Patients with CML developed HC at a higher rate than those with acute leukemia (22% vs 11%, p<.001). The factor most highly associated with the development of HC was the finding of adenovirus in the urine preceding the onset of hematuria (Relative Risk (RR)=6.1, p<.0001), but viremia was not associated with HC (p=.40). HC-related morbidity, and its associated increased hospitalization costs, frequently complicates BMT. Improved prophylactic measures, perhaps including the use of MESNA, are needed, at least for allogeneic BMT patients, especially those receiving high dose Cy, and those with known adenoviruria.

C 525 PHASE I/II TRIAL OF rhGM-CSF AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT). JW Singer, J Nemunaitis, CD Buckner, CD Epstein, CS Higano, R Storb, ED Thomas, FR Appelbaum. Fred Hutchinson Cancer Research Center, Immunex Corporation, Seattle.
To evaluate toxicities associated with rhGM-CSF in allogeneic BMT recipients and to determine effects on the rate of engraftment, 14 patients were treated with rhGM-CSF (30 to 120 μg/m2/day) by two hour infusions from day 0 to 20 after HLA-identical allogeneic BMT. The preparative regimens included cyclophosphamide plus TBI and GVHD prophylaxis was cyclosporin A (CyA) alone or CyA plus prednisone. The mean age was 31 years; 2 patients had ANL in remission, 11 had lymphoid malignancies and 1 had CML in relapse following a prior BMT. No significant GM-CSF toxicities were noted. Two patients died within 30 days of their BMT, one of pulmonary aspergillosis and one of pseudomonas pneumonia. The neutrophil recovery of the study patients compared to 185 historical controls are described below:

	Day ANC>100/mm^3	Day ANC>500/mm^3	Day ANC >1000/mm^3
GM-CSF Patients	3.6 ± 4.5	10.7 ± 3.9	12.9 ± 2.6
Control Patients	11.9 ± 5.5	---	21.2 ± 6.8

Five study patients reached and maintained an ANC greater than 100/mm^3 within 3 days after BMT compared to none of the controls. Three patients had greater than 5 febrile days, 7 patients had 1 to 4 febrile days and 4 never developed fevers. No patient developed severe mucositis and none relapsed. No patient developed grade II or greater GVHD during GM-CSF infusions, however, 2 patients developed grade III-IV GVHD 3 weeks after discontinuation of GM-CSF. These results suggest that GM-CSF is well tolerated and probably does not increase the incidence of severe GVHD following allogeneic BMT and may substantially decrease the period of neutropenic risk. Randomized trials with GM-CSF in allogeneic BMT are indicated.

C 526 IMPROVED PLATELET RECOVERY FOLLOWING 5-FU MODULATION OF HUMAN BONE MARROW, F. Marc Stewart, Daniel S. Temeles, Ian K. McNiece, David L. Meyer, Karin E. Eshleman, and Peter J. Quesenberry, University of Virginia Health Sciences Center, Department of Internal Medicine, Charlottesville, VA 22908. We have reported the appearance of high proliferative potential colony-forming cells (HPP-CFC) in marrow from 3 patients treated with 5-Fluorouracil (5-FU) prior to bone marrow harvest for autologous transplantation. Twenty patients have been treated with 5-FU (15 mg/kg-45 mg/kg) IV for 1 to 3 days given 7-21 days prior to bone marrow harvest. Post 5-FU marrow has been infused into 9 patients following high dose chemotherapy (CBV) or chemoradiotherapy (CY-TBI). Median platelet recovery to 50,000 (20 days) and to 100,000 (22 days) was significantly shortened compared to historical controls treated with the same regimens (34 and 48 days, $p=0.01$, $p=0.003$, respectively). Granulocyte recovery to 500, 1,000, and 1,500 was unaffected although a trend was in favor to improved recovery with the post 5-FU group. *In vitro* culture with combinations of 1 to 7 growth factors (IL-1, IL-3, IL-4, IL-6, CSF-1, GM-CSF, G-CSF) were performed. Pre and post 5-FU marrow were compared showing CFU-C stimulated by GM-CSF + IL-3 increased a mean of 615% (10 patients) at 14 days and 107% (3 patients) at 28 days. GM-CSF + IL-3 responsive HPP-CFC at 14 days were increased from pre-transplant values of $0.03/10^5$ (10 patients); at 28 days, the values increased from $0.11/10^5$ cells to $1.72/10^5$ cells (3 patients). HPP-CFC were seen in 50% and 76% and 28 day samples, respectively. Multifactor combinations, especially, GM-CSF + G-CSF + IL-3 + IL-6 + IL-1 + CSF-1 did not increase total colony count or classic HPP-CFC, but did result in altered morphology, producing huge loose colonies. The marrow from patients pre-treated with 5-FU is enriched with multifactor responsive HPP-CFC and provides superior *in vivo* platelet recovery.

C 527 THALIDOMIDE IN THE TREATMENT OF REFRACTORY CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION. **Szer, J., Atkinson, K., Vowels, M. Waters, K.** *for the* **Australasian Bone Marrow Transplant Co-operative Study Group (ABMTCSG),** *Alfred Hospital, Prahran 3181; St Vincent's Hospital, Darlinghurst NSW 2010, Prince of Wales Children's Hospital, Randwick NSW 2031 and Royal Children's Hospital, Parkville VIC 3050, AUSTRALIA*
The recent development of increasingly effective immunoprophylaxis has significantly reduced the incidence and severity of acute GVHD after HLA-identical, sibling-donor BMT. Unfortunately, there does not appear to have been as significant an impact on chronic GVHD. Some patients with this form of GVHD fail to respond to standard therapy (glucocorticoids, cyclosporine, azathioprine or combinations) or suffer unacceptable toxicity from prolonged therapy with these agents. In July of 1988, the ABMTCSG launched an open study of *thalidomide* in the treatment of patients with otherwise untreatable chronic GVHD. To date, 16 patients have received thalidomide and the results are summarized below. Eleven adult and 5 pediatric patients have been treated with a maximum daily dose of 400 to 1600mg. Duration of therapy was 1 week to 12+ months. Seven of 13 evaluable patients have demonstrated responses: 3 with skin disease alone, one with skin and oral disease, one with liver disease, one with oral disease and one with interstitial lung disease. Of the 6 clear non-responders, 2 had skin and oral disease, one had eye, mouth and lung disease, 2 had interstitial lung disease and one had a wasting syndrome. Three patients were not evaluable due to death within one month of starting therapy (2 patients) and inadequate follow-up time (1 patient). Toxicity data are available for 12 patients. Seven developed sleepiness which was tolerable in all, 4 developed constipation, one had an asymptomatic sensory neuropathy and one developed a pericardial effusion temporally related to commencing the drug. We conclude that thalidomide is an effective form of therapy for some patients with refractory chronic graft-versus-host disease.

C 528 EXPANSION OF CIROCULATING CFU-GM INTERLEUKIN-3 AND 6 IN SUSPENSION CULTURE, Shuichi Taniguchi, Shigeyoshi Makino, Mine Harada, Kpichi Akashi, Hisashi Gondo, Takanori Teshima, Yasushi Takamatsu, Tsunefumi Shibuya, Yoshiyuki Niho, The First Department of Internal Medicine, Kyusyu University, Fukuoka 812, Japan. Teh purpose of the present study is to clarify whether interleukin 3 and 6 (IL-3, IL-6) could expand circulating CFU-GM in short term suspension culture. Peripheral blood mononuclear cells (PBMNC) were obtained when white blood cells were recovered to 1,000-2,000/microL after marrow supresive chemotherapy. 2 x 10^5 PB non-adherent MNC were cultured in 1ml of Iscove's Medium for 2-6 days. IL-3 and IL-6 were added to the suspension cultures as follows. In Experiment 1 (Exp. 1), only 50U/ml of IL-3 was added (IL-6 was free). In Exp.2, 50U/ml of IL-3 and 50U/ml of IL -6, in Exp.3, 50U/ml of IL-3 and 100U/ml of IL-6, in Exp.4 50U/ml of IL-3 and 200U/ml of IL-6. Medium only was served as control, Clonal assays for CFU-GM were performed after 2, 4, 6 days of culture. The number of CFU-GM increased in all experiments. After 6 days culture, in Exp.1 CFU-GM increased approximately 3.2 folds, in Exp.2 3.3 folds, in Exp.3 3.4 folds, in Exp.4 3.5 folds, in Exp.5 3.5 folds and 1.7 folds in control. The same experiments were performed in bone marrow MNC obtained on the same day as PBMNC. In bone marrow MNC, the number of CFU-GM increased 2.4 folds in Exp.1, 2.9 folds in Exp.2, 2.9 folds in Exp.3, 3.1 folds in Exp.4 and 0.9 folds in control. THese data indicates that there are some stem cells responding to IL-3 and IL-6 in circulating MNC as same as in bone marrow MNC and that we can use these expanded CFU-GM as clinical use in patients whose harvested CFU-GM was too small.

C 529 METABOLIC DERANGEMENTS FOLLOWING BONE MARROW TRANSPLANTATION (BMT): AN INTEGRATED ANALYSIS. A. Taveroff, A.H. McArdle, W.B. Rybka. The Montreal General Hospital, Montreal, Que, Canada, H3G 1A4.
The aim of this study was to describe the metabolic impact of cytotoxic therapy in BMT patients, with a view toward explaining and improving the response to nutritional support. Stool, urine and serum biochemistry were studied in 10 BMT patients for 25 days, starting 1 day before cytoreduction (day 0). TPN (35 kcal/kg) began on day 7. Fecal Na+ concentration increased 200% ($p<.001$), while K+ concentration decreased 50% ($p<.01$). Examination of serum electrolytes revealed hyponatremia concurrent with hyperkalemia ($r=-0.55$, $p<.01$) from days 9-17, with Na+ and K+ reaching 126.9±1.3 and 5.0±0.21 mmol/L on day 14. A decrease in nitrogen balance (0.9 g/day, $p<.001$) and serum albumin (0.7 g/L/day, $p<.01$) was observed from day 10-18, immediately following the disturbances in serum Na+ and K+; improvement in nitrogen balance and serum albumin began on day 18, immediately following the resolution of the Na+ - K+ inversion. Since glucose-based TPN can influence Na+ balance, a "low-TPN" regimen (25 kcal/kg) was instituted in 5 BMT patients. Serum Na+ fell to only 135.0±1.1 ($p<.01$) and K+ rose to only 4.24±0.27 ($p<.05$). Nitrogen balance ($p<.001$), serum albumin ($p<.001$) and lean body mass ($p<.05$) all improved dramatically. Thus, delivering TPN below conventional rates can minimize metabolic derangements following BMT.

C 530 REGIMEN RELATED TOXICITY (RRT) IN PATIENTS UNDERGOING ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) FOLLOWING PREPARATION WITH BUSULFAN AND CYCLOPHOSPHAMIDE (BU4/CY2), J. Thompson, M. Daly, J. Giguere, E. Harden, G. Harmon, D. Johnson, R. Leff, R. Mercier, and G. Messerschmidt, Wilford Hall USAF Medical Center, San Antonio, TX 78236-5300. Between 10/86 and 6/89 58 patients (pts) have undergone allogeneic BMT using BU4/CY2 [BU 4mg/kg POx4 (-7,-6,-5,-4) and CY 60mg/kg IVx2 (-3,-2)] with support considerations as described by Tutschka et al (Blood 70:1382-1388, 1987) in an attempt to corroborate the lesser morbidity reported. RRT, as defined and reported by Bearman et al (J Clin Oncol 6:1562-1568, 1988), was assessed for our 58 pts and was then compared to the published cohort prepared with CY/TBI. The percentage of patients with maximum single organ toxicity grades (GR) using BU4/CY2 compared with (CY/TBI) were: GR I: 36% (17%), GR II: 50% (68%), GR III: 11% (10%), GR IV: 3% (5%). Overall RRT by organ for BU4/CY2 was:

GRADE	CNS	RENAL	BLADDER	HEART	GI	MOUTH	LIVER	LUNGS
0	92%	68%	82%	83%	47%	21%	34%	100%
I	0%	18%	15%	5%	47%	68%	42%	0%
II	0%	11%	3%	3%	6%	11%	21%	0%
III	8%	3%	0%	0%	0%	0%	0%	0%
IV	0%	0%	0%	0%	0%	0%	3%	0%
	(>)	(=)	(=)	(=)	(<)	(<)	(<)	(<)

BU4/CY2 appears to be associated with less RRT than CY/TBI for organ systems (<), equivalent RRT (=), and more RRT (>) when compared to the CY/TBI cohort.

C 531 A NOVEL METHOD OF EVALUATING POSTTRANSPLANT CHIMERISM USING PCR AMPLIFICATION OF VNTRs AND OLIGONUCLEOTIDE HYBRIDIZATION. Luis Ugozzoli, Priscilla Yam, Lawrence Petz, G.B. Ferrara and R. Bruce Wallace. University of California, Los Angeles, CA; City of Hope National Medical Center, Duarte, CA; Instututo Nazionale per la Ricerca sul Cancro, Genoa, Italy. Specific oligonucleotides for hybridization were synthesized homologous to tandemly repetitive core sequences of the following regions with a variable number of tandem repeats (VNTRs): 33.1, 33.4, 33.6 (Jeffreys et al), 3'HVR-globin gene (Jarman et al), H-ras (Capron et al), and YNZ-22 (Wolff et al). Polymorphisms at such loci result from allelic differences in the number of repeats. Primers flanking the repeat region of each of the corresponding VNTRs were used for amplification. Recipient (R) and donor (D) pretransplant DNA and R posttransplant DNA were amplified. The resultant fragments were analyzed after gel electrophoresis either by hybridization in-gel or after Southern transfer. We compared these findings with standard assays of restriction fragment length polymorphisms (RFLP). Evaluation of 12 selected cases indicated mixed chimerism (7), complete chimerism (3), and endogenous repopulation of hematopoiesis (2) following BMT. Sensitivity of the method was determined by mixing various proportions of R and D DNA; the limit of detection of the minor component in a mixture was 0.2%. PCR data correlated with RFLP data in all cases except one in which PCR proved more sensitive than RFLP. PCR amplification of VNTRs combined with oligonucleotide hybridization is a novel technique for documenting posttransplant chimerism and has advantages over RFLP analysis; high sensitivity, use of small amounts of DNA (250 ng), ease of preparation of DNA, elimination of need for restriction enzymes, and the ability to complete studies in two days.

C 532 AUTOLOGOUS BONE MARROW TRANSPLANTATION IN ACUTE LEUKEMIA AFTER IN VITRO PURGING WITH ALKYL LYSOPHOSPHOLIPIDS (ALP), Vogler W.R., Berdel W., Okamoto S., Olson A.C. Winton E F , Liotta D., Gordon D.S., and Heffner L.T., Department of Medicine, Emory University School of Medicine, Atlanta, GA 30322

ALP represents a new family of cancer drugs which are selectively cytotoxic to leukemic cells and has been shown to be an effective agent for in vitro purging of leukemic marrow in a murine model (Blood 64:1288). In vitro studies demonstrated that a 4 hour incubation with 50 μg of ALP eliminated HL60 cells and spared normal marrow progenitor cells. Therefore, a phase I study was initiated using 50 μg/ml as the initial purging dose. Marrow was harvested from adult patients with acute leukemia in first or subsequent remission and incubated with ALP (ET-18-OCH_3) for 4 hours. The cells were washed once and cryopreserved in 10% DMSO. Patients in second or subsequent remission were given either high dose cytosine arabinoside (3 gm per sq m q 12h X 6 days) or cyclophosphamide (60 mg/kg X 2) followed by fractionated total body irradiation (12 gy) over 3 days. The marrow was thawed at the bedside and infused. Eight patients have been transplanted (2 ALL, 6 AML). There were 5 males and 3 females. Ages ranged from 27 to 57 years. Three patients relapsed at 38, 48 and 165 days and 5 remain in remission from 28 to 486 days. Marrow recovery occurred in all patients. Median time to absolute granulocyte count >500 was 24 days and platelets >50,000 was 50 days. These encouraging results indicate that 50 μg/ml of ALP is safe and the dose has been escalated to 75 μg/ml.