ADVANCES AND CONTROVERSIES IN BONE MARROW TRANSPLANTATION

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Public Policy and BMT

G 001 STANDARDS FOR BONE MARROW PROCESSING, Scott D. Rowley, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

The evolution of medical care from initial research concept to routine application occurs because the indications for and administration of a therapy have been defined. This definition of indications and technique encompasses what is known as "standard of practice," to which all medical practitioners (outside of properly organized and conducted research protocols) must comply or face the prospects of professional censure and legal liability. Many aspects of bone marrow transplantation are widely recognized as standard practice, such as administration of broad-spectrum antibiotics for neutropenic fever, and irradiation of blood products to prevent transfusion-induced GVHD. Moreover, allogeneic bone marrow transplantation itself has become the standard of care for some patients such as those with CML in chronic phase who are of appropriate age and have an HLA-compatible, related donor. Standard practices are also easily defined for the bone marrow product itself. These standards encourage the use of marrow cells from unrelated donors by facilitating the transportation of products between centers. The expectation that adequate cells will be harvested from a donor of appropriate health status, and that the product will be labelled and transported without delay in a manner to maintain cell viability enable this interaction between donor and transplant centers, and between donor and recipient. Likewise, patients who store cells for autologous transplantation expect that the cells will be properly maintained because these cells belong to the patient and not to the harvesting facility. Failure to follow accepted cryopreservation techniques exposes the storage facility to potential legal liability if the cells are damaged. Again, as with the unrelated marrow donor, definition of standards for storage facilitates the use of autologous cells harvested and stored at another facility. Regulations differ from standards in that the former are law that must be complied with and carry penalties for violation. Standards are defined by practioners, usually through a professional organization, and are voluntary, although standardization of practice carries the threat of tort (malpractice) action if violated resulting in harm to the patient. Definition of standard practice has advantages compared to regulation in that standards may more quickly evolve to changing medical practice, and are defined by recognized experts in the field. Standards should be viewed as enabling, not punitive; compliance to standards protects the patient, physician and facility. Standards define a minimum to which all facilities must comply, but allow multiple, validated techniques to reach that level. Compliance to standard marrow processing and transplantation techniques does not hinder research because proper clinical trial design will incorporate good laboratory procedures and protection for human subjects. Standards for hematopoietic progenitor cell processing include easily defined laboratory standards such as space, personnel qualification and training, equipment maintenance, product labelling, and records. Non-compliance to these laboratory practices cannot be defended. Product standards include quality assurance testing such as cell counts and measurement of viability, donor selection criteria, proper storage techniques including cryoprotectant concentrations and temperature of storage. Some issues, such as the minimum number of cells to be transplanted cannot be easily defined. Bacterial cultures should be obtained on all bone marrow products because of the open harvest techniques used, but standards cannot dictate that contaminated products be destroyed. Product standards, therefore, must acknowledge that the use of products that do not meet the standards may still be of benefit to the transplant recipient, and allow for the use of such products after informed consent has been obtained from the recipient.

G 002 QUALITY OF LIFE AND LONG-TERM COMPLICATIONS FOLLOWING BONE MARROW TRANSPLANTATION, Keith Sullivan, Claudio Anasetti, Nigel Bush, Mary Flowers, Jean Sanders, Muriel Siadak, Karen Syrjala and Robert Witherspoon, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

Chronic graft-versus-host disease (GVHD) is the major determinant of late morbidity and mortality following allogeneic bone marrow transplantation (BMT). Among day 150 survivors, approximately 33% of recipients of unmodified HLA-identical sibling marrow, 50% of HLA-nonidentical related marrow and 66% of unrelated donor marrow will develop this complication. Without treatment, fewer than 20% of patients survive free of disability with Karnofsky performance scores (KPS) >70%. Based on prospective randomized trials of immunosuppressive therapy, an alternating-day regimen of cyclosporine and prednisone has reduced treatment failure, i.e., non-relapse mortality (NRM) or progression to secondary therapy, compared with either single agent used alone. Multivariate analysis demonstrates an increased risk of treatment failure with progressive onset of chronic GVHD, increased patient age, bilirubin and number of disease sites and single-agent therapy. Infection is the leading cause of death in chronic GVHD and 2-year NRM is independently associated with advanced stage malignancy, HLA-nonidentical or unrelated donors, and early onset or progressive onset of chronic GVHD. Those achieving a complete response after 9-18 months of treatment have a 3-4% probability of NRM, while those failing therapy have a 28-48% mortality. Disabling morbidity, (i.e., current KPS ≤70%) among current survivors with chronic GVHD is 10% for 196 HLA-identical recipients and 9% for 125 HLA-nonidentical/unrelated marrow recipients treated from 1987 to 1992.

Cross sectional studies from several transplant centers have evaluated morbidity and quality of life (QOL) between 1 and 4 years after BMT. Among autologous recipients, QOL at 1 year was reported by the Stanford group to be above average to excellent in 88% of patients. We prospectively studied 67 patients before and after allogeneic BMT and found that severe chronic GVHD and pretransplant family conflict predicted subsequent impaired physical and emotional recovery. In this cohort of patients, 68% returned to full-time work by 2 years and 91% by 4 years posttransplant. Recently 125 adult survivors of allogeneic (87%) or syngeneic/autologous (13%) marrow grafting were studied 6-18 (mean 10) years after transplantation in Seattle to determine the QOL beyond the time of perceived cure of the underlying malignancy. Seven wide-ranging tests measured physical, psychological, social functioning and disease/treatment symptoms: 80% rated their QOL as good to excellent while 5% rated it as poor. The most frequently cited demand of recovery was a perceived lack of social support from family and friends. Although lingering complaints such as fatigue, sexual dysfunction and sleep disturbances were noted, most survivors judged these of low severity and 88% of the 125 patients said the benefits of marrow transplantation outweighed the side effects.

Histocompatibility and Alternative Donors

G 003 UNRELATED AND HLA-PARTIALLY MATCHED RELATED DONOR TRANSPLANTS, Claudio Anasetti, Ruth Etzioni, Effie W. Petersdorf, *Paul J.Martin* and John A. Hansen, *Immunogenetics Program, Clinical Division, Fred Hutchinson Cancer Research Center, Seattle, WA.*

Despite intense regimens of pre and post-transplant immunosuppression marrow transplants from related donors who share one HLA haplotype and are variably matched for the HLA-A, B, or D antigens of the unshared haplotype have an increased risk of graft failure and graft-versus-host disease that correlates with the degree of HLA mismatch. Survival of one HLA-A, B or D antigen incompatible transplants, however, are not significantly different than after HLA identical sibling transplants. Available immunosuppression has not allowed as favorable survival for two or three locus incompatible transplants. Currently, less than 30% of patients in the western hemisphere have an HLA-matched sibling and only 3-5% have a one HLA-locus mismatched relative. Therefore, most patients who could benefit from an allogeneic marrow transplant are in need of an alternative donor. Since clinical trials have shown that donor HLA matching is critical for successful transplant outcome, it was rational to test the hypothesis that HLA-matched unrelated volunteers could be used successfully as marrow donors. Case reports between 1973 and 1986 demonstrated that unrelated donor transplants can cure patients with leukemia or other disorders of the lympho-hemopoietic system. Given the extreme polymorphism of HLA, transplantation of a large number of patients was feasible only with the development of precise and effective HLA typing techniques and accumulation in coordinated registries of large numbers of HLA typed volunteers willing to donate marrow. Nowadays, patients can look for a match among 1.4 million volunteers available worldwide through an international network of regional and national registries. The probability of finding an HLA-A, B, DR match at the initial search in the U.S. National Marrow Donor Program (NMDP) registry has increased from 10-15% in 1987 to 50-52% in 1993. As of October 1993, NMDP has facilitated more than 2,000 transplants which have allowed long-term disease-free survival for an increasing number of patients with a variety of hematological disorders. Despite HLA-matching, unrelated transplants have an increased incidence of graft failure and GVHD compared to HLA-identical sibling transplants. This may be due to disparity for undetected HLA determinants or non-HLA linked minor histocompatibility antigens. Outcome after unrelated transplantation is affected primarily by the type and stage of disease at the time of treatment. At our center all patients have received unmodified marrow and cyclosporine plus methotrexate for GVHD prophylaxis. Three to five year survival is 50-60% for CML-CP, 30-35% for SAA or RA, 40-45% for acute leukemia in first or second remission and 10-20% for CML-BC or relapsed acute leukemia. Patients with advanced disease have an increased risk of death from transplant-related complications and relapse, indicating the need to transplant patients earlier in the course of their disease. Among patients with advanced disease, older age is associated with a further decrease in survival. Graft failure has occurred in 2% of HLA matched and 3% of HLA "minor" mismatched transplants. Use of rhGM-CSF to facilitate engraftment has not had a measurable benefit on transplant outcome. The probability of grades II-IV acute GVHD has ranged from 75% for matched to 93% for mismatched transplants. The cumulative incidence of chronic extensive GVHD has been 60% for matched and 74% for mismatched transplants. Strict compliance with methotrexate and cyclosporine prophylaxis during the first month is associated with better control of acute GVHD and better survival. A limited degree of HLA mismatching has not impaired the chance of a successful transplant in patients with hematological malignancy. Opportunistic infections have been a frequent cause of death. Patients with positive CMV serology have had an increased risk of death compared to those with negative serology, but gancyclovir prophylaxis has decreased CMV morbidity and mortality. Future research in unrelated transplantation must develop methods that can reduce morbidity and improve survival even when some degree of HLA disparity is present.

G 004 RACIAL DIFFERENCES IN HISTOCOMPATIBILITY, IMPLICATIONS FOR UNRELATED DONOR BMT, Patrick G. Beatty[1], Motomi Mori[1], Edgar Milford[2], Mihir Mehta[1], [1]University of Utah Health Sciences Center, Salt Lake City, Utah 84132, [2]Brigham and Women's Hospital, Boston, MA 02115.

For the last ten years, the use of stem cells from HLA-matched but unrelated donors has evolved into an acceptable form of hematopoietic rescue in patients who do not have an acceptable donor within their family. Initially, only a small percentage of patients were successful in locating such an HLA-matched donor. This problem was the impetious to initiate an ambitious program to recruit and tissue type large numbers of volunteers. For instance, in the United States currently over one million individuals have been recruited and HLA typed for the National Marrow Donor Program. With this current registry size, over 50% of Caucasian patients are successful in locating HLA-A, B, DR matches. It has become apparent, however, that members of racial minorities have a far lower probability of finding donors. We therefore analyzed HLA polymorphism within and between racial groups in hopes of developing an optimal strategy for donor recruitment such that all patients irrespective of their race might have comparable probabilities of finding a match. Using simulation techniques based upon calculated haplotype frequencies, we found that for Caucasians, Native Americans, Hispanics, and Orientals, that the probabilities of finding a donor from within their racial group depended exclusively upon the number of donors who have been recruited, and furthermore that for a given size of registry made up of their own race, the probabilities for finding donors of their own race were comparable. This was not true, however, for Black patients. Even if equal numbers of Blacks and Caucasians were recruited into the registry, Blacks would still have a much lower probability of finding a donor. Our analysis indicates that the reasons for this are at least three-fold, in descending order of importance: an overall much more diverse HLA gene pool among Blacks; a significant admixture of "classical" white haplotypes amongst the Black population; and a higher rate of typing error in patients and donors of Black origin. With respect to an optimal donor recruitment strategy, it appears that the needs of Native American and Hispanics may well be met by recruiting donors from amongst any of three groups: Caucasian, Native American, and Hispanics. This is due to the relatively small genetic distance between these groups with respect to their HLA phenotypes. However, Blacks and Orientals are quite distant genetically from this three group cluster, and from each other, thus necessitating focusing on these two specific groups for recruitment. The ultimate solution to the problem of finding donors for Black patients may rest not so much with recruiting large numbers of Black donors, as this may well prove to ultimately be impossible. Rather, it will be necessary to develop technology that would allow transplantation across one antigen mismatches in the unrelated donor setting. Indeed, our calculations indicate that if such were acceptable, the current registry size may well prove adequate for the vast majority of patients.

G 005 MINOR HISTOCOMPATIBILITY ANTIGEN MATCHING: ACTUAL FACT OR WISHFUL THINKING?
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It is well believed that disparities for minor Histocompatibility antigens (mHag) between bone marrow donor and recipient create a potential risk for GvHD or graft failure. Assuming that as in the mouse, the human genome has an abundancy of mH loci, identification of the "majors" under the "minors" is a prerequisite. In an attempt to do so we studied the male specific mHag H-Y and five non-sexlinked mHag (designated HA-1 to HA-5) at three levels i.e. immunogenetics, immunogenicity and tissue distribution.
Immunogenetic studies revealed that some mHag appeared frequent (69-95%), others occurred with lesser frequencies (7-16%) in the healthy population. An analysis of the genetic traits demonstrated a Mendelian mode of inheritance independent of HLA.
Three sets of data are indicative for a hierarchy in *immunogenicity* among mHag. Firstly, CTL clones reactive to the same mHag HA-1 were obtained from peripheral blood lymphocytes of 3 out of 5 individuals each transplanted across a multiple and probably distinct mH barrier. Secondly, preliminary results indicate that the latter HA-1 specific CTL clones derived from different individuals, all seemed to use an identical TCR Vβ, but distinct Vα and γ segments. Thirdly, in a retrospective study, comprising 148 bone marrow donor/recipient pairs, investigating the influence of mHag HA-1 to HA-5 mismatching on the development of GvHD, we observed a significant correlation between mHag HA-1 mismatch and chronic GvHD.
Tissue distribution studies revealed differential expression: some mHag (i.e. H-Y, HA-3 and HA-4) are expressed on haematopoietic as well as on non-haematopoietic cells, while the expression of other mH antigens (HA-1 and HA-2) is limited to cells of the haematopoietic lineage only. Moreover, all mH antigens are present on clonogenic leukemic precursor cells as well as on circulating leukemic cells of lymphocytic and myeloid origin.
Finally, we are aiming at the *biochemical characterization* of human mHag. Indeed, HPLC separation of low Mr molecules obtained from acid treated HLA-A2.1 molecules appeared successful. mHag specific peptides containing fractions can be repeatedly obtained. Up to date however, the amino acid sequence of the T cell epitopes of these classical mHag are not available. Attempts are currently made to sequence the relevant peptides by means of tandem mass spectrometry.
Although the number of mH systems is expected to be large, only a limited number will fulfil the criteria (i.e. frequency, immunogenicity, tissue distribution) for being a risk factor for GvHD or rejection. Once the nature of the human mHag is established the search for mH families will hopefully accelerate.

G 006 HLA MATCHING AND UNRELATED DONOR MARROW TRANSPLANTATION. John A. Hansen, Claudio Anasetti, Effie Petersdorf, Eric M. Mickelson and Paul J. Martin. Fred Hutchinson Cancer Research Center and the University of Washington, Seattle, WA.

Although access to more than one million HLA typed volunteers world wide has lead to a substantial increase in unrelated donor (URD) transplants, many patients still fail to *match*. This has provided impetus for identifying the limits of mismatching. In our center a *match* is defined as phenotypic identity for HLA-A, B & DR by serology and identity for DRB1 alleles by DNA oligo-typing or sequencing. In the last 12 months 98% of our patients have had at least one HLA-A & B match and 51% at least one A, B & DR match during the initial search of the NMDP. An additional 15% of patients have found a DR match when available HLA-A, B identical donors have been DR typed. After DNA typing, 70% of patients with at least one HLA-A, B, DR match have found a donor matched for DRB1 alleles. Limited HLA disparity for one locus *minor mismatches* have been allowed for patients less than 36 years of age. HLA-A or B *minor mismatches* are antigens which belong to the same crossreactive group or CREG (as defined by NMDP) and DR *minor mismatches* are DRB1 alleles which encode antigens of the same DR specificity (e.g. DRB1*0401, 0402, etc.). Matching for HLA-A, B , DRB1 does not guarantee matching for other HLA genes. When 194 HLA-A, B, DRB1 identical pairs were analyzed retrospectively 5% were mismatched for DRB3, 0% for DRB5, 11% for DQB1 and 91% for DPB1. Retrospective sequencing of C-locus genes in 60 HLA-A, B, DRB1 identical pairs revealed 20% mismatched for C-locus alleles. Presumably, some transplants classified as HLA-A,B,DRB1 identical are mismatched for A- or B-locus alleles, however the frequency of these mismatches is currently unknown.

Clinical significance of HLA matching has been analyzed in 267 URD transplants treated for hematological disorders. All received cyclophosphamide and total body irradiation prior to transplantation of unmodified (non-T cell depleted) marrow, and methotrexate and cyclosporine for GVHD prophylaxis. HLA matching characteristics were: 190 (71%) identical for HLA-A, B, D(HTC-defined) or DRB1; 24 (9%) A-locus mismatched; 18 (7%) B-locus mismatched; and 36 (13%) mismatched for D/DRB1. Graft rejection occurred in 1.6% of matched and 2.5% of mismatched transplants ($p > 0.5$). Acute GVHD grades II-IV occurred in 76% of HLA matched, 82% of B-locus mismatched, 91% of A-locus mismatched and 94% of D/DRB1 mismatched transplants ($p< 0.05$). Acute GVHD grades III-IV occurred in 50% of HLA matched, 57% of B-locus mismatched, 58% of D/DRB1 mismatched and 62% of A-locus mismatched transplants ($p< 0.05$). Probability of non-relapse mortality was 44% in matched, 29% in A-locus mismatched, 39% in B-locus mismatched and 60% in D/DRB1 mismatched transplants ($p > 0.5$). Probability of survival at 2 years was 36% for HLA matched, 60% for A-locus mismatched, 27% for B-locus mismatched and 25% for D/DRB1 mismatched transplants ($p=0.1$). The clinical significance of DP matching has been analyzed in 129 URD transplants matched for HLA-A, B, DRB & DQB: there was no DPB1 incompatibility in 22%; one DPB1 mismatched allele in 56%; and two mismatched alleles in 22%. Although the degree of DPB1 mismatching correlated with MLC reactivity, there was no significant difference in risk of acute GVHD for 0, 1 or 2 mismatched DPB1 alleles and no significant effect on survival. In summary, the relatively high non-relapse mortality of URD transplants has restrained further relaxation of HLA matching requirements. Improvements in GVHD prevention and supportive care should occur before attempting more broadly mismatched URD transplants.

G 007 STRUCTURAL AND FUNCTION OF CLASS I HLA POLYMORPHISM, Peter Parham, Departments of Cell Biology and Microbiology & Immunology, Sherman Fairchild Building, Stanford University, Stanford, CA. 94305-5400.

Current knowledge of the structure, function and polymorphism of class I HLA genes and proteins will be reviewed in the context of their relevance to Bone Marrow Transplantation. Emphasis will be placed on the diversity of class I molecules within human populations and the difference between populations. The role of the HLA polymorphism in determining peptide binding specificities and the alloreactive T cell response will be discussed.

Tolerance and Alloreactivity

G 008 A FAMILY OF B7 MOLECULES COSTIMULATES T CELL PROLIFERATION, Gordon J. Freeman[1], Vassiliki Bousiotis[1], Gary Gray[2], John G. Gribben[1] and Lee M Nadler[1]. [1]Dana-Farber Cancer Institute, Boston MA 02115 and [2]The Repligen Corporation, Cambridge MA 02139.

Although presentation of antigen to the T-cell receptor is necessary to initiate an immune response, second signals delivered by costimulatory molecules expressed on antigen presenting cells are essential to prevent the induction of anergy. B7-1 (originally termed B7) is one such costimulatory molecule that induces T cell proliferation and regulates secretion of cytokines, including IL-2, by signaling via its T cell counter receptors CD28/CTLA-4. Recent studies have demonstrated the importance of the B7:CD28/CTLA4 pathway in the induction of tumor specific immunity, prevention of tolerance and the pathogenesis of autoimmunity. Although B7-1 appears to be a major CD28/CTLA4 counter-receptor, activated human B cells express at least two other CTLA4 counter-receptors. At 24 hours after activation, B cells express a molecule (B7-2) that binds CTLA4-Ig, induces T cell proliferation and IL-2 production that is not inhibited by anti-B7 mAb. At 48 to 72 hours after activation both B7 positive and B7 negative B cells bind CTLA4-Ig and B7 negative B cells bind BB1 mAb. Activated B7 negative B cells express a molecule (B7-3) that induces T cell proliferation, without detectable IL-2 accumulation. This proliferation is inhibited by CTLA4-Ig and BB1, but not by anti-B7 mAb. We cloned the human B7-2 gene by COS cell expression using CTLA4-Ig. B7-2 cDNA transfectants bind CTLA4-Ig but not anti-B7 (133) or BB1 mAb. In contrast, B7-1 transfectants bind CTLA4-Ig, B7-1 (133) mAb and BB1 mAb. Therefore, B7-2 encodes a CTLA-4 counter-receptor that is distinct from B7-1 and B7-3. Sequence analysis reveals that B7-2 molecule is a type I Ig superfamily membrane proteins. Comparison of both nucleotide and amino-acid sequences of B7-2 yielded homology with the human and murine B7-1 with 25% identity at the amino acid level. By RNA blot analysis B7-2 is expressed in unstimulated human splenic B cells whereas B7-1 mRNA is not. Both increase following activation. Both B7-1 and B7-2 COS transfectants costimulate T cell proliferation and IL-2 production. Anti-B7-1 mAb and BB1 completely inhibit proliferation and IL-2 secretion induced by B7-1 but had no effect upon costimulation induced by B7-2 transfected COS cells. Both anti-CD28 Fab and CTLA4-Ig inhibited proliferation and IL-2 production induced by either B7-1 or B7-2 COS transfectants. We conclude that B7-2 is an alternative counter-receptor for the CD28 and CTLA-4 T cell surface molecules. Both proteins are similar in that they are members of the Ig supergene family exhibiting 25% amino acid identity in these domains, and costimulate T cells to produce IL-2 and proliferate. However, B7-2 mRNA is constitutively expressed in unstimulated B cells whereas B7-1 mRNA does not appear until 4 hr and cell surface protein is not detected until 24-48 hr. Since unstimulated B cells do not express CTLA4 counter-receptors on their cell surface and do not costimulate T cell proliferation, the expression of B7-2 mRNA in unstimulated B cells likely facilitates rapid expression of B7-2 protein following activation. The early appearance of B7-2 provides one mechanism for B cell-induced costimulation before the induction of B7-1 and suggests that it may provide a critical costimulatory signal involved in the decision between immunity and anergy that is made by T cells within 24 hrs post activation. Therefore, B7-2 is likely to be a critical early costimulatory signal and its function important in the regulation of tolerance and induction of tumor immunity.

G 009 THE ROLE OF CYTOKINES IN TRANSPLANTATION TOLERANCE, Maria Grazia Roncarolo[1], Dominique Schols[1], Rosa Bacchetta[1], and Reiko Namikawa[1], [1]DNAX Research Institute of Molecular and Cellular Biology, 901 California Avenue, Palo Alto, CA 94304-1104.

The role of cytokines in mediating tolerance versus rejection after allogeneic transplantation was studied in Severe Combined Immunodeficient (SCID)-hu mice and in SCID patients successfully transplanted with allogeneic fetal liver or bone marrow stem cells.

A complete repertoire of mature $CD4^+$ and $CD8^+$ T cells develops in the human thymus of SCID-hu mice constructed with fetal liver and fetal thymus. These T cells not only mediate normal allogeneic responses *in vitro*, but also infiltrate and destroy fetal pancreas transplants of unrelated donors. The T cells infiltrating the pancreas secrete levels of IL-2 and TNF-α much higher than those produced by their counterparts in the thymus, suggesting that enhanced production of these cytokines is associated with transplant destruction.

In SCID-hu mice transplanted with fetal liver and fetal thymus from two HLA mismatched donors, fetal liver-derived T cells become tolerant toward the allogeneic (host) thymus in which they mature. This tolerance is due to clonal anergy and not to clonal deletion of thymic (host) reactive T cells. The anergic T cells do not proliferate or produce IL-2 following antigen-specific activation *in vitro*. This antigen-specific unresponsiveness is reversed by exogenous IL-2. A similar state of anergy can be induced by *in vivo* treatment of SCID-hu mice with the superantigen *Staphylococcal enterotoxin B*.

We have made a similar observation in SCID children transplanted with HLA mismatched fetal liver or bone marrow stem cells. Despite the absence of any sign of graft-versus-host-disease in these patients, host-reactive T cells are not clonally deleted from the repertoire. Donor-derived T cells specific for the HLA antigens of the host can be isolated at high frequencies *in vitro*. These host-reactive T-cell clones produce very low levels of IL-2 and very high levels of IL-10 after antigen-specific activation. The high IL-10 production by these cells is suppressing their proliferative activity. In these patients, high IL-10 levels were also detected *in vivo*, both in the T and in the monocyte compartments, suggesting that low levels of IL-2 and high levels of IL-10 are present when tolerance is achieved after allogeneic stem cell transplantation.

G 010 TRANSPLANTATION TOLERANCE -- REVIEW AND NEW APPROACHES, David H. Sachs, Megan Sykes, David Emery, Tomasz Sablinski, Christian LeGuern, Tatsuo Kawai and A. Benedict Cosimi, Mass. General Hospital and Harvard Medical School, Boston, MA.

Bone marrow transplantation (BMT) provides a means for inducing tolerance across MHC barriers, permitting acceptance of subsequent organ or tissue transplants from the same donor. In contrast to the use of BMT for treatment of leukemia, complete ablation of host lymphohematopoietic elements is neither necessary nor desirable when BMT is utilized as a tolerance-inducing regimen. Instead, it is advantageous to achieve a state of mixed chimerism, in which the presence of certain donor-derived elements induce specific tolerance, while host-type antigen presenting cells maintain normal immunocompetence. We have previously described a non-myeloablative preparative regimen capable of inducing mixed chimerism in a murine model[1]. This regimen involves pre-treatment of recipients with monoclonal antibodies directed against mature T cell subsets and administration of 3 Gy whole body irradiation and 7 Gy local thymic irradiation, followed by allogeneic BMT. Recipient mice were shown to be stable mixed chimeras and were specifically tolerant as demonstrated by long-term acceptance of donor strain skin grafts and prompt rejection of third-party grafts. We have now attempted to extend this protocol to the non-human primate model of fully MHC mismatched cynomolgus monkeys. In preliminary experiments using ATG in place of monoclonal anti-T cell antibodies, and adding Cyclosporin (CyA) treatment for one month after transplantation to further inhibit T cell immunity, we have achieved a stable, low level of lymphoid and myeloid chimerism and long-term acceptance of a renal allograft from the bone marrow donor.

We have also demonstrated that tolerance can be induced by primarily vascularized renal allografts in miniature swine if class II antigens of donor and recipient are matched and if a short course of CyA is administered. The mechanism of such tolerance is probably different from that of mixed bone marrow chimerism, since in the former case tolerance is dependent on the continued presence of the renal allograft, while in the latter case tolerance is maintained with or without the organ graft. We have recently combined these two approaches, using BMT to induce tolerance to class II antigens and a short course of CyA to induce tolerance across class I and minor antigens. By this means, tolerance across a full MHC and minor antigen barrier has been achieved[2]. Present studies are directed toward a genetic engineering approach in which induction of tolerance in this protocol may be achieved by replacing allogeneic class II bearing cells by autologous cells carrying allogeneic class II genes.

1. Sharabi Y, Sachs DH: J Exp Med 169:493, 1989
2. Smith CV, Nakajima K, Mixon A, et al: Transplantation 53:438, 1992

G 011 ROLE OF $CD4^-CD8^-\alpha\beta^+$ SUPPRESSOR T CELLS IN BMT, S. Strober, V. Palathumpat, S. Dejbakhsh-Jones, O. Liang, L. Cheng, H. Wang, T. Zimmer, Stanford University, Stanford.

The predominant T cell subset in the normal mouse bone marrow is $CD4^-CD8^-\alpha\beta^+$. These cells are generated in situ in the bone marrow, and do not require the thymus for their maturation. Fresh $CD4^-CD8^-\alpha\beta^+$ bone marrow T cells as well as cloned $CD4^-CD8^-\alpha\beta^+$ T cell lines are able to inhibit the mixed leukocyte reaction in vitro as well as acute lethal graft versus host disease in vivo. Studies of the cloned cell lines indicate that suppression is mediated by a unique 20 Kd cytokine which inhibits the function of antigen presenting cells.

The $\alpha\beta$ TcR genes of the cloned cell lines have been sequenced, and analysis of the junctional regions show that α and β genes from independently derived clones are identically rearranged. These invariant TcR's recognize autologous activated B cells in the context of Class II MHC antigens. These cells may provide an important regulatory network stimulated by polyclonal immune cell activation during GVHD.

Low density bone marrow cells are enriched for $CD4^-CD8^-\alpha\beta^+$ cells, and can mediate GVL activity in the absence of GVHD.

G 012 SEPARATING T CELL ACTIVITIES THAT MEDIATE GRAFT-VS-LEUKEMIA EFFECTS AND GRAFT-VS-HOST DISEASE, Megan Sykes, David H. Sachs, Tomasz Sablinski, Janos Szebeni, Minguang Wang, Massachusetts General Hospital/Harvard Medical School, Boston, MA 02129.

Graft-vs-host disease (GVHD) currently prohibits the application of BMT across wide HLA barriers. Removal of T cells from the donor marrow has unfortunately been associated with increased rates of engraftment failure and leukemic relapse. In contrast, a short (2 day) course of high dose IL-2, commenced at the time of BMT, inhibits both acute and chronic GVHD in mice, especially when administered with T cell-depleted host-type marrow. Alloengraftment is not impaired by this treatment, and graft-vs-leukemia (GVL) effects of allogeneic T cells against the EL4 leukemia/lymphoma were preserved. GVL in this model was mediated exclusively by donor $CD8^+$ T cells independently of $CD4^+$ cells. $CD4^+$ cells were required for the induction of GVHD, and the GVHD-inducing capacity of $CD4^+$ cells was inhibited by IL-2. This selective inhibition by IL-2 of the CD4 and not the CD8 subset demonstrated one mechanism by which GVHD and GVL effects can be dissociated by IL-2 treatment.

Since many human leukemias express class II MHC molecules, allogeneic $CD4^+$ cells might mediate GVL effects in man. To evaluate the possible effect of IL-2 on CD4-mediated GVL, we developed a murine class II MHC-positive pro-monocytic leukemia model. Lethal irradiation and allogeneic marrow were administered one week after leukemia had been established in recipients. Both allogeneic $CD4^+$ and $CD8^+$ T cells mediated GVL effects against this tumor, and the magnitude of GVL mediated by either subset was not reduced by IL-2 treatment. At the same time, IL-2 inhibited CD4-mediated GVHD in these mice. The failure of IL-2 to inhibit CD4-mediated GVL while it inhibited CD4-mediated GVHD suggests that these two activities of the CD4 subset are functionally distinct, and are separable by IL-2.

We have therefore examined the effect of IL-2 on T cell functions. IL-2 treatment does not inhibit the activation or marked expansion of host-reactive Th and CTL that occurs in the first week of GVHD. However, the pattern of cytokines produced by these host-reactive T cells is altered by IL-2 treatment. A marked reduction in IFN-γ production by spleen cells and reduced serum IFN-γ levels are observed at day 3 and 4 post-BMT in IL-2-treated mice. Preliminary data suggest that early IL-4 production may be increased among T cells of IL-2-treated compared to GVHD control mice. The possibility that these alterations in cytokine production could explain the inhibition of CD4-dependent GVHD is currently being explored.

Large animal studies have confirmed the ability of IL-2 to inhibit GVHD. In a model involving BMT between miniature swine differing at minor loci and at one haplotype for the entire class II region, grade 3 GVHD was observed in 6 of 6 control recipients, of which 2 died. Three IL-2-treated pigs, on the other hand, showed either no GVHD or mild (grade 1-2) GVHD and showed excellent survival. This result suggests that IL-2 may provide effective GVHD prophylaxis for class II-mismatched clinical BMT.

GVHD, GVL and Immunotherapy

G 013 T-CELL DEPLETION -THE FUTURE OR THE PAST FOR ALLOGENEIC BMT, Richard Champlin, M.D., University Texas M.D. Anderson Cancer Center, Houston, Texas.

GVHD results from reactivity of immunocompetent donor cells against recipient (host) tissues. This process is largely mediated by T-lymphocytes although other cell populations may participate. CD4 and CD8-positive cells are involved. T-cell depletion from the donor marrow is effective to prevent GVHD across major or minor histocompatibility barriers. In humans, antibody based depletions which include or exclude natural killer cells had similar reduction of GVHD. When engraftment occurs, hematopoietic recovery is prompt and immune reconstitution occurs from undifferentiated progenitors or pre-T cells present within the transplanted marrow. The incidence of acute GVHD is related to the number of residual t-cells or their precursors present in the transplanted marrow. The risk of graft rejection is increased in recipients of T-cell depleted transplants. There is an interaction between the intensity of conditioning, the transplanted cell dose and the T-cell content of the marrow. Subsets of CD8-positive T-cells may facilitate engraftment. High doses of total body irradiation do not completely ablate host immunity; viable T-cells persist but can be overcome by higher cell doses or intensification of the preparative regimen. In animals, addition of ALG, busulfan or thiotepa enhances engraftment; these agents are undergoing study in man. T-cells in the transplanted marrow participate in the beneficial graft-versus-leukemia (GVL) effect. In leukemia patients transplanted from an HLA-identical sibling, the beneficial effect of T-cell depletion reducing GVHD has been offset by an increased risk of graft failure and leukemia relapse, abrogating improvement in leukemia free survival in controlled trials and large registry analyses. For transplants from unrelated or HLA- nonidentical donors, graft-versus-host disease is a greater problem. In these patients, use of T-cell depletion markedly reduces the incidence of acute GVHD and may improve short term survival; controlled trials are necessary. Innovative approaches being studied to improve results of T-cell depleted BMT including intensification of the preparative regimen, subtotal T-cell depletion, late add back of T-cells, selective depletion of CD8-positive cells and altering immunoregulatory cells. Ultimately, the control of GVHD will likely result from optimizing the cellular composition of the transplanted marrow and posttransplant immunoregulatory therapy.

G 014 IMPROVED PROPHYLAXIS FOR ACUTE GRAFT-VERSUS-HOST DISEASE WITH CYCLOSPORINE, METHOTREXATE, AND PREDNISONE COMPARED TO CYCLOSPORINE AND PREDNISONE: RESULTS OF A PROSPECTIVE RANDOMIZED STUDY. Nelson J. Chao[1], Gerhard M. Schmidt[2], Joyce C. Niland[3], Michael D. Amylon[1], Andrew C. Dagis[3], Gwynn D. Long[1], Auayporn P. Nademanee[2], Robert S. Negrin[1]., Margaret R. O'Donnell[2], Pablo M. Parker[2], Eileen P. Smith[2], David S. Snyder[2], Anthony S. Stein[2], Ruby M. Wong[4], Karl G. Blume[1], and Stephen J. Forman[2], [1]Bone Marrow Transplantation Program and [4]Department of Health Research and Policy, Stanford University, Stanford, CA and [2]Department of Hematology and Bone Marrow Transplantation, and [3]Department of Biostatistics, City of Hope National Medical Center, Duarte, CA.

Acute graft-versus-host disease (GVHD) remains a serious problem following allogeneic bone marrow transplantation. In a clinical trial, we have tested a new drug combination for the prevention of GVHD. One hundred and fifty patients with either acute leukemia in first complete remission, chronic myelogenous leukemia in first chronic phase, or lymphoblastic lymphoma in first complete remission were entered on this study. All patients were prepared with fractionated total body irradiation (1320 cGy) and etoposide (60 mg per kilogram) followed by bone marrow grafting from genotypically histocompatible donors. For GVHD prevention, patients were randomized to receive either the combination of cyclosporine, methotrexate, and prednisone or cyclosporine and prednisone without methotrexate. All patients received standardized supportive care after bone marrow transplantation, including intravenous gamma globulin. Patients receiving cyclosporine, methotrexate, and prednisone had a significantly lower incidence of grade II-IV acute GVHD (9 percent) compared to those receiving cyclosporine and prednisone (23 percent, $P = 0.02$). Multivariate Cox regression analysis demonstrated an increased risk of acute GVHD associated with an elevated creatinine ($P = 0.006$) and randomization to cyclosporine and prednisone ($P = 0.02$). This lower incidence of acute GVHD did not result in a higher relapse rate. There was no statistically significant difference in disease-free survival at three years between the two treatment arms (64 percent for the triple drug regimen vs. 59 percent for the two drug combination, $P = 0.57$). The combination of cyclosporine, methotrexate, and prednisone was effective in reducing grade II-IV acute GVHD to 9 percent.

G 015 CONSOLIDATIVE IMMUNOTHERAPY WITH IL-2 ± LYMPHOKINE ACTIVATED KILLER (LAK) CELLS AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ACUTE MYELOGENOUS LEUKEMIA AND MALIGNANT LYMPHOMA, Alexander Fefer[1,2], Mark C. Benyunes[1,2], Andrea York[2], Dean Buckner[1,2], Finn B. Petersen[2], John A. Thompson[1,2], [1]Division of Oncology, University of Washington, Seattle, WA, 98195, and [2]Fred Hutchinson Cancer Research Center, Seattle, WA, 98104.

Patients who undergo autologous bone marrow transplantation (ABMT) for advanced hematologic malignancies experience a high relapse rate. Therapy with Interleukin-2 (IL-2) ± lymphokine-activated killer (LAK) cells has induced clinical responses in some patients with advanced malignant lymphoma (ML) or acute myelogenous leukemia (AML). It is postulated that IL-2 ± LAK cells represents a potentially non-cross-resistant therapeutic modality which might induce a GVL effect and prevent or delay relapses if used as consolidative immunotherapy against the minimal residual disease which exists after ABMT. It is also postulated that if IL-2 exerts an effect against tumor not eradicated by chemoradiotherapy, then it should also act as an *in vivo* purging agent against any tumor contaminating infused marrow. Since relapses after ABMT tend to occur early, we first studied the reconstitution of IL-2-responsive LAK precursor cells after ABMT and found them in the circulation as early as three weeks after ABMT. A Phase Ib clinical trial was then performed which identified an IL-2 regimen which could be tolerated early after ABMT and which could induce immunomodulatory effects. We then initiated a clinical trial to determine the feasibility of generating and administering autologous LAK cells using this IL-2 regimen after ABMT for patients with ML and AML. The results showed that IL-2/LAK therapy early after ABMT was feasible but was more toxic than IL-2 alone. Patients with AML on the Phase I IL-2 trial or the IL-2/LAK trial and patients with ML on the IL-2/LAK protocol were evaluated for tumor status. Of 14 patients with AML in first relapse or at a later stage who underwent ABMT and therapy with IL-2 ± LAK cells, three relapsed, one died during IL-2 therapy, while ten remain in continuous complete remission (CR) 13^+ to 48^+ (median = 34^+) months after ABMT. Of 16 patients with ML who underwent ABMT but were considered at high risk for relapse and who therefore received IL-2+LAK cells, seven have relapsed, one died in CR of infection at 14 months, and eight remain in continuous CR at 17^+ to 32^+ (median = 21^+) months after ABMT. The results in both trials compare favorably with those of non-randomized historical controls at our institution and are sufficiently encouraging to justify a Phase III trial of IL-2 versus observation after ABMT for patients with ML and AML at high risk for relapse.

Stems Cells I

G 016 CLONAL HEMATOPOIESIS AFTER TRANSPLANTATION, Janis Abkowitz[1], Monica Persik[1], Richard Ott[2], and Peter Guttorp[2], [1]University of Washington, Seattle, WA 98195, and [2]Washington State University, Pullman, WA.

The behavior of hematopoietic stem cells after marrow transplantation has been studied extensively in mice. For example, studies of Jordan and Lemischka (Genes Dev. 4:220, 1990) demonstrated that the contributions of individual stem cell clones varied for 2 – 6 months after transplantation when lethally-irradiated mice were given small numbers of syngeneic marrow cells. Certain clones contributed to hematopoiesis then stopped and other clones emerged. After this time, however, hematopoiesis was stably maintained by single or few clones. These data have led to the assumption that a similar kinetics will occur in man. However, the hematopoietic demand of a mouse is small (20 – 25 g, 1.4 – 1.8 ml blood volume, 1 – 2 year lifespan). The number of red cells that a mouse requires for its lifetime (2×10^{11}), for example, is equivalent to that required by man during one day or by a cat during 8 days. For this reason, we have developed a large animal model to study stem cell kinetics following transplantation. Female Safari cats (F_1 offspring of domestic and Geoffroy cat parents) are heterozygous for the X-chromosome -linked enzyme glucose-6 phosphate dehydrogenase (G-6-PD). These cats are cellular mosaics and individual stem cells express either a domestic or Geoffroy G-6-PD phenotype, but not both. Similarly, differentiated cells express the G-6-PD phenotype of the stem cells from which they originate. Using this marker system, we investigated hematopoiesis after autologous marrow transplantation with limiting numbers ($1 – 2 \times 10^{-7}$/kg) of buffy coat marrow cells. By 10 weeks after transplantation, the peripheral blood counts, the frequencies of marrow progenitor cells, and their cell cycle kinetics were normal, suggesting that the progenitor cell compartment was entirely reconstituted. However, contributions of stem cells to hematopoiesis varied, as significant fluctuations were seen in the G-6-PD phenotype of BFU-E and CFU-GM with repeated observations over time. In some animals, the G-6-PD phenotype of progenitors varied as widely as from 4% to 98% domestic phenotype. We now have observed 3 cats for 5 – 6 years after transplantation and 4 additional cats for shorter intervals. This unstable phase of stem cell kinetics is very long (e.g., 1 – 4 years). After this time, cells with a single G-6-PD phenotype can dominate hematopoiesis, suggesting that the hematopoietic reserve can be reconstituted by the replication of one or few stem cells, and that an individual stem cell has an enormous proliferative potential. These data challenge the biological strategies for gene therapy and the transplantation of limited numbers of stem cells in man, as by extrapolation, the period of unstable stem cell kinetics may be extremely long in this setting. The implications of these observations for gene therapy will be discussed.

G 017 COMBINATION OF CEPRATE™ CD34 COLUMN SELECTION FOLLOWED BY A SECOND AVIDIN-BIOTIN NEGATIVE DEPLETION RESULTS IN ENHANCED PURGING OF TUMOR CELLS OR B-CELLS. R.J. Berenson, S.R. Corpuz, K.M. Stray, M.A. Colter and S. Heimfeld. CellPro Incorporated, Bothell, Washington.

Previous work has demonstrated that CEPRATE™ SC positive selection of CD34+ cells is effective in providing an engrafting cell population which is substantially depleted (1.5-4 logs) of contaminating tumor and other cell types.[1] Further strategies are being evaluated to obtain even more efficient purging by using a combination of CD34 selection followed by a second avidin-biotin immunoadsorption column for cell-type specific negative depletion. Model systems to evaluate purging efficiency were developed using either breast cancer cell lines spiked into ficolled blood or the specific removal of CD19+ B-cells. Flow cytometry was used to detect residual target cells. Using monoclonal antibodies specific for breast cancer cells or CD19, a single pass through a column of avidin coated beads resulted in a 1-2 log removal of contaminating tumor cells or lymphocytes with a 70-90% recovery of the non-target cells.

Target Cell	Log removal of Target Cells Mean (range)	Percent Recovery of Non-Target Cells Mean (range)
B-Cells (CD19+)	1.3 (1.1-1.9)	89.2% (80.2%-95.2%)
Breast Cancer Cell Lines	1.7 (1.0-2.1)	84.3% (73.6%-89.9%)

Preliminary experiments have been done combining this negative selection model with CD34 positive enrichment. Those results suggest 5 logs of purging efficiency with an overall recovery of 35-50% of the CD34 progenitor cells and colony forming activity.

1. Shpall et al, Blood 1992

G 018 ONTOGENY-RELATED FUNCTIONAL CHANGES IN VERY PRIMITIVE HEMATOPOIETIC CELLS, Peter M. Lansdorp[1,2], Wieslawa Dragowska[1], Homayoun Vaziri[3], Calvin B. Harley[3], Vivienne Rebel[1], Connie J. Eaves[1,2], R. Keith Humphries[1,2], and Hector Mayani[1], [1]Terry Fox Laboratory, B.C. Cancer Agency, [2]University of British Columbia, Vancouver, B.C., Canada and [3]Geron Corporation, Menlo Park, CA.

The mechanism by which hematopoiesis is maintained throughout the life-span of an individual is incompletely understood. Two competing models postulate that hematopoiesis is maintained by 1) self-renewal divisions or 2) clonal succession of a population of hematopoietic stem cells. The distinction between these two models is important for a variety of experimental therapeutics strategies involving e.g. gene transfer and transplantation. In order to address this issue, we have studied the behaviour of highly purified stem cell "candidates" cultured in different well-defined culture conditions. The results of these studies can be summarized as follows. Primitive $CD34^+CD45RA^{lo}CD71^{lo}$ hematopoietic cells purified from adult bone marrow, umbilical cord blood and fetal liver show striking differences in their ability to produce $CD34^+$ cells in cytokine supplemented serum-free cultures. Both the fraction of responding cells and their proliferative potential decrease markedly with the age of the cell donor. These results suggest that the actual *production* of very primitive hematopoietic cells may be restricted to early stages of development. Selection of Thy-1^+ among $CD34^+CD45RA^{lo}CD71^{lo}$ bone marrow cells did not alter the major conclusion of the previous studies: no net production of primitive hematopoietic cells in culture. Similar findings have been obtained in a murine model in which $Sca^+Lin^-WGA^+$ adult bone marrow cells gave rise to large numbers of daughter cells including CFU-S and cells with a $Sca^+Lin^-WGA^+$ phenotype without a net increase (or decrease) in the number of cells with the ability to reconstitute long-term lympho-myelopoiesis *in vivo*. In agreement with the notion that stem cells in the adult may have limited or no "self-renewal" potential are recent observations obtained from telomere analysis. Loss of telomeric DNA at chromosome ends upon cell division has been linked to cellular senescence and aging and is thought to normally limit the proliferative potential of somatic cells. Initial studies have revealed a striking decrease in the average telomere length of hematopoietic cells from adult bone marrow compared to those from fetal liver indicating that telomere loss occurs during hematopoiesis *in vivo*. We furthermore found that cell divisions of purified $CD34^+CD45RA^{lo}CD71^{lo}$ cells *in vitro* is associated with a predictable decrease in the mean telomere length in their progeny. Taken together, these observations suggest that the proliferative potential of hematopoietic (stem) cells is not unlimited and that loss of telomeric DNA during successive cell divisions may contribute to the loss of this potential. From a practical point of view, these observations favour the use of relatively large numbers of adult hematopoietic cells for transplantation or the use of fetal or cord blood cells assuming that ontogeny-related differences in the function of cells are restricted to hematopoietic cells and do not also apply to the cells of the microenvironment.

G 019 **Characterization of fetal bone marrow progenitors** Leon.W.M.M. Terstappen, Johanna Olweus, Shiang Huang, Bob Hoffman, Edmund Waller, Fridtjof Lund-Johansen, Louis Picker. Becton Dickinson Immunocytometry Systems, San Jose, CA.

Hematopoietic and stromal bone marrow progenitors express the CD34 antigen. The majority of the $CD34^+$ cells are lineage committed, expression of the CD38 antigen on $CD34^+$ bone marrow cells indicates commitment into the hematopopietic cell lineages. In fetal bone marrow, cells which express CD34 and lack CD38 comprise approximately 0.6% of low density fetal bone marrow suspensions. This cell population is, however, still heterogenous in that they contain common stem cells (CSC), hematopoietic stem cells (HSC) and stromal stem cells (SSC). To explore whether these functional distinct cell populations within the $CD34^+$, $CD38^-$ cells were confined within phenotypically distinghuishable cell populations the antigenic profile of the $CD34^+$, $CD38^-$ cells was assessed in four color immunofluorecence experiments utilizing an experimental flowcytometer with a sixfold increased sensitivity. Heterogeneity within the $CD34^+$, $CD38^-$ cell population could indeed be obtained with a wide variety of cell surface antigens as HLA-DR, GMCSFR, IL-6R, Thy-1, L-selectin, CD7, CD13, CD31, CD33, CD49b and CD50. Cell sorting experiments indeed showed that CSC, HSC and SSC could be obtained with higher or lesser purity utilizing various combinations of these cell surface antigens. An important observation we made is that cross linking of receptors with monoclonal antibodies used to distinguish and separate cell populations can not always be done without influencing the outcome of the experiments. We have found that the leukocyte adhesion molecule L-selectin is expressed by a subset of human multipotent $CD34^+/CD38^-/HLA\text{-}DR^+$ progenitor cells in fetal bone marrow, and have found evidence that ligation of this receptor by either L-selectin specific monoclonal antibodies or one of its natural glycoprotein ligand(s) -- the peripheral lymph node addressin -- reproducibly increases the plating efficiency of the $CD34^+/CD38^-/HLA\text{-}DR^+$ cells in liquid culture. L-selectin ligation thus induces the production of clonal progeny from stem cells that are otherwise not responsive to a broad cocktail of hematopoietic cytokines.

Stroma and Engraftment

G 020 T CELLS AND ENGRAFTMENT, Paul J. Martin. Fred Hutchinson Cancer Research Center, Seattle, WA.

Numerous experimental models have demonstrated that GVHD does not occur when the hematopoietic stem cell graft does not contain mature, immunocompetent T lymphocytes, but clinical studies have shown that removal of T cells from the donor marrow can be associated with a greatly increased risk of graft rejection which nearly always causes death, thereby offsetting any benefit brought about by a reduced risk of GVHD. Studies in canine and murine marrow transplant models have demonstrated the importance of T cells in allogeneic marrow engraftment. One possible mechanism by which donor T cells might facilitate engraftment is through production of lymphokines or cytokines that promote proliferation and differentiation of hematopoietic stem cells. In fact, however, we have found that parental T cells capable of causing GVHD in F1 recipient mice slowed the rate of initial engraftment as measured by the number of nucleated cells in the marrow between 7 and 14 days after transplantation, while F1 T cells had no effect compared to controls transplanted without T cells in the graft. Thus deficiency of T cell derived cytokines does not represent a likely explanation for the graft failure that can occur when T cells are removed from allogeneic marrow to prevent GVHD. Circumstantial evidence from clinical studies has strongly suggested that at least in some cases, graft failures have been caused by small numbers of host lymphoid cells that survived the pretransplant conditioning regimen of chemotherapy and TBI. These observations have led to the hypothesis that donor T cells may help to eliminate or inactivate any residual host cells that could otherwise cause rejection. This hypothesis has been corroborated by experiments showing that donor CD8 cells could prevent allogeneic marrow graft rejection in mice (1). Further evidence has indicated that this effect results from the generation of donor-derived cytotoxic T cells that recognize residual host T cells containing effectors capable of causing rejection. These results suggest that successful engraftment of T cell depleted marrow will require the development of more effective, but tolerable pretransplant conditioning regimens. Alternatively, it may be possible to adjust the numbers and types of T cells in the graft so as to decrease the risk of GVHD without increasing the risk of rejection.

(1) Martin PJ: Donor CD8 cells prevent allogeneic marrow graft rejection in mice: potential implications for marrow transplantation in humans. J. Exp. Med. 178:703-712, 1993.

G 021 MARROW TRANSPLANTATION WITHOUT MYELO-ABLATION: STROMAL CELL CYTOKINE SUPPORT, Peter J. Quesenberry[1], Marc Stewart[1], Philip Lowry[1], Sudir Rao[1], and Rowena Crittenden[2], [1]University of Massachusetts Medical Center, Worcester, MA 01655, [2] University of Virginia Health Sciences Center, Charlottesville, VA 22908.

Lymphohemopoiesis occurs in close approximation with marrow supported or stromal cells. These cells produce cytokines, and intergrins and presumptively modulate baseline lymphohemopoiesis. We have utilized the long-term murine Dexter marrow culture system as a model system for studying the production of cytokines by marrow stroma and the capacity of stroma to support hemopoiesis. In this system we have found that a irradiated stromal cells from a variety of different murine strains produce G-CSF, GM-CSF, CSF-1, IL-1, IL-6 and Steel factor. This production is constitutive except that G and GM-CSF production are generally seen only after feeding of the cultures, a major inductive stimulus. In addition on-going studies have revealed that different lots of horse sera differentially support cytokine production, which in general does not correlate with the ability of stroma to support hemopoiesis. In detailed studies using standard factor dependent cell line assays and Northern blot approaches we have been unable to detect Interleukin-3, Interleukin-4 or Interleukin-5 in irradiated murine Dexter stroma. However we routinely detect Interleukin-3 by reverse transcriptase-PCR techniques and find that up to 20% of stromal cells are producing Interleukin-3 as detected by in-situ hybridization with ^{35}S labelled antisense IL-3 oligonucleotides. This "subliminal" production is biologically significant in that antibodies to Interleukin-3 will block the growth of Interleukin-3 dependent cell lines on stroma. These data however are further complicated by the observations that at high density these Interleukin-3 dependent target cells may evidence autocrine production of Interleukin-3.

As an alternate approach to the study of stromal cell/hematopoietic stem cell interactions we have utilized the non-myeloablated BALB/c female host and studied transplantation of male BALB/c marrow into this recipient host female mouse. Engraftment of normal male BALB/c marrow was quantitated by analysis of host tissue DNA for sequences unique to the Y chromosome (PY2 cDNA probe). Using this approach and transplanting 40 million cells per day for 5 days or 2 tibia and 2 femur equivalents, we have demonstrated long-term engraftment of male cells into female BALB/c marrow, spleen and thymus. Analyzing tissue DNA by Southern blot and PY2 probing with densitometry the percent of male marrow in female hosts at 21 to 25 months after transplantation ranged from 15 to 40 percent. When marrow from mice pre-treated 6 days before sacrifice with 5FU (150 mgs per Kg) was compared with normal marrow for its capacity to engraft in a normal host, the post-5FU marrow was markedly inferior; mean engraftment at 1-3 months for 6 mice receiving normal marrow was 38%, whereas for 6 mice receiving post-5FU marrow it was 8% at 10 to 12 months. Further data has indicated that the key to the high rates of engraftment seen when normal marrow is transplanted into normal female hosts is the schedule of transplantation. When 200 million cells are administered over 5 or 10 injections with at least 24 hours separating each injection the level of engraftment is superior to that seen when the same total number of cells is given in a single injection. Altogether these data suggest a model of fluctuating niche availability in marrow stroma in which a set percentage of niches may be available for engraftment (5 to 10%), support of the engrafting cells presumptively being mediated in part by the production of different stromal based cytokines.

G 022 HUMAN BONE MARROW STROMAL CELL PRECURSORS: IDENTIFICATION & DEVELOPMENTAL POTENTIAL, Paul J. Simmons[1], Stan Gronthos[1], Andrew Zannettino[1], Steve Graves[2], [1]Division of Haematology, Hanson Centre for Cancer Research, Adelaide, SA 5000 and [2]Department of Orthopaedics, Royal Adelaide Hospital, Adelaide 5000, Australia.

The regulation of haemopoiesis in the bone marrow (BM) is critically dependent upon interactions with a heterogeneous population of stromal cells. Despite intensive interest in the stromal tissue of the BM, several fundamental aspects of stromal cell biology remain unclear, including the ontogeny of these cells during development, their proliferative potential and requirements for growth and differentiation. The design of experimental strategies to address these issues is complicated both by the diversity of BM stromal elements and by the paucity of markers for these cells and their precursors. Monoclonal antibody STRO-1 was previously shown to identify 100% of clonogenic fibroblast colony-forming cells (CFU-F) in aspirates of normal human adult BM, including cells with the capacity to transfer the haemopoietic microenvironment (HM) in-vitro. We now provide further phenotypic and functional characterisation of the CFU-F population. By means of 2-colour fluorescence-activated cell sorting (FACS) and clonogenic assay, CFU-F were found to lack CD45 and demonstrated no detectable expression of T-cell (CD3), B-cell (CD19), myeloid (CD33) or erythroid (glycophorin A) specific antigens. Essentially all CFU-F, however, expressed the endopeptidase enzymes CD10 and CD13. A broad range of cell adhesion molecules (CAMs) were identified on CFU-F including various members of the immunoglobulin (ICAM-1, VCAM-1, Thy-1), selectin (L- and P-selectin) and integrin (β_1, β_2 and β_3 families) superfamilies. The 'endothelial'-like nature of CFU-F was illustrated by their expression of CD31, CD34, CD62, thrombomodulin and STRO-1 (itself a specific marker of vascular endothelium in many organs) and by the preferential adhesion of CFU-F to basal lamina ECM components, collagens IV, V and laminin. Clonogenic growth of CFU-F under serum-deprived conditions in response to a large panel of cytokines demonstrated absolute dependence upon corticosteroid and ascorbate. EGF and PDGF elicted colony growth equivalent to 20% FCS while other factors, IL-1, IFNγ, TNFα and, significantly, IL-6, IL-11 and LIF stimulated colony-formation at a frequency 10-20% of control. STRO-1$^+$ cells were also shown to contain osteogenic precursors as demonstrated by Vit D_3-dependent osteocalcin synthesis and generation of hydroxyapatite mineral in vitro. These data provide a basis for establishing the cellular hierarchy of BM stromal elements and for manipulating their differentiation in vitro. We are currently examining the existence of multipotential stromal stem cells in adult human BM by ectopic transplantation in SCID mice.

Leukemia

G 023 WHAT IS THE BEST THERAPY OF LEUKEMIA? Robert Peter Gale Salick Health Care, Inc., Los Angeles

The best therapy of leukemia is controversial. In this analysis I compare results of three therapies: chemotherapy, allo- and autotransplants. Three types of leukemia are considered acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML). Also considered are results in different remission states.

Stem Cells and Growth Factors

G 024 STUDIES OF C-KIT LIGAND IN PRECLINICAL TRANSPLANT MODELS, Friedrich G. Schuening[1], H. Joachim Deeg[1], Robert G. Andrews,[1] Frederick R. Appelbaum[1], Ted Gooley[1], Ian K. McNiece[2], and Rainer Storb[1], [1]Fred Hutchinson Cancer Research Center, Seattle, WA, and [2]Amgen, Thousand Oaks, CA.

The effects of recombinant c-kit ligand (rkit) on hematopoiesis were studied in dogs and baboons. Normal dogs treated with recombinant canine kit (rckit) at 200 μg/kg/day showed a twofold increase in neutrophils. Other peripheral blood counts were not changed. Marrow showed panhyperplasia. Ten dogs were given an otherwise lethal dose of 400 cGy TBI, no marrow infusion, and 200 μg rckit/kg/day for 21 days. Five dogs showed hematopoietic recovery and survived, as compared to 1 of 28 control dogs ($p < .005$). Survival and neutrophil recovery in rckit-treated dogs was not different from that of rG-CSF-treated dogs, but platelet recovery was faster in rckit-treated animals ($p = .06$). Two of 4 dogs given 500 cGy and 0 of 5 given 600 cGy TBI survived (comparable to G-CSF). Next, 10 dogs were given DLA-identical littermate marrow grafts after 920 cGy TBI followed by 200 μg rckit/kg/day from days 1 through 10 after TBI. Results were compared with those of 16 dogs not administered growth factor and 10 dogs treated with 100 μg rhG-CSF/kg/day. Neither group of dogs received GVHD prophylaxis. Median time to 1,000 neutrophils/mm^3 was 9 days with rckit, 8 days with G-CSF, and 14 days in controls ($p = .0002$). Median time to 100 monocytes/mm^3 was 15 days with rckit, 17 days with G-CSF, and 35 days in controls ($p = .0001$). Platelet and lymphocyte recovery, incidence of GVHD and graft failure, as well as survival at day 100 were not different from the control. Results obtained with rckit were comparable to G-CSF except that lymphocyte recovery to 500/mm^3 was faster with G-CSF (15 vs. 21 days; $p = .03$). Rckit was also given at 200 μg/kg/day for 21 days after 920 cGy TBI and DLA-identical littermate marrow transplants treated with L-Leucyl-L-Leucine Methyl Ester (Leu). Leu eliminates cytotoxic T lymphocytes, monocytes, and natural killer cells. Treatment of DLA-identical littermate marrow grafts with Leu before transplantation led to graft failure in 6 of 8 dogs as compared to 0 of 7 controls receiving untreated grafts. Rckit treatment decreased graft failure to 5 of 10, but this was not significant ($p = .37$). Next, rckit was tested in a different model of graft failure. While 6 of 7 dogs given 920 cGy TBI, autologous marrow, and anti-MHC class II monoclonal antibody H81.9 posttransplant died with graft failure, all 6 dogs given rckit, 200 μg/kg/day for 7 or 21 days concurrently with the antibody showed complete and sustained engraftment. Finally, peripheral blood mononuclear cells (PBMNC) were collected by leukapheresis from 3 baboons on day 10 or 11 of treatment with 200 μg recombinant human kit (rhkit)/kg/day and from 4 untreated controls. Animals received 1020 cGy TBI and infusion of 1.00 to 1.04 x 10^8/kg of cryopreserved PBMNC 24 to 38 days after the last dose of rhkit. The 3 baboons receiving rhkit-mobilized PBMNC engrafted but none of the 4 untreated control animals. Conclusions: Rkit treatment improves neutrophil and platelet recovery and survival after lethal TBI, accelerates neutrophil and monocyte recovery after DLA-identical littermate marrow transplant, prevents graft failure due to anti-MHC class II antibody treatment but not due to T-cell depletion, and mobilizes cells into the circulation that rescue lethally irradiated baboons.

Dose Intensity and Lymphoma

G 025 TWO-HUNDRED SIXTY AUTOTRANSPLANTS (TX) FOR MULTIPLE MYELOMA (MM) - PROGNOSTIC FACTOR ANALYSIS. B. Barlogie, S. Jagannath, D. Vesole, G. Tricot and J. Crowley. Universities of Arkansas, Little Rock, AR; Texas, Houston, TX; and Washington, Seattle, WA.

Since 1985, 260 patients (pts) with symptomatic MM have received myeloablative therapy with Tx; 55 had total body irradiation (TBI, 850 cGy) + melphalan (MEL) 140 mg/M^2 or thiotepa 750 mg/M^2 and autologous bone marrow (ABMT); since 1989, MEL 200 mg/M^2 and both peripheral blood stem cells (PBSC) + ABMT were given once (87 pts) or twice (118 pts), sometimes with MEL 140 mg/M^2 + TBI. Median age was 50, 35% had stage III at diagnosis (Dx); 55% had > 12 mos of prior therapy, and 41% were resistant to multiple standard therapies. With added PBSC, the median times post-Tx to granulocytes > 500/μl and platelets > 50,000/μl were 11 and 13 days, resp., as compared to 20 and 28 days, resp., when only ABMT was employed ($p < 0001$). Tx-related mortality was 3%; 27% achieved true CR; median durations of event-free survival (EFS) and overall survival (OS) post-Tx were 21 and 34 mos, resp. Mortality was 7% among 106 pts with refractory MM versus 1% with sensitive disease. Multivariate analysis (MV) identified 3 independent favorable pretreatment variables (FV): beta-2-microglobulin (B2M) ≤ 2.5, ≤ 12 mos from Dx and non-IgA MM. CR rates decreased from a high of 40% with 3 FV to a low of 6% with 0 FV ($p < .0001$). EFS and OS shortened progressively from 37 and 66 mos in the presence of 3 FV to 6 and 8 mos, resp, when 0 FV was present ($p < .0001$). A 6 mos landmark was chosen to determine the added benefit of a 2nd Tx (most significant on MV for EFS) applied within 6 mos. Two Tx improved clinical outcome markedly among both good risk (135 pts with > 1 FV) and poor risk groups (67 pts with < 2 FV). Thus, drug resistance in MM can be overcome by dose-intensive therapy with Tx.

G 026 MINIMAL DISEASE AND PURGING IN LYMPHOMA, John G. Gribben, Arnold S. Freedman and Lee M. Nadler, Division of Hematologic Malignancies, Dana-Farber Cancer Institute, Boston MA 02115

The major obstacle to the use of autologous bone marrow transplantation (ABMT) is that the infusion of occult malignant cells harbored within the harvested marrow will result in rapid relapse of disease. It has been demonstrated clearly that marrow with no morphologic evidence of lymphoma may contain a significant number of lymphoma cells when assessed by more sensitive techniques. A variety of techniques have been developed that are capable of selectively depleting contaminating lymphoma cells while sparing normal hematopoietic tissue. Although the rationale for removing such contaminating lymphoma cells appears compelling, the issue of bone marrow purging remains highly controversial. In particular, since the majority of patients relapse at sites of prior disease the widespread view has been that purging of autologous marrow could contribute little to subsequent outcome after ABMT. We used PCR amplification of the bcl-2/IgH translocation to detect minimal residual disease in the bone marrow and peripheral blood of patients with B-cell NHL undergoing ABMT. A total of 326 patients with B-cell NHL had been treated at our institution with an identical protocol that included treatment to a protocol eligible minimal disease state followed by total-body irradiation, high dose cyclophosphamide and immunologic purging of autologous marrow before transplantation. Immunologic purging was performed for three successive rounds of treatment using a cocktail of anti B-cell monoclonal antibodies and complement mediated lysis. The bcl-2/IgH translocation was identified in diagnostic tissue obtained from 212 patients (167 at the major breakpoint region and 45 patients at the minor cluster region). At bone marrow harvest, although 96 patients (45%) had morphologic evidence of bone marrow infiltration, PCR analysis detected marrow infiltration in 211 of these 212 patients (99.5%). Only one patient had no evidence of residual bone marrow infiltration by PCR analysis in any of four bone marrow samples analyzed. Bone marrow samples after immunologic purging were available for analysis from 202 of patients. 91 patients (45%) had no PCR detectable residual lymphoma cells after purging and 111 patient (55%) had residual PCR detectable lymphoma after purging. The detection of residual lymphoma cells after immunologic purging was associated with a highly significantly increased risk of relapse after ABMT ($p=0.001$). Peripheral blood samples were available for analysis from only 45 of these patients at the time of bone marrow harvest. PCR detectable lymphoma cells were found in the peripheral blood in 22 of these patients (49%) suggesting that a significant number of peripheral blood stem cell collections may be contaminated with lymphoma. We prospectively analyzed peripheral blood samples immediately before and at two hours after infusion of autologous bone marrow in 56 patients. Residual lymphoma cells were found in the peripheral blood of 8 patients (14%) immediately before infusion of marrow, by two hours after infusion of marrow circulating lymphoma cells were detected by PCR analysis in 33 patients (60%). All 25 patients who had circulating lymphoma cells detected after, but not before, infusion of bone marrow were infused with marrow that contained residual lymphoma cells after purging. These data suggest that residual lymphoma cells infused with the autologous bone marrow circulate in the peripheral blood after infusion and may be capable of homing back to sites of prior disease and contributing to subsequent relapse.

G 027 MYELOABLATIVE THERAPY WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN LOW GRADE LYMPHOMA , Rohatiner AZS[1], Johnson PWM[1], Mahmoud M[3], Arnott SJ[2], Amess JAL[3], Norton AJ[4], Adams K[1], Matthews J[1], Whelan JS[1] & Lister TA[1]. [1]ICRF Dept of Medical Oncology, Depts of [2]Radiotherapy, [3]Haematology and [4]Histopathology, St Bartholomew's Hospital, London, UK

Low grade lymphoma is incurable with conventional treatment. The use of very intensive therapy supported by ABMT results in long term survival for a proportion of patients with intermediate and high grade lymphoma in whom conventional therapy has failed. Myeloablative therapy with ABMT was therefore used in younger patients with low grade B cell lymphoma in the hope of prolonging remission duration and survival.

Since June 1985, 83 patients with low grade B cell lymphoma (74 follicular, 9 low grade B cell diffuse) have received Cyclophosphamide: 60mg/kg x 2 and total body irradiation: 200cGy x 6, supported by ABMT as consolidation of second or subsequent remission. The marrow mononuclear cell fraction was treated in vitro with 3 cycles of the monoclonal antibody anti CD-20 (Coulter Immunology, Florida) and baby rabbit complement (Pel-Freez, Wisconsin) prior to cryopreservation. At the time of treatment, 37 patients with follicular lymphoma were in complete remission (CR), 37 had residual disease present. Five of the nine patients with low grade B cell diffuse lymphoma were in CR.

The median time to engraftment was 28 days (range 15 to 46 days) for both neutrophils >0.5 x 10^9/l and platelets >20 x 10^9/l. One patient did not engraft and died at 12 weeks and 6 patients (excluded from the range) have had delayed recovery (>6 months) of red cells and platelets. 69/83 patients are alive (62 follicular, 7 low grade B cell diffuse): 3 died as a consequence of the transplant procedure; 2 died in remission from other causes and 9 have died of recurrent lymphoma (7 follicular, 2 low grade B cell diffuse). Four of the nine patients with low grade B cell diffuse lymphoma remain well without recurrence. 43/74 patients with follicular lymphoma continue in remission between 5 months and 8 years, with a median follow up of 31/2 years. Twenty seven have developed recurrent lymphoma, 5 with evidence of transformation to high grade histology. These results are preliminary but encouraging; it remains to be established whether this treatment prolongs survival.

Gene Therapy/Non Malignant Diseases

G 028 GENE THERAPY FOR CONGENITAL HEMATOLOGIC AND IMMUNE DISORDERS. Donald B. Kohn. Division of Research Immunology/Bone Marrow Transplantation, Childrens Hospital Los Angeles, USC School of Medicine, Los Angeles.

Genetic disorders of lymphohematopoietic cells, including hemoglobinopathies, immune deficiencies and storage diseases, may be treated by allogeneic BMT. Autologous transplant of genetically-corrected stem cells (gene therapy) may have the same benefits, without the immunologic barriers of allogeneic transplant. Retroviral vectors have been most extensively used for gene insertion into bone marrow. Conditions which cause proliferation of stem cells are needed for retroviral-mediated transduction to occur; recombinant growth factors and marrow stroma are effective for murine stem cells and human progenitors *in vitro*. Gene transfer into stem cells of large animal models have been less successful (0.1-2%). Expression of a variety of clinically relevant genes have been achieved in murine gene transfer/BMT models. Using the human glucocerebrosidase (GC) gene (relevant to gene therapy of Gaucher disease) as an immunohistochemical marker, we have documented progressive replacement of a fraction of the fixed tissue macrophages and microglial cells of murine recipients. Serial transplants of GC-transduced marrow has shown a high frequency of expression failure from the Moloney LTR associated with DNA methylation in secondary recipients. New vectors to overcome this inactivity are being studied. We have used retroviral-mediated gene transduction techniques to insert the normal human ADA cDNA into umbilical cord blood cells of three neonates diagnosed *in utero* with ADA-deficient SCID. The results of this study will be presented. A similar protocol to study transfer of the human GC cDNA into CD34+ cells from bone marrow or peripheral blood stem cells of patients with Gaucher disease has been approved by the NIH RAC and will be performed coordinately with Drs. Karlsson and Dunbar at the NIH. These studies represent the initial steps towards application of gene therapy for genetic diseases.

Breast Cancer

G 029 ARE THE RESULTS OF HIGH DOSE CHEMOTHERAPY IN BREAST CANCER REALLY BETTER THAN STANDARD TREATMENT? Gabriel N. Hortobagyi, University of Texas, M. D. Anderson Cancer Center, Houston, Texas 77030.

The concept of dose intensity has ample preclinical support in mammary carcinoma and other solid tumors. Retrospective analyses of chemotherapy trials have suggested a linear dose-response and dose-survival correlations. There are few prospective trials performed to evaluate this concept. Of those published, many show that higher dose-intensities are related to higher response rates, and a few, with prolonged survival. The slope of the dose-response curve is much steeper than the dose-survival curve. This might explain why survival is altered in very few clinical trials, while response rate increases with increased dose intensity. All prospective randomized trials have been conducted within a relatively narrow range of doses possible without stem cell support.

The high-dose chemotherapy programs reported since the introduction of autologous bone marrow and/or peripheral stem cell support have shown a markedly increased overall response rate and complete response rate. In metastatic breast cancer, the median time to progression for these regimens varies from 4 to 8 months and the median survival from 8 to 12 months. This is no different, and possibly inferior to survival figures obtained with standard dose combination chemotherapy. Several high-dose chemotherapy trials reported two and three-year progression-free survival rates from 15% to 25%. Many standard chemotherapy trials show no long term disease-free survivors with metastatic disease, while some show that up to 10% of patients who achieve a complete remission remain progression-free for extended periods of time exceeding five and ten years. The extensive selection process for high-dose chemotherapy and autologous bone marrow/stem cell transplantation protocols precludes any reasonable comparison with standard therapies. Selection by age, performance status, organ function, and much more extensive staging workup, as well as exclusion of non-responding patients from high dose therapy, suggests that the minimal perceived advantages of high-dose chemotherapy are probably related to patient selection. While prospective randomized trials are indicated to assess the relative benefits and toxicities of high-dose and standard regimens, it is doubtful that with current preparatory programs much long-term benefit will be demonstrated. High-dose chemotherapy programs for high-risk breast cancer appear more promising. A recent prospective randomized trial within the "standard" dose intensity ranges demonstrated a definite correlation between dose intensity and disease-free and overall survival. Two substantial Phase II pilot studies in patients with 10+ axillary lymph nodes suggested an early disease-free survival benefit when compared with historical controls. However, these trials also suffer from major selection bias. Fortunately, there are four ongoing randomized trials to test the dose intensity hypothesis in patients with high risk primary breast cancer. Until these trials are completed, however, there is no scientifically acceptable evidence that dose intensive therapy is superior to standard therapy.

G 030 HIGH-DOSE CHEMOTHERAPY AND PURGED AUTOLOGOUS BONE MARROW SUPPORT FOR PATIENTS WITH METASTATIC BREAST CANCER, James J. Vredenburgh[1], Maureen Ross[1], Atif Hussein[1], Robert C. Bast, Jr.[1], Elizabeth J. Shpall[2], Christy Tyer[1], and William P. Peters[1]. [1]Duke University Medical Center Bone Marrow Transplant Program, Durham, NC 27710, [2]University of Colorado Health Sciences Center, Denver, CO 80262.

High-dose chemotherapy and hematopoietic support produces high response rates and prolonged, disease-free survival for some patients with metastatic breast cancer. Bone marrow involvement or multiple boney metastases generally precludes patients from treatment with high-dose chemotherapy. There are a variety of ways to purge the hematopoieic support, including pharmacologic and immunologic purging or CD34 positive cell selection. We treated 76 breast cancer patients involving the bone marrow or more than 3 bone sites with AFM induction chemotherapy followed by high-dose cyclophosphamide, cisplatin and BCNU with purged autologous marrow support. The bone marrow was purged with 4-HC at 80 mcg/ml, 5 anti-breast cancer monoclonal antibodies and immunomagnetic beads. The initial 9 patients received no growth factor support, and took a median of 36 days to reach an ANC >500, and 38 days to platelet transfusion independence. Subsequent patients received IL-3, GM-CSF, IL-3 + GM-CSF, or G-CSF, and each cohort had a shortened time to an ANC >500, but no significant decrease in the time to platelet independence. The response rate (PR + CR) to AFM was 70%, with 16% CR's, and the response rate to the high-dose chemotherapy was 86%, with 54% CR's. Fifteen of the 55 patients (27%) treated more than 2 years ago remain alive, and 10(18%) are progression-free. For patients with metastatic breast cancer and bone marrow involvement or a high-risk of marrow involvement, high-dose chemotherapy and immunopharmacologically purged bone marrow support produces durable engraftment, high response rates and in some patients, prolonged disease-free remission. The determination of the role of purging is not possible in the metastatic setting because the patients generally recur in previous sites of disease. A randomized purge versus no purge trial in high-risk adjuvant patients may be an ideal setting to determine the role of purging.

Minimal Disease and Purging; Solid Tumors Other Than Breast; CMV, HHV6 and Other Infection; Acute Leukemia and MDS; Toxicity, Late Effects and Supportive Care

G 100 EX VIVO EXPANSION OF HUMAN HEMATOPOIETIC STEM/PROGENITOR CELLS RESISTANT TO TREATMENT WITH 4-HYDROPEROXYCYCLOPHOSPHAMIDE, R. D. Armstrong, S. A. Rummel, S. G. Emerson, and G. Van Zant. Aastrom Biosciences, Inc., Ann Arbor, MI 48106.

The use of 4-HC for *ex vivo* purging prior to autologous transplantation is limited by the resulting prolonged time to engraftment. In this study, 4-HC resistant stem/progenitor cells were expanded in a continous perfusion bioreactor system that has been shown capable of generating a sufficient number of colony forming units--granulocyte-macrophage (CFU-GM) from a small bone marrow aspirate. Bone marrow cells were treated with 4-HC, resulting in an initial 95% decrease of CFU-GM and a 26% decrease in long-term culture initiating cells (LTC-IC). Cells were subsequently placed into perfusion bioreactors and grown (14-21 days) in the presence or absence of allogeneic, irradiated stroma. The number of cells generated from 4-HC treated samples was greater in cultures with exogenous stroma than without (8.9 million and 4.9 million, respectively) but was less than in cultures of untreated cells (24.0 million). While the number of CFU-GMs from untreated samples expanded 10-fold (138,000 total) during the first 2 weeks of growth, CFU-GMs from 4-HC resistant cells expanded 60-fold (49,000 total) when grown without exogenous stroma and 146 fold (118,000 total) when grown in the presence of exogenous stroma. The number of LTC-ICs from untreated samples expanded greater than 4 fold (465) following 2 weeks growth in bioreactors, while the number of LTC-ICs remained essentially the same in 4-HC treated cultures (59 without exogenous stroma and 69 with). However, LTC-IC from 4-HC treated marrow cultured in the presence of exogenous stroma generated 4-fold more CFU-GM per LTC-IC (4.4) than did LTC-IC harvested from either control reactors (1.3) or 4-HC treated cells cultured without exogenous stroma (1.9), attesting to their greater proliferative potential and more primitive state. These results suggest that *in vitro* expansion of 4-HC resistant primitive progenitor cells in a continuous perfusion system may be able to provide a clinically relevant number of hematopoietic precursors necessary for early engraftment.

G 101 PURGING OF HEMATOPOIETIC MALIGNANCIES VIA ISOLATION OF SBA-CD34+ STEM CELLS FROM BONE MARROW, Cristina Gasparetto[1], Clay Smith[1], Michael Flasshove[2], Carmelo Bengala[2], Ravi Verma[2], Andrew Zelenetz[2], Jane Lebkowski[3], and Malcolm AS Moore[2], [1]Duke University Medical Center, Durham, NC 27710, [2]Memorial Sloan Kettering Cancer Center, NY, NY and [3]Applied Immune Sciences, Santa Clara, CA.

The SBA-CD34+ marrow population is enriched for hematopoietic stem cells (Smith, et al, Blood, 77, p2122). Since a wide spectrum of hematopoietic malignancies are SBA+CD34-, isolation of SBA-CD34+ hematopoietic stem cells may be useful for purging. We have explored the utility of this approach to purging B-cell lymphoma and acute promyelocytic leukemia (APL) by depleting SBA+ cells and subsequently recovering CD34+ cells using polystyrene flasks containing covalently attached SBA and CD34. In models of B-cell lymphoma and APL, a 2-3 log depletion of malignant cells was observed in PCR based assays. Addition of an additional step employing removal of CD33+ cells resulted in elimination of APL cells. Isolation of SBA-CD34+ cells from patients in histologic remission following salvage chemotherapy for B-cell lymphoma resulted in substantial enrichment for CFU-GM and precursors to CFU-GM while isolation of SBA-CD34+CD33- cells from patients with APL yielded substantial enrichment for precursors to CFU-GM. These results indicate that isolation of SBA-CD34+ cells or further subsets may be a useful method for purging hematologic malignancies while yielding cells capable of initiating and sustaining engraftment.

G 102 ISOLATION OF HUMAN CD34$^+$ CELLS: PURGING OF TUMOR CELLS, Jane S. Lebkowski, Annemarie Moseley, Axel Fauser*, Robert Collins$^+$, and Thomas B. Okarma, Applied Immune Sciences, Inc., Santa Clara, CA 95054, *Institute Albert Ludwig University Clinic, Freiburg, Germany, $^+$Baylor Hospital, Dallas, Texas

The purification of human CD34$^+$ cells could offer a widely applicable method to purge tumor cells from bone marrow and peripheral blood autografts. To facilitate the clinical scale isolation of CD34$^+$ cells we developed a solid phase cell selection system based on the sequential passage of bone marrow or peripheral blood mononuclear cells on polystyrene surfaces containing covalently immobilized soybean agglutinin (SBA) or the CD34 monoclonal antibody, ICH3. Soybean agglutinin is a plant lectin that binds N-acetylgalactosamine to derivatives, and as a result, binds many cell types including tumor cells. CD34$^+$ cells isolated using this system are in general 65-95 % pure depending on the input cell sources, and lack any bound mouse immunoglobulin. Our previous studies using normal human bone marrow spiked with breast, neuroblastoma, lung, and leukemia cell lines indicated that the soybean agglutinin device purged up to 1 log of the tumor cells with a further 2 log depletion upon capture of the CD34$^+$ cells. Our subsequent preclinical and clinical studies using actual breast cancer patients' samples confirmed and extended these results. In these investigations, up to 4 logs depletion of breast tumor cells was observed upon soybean agglutinin binding alone with a total of up to 5 log depletion of tumor cells seen throughout the entire process. Furthermore, in clinical samples having identified tumor contamination, the transformed cells were eliminated from the final engraftable fraction by the completion of the SBA binding step. Upon examination of bone marrow samples from lymphoma patients, serial passage over SBA produced greater than 2 logs depletion of CD19$^+$ cells. These studies demonstrate the utility of CD34$^+$ cell isolation and especially SBA depletion for the elimination of tumor cells from autografts.

G 103 EX VIVO EXPANSION OF PURGED BONE MARROW FROM NEUROBLASTOMA PATIENTS, S. A. Rummel[1], C. P. Reynolds[2], R. C. Seeger[2], R. J. Hutchinson[3], S. G. Emerson[4] and G.Van Zant[1], [1]Aastrom Biosciences, Inc., Ann Arbor, MI 48106,[2]Division of Hematology-Oncology,Children's Hospital Los Angeles, CA and Depts. of [3]Pediatrics and [4]Internal Medicine, University of Michigan, Ann Arbor, MI 48109.

We have evaluated the effect of purging on the expansion of normal stem/progenitor cells cultured in a continuous perfusion bioreactor system. Bone marrow was purged using immunomagnetic beads, after which treated and untreated marrow cells were inoculated into bioreactors and expanded in Dexter medium with IL-3 (2 ng/ml), GM-CSF (5 ng/ml), EPO (0.1 U/ml), with or without c-kit ligand (KL; 10 ng/ml). The presence of tumor cells was analyzed by immunocytochemistry (IC). Before expansion, IC revealed the presence of tumor cells in 1 of 4 patients. Equivalent numbers of cells were generated from unpurged and purged marrow, with the addition of KL resulting in a greater cell expansion (9-15 fold versus 2-5 fold). CFU-GM expansion was also similar in unpurged and purged samples (3-11 fold), although the response of individual samples to KL was variable, perhaps as a result of prior chemotherapy. After expansion, no tumor cells were detected by IC in either purged or unpurged samples; thus tumor cells appeared to be passively purged during the expansion process. These studies demonstrate that marrow can be effectively expanded after purging with immunomagnetic beads.

G 104 PURGING OF MINIMAL RESIDUAL DISEASE FROM AUTOGRAFTS WITH THE PHOTOSENSITIZER BENZOPORPHYRIN DERIVATIVE MONOACID RING A (BPD-MA). Sorrenti R, Chadderton T, Glück S, Gulati S, Ho A, Hornby A, Jamieson C, Knizewski M, Levy J, Mitchell D, and Richter A. Quadra Logic Technologies and The Univ of B.C., Vancouver, B.C., Canada; NE Ontario Regional Cancer Center, Sudbury, Ontario, Canada; Memorial Sloan-Kettering Cancer Center, New York, N.Y.

Under treatment conditions in which BPD-MA and red light were found to spare normal stem and committed progenitor cells, a 4- to 6-log decrease in malignant cell survival was observed. The uptake of BPD-MA and subsequent light-activated toxicity was shown at both the cellular and molecular level to be selective for malignant cells. Following therapeutic treatment, early progenitor cells ("stem" cells and/or CD34+ cells) were functional in both in vitro long-term cultures and in an in vivo reconstitution model. These data suggest that the use of BPD-MA in photodynamic treatment of autologous bone marrow or enriched hematopoietic products may produce a safe product for reconstitution of conditioned patients. A Phase I clinical trial in patients with acute leukemia is in process.

G 105 AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) FOLLOWING CYTOREDUCTION WITH CYCLOPHOSPHAMIDE (Cy), CARBOPLATIN (Cb), AND MELPHALAN FOR RECURRENT OR PROGRESSIVE CENTRAL NERVOUS SYSTEM (CNS) MALIGNANCIES A. Billett, R. Duerst, P. Savina and A. Guaspari. Univ Rochester Med Ctr, Rochester, NY, Dana-Farber Cancer Institute, Boston, MA and Burroughs-Wellcome, Research Triangle Park, NC

We have treated 11 patients (3-21 yo) with recurrent or progressive CNS malignancies according to the following schedule: Cy 1800 mg/m^2 days -4 & -3, Cb 400-600 mg/m^2 days -4 & -3 and Melphalan 140 mg/m^2 day -2. Diagnoses included: medulloblastoma, 3; glioblastoma multiforme, 3; glial tumor, 2 and 1 each choroid plexus carcinoma, ependymoma, pinealblastoma. All patients had received prior radiotherapy (RT, 4950-6960 cGy, mean 5750 cGy) to the primary tumor and 5 patients had also received spinal RT (2340-3600 cGy). 10 of the 11 patients had received chemotherapy prior to ABMT. The interval from initial diagnosis to ABMT was 4 mo-10 yr (median, 18 mo). The regimen has been generally well tolerated but there was one toxic death (combined pancreatitis, renal failure and ARDS) at the initial dose-level of Cb. Nine patients had tumor masses evaluable for response. No complete responses were observed. Five patients developed progressive disease 2-4 mo post ABMT. 5 patients are surviving without evidence of progression for 2, 4, 8, 9 and 26 mo post-ABMT. These initial results support the use of high-dose chemotherapy for patients with recurrent or progressive CNS tumors. Further enrollment and evaluation will be necessary to determine the maximally tolerated dose of Cb in this cytoreduction regimen. Also, the effect of this regimen on tumors of a specific histology or the role of residual tumor mass at the time of high-dose chemotherapy will require additional study.

G 106 ADENOVIRUS INFECTIONS IN T-CELL DEPLETED ALLOGENEIC BONE MARROW TRANSPLANTS,
Charles Blanke[1], Ann Hedderman[1], Isabel Cunningham[1], Guido Tricot[2], Kenneth Cornetta[1], E. Randolph Broun[1], Cari Clark[1], and Robert Hromas[1], [1]Indiana University Bone Marrow Transplant Section, Indianapolis, IN 46202, [2]University of Arkansas for Medical Services, Little Rock, AK 72205

Serious viral infections are common after allogeneic bone marrow transplant (BMT). With the development of effective therapy for CMV and other herpes viruses, adenovirus has emerged as a life threatening pathogen in our population of T-cell depleted BMT patients. Ten of seventy-four patients undergoing T-cell depleted allogeneic BMT were found to have adenovirus infection. This represents an incidence of 13.5%, which is higher than that described in other studies published. We have found four defined adenovirus syndromes based on location of infections: 1) lung infection with result and interstitial pneumonia, 2) bladder infections with hematuria, 3) intestinal infection with diarrhea and bleeding, 4) asymptomatic throat infections. The most common serotype isolated was adenovirus 11 (5 patients), adenovirus 1 in two patients, and adenovirus 12 in one patient. The remaining two patients could not be typed. Six of these patients died, with five of the deaths attributable to adenovirus. Four patients were treated with intravenous ribavirin without any evidence of response. All invasive adenovirus infections were fatal. There was no statistically significant difference in age, sex, GVH, unrelated versus related transplants or co-morbid infections between the adenovirus infected patients and the rest of the allogeneic population. The diagnosis of adenovirus should be considered in BMT patients presenting with any of these clinical syndromes.

G 107 CYTOMEGALOVIRUS (CMV) ANTIGENEMIA FOR RAPID DIAGNOSIS AND MONITORING OF CMV-ASSOCIATED DISEASES AFTER BONE MARROW TRANSPLANTATION,

Hisashi Gondo1),Toshio Minematsu2), Mine Harada1), Shin Hayashi3), Shuichi Taniguchi4), Kazuo Yamasaki4), Tetsuya Eto1), Koji Nagafuji1), Shin-ichi Mizuno1), Kenji Hosoda5), Ryoichi Mori6), Yoichi Minamishima2), and Yoshiyuki Niho1)
1)First Dept of Int Med,Kyushu Univ, 2)Dept of Microbiol, Miyazaki Med College, 3)Dept of Hematol, Harasanshin Hosp, 4)Dept of Int Med,Hamanomachi Hosp, 5)Institute for Bio-med Res,Teijin, 5)Dept of Virol,Kyushu Univ. JAPAN

A technique for the rapid detection of cytomegalovirus (CMV) antigen-positive blood leukocytes (CMV antigenemia) was evaluated in 15 marrow transplant patients as a means of diagnosis and for monitoring CMV-associated disease. CMV antigenemia was determined by direct immunoperoxidase staining of leukocytes with a peroxidase-labeled monoclonal antibody, HRP-C7, which binds an immediate-early antigen of human CMV.

CMV antigenemia occurred in 7 out of 15 marrow transplant patients (47%) and was initially detected between 4 and 6 weeks after transplantation. CMV-associated disease developed in 3 of 15 patients (20%). All patients with CMV-associated disease had a relatively large number of CMV antigen-positive leukocytes, exceeding 10 per 50000 WBCs. In the remaining 12 patients, CMV antigen-positive leukocytes were less than 10 per 50000 WBCs or were undetectable. CMV-associated disease did not develop in these patients during the period of monitoring. CMV antigen-positive leukocytes were detected more frequently in patients who developed GVHD or hemorrhagic cystitis than in those without such complications. CMV antigen were detectable from 1 to 4 weeks before the onset of CMV-associated disease which allowed initiation of ganciclovir treatment at an early stage. The degree of CMV antigenemia paralleled the clinical symptoms and signs, higher degree of antigenemia being associated with more significant disease. Thus, the detection of CMV antigen-positive blood leukocytes is useful for the diagnosis and monitoring of CMV-associated disease following BMT.

G 108 THE PATHOLOGY OF IDIOPATHIC PNEUMONIA AFTER BONE MARROW TRANSPLANTATION,
Robert C. Hackman, David Myerson, and Stephen W. Crawford, Clinical Research Division, Fred Hutchinson Cancer Research Center; Department of Pathology and Pulmonary and Critical Care Division, University of Washington School of Medicine, Seattle, WA

Idiopathic pneumonia (IP) continues to be a frequent and sometimes severe complication of bone marrow transplantation. Suggested causes have included irradiation and chemotherapy, occult cytomegalovirus infection and a graft-versus-host (GVH) reaction. This retrospective study of 39 open lung biopsies obtained during a 6 year period prior to routine diagnostic use of bronchoalveolar lavage tested 2 hyphotheses. The first is that occult CMV infection, negative by culture but detectable by in situ hybridization (ISH), is associated with IP. There was no support for this hypothesis, since all biopsies were ISH negative. The second is that the histological patterns [such as diffuse alveolar damage (DAD) and idiopathic interstitial pneumonia (IIP)] or specific histological abnormalities (such as pulmonary fibrosis, hemorrhage, cellular atypia, and bronchiolar inflammation) will correlate with characteristics of the clinical presentation, course or outcome to indicate a cause for the pneumonia. No correlations were identified to support this hypothesis. There was a suggestion that DAD and IIP have different etiologies. Thus these 2 patterns tended to persist to the time of death unless fungal or viral infection developed after biopsy, when the interstitial pneumonia pattern frequently evolved to diffuse alveolar damage. Pulmonary hemorrhage was associated with DAD ($p=0.0001$), indicating that it is a manifestation of the clinical adult respiratory distress syndrome rather than being a separate clinical entity. In summary, neither ISH for CMV nor clinical-histological correlations indicated the etiology of post-transplant IP.

G 109 LOW DOSE GANCICLOVIR (DHPG) PROPHYLAXIS AGAINST CYTOMEGALOVIRUS (CMV) PNEUMONITIS IN PATIENTS UNDERGOING ALLOGENEIC BONE MARROW TRANSPLANTATION.
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CMV pneumonitis is a major cause of morbidity in patients receiving an allogeneic BMT. Prophylaxis with DHPG reduces the risk of CMV disease but can be associated with significant, even fatal, myelosuppression. The DHPG prophylaxis study group consists of all patients transplanted between 1 March 92 and 4 May 93, receiving an allogeneic BMT with chemotherapy only (no TBI) using cyclosporine with or without methotrexate GVHD prophylaxis. Additionally, all patients were (1)CMV IgM(-); (2)CMV IgG(+) or donor CMV IgG(+); and (3)alive beyond day +30. If a patient developed grade II-IV aGVHD requiring treatment with corticosteroids, prophylaxis was started with DHPG 5 mg/kg IV BID x 5 days to load, then 5 mg/kg/d IV 3x weekly maintenance until at least d+100. DHPG was held temporarily if the ANC was less than 1500 cells/mm^3. The control group, which received no DHPG prophylaxis, consisted of all patients with the same inclusion criteria transplanted between 1 June 89 and 28 Feb 92. Both study and control groups received the same standard supportive care including acyclovir 250 mg/m^2 IV TID until engraftment then 200 mg PO TID until d+60 if HSV positive (96% study group vs 92% controls), and IVIG 500 mg/kg Q 2 wk. Weekly CMV cultures (throat and urine) and chest radiographs were obtained in both groups. The study group consisted of 28 patients (10 female, 18 male) with a median age of 38(range 22-57). The underlying diseases include CML-12, AML-4, MDS-3 and other-9. The preparative regimen was BuCy2 in 25(89%) patients. The control group consisted of 38 patients (16 female, 22 male) who underwent 39 BMT's. The median age was 39(range 19-63). Underlying diseases included CML-17, AML-12, MDS-3, and other-7. BuCy2 was the preparative regimen in 36(92%) of BMTs. CMV pneumonitis occurred in 0/28(0%) patients in the DHPG prophylaxis group vs. 6/39(15%) in the control group ($p= 0.036$). Acute GVHD grade II-IV occurred in 15/28(54%) patients in the study group vs.18/39(46%) in the control group (p = NS). Of these patients with aGVHD, 0/15 in the study group developed CMV pneumonitis vs. 6/18 (33%) in the control group ($p = 0.02$). Non-CMV interstitial pneumonitis during the first 180 days post-BMT occurred in 1/28 (4%) in the study group vs. 3/39 (8%) in the control group. The 180 day survival was 75% in the study group vs. 71% in the control (p= NS). Toxicity of prophylaxis included grade III neutropenia in 2 patients and multifactorial renal insufficiency in 2 patients. Low dose DHPG prophylaxis directed at those with aGVHD and additional immunosuppression appears effective at decreasing the incidence of CMV pneumonitis in this population.

G 110 CHRONIC BONE MARROW SUPPRESSION IN BMT PATIENTS ASSOCIATED WITH PERSISTENT MARROW INFECTION WITH HUMAN HERPESVIRUS 6 (HHV-6). Konstance Kehl Knox and Donald R. Carrigan. Department of Pathology. Medical College of Wisconsin. Milwaukee, Wisconsin. 53226. HHV-6 is a ubiquitous herpesvirus that is a significant pathogen in bone marrow transplant patients causing interstitial pneumonitis and post transplant marrow suppression. In vitro virus isolation techniques have been used to show that this marrow suppression is caused by direct bone marrow infection by HHV-6 (Drobyski et al.; J Infect Dis 167:735-739; 1993). The above studies identified four BMT patients with HHV-6 associated bone marrow suppression. Using established immunohistochemical staining techniques, recent studies of trephine bone marrow biopsies obtained from these four patients at the same time as the marrow aspirates used for the virus isolations have confirmed the presence of HHV-6 infected cells in the bone marrows of three of the patients. Additional marrow biopsies were available for study from two of the three patients whose bone marrows were virus positive by immunohistochemical staining. In one of these (UPN 356) a biopsy taken 51 days before the culture positive marrow was obtained stained positively for HHV-6 infected cells. In the second patient (UPN 399) biopsies taken 7 and 27 days prior to the virus culture positive biopsy contained HHV-6 infected cells. The earlier of these two biopsies was taken on the seventh day after transplant. For both of these patients, the biopsy positivity for HHV-6 correlated with periods of persistently declining bone marrow function. These studies demonstrate that HHV-6 infection of bone marrow can occur very early in the post transplant period, that chronic marrow infection with the virus can be associated with progressive decline in marrow function, and that direct immunohistochemical staining of bone marrow biopsies for HHV-6 infected cells may be a useful diagnostic tool for the detection of marrow infections by HHV-6.

G 111 Long-term immunity to measles, mumps and rubella after allogeneic bone marrow transplantation. Per Ljungman, Ilona Levensohn-Fuchs, Viera Hammarström, Johan Aschan, Per Bolme, Lena Brandt, Berit Lönnqvist, Olle Ringdén, Gösta Gahrton. Bone Marrow Transplant Program. Huddinge University Hospital, Karolinska Institute, Huddinge, Sweden.

The aims of this study were to examine antibody levels to measles and eventual late effects of immunization with attenuated measles vaccine in long-term survivors after allogeneic BMT.

Patients and methods: 124 patients who had survived at least two years after allogeneic BMT were studied. The median follow-up of the patients was 6.5 ys (range 2-13.5 ys). Serum samples were collected at least once yearly. IgG antibodies were determined by ELISA for measles and mumps. Rubella antibodies were analyzed by hemagglutination inhibition. Antibody levels were interpreted as representing immunity, seronegativity, or uncertain immunity. Immunization with live, attenuated measles, mumps, and rubella vaccine (MMR) were performed at two years after BMT in children and young adults who did not have active chronic GVHD or ongoing immunosuppression. Immunity and seronegativity was defined according to previously determined laboratory standards. Proportions of patients remaining immune was calculated according to the Kaplan-Meier technique. Patients were censored from the analysis at the time of MMR vaccination.

Results: The calculated probabilities of being immune to measles at 3, 5 and 7 ys from BMT were 47%, 27%, and 20%, respectively. The corresponding probabilities for mumps were 37%, 12% and 6%; and for rubella 47%, 33%, and 28% respectively. The probabilities for being seronegative for measles, mumps and rubella at 5 ys after BMT were 60%, 73%, and 52%, respectively. The only factor being important for immunity to measles after BMT was whether the patient had experienced previous natural measles or had been immunized ($p<0.05$). Forty-five patients were immunized with MMR vaccine, 8 of these patients were immunized twice. 40 of 42 immunized patients were seropositive to measles in the follow-up sample with a median follow-up of 6.5 ys (range 0 - 10 ys) from immunization. A few cases of low-degree fever and mild rashes were noted after immunization but no late side-effects were seen.

We conclude that during extended follow-up after allogeneic BMT most patients will lose immunity to measles, mumps, and rubella.. Immunization with live attenuated MMR vaccine is effective and safe in allogeneic BMT recipients without chronic GVHD or ongoing immunosuppression.

G 112 CYTOKINE USAGE IN ASPERGILLUS INFECTIONS: A NEEDED THERAPEUTIC ADJUVANT, Michael L. Nieder and Katherine H. Bentley, Department of Pediatrics, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

All cases of invasive aspergillosis in pediatric oncology patients at Rainbow Babies and Childrens Hospital from 1984-92 were reviewed. All patients were treated with Amphotericin B and received aggressive surgical debridement when possible. Only three of nine patients survived the infection. The important features of the cases included:

UNDERLYING DISEASE	SITE OF INFECTION	DURATION ANC<500	USE OF GM-CSF	OUTCOME
ANLL	Sinus/Brain	44 days	No	Died
NBL	Lung	70 days	No	Died
ALL	Lung/Brain Kidney	47 days	No	Died
CML	Sinus	35 days	No	Died
ANLL	Nose	53 days	No	Died
ALL	Sinus	40 days	No	Died
ANLL	Epidural/ Lung	26 days	No	Alive
ALL	Lung	20 days	GM-CSF	Alive
ALL	Nose	33 days	GM-CSF	Alive

In this small series, only 1/7 patients survived without the use of cytokines, while 2/2 patients who received GM-CSF recovered from the invasive aspergillosis. Clearly, cytokine-induced resolution of neutropenia was an important feature of survival. The enhancement of neutrophil function associated with GM-CSF usage may have also contributed to the successful eradication of the aspergillus infections. Preliminary results of current studies using G-CSF and M-CSF also reveal the survival advantage afforded by cytokine usage in patients with invasive aspergillosis and neutropenia.

G 113 LONG-TERM INFECTION PROPHYLAXIS WITH INTRAVENOUS IgG (IVIG) AFTER BONE MARROW TRANSPLANTATION (BMT). Jan Storek, Jane Jocom, Kenneth J. Kopecky and Keith M. Sullivan, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

Immunodeficiency is a major problem in long-term BMT survivors. To determine whether administration of IVIG from day 90 to day 360 after BMT results in decreased infection rates, we analyzed results from days 100-365 post BMT among patients (pts) in a clinical trial of IVIG vs. no IVIG. Results of infection prevention with weekly IVIG for days 0-100 have been previously reported (NEJM 323:705, 1990). After day 100, 123 pts received IVIG prophylaxis (500 mg/kg monthly) and 127 pts received no IVIG. The pt groups were similar in age, marrow source, CMV serostatus prior to BMT, donor/pt sex, conditioning regimen, acute GVHD and infection prophylaxis other than with IVIG; however by chance the IVIG group included more pts with advanced-stage malignancy (57% vs. 40%). The incidence of bacteremia or sepsis per 100 pt-days was 0.10 for the IVIG and 0.11 for the control pts. The incidence of localized infections was somewhat lower with IVIG compared to controls: 0.24 vs. 0.44 per 100 pt-days, respectively ($p = 0.07$). Multivariate analyses of day 100-365 data showed that IVIG had no significant effect on survival ($p = 0.89$), non-relapse mortality ($p = 0.83$), or the incidence of chronic GVHD ($p = 0.85$). We conclude that monthly IVIG may reduce infectious morbidity but has no effect on mortality or chronic GVHD.

G 114 ANTIFUNGAL PROPHYLAXIS IN BONE MARROW TRANSPLANT RECIPIENTS. RESULTS OF A DOUBLE-BLIND RANDOMIZED STUDY OF LIPOSOMAL AMPHOTERICIN B (AMBISOME) VS PLACEBO.

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Invasive fungal infections (FI) remain a major cause of morbidity and mortality in patients undergoing allogeneic or autologous bone marrow transplantation (BMT). In order to determine if a liposomal formulation of amphotericin B (AmBisome) is safe and can prevent FI we performed a placebo-controlled double-blind randomized prophylactic trial. AmBisome at a dose of 1 mg/kg/day or equal volume of placebo was given for a time period of 30-60 min. each day. The study drug was administered after conditioning when neutrophil count had decreased $< 0.5 \times 10^9/l$ and was continued until neutrophiles recovered to this level or an infection or toxicity endpoint was reached. 84 patients, 69 allogeneic and 15 autologous BMT recipients were randomized in the study. All patients were evaluated for safety; for efficacy evaluation the patients had to have completed at least 7 days of prophylaxis.

Results. 75 patients could be evaluated for efficacy: 36 received AmBisome and 39 received placebo. There were no significant differences in patient characteristics, prognostic factors or clinical outcome. During the study fungal colonization decreased in the AmBisome group while it continuously increased in the placebo group. By the end of prophylaxis 8 of 24 (33%) patients receiving AmBisome had a positive culture at any site compared with 18 of 28 (64%) placebo treated patients ($p < 0.05$). Five (14%) and 8 (21%) patients on AmBisome or placebo were withdrawn due to a presumed FI. None of the AmBisome treated patients that were withdrawn died with proven FI compared to two placebo treated patients. In total, autopsy documented FI occurred in 1 (3%) patient receiving AmBisome (*Candida guilliermondii*) compared to 3 (8%) patients receiving placebo (*C. guilliermondii*, 2; *C.albicans*, 1). AmBisome was well tolerated. Three patients experienced allergic reactions at first dose and was withdrawn from the study.

Conclusion. Prophylactic treatment with AmBisome during BMT is well tolerated, reduces fungal colonisation and may decrease the incidence of systemic FI.

G 115 VALUE OF DETECTION OF GALACTOMANNAN ANTIGEN IN THE DIAGNOSIS OF ASPERGILLOSIS AND IN THE MONITORING OF TREATMENT IN HIGH RISK PATIENTS, Etienne VILMER, Patricia MARIANI, Pierre ROHRLICH, Jacqueline SARFATI, Catherine DE ST MARTIN, Jean-Paul LATGE, Service d'hématologie et de microbiologie, Hôpital Robert Debré; unité de mycologie, Institut Pasteur, Paris, France.

Invasive aspergillosis infection (IA) remains a frequent cause of death in BMT recipients or in other high risk patients (pts). The difficulty of establishing an early diagnosis may explain the frequent failure of antifungal therapy started too late in the course of disease. Circulating Galactomannan (CGM) is an aspergillus antigen detected in sera from pts with IA. Up to now limits of sensitivity of available tests was 10ng/ml of CGM.

We developped an ELISA sandwich test using a monoclonal antibody recognizing the galacto-furan chain of GM. This method allows the specific detection of 1ng/ml of CGM in sera. 20 children with high risk of IA were prospectively studied (11 BMT pts and 9 pts with prolonged period of neutropenia). The detection of CGM was associated with non specific symptoms in 5 pts and were completely asymptomatic in one pt. In this latter these results led to diagnose an aspergillus sinusitis. CGM concentrations varied according to the presence and the severity of predisposing factor (Neutropenia, steroids...) and also to the antifungal therapy. Conventional or other forms of amphotericin B (liposomal, mixing with intralipid...) administered as soon as CGM was detected, induced the decrease of CGM concentrations, to undetectable levels provided that pts received sufficient daily doses of amphotericine B (05-1mg/kg). In pts in whom CGM levels remained negative, no case of invasive or limited forms of aspergillosis was diagnosed. The variations of CGM levels paralleled the clinical improvement. This strongly suggests that CGM is an accurate marker to monitor an early treatment of aspergillosis before recognizable invasive disease.

G 116 EPSTEIN-BARR VIRUS (EBV) INFECTION IN BONE MARROW TRANSPLANT (BMT) PATIENTS, John R. Wingard, Seh-Ching Lin, Eng-Chun Mar, Steven M. Devine, Carol C. Pascuzzi, Suzanne P. Dix, Rein Saral, Jung-Chung Lin, Department of Medicine, Emory University School of Medicine and Centers for Disease Control, Atlanta, GA 30322

Saliva specimens were screened before and weekly after BMT in 19 patients (17 allogeneic, 1 syngeneic, and 1 autologous BMT) and their donors for EBV using polymerase chain reaction (PCR) amplication to detect the presence of free and cell-associated viral DNA. Blood specimens were similarly screened in 7 patients and their donors. The type of EBV was determined using probes for EBNA-3c and EBNA-2. EBV was detected in the saliva of 16 of 19 (84%) and in the blood of 6 of 7 screened patients (86%). EBV was detected in the saliva of 7 of 18 (39%) and in the blood of 1 of 6 marrow donors (17%). EBV was first recovered before BMT in the saliva of 11 (58%) and in the blood in 5 (71%) patients. The median onset of salivary excretion was -1 day (-29 to +11 days after BMT) and its duration was 33 days (7-71 days). The median onset of viremia was -4 days (-23 to +11 days after BMT) and its duration was 34 days (21-49 days). The type of EBV recovered was Type A in 10, Type B in 1, and both types in 5 patients. No definite clinical manifestations were ascribed to EBV infection. EBV salivary excretion and viremia did not cease in 12 of 14 and in 3 of 4 patients respectively during acyclovir therapy. These data suggest there is a high prevalence of EBV infection in BMT patients, onset frequently occurs before BMT, Type A virus is most common but some patients have dual infection by both Types A and B virus, and acyclovir does not eliminate virus in most patients.

G 117 THE ONTARIO BONE MARROW TRANSPLANT NETWORK (OBMTN): A MODEL FOR OPTIMAL RESOURCE UTILIZATION, C.N. Bredeson, Division of Hematology, University of Ottawa, for The OBMTN, Canada.

As the economies of health care continue to become more restrictive and the indications for high dose therapy supported by stem cell rescue continue to broaden, increasing demands are being placed on the resources of Ontario's bone marrow transplantation (BMT) programs. At the same time, the provincial health ministry (MOH) of Ontario, the payer agency for health care in Ontario, expressed its interest in becoming better informed regarding the overall status of BMT in the province. Areas of interest to the MOH included details regarding current transplantation practices (indications, number of transplants, waiting lists and their effects on patient well being, costs, resource utilization) as well as evidence of maximal utilization of existing resources through inter-transplant program/inter-university cooperation. In an attempt to address both the difficulty of limited resources, manifested as lengthening waiting lists and the expressed interests of the provincial MOH, the directors of the 8 transplant centres in Ontario met and over a year developed a working inter-program network, The OBMTN.

The objective of the OBMTN as outlined in their working document includes the above mentioned items of interest to the MOH. As well, an urgency scoring system was developed in an attempt to prospectively prioritize patients on the waiting list based on medical criteria. The urgency score is determined from a series of sub-scores assigned according to diagnosis, stage of disease, age (for allo transplants), performance status, optimal time to transplantation and strength of indication. One of the functions of the network is to try and validate such a scoring system. The OBMTN working document, including the urgency scoring system, has undergone external review both nationally and internationally by other transplant centres.

All transplant candidates once identified at any of the OBMTN centres are entered onto a central database administered by the BMT program in Ottawa. Monthly summary reports are distributed to each centre and reviewed during a monthly teleconference. During these, patient status is updated and individual patient urgency scores are revised. Referral of urgent patients to other transplant centres within the OBMTN is arranged, based on resource (bed) availability.

The OBMTN began registering patients May 1, 1993. Data on the network functioning for the initial 250 patients entered will be presented.

It is hoped that data generated from this program will lead to more effective utilization of available resources and in doing so may provide a model for other large health providers, such as, health maintenance organizations or the recently proposed Health Alliances.

G 118 AUTOLOGOUS BMT AND ACUTE LYMPHOBLASTIC LEUKEMIA IN 2nd OR SUBSEQUENT REMISSION: DATA FROM THE AIEOP (ITALIAN ASSOCIATION OF PEDIATRIC HEMATO-ONCOLOGY) BMT REGISTRY, Paolo Colleselli, Flavio Rossetti, Marco Vignetti, Giorgio Dini, Roberto Miniero, Marino Andolina, Franco Locatelli, Cornelio Uderzo, Claudio Favre, Augusto Amici, Fulvio Porta, Roberto Rondelli, Chiara Messina, Maria Vittoria Gazzola, Paolo Paolucci for the AIEOP-BMT Group.

Data from 129 patients who underwent autologous BMT following a relapse of childhood ALL were analyzed. ABMT were performed between December 1984 and May 1992 in the following BMT Centers: Rome, Padua, Genoa, Turin, Trieste, Pavia, Monza, Pisa, Perugia and Brescia. Eighty-four were males and 45 females. The median age at transplant was 10 (range, 3-18) years. Nineteen children were transplanted following an isolated extramedullary relapse while in 1st bone marrow CR, 56 were in 2nd CR and 54 were in 3rd or subsequent remission. The bone marrow of 87 patients were purged with mafosfamide (n=55) or vincristine + prednison (n=30) or monoclonal antibodies (n=2). Forty-two harvests were not processed. The conditioning regimens included TBI for 64 patients, and only chemotherapeutic agents for 65 patients. Thirteen children (10%) died of regimen related toxicity. Seventy-six patients relapsed 1-40 months post-ABMT. Forty-five children survive in continous CR from 6 to 77 months post-ABMT. The overall 5-year DFS resulted 24%. Bone marrow purging, the 1st CR duration (<30 vs >30 months) and the remission status at transplant (2 CR vs >2 CR) did not show any significant impact on DFS. On the other hand, TBI-containing regimens resulted in a better DFS than non-TBI regimens (40% vs 11%, p= .008). Also, the relapse site influenced the DFS (isolated extramedullary, 45% vs bone marrow +/- other site(s) 16%; p= .04). In conclusion, from this analysis, ABMT for an isolated extramedullary relapse of childhood ALL may be a good option compared with other therapeutic choices. Moreover, a TBI-containing regimens seem to be superior to only-chemotherapy conditionings.

G 119 G-CSF-COMBINED CONDITIONING REGIMEN FOR ALLOGENEIC BONE MARROW TRANSPLANTATION OR AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION IN THE TREATMENT OF MYELOID LEUKEMIA

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We conducted high-doses of granulocyte colony-stimulating factor (G-CSF)-combined conditioning regimen for allo-BMT or auto-PBSCT in attempt to increase the chemosensitivity of myeloid leukemia to cell cycle-specific drug, cytosine arabinoside (Ara-C). Six patients with AML (2nd CR;3cases, 3rd CR;1, 1st relapse;1, 2nd relapse;1) and 3 with CML(2nd CP;1, My-BC;2) received allo BMT from their HLA identical siblings, and 19 patients with AML (1st CR;13, 2nd CR;4, 3rd CR;1, Refractory phase;1) received PBSCT. The conditining regimen was BU(16mg/kg)/CY(120mg/kg)/Ara-C/G-CSF in 7 cases, and TBI/Ara-C/G-CSF in 2 who received BMT, and BU/VP-16(30mg/kg)/Ara-C/G-CSF in all cases who received PBSCT. Ara-C (100mg/sqm/d) was administered continuously for 7 days combined with intravenous infusion of G-CSF(5μg/kg for 5d, 10μg/kg for 2d, and 20μg/kg for 2d), and then high-dose Ara-C (3g/sqm/h x 2/d; for 2d) was administered. As for BMT, 6 of 9 cases are now continuing CR (follow up 3 to 25mo, median 6mo). Two of 3 cases who received 2nd BMT with G-CSF combined regimen have prolonged CCR duration up to 8 and 25 months, though they relapsed 3 or 4 months after 1st BMT with conditioning of only BU/CY. Concerning PBSCT, relapse-free survival at 2 years was 63% for all cases and 77% for cases transplanted in 1st CR. One case who was conducted G-CSF-combined regimen for 2nd PBSCT kept 2nd CR for 27 months, though he relapsed 8 months after 1st PBSCT without combination of G-CSF. This conditioning regimen presented here was well-tolerated, with mucositis, the predominant toxicity. Althogh our results are preliminary, G-CSF-combined regimen is worth applying as a conditioning for PBSCT, allo or auto-BMT in the treatement of myeloid leukemia.

G 120 DONOR CELL DERIVED ACUTE MYELOGENOUS LEUKEMIA (AML) DEVELOPING POST ALLOGENEIC BONE MARROW TRANSPLANT (BMT) FOR A PATIENT WITH CHRONIC MYELOGENOUS LEUKEMIA (CML): G.M. Fyles, M.D. Minden, J.M. Meharchand, J.H. Lipton, H.L. Atkins, I. Tejpar and H.A. Messner, The Ontario Cancer Institute, Toronto, Canada.

A 44 year old male underwent allogeneic BMT for Philadelphia (Ph) chromosome positive CML in accelerated phase. The donor, his 48 year old sister, had been successfully treated 8 years before for stage 3B malignant melanoma. Engraftment with donor cells was established by a switch in blood group from recipient to donor. Seven months after BMT, marrow examination showed relapse with 23 of 25 cells examined Ph positive XY and only 2 normal female. Alpha interferon (IFN) was started at a dose of 3.5 million units given three times weekly. After three months of IFN the patient became aplastic and remained so for 10 months, following which marrow recovery occurred exclusively with female, Ph negative cells. Five months later, cytogenetic testing showed that 5 of 16 cells examined were 45XX with the new finding of a monosomy 7 (-7), while the remaining 11 were 46XX. Twelve months after marrow recovery all 25 metaphases examined were 45XX, -7, and 15 months later, marrow examination showed 20% myeloid blasts. The BCR-ABL translocation was not detected by Southern blot analysis at this time, nor were transcripts found by RT-PCR. Shortly thereafter, the percentage of blasts in the marrow increased to 50% and metaphase analysis became increasingly abnormal with 4 of 10 cells presenting with 45XX,-7, and 6 of 10 45XX, -7 with an additional deletion of one chromosome 5 (-5). Again, no recipient cells were found and Southern blot analysis did not detect the BCR-ABL translocation. The patient eventually required induction therapy for AML, but succumbed to sepsis more than 4 years post BMT. The donor remains well with no evidence of leukemia. We believe this to be the first report of a new, Ph negative acute myeloid leukemia developing in female donor cells following allogeneic BMT for CML.

G 121 Interleukin-2 (IL-2) pre and/or post Autologous BMT for pediatric acute leukemia patients, Chiara Messina, Renato Zambello^, Flavio Rossetti, Stefania Varotto, Maria Vittoria Gazzola, Giuseppe Basso, Paolo Colleselli, Luigi Zanesco, Centro Leucemie Infantili, Dept. of Pediatrics; and Istituto di Medicina Clinica, Clinica Medica I^, University of Padova. ITALY.

The role of Autologous Bone Marrow Transplantation (ABMT) in the treatment of Acute Leukemia patients with poor prognosis is still controversial because of the high risk of relapse. Since immunological factors may play an important role in preventing relapse post-BMT, we attempted to obtain an antitumor effect administering IL-2 pre and/or post ABMT. Clinical and biological data will be reported on 9 pediatric patients: 1 Acute Mieloid Leukemia (AML) late-responder and 8 Acute Lymphoblastic Leukemia (ALL) patients in second or subsequent complete remission (CR) who received ABMT and IL-2. In particular, 5 patients (Group A) received continous infusion (c.i.) IL-2 at $6x10^6$ U/m^2/day (120 hr/week x 2 weeks) after ABMT, beginning from day +63 to +144. Four patients (GROUP B) received c.i. IL-2 $6x10^6$ U/m^2/day (120 hr/week x 1 week) pre-bone marrow harvest. They were scheduled to receive the above mentioned rIL-2 treatment after engraftment. In group B, no delayed bone marrow take was observed. Two patients began IL-2 treatment 46 and 65 days post-ABMT. All the cycles were tolerated without side effects greater than grade 2 (WHO system). Two of 9 patients completed the designed treatment. Reasons for therapy withdrawal were: parental decision (n=2), relapse (n=5). One patient in group A and 1 patient in group B are still in CCR 39 and 28 months after ABMT. Six patients relapsed from 3 to 14 months after ABMT. Two of 6 patients who relapsed, obtained a new CR and are still alive 13-20 months from relapse. This IL-2 (pre and/or post ABMT) scheme of treatment, seems feasible but further studies are required to assess its efficacy.

G 122 Evaluation of leukemic contamination in peripheral blood stem cell harvests.
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A major issue in autologous blood stem cell transplantation (ABSCT) for leukemia is whether peripheral blood stem cell (PBSC) harvests are less contaminated with leukemic cells than bone marrow mononuclear cells (BMMNC). We compared leukemic cells contamination in PBSC harvests and BMMNC, obtained simultaneously, by using reverse transcriptase polymerase chain reaction (RT-PCR), in three patients with Philadelphia chromosome (Ph)-positive acute lymphoblastic leukemia (ALL), one with Ph-positive acute myelogeneous leukemia (AML), and two with acute promyelocytic leukemia (APL). In three of four patients with Ph-positive ALL and AML, we detected leukemic contamination in both PBSC harvests and BMMNC. In the remaining patient with ALL, both PBSC harvests and BMMNC were PCR negative. Both PBSC harvests and BMMNC from one patient with APL were PCR-positive. In contrast, PBSC harvests from another patient with APL, whose BMMNC could not be obtained because of bone marrow necrosis, were PCR-positive after the first course of consolidation chemotherapy, but became PCR-negative after the second course. The present study does not support the hypothesis that PBSC harvests are less contaminated by leukemic cells than BMMNC, but suggests that PBSC harvests are contaminated when BMMNC are contaminated.

G 123 ALLOGENEIC TRANSPLANTATION IN CHILDREN WITH ACUTE MYELOID LEUKEMIA (AML) IN FIRST COMPLETE REMISSION USING BUSULFAN (BU) AND CYCLOPHOSPHAMIDE (CY), Ogden AK, Parsons SK, Ravindranath Y, Krischer JP, Yeager A, Graham-Pole J, Weinstein HJ, Depts of Pediatrics Baylor College of Medicine, Houston TX 77030, Dana Farber Cancer Institute, Boston MA 02115, Wayne State Univ, Detroit MI, 48201, POG Statistical Office, Gainesville FL 32608, Johns Hopkins, Baltimore MD,21287, Univ of Florida, Gainesville FL 32610

Between 5/88 and 10/92, 53 patients (pts) age 2 months-16 11/12 years (median 8 1/12 yrs) were transplanted at various POG institutions (35 pts) and Dana Farber Cancer Institute (18 pts) using similar induction programs and the same preparative regimen to determine the role of allogeneic bone marrow transplantation (BMT) in childhood AML in first remission. All patients received induction chemotherapy with daunorubicin, Ara C, 6-TG, +/- VP16. FAB subtypes were M0 -2 pts, M1-9 pts, M2-14 pts, M3-5 pts, M4-11 pts, M5-7 pts, M6-0 pts, M7-1 pt, unknown 4-pts, myelodysplastic syndrome-1 pt.. All donors were HLA identical (51 siblings, 1 twin, 1 parent). The median interval from diagnosis to BMT was 97 days (range 67-194). All patients were cytoreduced with Bu 16 mg/kg and Cy 200 mg/m^2. The method of graft-vs-host disease (GVHD) prophylaxis was variable: cyclosporine (CSA)/prednisone (Pred) 17 pts, CSA/methotrexate (MTX) 11 pts, CSA/MTX/Pred 7 pts, CSA 7 pts, MTX 6 pts, MTX/Pred 2 pts, Pred 2 pts, none 1 pt. The incidence of acute GVHD was low with grade 2-4 acute GVHD in 8 pts (15.4 %), of which 5 were grades 3 and 4 (9.6 %). 47 pts were evaluable for chronic GVHD (6 severe, 4 moderate, 4 mild, 33 none). 32 pts (60.4%) remain in remission 11-60 months post-transplantation (median 27 months). With current chemotherapy programs, matched allogeneic using BMT Bu and Cy is an effective therapeutic alternative for pediatric patients with AML in first remission.

G 124 INCREASED RISK OF TRANSPLANT RELATED MORTALITY IN PATIENTS TREATED WITH BUSULPHAN COMPARED TO TOTAL BODY IRRADIATION. RESULTS OF A RANDOMIZED TRIAL IN ALLOGENEIC MARROW TRANSPLANT RECIPIENTS.
O. Ringdén, T. Ruutu, M Remberger, J. Nikoskelainen, L. Volin, L. Vindeløv, T. Parkkali, S. Lenhoff, B. Sallerfors, P. Ljungman, L. Mellander and N. Jacobsen for the Nordic Bone Marrow Transplantation Group.
BMT Units at Huddinge Hospital, Helsinki University Hospital, Turku University Hospital, Rigshospitalet, Copenhagen, Lund University Hospital and Östra sjukhuset, Gothenburg, Sweden, Finland and Denmark

205 patients with hematological malignancies conditioned with cyclophospamide (120 mg/kg) were randomized to additional treatment with either busulphan (16 mg/kg) (n=99) or total body irradiation (TBI, n=106). The two groups were comparable with regard to age, HLA compatibility, marrow cell dose, diagnosis and disease status. 74 patients had AML, 43 ALL, 59 CML, 4 lymphoma, 14 myeloma and 9 myelodysplastic syndrom. All patients received methotrexate and cyclosporin. Engraftment and transfusion requirements were the same in the two groups. The incidence of seizures was 5% in the busulphan group and none in the TBI group (p=0.02). The incidence of grade II-IV acute GVHD was 28% and 29% in the two groups, respectively. Chronic GVHD occurred in 72% in the busulphan group vs. 40% in the TBI group (p=0.06). The busulphan treated patients had an increased risk of veno-occlusive disease of the liver, 12% compared to 4% in the TBI group (p=0.02). Hemorrhagic cystitis occurred in 25% of the busulphan patients vs. 7% in the TBI patients (p=0.0002). Transplantation related mortality was 28% in the busulphan patients compared to 16% in the TBI patients (p=0.02). Including all patients actuarial 3-year survival was 62% in the busulphan group compared to 71% in the TBI patients. The incidence of relapse was 21% and 26%, respectively. Relapse-free survival of all patients, patients with early disease, those with AML, ALL and CML did not differ between the two groups. However, in patients with intermediate disease relapse-free survival at 3 years was 21% in the busulpan group vs. 58% in the TBI group (p=0.05). In adults (>17 yrs) actuarial patient survival at 3 years was 62% in the busulphan group (n=82) which was significantly worse than 75% in the TBI group (n=89, p=0.05). Among all HLA identical siblings those treated with busulphan (n=94) had a 3-year survival of 61% compared to 74% among those treated with TBI (n=94, p=0.04).

To conclude, patients treated with busulphan experienced more early toxicity and an increased transplant-related mortality.

G 125 MODULATION OF MULTIPLE DRUG RESISTANCE AND INDUCTION OF GRAFT VS. LEUKEMIA IN AUTOLOGOUS BONE MARROW TRANSPLANTS FOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL), Joseph Rosenthal, Joel Weinthal, Leonard Sender, Robert Sweetman, Renna Killen, Suzanne Serber, Rita Secola, and Mitchell S. Cairo, Children's Hospital of Orange County, Orange, CA 92668, and University of Louisville, KY 40202.

Autologous BMT for ALL is associated with a higher rate of relapse compared to allogeneic BMT (Kersey, et al, *N Engl J Med* 317:461, 1987). This higher relapse rate may be attributed to the combination of: 1) resistance of leukemic cells to the standard preoperative regimens following development of multiple drug resistance (MDR); 2) contamination of the autologous BM with leukemic cells due to ineffective purging ; 3) absence of the graft versus leukemia effect secondary to GVHD involved with successful ALLO BMT (Butturini, et al, *Bone Marrow Transplant* 2:233, 1987). Verapamil (VPL) has been shown *in vitro* to reverse MDR but is limited clinically by cardiac toxicity (Cairo, et al, *Cancer Res* 49:1063). VPL (10 uM) significantly reduced both Vincristine (V) and VP-16 IC_{50} of an MDR(+) T-ALL cell line ($p<0.05$), while increasing BM CFU-GM toxicity only by 11.1% ($p<0.001$). These studies suggest that VPL may potentiate the ability of V and VP-16 to chemopurge resistant ALL cells from BMT without enhancing toxicity (Cairo, et al, *AACR Proc* 33:199a, 1992). Cyclosporine A (CSP) has been demonstrated to induce a cutaneous GVHD syndrome following ABMT (Yeager, et al, *Blood* 78:223a, 1991). Alpha-interferon has also been shown to induce post ABMT GVHD (Klingmann, et al, *Blood* 78:3306, 1991). Thirteen pts with ALL (median age 7 yrs, range 1 $^{9}/_{12}$–19) (three 1st CR, 8 2nd CR, 1 4th CR) have undergone ABMT with chemopurged BM utilizing V (1.5 uM), VP-16 (50 ug/ml), and VPL (10 uM). The preparative regimen consisted of TBI (1200 cGY), VP-16 (1800 mg/m^2, 4 hr infusion), Cytoxan (60 mg/kg x 2), and VPL I.V., bolus (0.15 mg/kg + 0.005 mg/kg/min x 4 hr). The last 7 pts received in addition CSP (1 mg/kg/d, days 1-28) and alpha interferon (1 x 10^6 U/m^2/dose, M, W, F, x 6 months) after ANC recovery of >1000/mm^3 and platelet >10^5. Recovery of ANC >500 /mm^3 x 2 d (mean + SEM) was 25 ± 8.2 and for platelet >20,000/mm^3 31.8 ± 13.1. Of the 6 pts not receiving auto GVHD therapy, 2 pts relapsed 4 and 8 mo. post ABMT and both died with progressive disease (PD), 1 pt died +60 days with Aspergillosis, and 3 pts are with NED 17, 21, and 25 mo. post ABMT. Three of 7 pts receiving auto GVHD therapy relapsed 3, 10, and 11 mo. post ABMT, and all died with PD. The other 4 pts are with NED 4, 7, 8 and 11 mo. post ABMT. In summary, 6/13 patients are NED 3-25 months post ABMT. The use of chemopurging using V and VPL in combination with an induced modified auto GVHD/GVL response in ABMT remains a promising approach in treatment of high-risk or relapsed ALL.

G 126 "INTENT TO TRANSPLANT" PROTOCOL IN PEDIATRIC ACUTE NONLYMPHOBLASTIC LEUKEMIA. E.F. Saunders, S. Calderwood, M.H. Freedman, J. Doyle. Division of Hematology-Oncology. The Hospital for Sick Children,Toronto,Canada.

We adopted an "Intent to transplant" protocol in which all patients (pts) with newly diagnosed, primary ANLL underwent either allogeneic (allo) or autologous (auto) BMT in first clinical remission (CR1). Between 06/87 and 01/92, 26 pediatric pts were eligible for treatment on this protocol. Remission induction and consolidation were done according to the CCG213 protocol. Pts with an HLA matched sibling donor then received an allo BMT, while pts with no donor received an auto BMT. The preparative regimen consisted of Busulfan (16mg/Kg for pts >2 years, 20 mg/Kg for pts <2 years) and Cyclophosphamide (200 mg/Kg). Marrow was not purged.

Results. The overall survival of the group is 38% (FU 17-66 mo). Five pts (19%) failed to achieve remission and all died of progressive disease. Six pts (23%) received a allo BMT at a mean of 5.6 mo (range 3-7 mo) from diagnosis (dx). Two of these patients are alive in CR1 66 mo and 48 mo from dx. One pt relapsed 18 mo from transplant, but is now in second remission (CR2) 6 mo following a 2nd allo BMT. Two additional pts relapsed 6 and 4 mo from transplant and both died of progressive disease. One pt died of fungal infection in the immediate post transplant period (overall survival at 24-66 mo; 50%).

Fifteen pts were eligible for auto BMT. Of these, seven underwent the procedure at a mean of 8.4 mo (range 5-12 mo) from dx. All are alive in CR1 at a mean of 31 mo (range 17-57 mo) from dx. The remaining eight pts all relapsed prior to undergoing the procedure at a mean of 6.5 mo (range 508 mo), from dx. All died with progressive disease. (Overall survival; 47%).

Conclusions. In our series, the long delay from diagnosis to BMT resulted in a large percentage of auto BMT candidates relapsing prior to undergoing the procedure. This introduced a selection bias which made interpretation of the role of BMT in CR1 difficult. In an attempt to ensure that a majority of pts achieving CR1 undergo BMT, our protocol has been revised to perform the procedure at 4 mo from dx. This will allow better assessment of the contribution of BMT to overall survival in childhood ANLL.

G 127 IMPROVEMENT IN THERAPEUTIC RATIO OF TOTAL BODY IRRADIATION, Huibert M. Vriesendorp, The University of Texas M. D. Anderson Cancer Center Houston, Texas 77030

Total body irradiation (TBI) is an effective conditioning agent for bone marrow transplantation (BMT). The morbidity and mortality rate of BMT remains high and could be decreased by improved TBI applications. Further progress requires integration of physicists, radiobiologists and radiotherapists in human BMT teams.

Simplification and Dosimetric Control

High energy beam, long treatment distance, scatter plate, AP/PA orientation, rice bag bolus for missing tissue, flat beam profile at treatment distance, lung blocks are all providing simiplier TBI set ups with better dosimetric control.

Radiobiology

TBI can be optimized for acute side effects and immunosuppression. Fractionated TBI will have a higher therapeutic ratio than single fraction TB1. Due to a significant shoulder in the lymphocyte radiation survival curve, large fraction size TBI (>2 Gy per fraction) can be shown to be more effective. The highest possible dose rate is the most practical one for fractionated TBI. More than one fraction per day is not necessary. Treatment time can be limited to less than 4 days.

Interactions with Non-TBI BMT Parameters

Higher TBI doses are needed for T cell depleted grafts, HLA mismatched grafts. The concept of reciprocal interference between Host Versus Graft (HVG) and Graft Versus Host (GVH) reaction applies. Decrease in GVH reactions will require more HVG suppression by radiation. More HVG suppression will increase GVH reactions.

Dose Inhomogeneity

Uniform TBI has a lower therapeutic ratio than some types of inhomogenous TBI. The volumes in the body that require more or less radiation than the prescribed dose will differ for different clinical situations and remain to be defined. Radiolabeled immunoglobulin therapy can enhance TBI effects at an excellent therapeutic ratio. Intravenous administration of bone seeking radio isotopes as part of BMT conditioning is not expected to improve current results.

Conclusion

Important improvement in the therapeutic ratio of BMT can be obtained by a step by step rational adjustment of TBI parameters. Dosimetric control, radiobiologic modeling and deliberate dose inhomogeneity are prerequisites for success.

G 128 RETROSPECTIVE ASSESSMENT OF THE QUALITY OF LIFE OF ALLOGENEIC BONE MARROW TRANSPLANT SURVIVORS: G.B. Adams, Y. Hao, G.M. Fyles, M.D. Minden, J.M. Meharchand, J.H. Lipton, H.L. Atkins, I. Tejpar, H.A. Messner, Depts of Medicine, The General Hospital, St. John's, Newfoundland and The Ontario Cancer Institute, Toronto, Canada
We surveyed all surviving recipients of allogeneic BMTs performed at the Princess Margaret Hospital, for a variety of quality of life measures. As of mid April, 1993, 251 patients were eligible with a post BMT interval ranging from 1 to 252 months. Of these, 2 died prior to receiving the questionnaires. Of the 249 remaining, 3 were lost to followup, 3 refused to participate and 11 patients did not return their forms for unknown reasons. A total of 232 completed questionnaires were received (93%). The set of questionnaires included the Medical Outcome Survey Short Form - 36, the Satisfaction with Life Domains Scale - Cancer and a third tool devised by the investigators containing demographic items and a symptom experience report. For test re-test reliability assessment, a randomly selected subset of 100 patients were asked to complete the questionnaire on a second occasion after a 2 week interval. One patient died prior to receiving the 2nd questionnaires, and of the remaining 99, 2 were lost to followup at the time of the first set of questionnaires and 70 or 72% returned the questionnaires. Twenty-seven patients did not return the forms for unknown reasons. We will present the results.

G 129 Characterization of Human Primitive Hematopoietic Progenitors by Rhodamine 123 Staining.
Jesse Combs, Shirley Chen, and Nobuko Uchida, SyStemix Inc., 3155 Porter Drive, Palo Alto, CA 94304
The vital dye rhodamine 123 (Rh123) has been used to separate different subsets hematopoietic progenitors. Low levels of Rh123 staining ($Rh123^{lo}$) correlate with the primitive nature of hematopoietic progenitor cells. The Rh123 dye stains mitochondria preferentially, and is believed to reflect mitochondria numbers and/or activity. $Rh123^{lo}$ hematopoietic cells are thought to be quiescent. Chaudhary and Roninson showed, furthermore that Rh123 staining of human hematopoietic cells was inversely correlated with the expression of multidrug resistance (MDR1) gene product P-glycoprotein (P-gp) (Chaudhary and Roninson, Cell, 66, 1991). We have previously reported that a rare subset of Thy-1^+ Lin^- $CD34^+$ cells from human fetal bone marrow were highly enriched for candidate hematopoietic stem cells (Baum et al., PNAS, 89, 1992). We extended the phenotypic characterization of hematopoietic stem cells to adult bone marrow and mobilized peripheral blood. $CD34^+$ Thy-1^+ cells from adult bone marrow and mobilized peripheral blood contained substantial numbers of $Rh123^{lo}$ cells; high level expression of Thy-1 was inversely correlated with low levels of Rh123 uptake. We next analyzed the efflux of Rh123 by Thy-1^+ $CD34^+$ adult bone marrow cells. After being loaded with Rh123, Thy-1^+ $CD34^+$ cells were allowed to efflux Rh123 dye for various times. A drastic shift from $Rh123^{hi}$ towards to $Rh123^{lo}$ was observed by the Thy-1^+ $CD34^+$ subset. Within 3.5 hrs, Rh123 dye was completely dispersed from Thy-1^+ $CD34^+$ cells. The efflux was blocked by the P-gp inhibitor, verapamil, in a dose-dependent manner. We characterized the proliferative potential of $CD34^+$ Lin^- progenitors from adult bone marrow separated by Rh123 staining ($Rh123^{lo}$ vs. $Rh123^{hi}$). Both *in vitro* (LTBMC) and *in vivo* (SCID-hu bone engraftment) analysis revealed that "primitive" progenitor activity was highly enriched in the $Rh123^{lo}$ $CD34^+$ Lin^- subset and relatively depleted in $Rh123^{hi}$ $CD34^+$ Lin^- subset. We conclude that Rh123 dye exclusion by hematopoietic progenitors was almost certainly achieved by P-gp; and therefore that most primitive hematopoietic progenitors express high levels of P-gp or surrogate activity. In addition, we conclude that candidate human hematopoietic stem cell populations (Thy-1^+ $CD34^+$ Lin^- cells) from both adult bone marrow and mobilized peripheral blood are characterized by limited uptake of Rh123.

G 130 EXPRESSION OF CD33, CD2, CD7, CD10, OR CD19 ON $CD34^+$ BONE MARROW PROGENITOR CELLS INDICATES MULTILINEAGE POTENTIAL AND NOT LINEAGE-RESTRICTED COMMITMENT. Torstein Egeland, Geir E. Tjønnfjord, Ole P. Veiby and Rita Steen. Institute of Transplantation Immunology and Med. Dept. A, Rikshospitalet, The National Hospital, and Nycomed Bioreg, Oslo, NORWAY.
To determine lineage commitment of defined subsets of bone marrow progenitors, normal human bone marrow $CD34^+$ cells were sorted according to expression of CD34 and the myeloid-associated marker CD33, the T lineage-associated markers CD2 or CD7, and the B lineage-associated markers CD10 or CD19. $CD34^+$ cells coexpressing one of these markers could differentiation to mature myeloid and $TcR\alpha\beta^+$ and $TcR\gamma\delta^+$ $CD4^+$ and $CD8^+$ T cells *in vitro* after cultivation in the presence of adherent human thymus stromal cells. However, if the $CD34^+CD33^+$ cell subset also coexpressed the more mature myeloid-associated markers CD13, CD14, or CD15, only myeloid differentiation was achieved. Similarly, it was not possible to drive $CD34^+CD10^+$ or $CD34^+CD19^+$ cell subsets to T lymphoid or myeloid differentiation if they also coexpressed the later B lineage-associated marker CD20.

To conclude, normal bone marrow $CD34^+$ cells can still be multipotential when they express lineage-associated markers like CD33, CD2, CD7, CD10, or CD19. However, when the cells also gain expression of later lineage-associated markers like CD13, CD14, CD15 or CD20, i.e. belong to more mature subsets, they loose their multilineage potential and become committed for differentiation along fewer or only one lineage. Whether all or only certain subsets of maturing $CD34^+$ cell progenitors go through a stage of expression of one or more of the markers CD33, CD2, CD7, CD10, or CD19 *in vivo* is unknown, but the coexpression of these markers represents *per se* no defined lineage restriction.

G 131 IRON OVERLOAD WITH ABNORMAL LIVER FUNCTION IN LONG TERM SURVIVORS OF ALLOGENEIC & AUTOLOGOUS BMT HAS IMPLICATIONS FOR PERSISTING RISKS OF CHRONIC HEPATITIS & HEPATOMA.
I M Franklin., P McKay, J Murphy, S Cameron, A K Burnett, M Campbell, P Tansey. Bone Marrow Transplant Unit, Royal Infirmary, & Regional Virus Laboratory, Ruchill Hospital, Glasgow, U K.
We have traced and investigated sixty six patients who survived an autologous or allogeneic bone marrow transplant for a median of 36 months (range 7 - 141 months). They were assessed for liver dysfunction, iron overload (serum ferritin) and hepatitis C (HCV) exposure. There were 27 autografts and 39 allografts. GvHD was not considered an important cause of liver function abnormalities in the allografts because i) of the relatively long period elapsed after BMT and ii) twenty allografts (51%) were T-cell depleted and only 5 other patients had GvHD >grade 2 at any time. Fourteen patients are HCV antibody positive, all transplanted before September 1991. The range of serum ferritin levels was 139 - 5148, with a median of 1491 ng/ml. Liver dysfunction was assessed as hepatitis (ALT or AST >2 x normal) or abnormal (intermediate between hepatitis and normal). Ferritin >1000 ng/ml was found in 49 of 66 patients, and 12 of the HCV positive cases were in this group. Therefore 37 of 54 (68%) of HCV negative BMT survivors were significantly iron overloaded and of these 25 (68%) had abnormal or hepatitic liver function tests. The first four patients to have an adequate venesection trial have all had improved liver function as ferritin levels have reduced. Transfusion siderosis is a major cause of liver dysfunction in survivors of BMT. Iron loading in the pre- and peri-BMT period may have major implications for the quality of life of survivors, in terms of abnormal liver function, the need for iron depletion therapies and, probably, the very long term risk of hepatoma even in those negative for HCV. These issues need to be considered in the initial BMT work-up and counselling in order to minimise physical and psychologic complications.

G 132 CREATIVE ART IN A BONE MARROW UNIT,
John Graham-Pole, Mary Rockwood Lane and James Rodrigue, Departments of Pediatrics, Nursing, and Clinical and Health Psychology, University of Florida, Gainesville, FL 32610

We describe a project assessing the value of the creative arts in enhancing the overall health of patients undergoing bone marrow transplant (BMT). Our thesis is that giving these patients opportunity for artistic expression frees innate creative energy that can be a potent force for physical, emotional, and spiritual healing in such life-challenging situations.

The key component of this pilot project is the inclusion of professional artists in our BMT unit's healthcare team. Five artists have worked in the unit so far for 6-8 hours a week for periods of 2-3 months using fabric painting, sculpture and ceramics, music and song, poetry and prose-writing, and drama and clowning. They have begun their residencies with workshops at which artists and staff orient each other: the staff to the intensive care setting and the artists to their creative work. Further family workshops have followed before the artists have started working with patients in isolation one-to-one and with outpatients in small groups.

Assessments to date have included quantitative measures of patient functioning, behavior, compliance, depression, anxiety, pain, and quality of life; standardized evaluations by families, staff, and artists; and qualitative data from interviews and journals of patients, staff, and artists.

Measurable benefits have included a sense of accomplishment and self-validation; less stress, anxiety, and loneliness; improved morale and cooperation; staff leadership and advocacy in a new area of healthcare; and favorable publicity throughout the community. Remaining challenges include the need for thorough education of both staff and artists to each other's work; resistance to artistic self-expression by patients, families and staff; occasionally inappropriate dress and behavior on the part of artists; and limited funds and time for artists and staff.

G 133 DONOR SPECIFIC BONE MARROW (DSBM) INFUSION FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION. A RANDOMISED CONTROLLED STUDY. M.D. Hamon, A. Burroughs, B. Davidson, H.G. Prentice and K. Rolles. Liver Transplant Unit and Bone Marrow Transplant Unit, Royal Free Hospital, London, U.K.

Late complications of otherwise successful organ transplantation including opportunistic infection and secondary malignancy (lymphoma) are attributable to the immunosuppressive (IS) drugs given to prevent graft rejection. DSBM infusion following solid organ grafting has been shown to induce a state of tolerance/immuno hypo-responsiveness in a variety of animal models and more recently in man. At the Royal Free Hospital 9 of 25 patients were randomly selected to receive an infusion for cryo-preserved red cell depleted donor bone marrow (1.5-3.5 x 10^8 nucleated cells/kg) 5 days after completion of a 10 day course of ATG (Merieux), 2.5 mg/kg/day. Maintenance IS was with Cyclosporin A (whole blood levels between 50 and 150 nannograms/ml). 8 of the 9 patients (89%) receiving donor bone marrow are currently alive with the minimum follow up of 13 months which compares with 12 out of 16 control patients (75%). There have been 3 rejection episodes per patient in the bone marrow group compared with 3.125 in the control group. Studies of peripheral blood and bone marrow using fluorescent *in situ* hybridisation for the Y chromosome probe (male donor, female recipient), red cell antigen differences between donor and recipient demonstrated by flow cytometry, and nested polymerase chain reaction (pcr) using sequence specific primers for HLA Class II determinants have been used to look for evidence of donor engraftment (haematolymphoid chimerism). These methods have respectively sensitivities of approximately 1 cell in 30, 1 in 200 and 1 in 10^5. With the exception of one patient who showed transient evidence of donor lymphoid cells in the peripheral blood 2 days after bone marrow infusion (using nested pcr), all of the tests for chimerism have proven negative. No patient showed any features of graft-versus-host disease. Our results give no encouragement nor justification for the eventual withdrawal of immunosuppressive therapy in DSBM recipients (our initial objective).

G 134 MEGAKARYOCYTE PROGENITORS IN REMISSION BONE MARROWS IN PATIENTS WITH MALIGNANT BLOOD DISEASES: CLUES FOR THE SLOW PLATELET REGENERATION AFTER ABMT? Peter Hokland, Pia Mikkelsen & Marianne Hokland. Department of Hematology and Institute of Medical Microbiology, University of Århus, 8000 Århus, DK-Denmark.

It is well known that thrombocyte regeneration after autologous bone marrow transplantation (ABMT) is considerably slower than after syngeneic as well as allogeneic BMT. This seems to be especially true for acute myeloid leukemia, where recent studies have implicated poor marrow quality to either high-dose TBI or harvest of marrow during thrombocytopenia. We have sought to address this issue further 1) by measuring megakaryocyte precursor cells (CFU-Meg and CFU-GEMM) in patients in remission after high-dose chemotherapy for various malignant blood diseases, and 2) by determining precursor cells at all steps of bone marrow harvest separation- and cryopreservation procedures. Our results so far suggest that the megakaryocyte progenitor cell fractions are lower in ALL (28-34%) and AML (51-63%) patients compared to normal donors in steady-state. While these modest decreases do not explain the slow platelet recoveries in AML after ABMT, the normal levels of precursor cells in high-grade lymphoma patients are compatible with the relatively fast recovery of platelets in these patients. By trailing the absolute numbers of precursor cells during marrow harvests in hemolysed marrows, after buffy coat preparation, MNC preparation and in cryopreserved aliquots in more than 20 marrow harvests, we have found no evidence for selective loss of megakaryocyte progenitor cells in any patient group. Indeed, the Cobe blood separator procedures employed here seem to influence cells from acute leukemias and lymphomas equally, with the major part of CFU-GEMM/CFU-Meg losses happening during buffy coat preparation. While the accrual of more patients is clearly needed to better identify patients with low *a priori* precursor cell frequencies (e. g. those heavily treated with high dose Ara-C), we conclude at this stage that no obvious explanation is available for the slow platelet regeneration after ABMT for AML.

G 135 DOSE ESCALATION STUDY OF CARBOPROST FOR HEMORRHAGIC CYSTITIS (HC) AFTER BONE MARROW TRANSPLANTATION (BMT), Cindy Ippoliti, Donna Przepiorka, Rakesh Mehra, Joyce Neumann, James Wood, Koen Van Besien and Colin P. Dinney, Department of Bone Marrow Transplantation and Department of Urology, M.D. Anderson Cancer Center, Houston, TX 77030.

HC is a potentially life-threatening complication of high-dose chemotherapy and occurs in up to 13% of BMT patients despite hyperhydration and mesna. Because no effective therapy exists for patients who fail to respond to continouous bladder irrigation (CBI), we initiated a dose-escalation trial of carboprost bladder instillation for HC after BMT. Carboprost tromethamine (CT), a prostaglandin F_2-analog, has shown efficacy for refractory HC in a limited number of patients. It works by causing smooth muscle contraction of the blood vessels in the bladder wall. Eleven patients (allo-10, auto-1) with culture-negative grade 3 hematuria (clots) occurring 3-56 days after BMT were treated with CT. All patients had failed therapy with CBI and platelet transfusions and 2 patients failed formalin instillation. The duration of HC prior to CT was 2-104 days. CT was initiated 5-143 days post BMT. CT was instilled in the bladder x 60 minutes 4 times daily. CT was started at 0.2mg% and increased by 0.2mg% every 24 hours to a maximal concentration of 1.0mg% or until complete response occured. Response was defined as resolution of macroscopic hematuria x 48 hours. Treatment could not exceed 14 days. CBI with hydrocortisone 0.01% and aggressive platelet support was continued throughout CT therapy. 6/11 patients responded at 0.4mg%, 0.6mg%, 0.8mg%(3) and 1.0mg%. 5/6 relapsed 1-17 days after response. 2/5 patients who relapsed responded briefly to alum irrigation. Five non-responders did not respond to other measures. Overall, 5/11 died with HC. Bladder spasms requiring narcotic analgesics occured in all patients at all levels. No other toxicities were seen. CT has activity in the treatment of HC refractory to CBI. Further study is warranted with early initiation of CT at a dose of 0.8mg% for a phase II study.

G 136 LAPAROSCOPIC LIVER BIOPSY IN SEVERELY THROMBOCYTOPENIC BONE MARROW TRANSPLANT PATIENTS: A SAFE AND UNIQUE APPROACH, Hillard M. Lazarus, Richard J. Creger, Robert M. Fox, Brenda W. Cooper, Gretta Jacobs, Thomas A. Stellato, Departments of Medicine, Pathology, Surgery, Ireland Cancer Center of University Hospitals of Cleveland, Case Western Reserve University, Cleveland, Ohio 44106

The use of intensive cytotoxic drug therapy for malignant disorders often results in hepatic dysfunction, but neutropenia and thrombocytopenia may prevent performing liver biopsy to establish a cause. We prospectively evaluated the safety and utility of laparoscopic liver biopsy to assess the cause of hepatic dysfunction in 20 consecutive patients who were receiving intensive cytotoxic therapy with or without bone marrow transplantation, or who were being treated for severe aplastic anemia. One to three direct-vision laparoscopic liver biopsies were performed in each patient using a Tru-Cut® needle during general anesthesia. Platelet concentrate transfusions were given before, during, and immediately after the biopsy, and bleeding after removal of the biopsy needle was controlled with spatula electrocautery.

Platelet and white blood cell counts at the time of liver biopsy ranged from 1,000-83,000/uL (median: 23,500/uL) and 0-14,300/uL (median: 2,200/uL), respectively. Nineteen of 20 patients had platelet counts less than 68,000/uL. Bleeding at biopsy site was completely controlled during the procedure without any evidence of post-biopsy bleeding or complications. Biopsies demonstrated graft-versus-host disease, hepatic veno-occlusive disease, steatosis, cholestasis, hemosiderosis, or granuloma.

Laparoscopic liver biopsy is a safe procedure, even in the severely thrombocytopenic, immunocompromised patient. In several patients the knowledge derived from this invasive technique altered therapeutic strategy.

G 137 THE RELATIONSHIP BETWEEN HEMOLYTIC-UREMIC SYNDROME AND RADIOTHERAPY IN PATIENTS WITH NEUROBLASTOMA AFTER BONE MARROW TRANSPLANT, Hideo Mugishima [1], Toshiaki Shimada[1], Takashi Suzuki[1], Motoaki Chin[1], Takahito Fujisawa [1], Kensuke Harada[1], Ikuo Okabe[2], Toshio Maeno[3] and Eiichi Sanuki[3]
1)Dept. of Pediatrics, Nihon University School of Medicine, 2)Dept. of Pediatric Surgery, Nihon University School of Medicine, 3)Dept. of Radiology, Nihon University School of Medicine, Tokyo 173, JAPAN

Having identified hemolytic uremic syndrome(HUS) developed in patients with neuroblastoma(NB) after autologous bone marrow transplant(A-BMT), we discussed the relationship between HUS and radiotherapy in addition to the prophylaxis of HUS. Five of 30(17%) patients developed HUS within 4.5 months post A-BMT. The prognosis of these patients have various course ranging from those patients recovered by conventional therapy to the ones died of renal failure.
All patients developed HUS received CDDP and total body irradiation(TBI) for preconditioning regimen, however, no patients whose kidney were shielded from TBI developed this syndromde. The difference between the two proves to be statistically significant. (p=0.008) It is postulated that TBI be the most important factor causing HUS. It would be crucial to shield the kidney from TBI to prevent development of HUS.

G 138 **GLUTAMINE SUPPLEMENTATION IN BONE MARROW TRANSPLANTATION** Christopher H. Poynton, Tim S. Maughan, Jane Hanson and Una O'Callaghan. Departments of Haematology and Oncology, University Hospital of Wales, Cardiff, CF4 4XN, UK.

Glutamine is a major substrate for the intestinal mucosa providing up to 40% of its energy utilization. It is not routinely included in hyperalimentation regimens. In animal models, depletion of glutamine leads to mucosal ulceration, bloody diarrhoea and eventual death, whereas supplemental glutamine improves gastrointestinal mucosal cellularity and recovery after chemotherapy. In BMT, the breakdown of gastrointestinal integrity allows endotoxin to enter the systemic circulation, potentiating GVHD, and can lead to multiorgan damage. Preservation of gut integrity during the first few weeks after BMT is therefore critical.

We have administered the dipeptide Glycyl-L-glutamine (GLG) which is metabolised rapidly to glycine and L-glutamine, intravenously to patients undergoing autologous and allogeneic bone marrow transplantation. Patients received 50g GLG in 400ml 0.45% Saline daily over 12 hours or (double blinded) an isonitrogenous mixture of amino acids giving a total of 10.3g Nitrogen daily for up to 28 days or at discharge from the unit. The decisions to introduce standard parenteral nutrition in addition were made independently of this protocol. As well as clinical endpoints, gut integrity measurements with lactulose/mannitol, TNF/IL-6 profiles and endotoxin levels are performed.

Unlike previous studies, we elected to start the GLC/placebo supplement prior to receiving the high dose chemotherapy, in order to maximize the chance of detecting a real difference in efficacy. Since glutamine is also a substrate for lymphoid tissue, we investigated *in vitro* the protective affect of L-glutamine on tumour cell lines (WIDR, MOLT4, K562, DAUDI, RAJI) and patient's leukaemic cells, after *in vitro* exposure to radiation, melphalan and chlorambucil by MTT and colony forming assays. Although WIDR (colon carcinoma) showed a marked protective effect with glutamine after melphalan, there was no difference with the haematological cell lines.

In conclusion, GLG/placebo given to patients prior to and after BMT appears safe, and we have no evidence that L-glutamine protects the cells from the antitumour therapy in lymphoid malignancy. The analysis of the potential benefits of GLG supplementation vs placebo in BMT will be analysed at 6 monthly intervals during the study.

G 139 PATIENTS ≥ AGE 40 UNDERGOING AUTO- OR ALLO-BMT HAVE REGIMEN-RELATED MORTALITY RATES AND EVENT-FREE SURVIVALS COMPARABLE TO PATIENTS < AGE 40, Aaron P. Rapoport, Jacob M. Rowe, Peter A. Kouides, Beth A. Martin, Michael Sherman, Martin A. Tanner, and John F. DiPersio, Departments of Medicine and Biostatistics, University of Rochester, Rochester NY, 14642

Between Sep. 1987 and Aug. 1993, 390 transplants were performed at the University of Rochester Medical Center. The 86 allotransplants and 304 autotransplants included 101 for acute leukemia, 21 for CML, 54 for breast cancer, 143 for lymphoma, and 71 for a variety of other malignancies. Since a significant proportion of these patients were ≥ age 40 at the time of transplant, we examined whether age had a significant impact on regimen-related mortality (RRM) or event-free survival (EFS). Among the patients receiving allotransplants, 56 were < age 40 [range: 2-39, median: 27] and 30 were ≥ age 40 [range: 40-61, median: 46]. The 2-year actuarial EFS for the younger patients is 29% compared with 51% for the older age group (p=.13). The RRM was 25% for the younger patients and 17% for the older patients (p=.42) and the mortality rates from GVHD were 26% and 12% respectively (p=.22). Among the 143 autotransplants for lymphoma, 71 patients were < age 40 [range: 9-39, median: 30] and have a 4-year actuarial EFS of 48% compared with 49% for the 72 patients ≥ age 40 [range: 40-70, median: 48] (p=.96). The latter group included 24 patients ≥ age 55, and 11 patients ≥ age 60. The RRM for lymphoma patients < age 40 was 14% compared with 4.2% for patients ≥ age 40 (p=.046). The median age for all the patients receiving transplants for breast cancer was 44 [range:31-57] and RRM occurred in 2 patients (4%) aged 33 and 37. The incidence and morbidity from severe mucositis, VOD of the liver, GVHD, neutropenic infections, and interstitial pneumonitis were comparable in both age groups. While the older patients referred to transplant centers may represent a highly selected group, this database confirms other reports suggesting that high-dose chemoradiotherapy is as safe and efficacious in this group as it is in younger age groups.

G 140 CIRCULATING CFU-E DURING HEMATOPOIETIC RECOVERY AFTER BONE MARROW TRANSPLANTATION: RELATIONSHIPS TO ERYTHROID ENGRAFTMENT, Alessandro M. Vannucchi, Alberto Bosi, Alberto Grossi, Patrizia Bacci, Stefano Guidi, Riccardo Saccardi, and Pierluigi Rossi-Ferrini, BMT Unit, Dept Hematology, Univ of Florence, Florence, Italy

Colony-forming unit, erythroid (CFU-E) is the more mature erythroid progenitor forming hemoglobinized colonies in vitro, and unlike burst-forming unit (BFU-E) is normally found only in the bone marrow (BM) and not in the peripheral blood (PB). During studies of erythroid engraftment in pts undergoing allogeneic bone marrow transplantation (BMT), we observed the appearance of circulating CFU-E in 5/15 pts under study. PB CFU-E were found as early as day +14 after BMT (35 ± 29 x10^9 cells plated), reached the maximum at day +30 (92 ± 18), but were still present on day +60 (range 5-80) in 3/5 pts. Pts were divided into two groups according to the presence, or absence, of PB CFU-E, and compared each other about some engraftment parameters. Time to granulocyte or platelet engraftment, and number of platelet unit transfused was similar in the two groups. However, pts with PB CFU-E required 14 vs 17 days after BMT to reach a reticulocyte count $\geq 30 \times 10^9$/L ($p=0.04$), and the maximal number of reticulocytes within the first 30 days after BMT was higher (141,000 vs 98,000x10^9/L; $p=0.04$). There was also a trend towards both a faster erythroid engraftment (a Htc >30% was reached on day +18 vs day +23, $p=0.054$) and the attainment of transfusional independence (18 vs 23 days after BMT, $p=0.06$). Moreover, pts with PB CFU-E received only 2.0 vs 4.8 RBC unit transfusions ($p=0.027$) in the first month after BMT. The frequency of both PB and BM BFU-E during the study period was similar in the two groups. These data suggest that pts presenting with circulating CFU-E after BMT may have faster erythroid engraftment than the others.

G 141 CYTOCHROME P4503A ACTIVITY MAY PREDICT RISK OF HEPATIC VENOOCCLUSIVE DISEASE (VOD) AFTER BONE MARROW TRANSPLANTATION (BMT), Gary C. Yee, Rodney J. Franey, Paul B. Watkins, Gary L. Davis, Alan M. Miller, and Roy S. Weiner, Colleges of Pharmacy and Medicine, University of Florida, Gainesville, FL 32610 and College of Medicine, University of Michigan, Ann Arbor, MI 48109.

Marker drugs such as antipyrine have been reported to be useful indicators of liver function and predictors of outcome in patients with liver disease. The erythromycin breath test (ERMBT) is a noninvasive and specific test of the activity of cytochrome P4503A enzymes, which metabolize many drugs including anticancer drugs. P4503A activity may therefore correlate with the systemic exposure of anticancer drugs in the preparative regimen. We initiated a pilot study to identify factors that may influence P4503A activity and to determine whether the ERMBT can identify patients at high risk for hepatic VOD after BMT. Fifteen patients 19 to 52 years old (median: 36) undergoing autologous or allogeneic BMT have been studied. The ERMBT was performed before the preparative regimen was given (baseline) and at various times post-transplant. Median ERMBT increased from 2.30% at baseline to 3.42% on day 3 post-transplant ($p = 0.06$). Six patients developed hepatic VOD, and three of these patients have died. Five of seven patients with a baseline ERMBT value of < 2.25% developed hepatic VOD, compared with only one of eight patients with a baseline value of > 2.25% ($p = 0.04$). Predicted ERMBT (based on the 20 min sample) correlated with the actual ERMBT (based on samples collected over 60 min) ($r^2 = 0.98$), which shows that a single breath sample at 20 min is adequate. Our results suggest that P4503A activity is increased after high-dose chemotherapy and that patients with a ERMBT < 2.25% may be at increased risk for hepatic VOD. Additional studies are needed to confirm these preliminary results and to determine the mechanisms involved in these observations.

Unrelated Donors, Mismatched BMT; Genetic and Immunologic Diseases; Animal Models Engraftment and GVHD; Pediatric Malignancies; Myeloma

G 200 HLA SEQUENCING PROVIDES IMPROVED INFORMATION TO EXAMINE THE IMPACT OF HLA DISPARITY ON BONE MARROW TRANSPLANTATION, L.A. Baxter-Lowe, D. Dinauer, T. Daniels, N. Flomenberg and C. Keever. The Blood Center of Southeastern Wisconsin and The Medical College of Wisconsin, Milwaukee, WI 53223

Bone marrow transplantation is the treatment of choice for a variety of hematological disorders but use of this treatment alternative is often limited by lack of a suitable marrow donor. It is likely that this limitation can be ameliorated by understanding the relative immunogenicity of each HLA disparity. It has been difficult to assess the relative immunogenicity of each HLA disparity in unrelated individuals because conventional HLA typing does not have sufficient resolving power to detect all HLA disparity or to accurately assess the similarity of two disparate alleles. This study addresses this problem by unequivocally detecting HLA disparity through determination of the nucleotide sequences of each HLA allele. A solid-phase automated sequencing method was developed to make it feasible to determine the sequences of HLA alleles of marrow recipients and donors. Solid phase sequencing is achieved by selective amplification of individual HLA alleles using biotin-primers, capture of amplified DNA on streptavidin-coated magnetic beads, and use of the captured DNA as templates for primer extension in the presence of dideoxy terminators labeled with fluorescent dyes. Nucleotide sequences are determined using an ABI 373A automated sequencer. Partial sequence data from HLA genes of eight donor-recipient pairs have been determined. Disparity that was not evident using conventional HLA typing has been observed. The functional importance of these disparities is being investigated through the use of assays to determine the frequency and specificity of cytotoxic and helper T lymphocyte precursors. It is now feasible to precisely examine the impact of particular HLA disparities in order to identify those HLA disparities that may be tolerated. This information could increase the number of suitable donors by identifying compatible donors who are not HLA identical.

G 201 BONE MARROW TRANSPLANTATION (BMT) FOR APLASTIC ANEMIA (AA) USING UNRELATED DONORS (UD). Bruce Camitta, James Casper, Daniel Pietryga, Frederick Garbrecht, Robert Ash, Carolyn Keever, Kevin Murray, LeeAnn Baxter-Lowe, and Neal Flomenberg, Medical College of Wisconsin, Children's Hospital of Wisconsin, Milwaukee, WI 48226

Success of UD BMT for AA has been limited by high rates of graft rejection and graft vs host disease (GVHD). To overcome these problems we have used intensive conditioning (cytosine arabinoside [3gm/m^2 q 12 hr x 6, days -7 to -4], cyclophosphamide [45mg/m^2, days -6 and -5], total body irradiation [1320-1440 cGy/6-9 fractions/days -2 to 0]) and intensive GVHD prophylaxis (1.5 log T-cell depletion [anti-alpha/beta T-cell receptor monoclonal antibody T10B9 plus complement], cyclosporine, methylprednisolone). Sixteen children (ages 1-16[median 6] yr; 9 male/7 female) with severe AA were treated. Diagnosis to BMT interval was 3-90(median 9) months. 14/16 were heavily transfused. Prior therapy included antithymocyte globulin (14), cyclosporine (11), androgens (4), and GMCSF (2). HLA-A,B,D region typing was performed by serologic techniques and mixed lymphocyte culture (3 patients) or high resolution oligotyping (13 patients). Donors and recipients were HLA-A,B,D identical (5) or mismatched for one A (4) or B (7) antigen. Five donor-recipient pairs were sex mismatched. 15/16 patients engrafted. Acute GVHD >grade 1 occurred in 6/15 at risk (grade 2 [5], grade 3 [1]). Limited chronic GVHD confined to the skin occurred in 5/11 at risk. Ten patients survive 1^+-73^+ (median 26^+) months. Causes of death were: non-engraftment (day 58), venocclusive disease (day 86), GVHD (day 99), B-cell lymphoproliferative disease (day 210), Pneumocystis carinii pneumonia (day 127), and infection plus ARDS (day 170). Rates of engraftment, GVHD and survival with this intensive regimen are amongst the best seen for UD BM of AA. The contributions of intensive pretreatment and T-cell depletion to this success must be elucidated. UD BMT is an appropriate option for children with a matched sibling donor who fail an initial 4 month trial of immunosuppression.

G 202 BONE MARROW TRANSPLANTATION (BMT) FOR CHILDHOOD ACUTE LYMPHOCYTIC LEUKEMIA (ALL) USING UNRELATED DONORS. J. Casper, B. Camitta, L.A. Baxter-Lowe, N. Bunin, R. Ash, D. Pietryga, F. Garbrecht, K. Murray, C. Lawton, R.Truitt, C. Keever, N.Flomenberg. Medical College of Wisconsin, Children's Hospital of Wisconsin and the Blood Center of SE Wisconsin, Milwaukee, WI.
Combination chemotherapy can cure >65% of children with ALL. Patients who relapse during, or <6 months after stopping therapy, and patients with Ph+ ALL in first remission, have <10% survival with chemotherapy alone. Optimal treatment of these patients requires BMT but only 25% have an HLA matched sibling. Between April 1987 and July 1993, 54 children (ages 1-17 years [median 7]) received T-cell depleted BMT from unrelated donors for the treatment of ALL in CR1(10), CR2(19), >CR2(11 in remission, 14 in relapse). There were 31 males and 23 females. Unrelated donors were selected by HLA serology (HLA-A,B,DR, and DQ) plus MLC and/or high resolution oligonucleotide genotyping for DR and DQ. Mismatches occurred in 63% (34 cases): HLA-A(20), -B(10), -D(3) or multiple loci(1). Intensive conditioning included cytosine arabinoside [3gm/m^2 q 12 hr x 6, days -7 to -4], cyclophosphamide [45mg/m^2, days -6 and -5], and total body irradiation [1320-1440 cGy/6-9 fractions/days -2 to 0]. Higher risk patients also received busulfan (2-4mg/kg/day, x 2 days -9,-8). Graft versus host disease (GVHD) prophylaxis included 1.5 log in vitro T-cell depletion (anti αß T-cell receptor monoclonal antibody T_{10} B_8 plus complement) and post BMT cyclosporine. The median number of viable nucleated cells infused after T-cell depletion was 1.6 x 10^8 cells/kg (range 0.4 - 4.8 x 10^8 cells/kg). All 54 patients engrafted. The median time to PMN >500 /mm^3 was 18 days (range 12 - 42 days). Acute GVHD (≥ II) occurred in 15 pts. but was severe (III,IV) in only two. Chronic GVHD was limited(23) extensive(3) or absent(12) in the 38 evaluable patients. Overall event free survival (EFS) was 44 ± 7% with median follow-up of 16 months. EFS for patients in CR1(68%) or CR2(58%) was better than for patients >CR2 (23%) ($p < .03$). There was no significant difference in EFS between matched (50%) and mismatched transplants(40%). Primary causes of death included: toxicity(12), relapse(10), infection(4), lymphoproliferative disease(2) and GVHD(1). These results compare favorably with HLA matched sibling transplants for ALL and hopefully will encourage the earlier use of unrelated donor BMT for appropriate children with ALL in CR1 or CR2.

G 203 MARROW UNRELATED DONORS FOR THE TREATMENT OF ACUTE AND CHRONIC LEUKEMIA USING A HIGH DOSE CYCLOSPORINE A/PREDNISONE GvHD-PROPHYLAXIS PROTOCOL, A. A. Fauser*,J. Finke, J. Heinz, H. Bertz, R. Mertelsmann. Department of Hematology/Oncology, Med.Universitätsklinik Freiburg, Germany
Acute graft versus host disease (aGvHD) is a major cause of morbidity and mortality during the first months after allogeneic bone marrow transplantation. Despite of GvHD-prophylaxis with combinations of cyclosporine, methotrexate or steroids, aGvHD of grades II-IV develops in 30% to 40% of the recipient of nonmanipulated HLA-identical marrow grafts. 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 or even subtherapeutic. The objective of this study was to determine whether cyclosporine started at day -3 at a concentration of 3.5 mg/kg i.v., twice daily, over a period of 4 hours in combination with prednisone given at day 7 would reduce the incidence and severity of acute GvHD in HLA-identical family-unrelated transplants. 25 patients, 15-53 years old, with acute or chronic leukemia (8 AML, 3 ALL, 13 CML, 1 MDS), were transplanted. All patients received an HLA-identical family unrelated donor marrow. All patients tolerated the immunosuppressive therapy. At present time 15 patients are alive and well. 2 patients with AML transplanted in a second partial remission relapsed after 11 and 17 months post transplant. 8 patients died with uncontrolled GvHD. The results suggest that bone marrow transplantation with HLA-identical family unrelated marrow donors using a modified GvHD prophylaxis protocol might generate a similiar outcome compared with family related transplants.

G 204 THE SELECTION OF UNRELATED DONORS FOR BONE MARROW TRANSPLANTATION, Michel Jeannet, Eddy Roosnek, Jean-Marie Tiercy and Daniel Speiser, Transplantation Immunology Unit, Division of Immunology and Allergology, Hôpital Cantonal Universitaire, Geneva, Switzerland.
Histocompatibility between donor and recipient is of major importance for the successful outcome of bone marrow transplantion. Tissue typing by serology that is usually adequate to assess compatibility between sibling donors recipients is however able to type for a limited number of HLA antigens only. Therefore more discriminative techniques are needed to assess optimal histocompatibility between the patient and his unrelated potential donor. To obtain a compatibiliy testing of higher resolution we combined a "molecular class II typing" technique recently developed in our laboratory with a functional assay that detects the reactivity of donor cytotoxic lymphocyte precursors (CTLp) specific for recipient incompatible alloantigens. The results show that in about 50% of the donor/recipient combinations matched by serology, molecular typing still detected DR incompatibilites. Furthermore, among 30 % of the remaining truly compatible cases, a CTL-alloreactivity could be explained by serologically undetected class I polymorphism (subtypes of HLA-A2, B7, B35 and B44). Using these very strict histocompatibility criteria, it was possible to identify a "perfectly" matched donor for only 33% of the patients for whom a serologically ABDR matched donor was identified. It is therefore of utmost importance for the future, to discriminate acceptable from unacceptable mismatches using high resolution HLA typing and precise follow-up studies.

G 205 PROPOSED LOCALIZATION OF GENES ENCODING MINOR HISTOCOMPATIBILITY ANTIGENS, James C. Jenkin, Michael I. Gubarev, and Patrick G. Beatty, Bone Marrow Transplant Program, University of Utah Health Sciences Center, Salt Lake City, UT 84132
While the role of minor histocompatibility antigens in graft-versus-host disease has been appreciated for some time, aspects of their biology have limited knowledge of the number and polymorphism of loci involved. First, T-cells recognizing minor antigens generally require in-vivo priming, e.g. in transplantation. Second, T-cells recognize minor antigen in the context of a single HLA restricting antigen, making genetic analysis difficult. Our approach circumvents these limitations by cloning T-cells reactive against pretransplant patient EBV transformed lymphoblastoid cell lines (LCL) from the circulating blood of transplant recipients with documented GVHD. The restricting MHC antigen for these clones is determined by reactivity against a panel of LCL, after which the cDNA encoding the MHC restricting element can be transfected into LCL targets. From patient one (data below), we isolated two T-cell clones restricted by HLA-A1. Reactivity to a panel of HLA-A1 positive LCL shows that each clone recognizes a different minor antigen. Transfection with a plasmid containing the HLA-A1 cDNA renders some (not all) cells positive. Work is in progress to transfect LCL of several large families which have been saturation mapped for a number of polymorphic gene markers, thus allowing localization of the MHC loci with respect to these markers.

Percent Lysis of Targets by T-Cell Clones

Target	HLA Antigens	BB11	AD9
Patient	A1,26;B35,51	37%	34%
Donor	A1,26;B35,51	N	3
BM15	A1,1 ;B49,49	39	N
BM21	A1,1 ;B37,37	N	35
TF	A1,1 ;B8, 8	N	41
Sib1	A2,26;B35,51	N	N
Sib1+A1	A1,2,26;B35,51	32	3

G 206 STRATEGIES FOR THE INTERNATIONAL COMMUNICATION BETWEEN BONE MARROW DONOR HUBS – REPORT AND CONCEPTS DEVELOPED IN EMDIS, C. Müller, E. Fischer, S. F. Goldmann*, S. Cleaver, L. Canham**, A. Baouz and C. Raffoux***
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** The Anthony Nolan Research Centre, London, UK
*** France Greffe de Moelle, Paris, France

Due to the high degree of polymorphism of the MHC loci relevant in bone marrow transplantation, the international co-operation between all institutions involved in the search for unrelated donors has always been a crucial issue. EMDIS (European Marrow Donor Information System) is a project based on the joint efforts of 12 European Hubs that want to create an open, fully-automated communication system between their national computer systems in order to speed up searches, to reduce administrative overhead and to facilitate the co-operation through more standardized procedures. EMDIS consists of a system of individually exchangeable layers and modules all adhering to international standards (e.g. TCP/IP, UUCP, Unix). They are designed to connect heterogeneous databases through a loosely coupled system in an international WAN. To the participating hubs, EMDIS provides either the necessary front-end-software or tools to create a direct interface into the national computer system.

Currently, the system design as well as the selection and development of tools are complete and the project is in its implementation and test phase.

EMDIS is supported by the European Community (AIM A 2006) and by national grants.

G 207 DOES HIGH DOSE METHYLPREDNISOLONE (MP) REDUCE THE INCIDENCE OF CHRONIC GRAFT VERSUS HOST DISEASE (cGVHD) ASSOCIATED WITH MATCHED UNRELATED (MU) BONE MARROW TRANSPLANTATION (BMT)? Mary Beth Oblon, David J. Oblon, Lesley Myers, Departments of Nursing and Medicine, Roger Williams Hospital, Brown University, Providence, R.I. 02908, Department of Medicine University of Florida, Gainesville, Fl. 32610.

There is a high incidence of aGVHD and cGVHD associated with MU BMT. We treated aGVHD in 11 patients (pts) undergoing MU BMT with MP, an effective regimen for aGVHD in matched sibling BMT. The regimen was: MP [5mg/kg/day(D) x 4D]; if response R_x X 8 D, then taper 20% q8 D; if no response by D 4, then escalate to 10mg/kg/D. Evaluate as detailed above. Twelve pts received MU BMT. Median age was 29 years (range 21-36); male:female 8:4; diagnoses: chronic myelogenous leukemia: 10 pts, acute leukemia: 2 pts. The conditioning regimen was cytoxan/total body irradiation (1200 Gy for 10 nonT-Cell depleted pts and 1350 Gy for 2 partially T-Cell depleted pts). aGVHD prophylaxis was short course Cyclosporin and methotrexate for all pts. One pt died an early death; 11/11 pts at risk developed severe aGVHD (Grade II-IV). The median time of onset of aGVHD was 13 D (range 11-53). One pt died of sepsis at time of diagnosis of aGVHD. Ten of 10 pts responded to MP; 5/10 died before day +100. Of the 5 pts at risk for cGVHD, one developed severe cGVHD and died with sepsis. The remaining 4 pts are alive at 12+, 23+, 41+, and 43+ months respectively without any evidence of cGVHD.. Although the number is small we speculate that MP can reduce the incidence of cGVHD in MU BMT.

G 208 FACTORS INFLUENCING GRAFT FAILURE AFTER ALLOGENEIC BMT, J.A. Russell, C. Brown, A.R. Jones, B.A. Ruether, R.C. Woodman, M-C. Poon, Alberta Bone Marrow Transplant Program, Foothills Hospital and Tom Baker Cancer Centre, Calgary, Alberta, Canada

Between 1986 and 1993 92 patients (pts) with leukaemia (54 AL, 38 CML) were evaulable for engraftment after transplants from donors matched for 5 or 6 serologically defined antigens. Standard conditioning therapy was initially with busulfan 16 mg/kg plus cyclophosphamide 120 mg/kg (BuCy2) for related donor (RD) transplants and cyclophosphamide 180 mg/kg plus TBI 1200 cGy (Cy3TBI) for unrelated donor (UD) transplants.

Of 25 CML pts and 34 AL pts transplanted from fully matched RD graft failure occurred in a single AL pt who had persistent leukaemia after BMT. In 12 transplants from 5/6 antigen matched RD graft failure occurred in 0/7 AL pts and 3/3 CML pts conditioned with BuCy2. Two pts, one with AML arising from MDS and transplanted without induction chemotherapy and one with CML, received cyclophosphamide 120 mg/kg plus TBI 1200 cGy (Cy2TBI) with the expectation that this might be more immunosuppressive than BuCy2. Both failed to engraft. Thus of pts who had not received prior intensive chemotherapy and who were transplanted from 5/6 matched RD after BuCy2(3) or Cy2TBI(2) all five (4 CML, 1 AML) failed to engraft. Autologous recovery occurred in 3 (2 CML, 1 AML). Cy3TBI was followed by engraftment in all 18 pts (12 AL, 6 CML) transplanted from UD (10 6/6 matches, 8 5/6 matches). This regimen was therefore used to condition three pts with CML transplanted from 5/6 antigen matched RD all of whom engrafted. We conclude that conditioning with BuCy2 or Cy2TBI may be inadequate to allow engraftment in a subgroup of pts transplanted from single antigen mismatched RD. Patients with CML appear to be particularly at risk. Although the disease process may be responsible it is also possible that the absence of prior immunosuppressive chemotherapy may add to the effect of histoincompatability in preventing engraftment. Our preliminary experience suggests that conditioning with Cy3TBI may allow engraftment in these pts.

G 209 TWO BROTHERS WITH WISKOTT-ALDRICH SYNDROME (WAS) TRANSPLANTED WITH MARROW FROM THE SAME UNRELATED MATCHED DONOR. Anders Fasth and Sólveig Óskarsdóttir, Department of Pediatrics, University of Göteborg, East Hospital, S-416 85 Göteborg, Sweden

Haploidentical marrow transplantation for WAS using the EBMT/EGID protocol and T-cell depleted marrow has a less favorable prognosis with a high rejection rate and mortality. As the severity and progress of the disease usually give time for the search of a HLA-matched volunteer donor we used that option for two brothers with WAS. The two brothers were HLA-identical and the donor volunteered to donate twice with two years between the donations. The brothers were 2.9 and 0.3 years of age, respectively at the time of transplantation. Both were prepared with busulfan (500 mg/m^2) + cyclophosphamide (200 mg/kg). The marrow was *not* T-cell depleted and the dose was for both 5.2 x 10^8 nucleated cells/L. GvHD prophylaxis was cyclosporin and short course methotrexate. G-CSF (Neupogen®, Amgen-Roche) was given from day +3. Neutrophils >0.5x10^9/L were for both achieved at day +10. The last thrombocyte transfusion was given for the older brother at day +37, and for the younger at day +15. The clinical course for the older brother was complicated by acute GvHD grade III from day +8 with biopsy-verified skin, duodenal and rectal involvement. Methylprednisolone was given with prompt resolution. No chronic GvHD developed. From day +6 the younger brother had progressive respiratory failure with localized pulmonary infiltrates and isolation of Respiratory syncytial virus in tracheal secretions. He was successfully treated with ribavirin (Virazole®, Swedish Orphan) given as continuous inhalation for 6 days.

The older brother is today, >2 years post BMT, free from infections and allergy. He has platelets of normal number and size. Serum IgE is not detected. The younger brother is presently 2 month post BMT with subnormal platelet numbers, but the thrombocytes have normal size. He no longer has any bleeding and the skin is free from eczema.

The two brothers show that marrow transplantation from a matched volunteer donor can successfully be done in WAS. They also represent a rare occasion where a volunteer donor has given marrow twice for siblings with the same disease.

G 210 T-CELL DEPLETED BONE MARROW TRANSPLANTATION (BMT) FOR WISKOTT-ALDRICH SYNDROME (WAS) USING UNRELATED DONORS. Daniel Pietryga, Bruce Camitta, James Casper, Frederick Garbrecht, Robert Ash, Nancy Bunin, Carolyn Keever, Kevin Murray, LeeAnn Baxter-Lowe, Neal Flomenberg, Medical College of Wisconsin, Children's Hospital of Wisconsin, Milwaukee, WI 53226

Since 1988, 3 patients with WAS have received T-cell depleted bone marrow grafts from unrelated donors. Two patients were serologically identical with their donors and MLC non reactive. A third pt. had a single serological DR disparity with his donor and a positive MLC in the donor vs. recipient direction. Conditioning consisted of cytosine arabinoside (3 gm/m^2 q 12 hrs x 6 day -7 to -4), cyclophosphamide (45 mg/kg days -6 & -5) and total body irradiation (1400 Gy/9 fractions/3 days). Graft versus host disease prophylaxis consisted of partial T-cell depletion with monoclonal antibody T_{10} B_9 plus complement, a short course of methylprednisolone and cyclosporine. Bone marrow nucleated cell dose was 1.95, 2.1, 4.0 x 10^8 nucleated cells/kg. A sustained absolute neutrophil count of 0.5 x 10^9/L was obtained at 11, 14 and 23 days. An unsupported platelet count of >25 x 10^9/L was obtained 10, 13 and 24 days and the time to platelet count >100 x 10^9/L was 19, 73 and 389 days. Grade I acute graft vs. host disease involving the skin only occurred in all 3 patients with mild, limited chronic GVHD occurring in a single patient. All patients are alive at 1825, 1157, and 700 days post BMT. T-cell numbers and mitogen/alloantigen-responses are normal in all patients. Natural killer (NK) cell percentages are normal in 2 patients and slightly decreased in one. Two patients have normal numbers of B cells and normal serum levels of IgG, IgA and IgM. The most recently transplanted patient has an elevated percentage of B cells, a normal serum IgG level, but elevated serum IgA and IgM levels. The use of TCD unrelated donor marrow is a reasonable option in the treatment of WAS.

G 211 SUCCESSFUL BONE MARROW TRANSPLANTATION OF CHILDREN WITH X-LINKED SEVERE COMBINED IMMUNODEFICIENCY USING BONE MARROW DEPLETED OF T-CELLS BY CENTRIFUGAL ELUTRIATION. John W. Sleasman, Charles E. Hutcheson, Roy S. Weiner, and Alan M. Miller, University of Florida College of Medicine, Department of Pediatrics, Gainesville, FL 32610 and Tulane Medical School Bone Marrow Transplant Program, New Orleans, LA. X-linked Severe Combined Immunodeficiency (XSCID) is a lethal immunodeficiency unless cured by bone marrow transplantation. Infants lacking a HLA identical donor have successfully received T-cell depleted haploidentical transplants to restore immunity. However, current T-cell depletion strategies have resulted either in delayed engraftment or graft versus host disease (GVHD). We sought to accelerate stem cell engraftment by titrating the dose of mature donor T-cells (2-5 x 10^5 cells/kg) returned to the recipients receiving bone marrow transplant for XSCID. We successfully restored T-cell immunity in three male infants with XSCID using haploidentical T-cell depleted donor bone marrow obtained from either the mother or father. Two children had maternally derived GVHD prior to transplant and received a preconditioning regimen of busulfan and cyclophosphamide. A third infant who had no evidence of maternal GVHD, received no prior conditioning. T-cell depletion was carried out by centrifugal elutriation of harvested bone marrow. An average of 1.53 +/- 0.9 x 10^8/kg nucleated cells containing 3.67 +/- 1.5 x 10^5/kg T cells was infused following elutriation. Engraftment of donor cells occurred at 14 days, 11 days, and 12 days respectively. Grade I GVHD was evident in only one child. The child who did not receive pre-transplant conditioning failed to engraft the donor B cells. Our strategy to provide a limited number of donor T-cells using elutriated marrow depleted of T-cells is an effective therapy for the treatment of XSCID.

G 212 MODULATION OF REJECTION RESPONSES WITH rIL4 PLUS rIL2 TREATMENT IN VIVO, Nobuyuki Aotsuka, Daniel H. Fowler, and Ronald E. Gress, Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

T helper cells and T cytotoxic cells participate in the host immune response to foreign tissue grafts. T helper cells include those which produce IL-2 and gIFN (Th1) and those which produce IL-4 and IL-10 (Th2). Further, it is known that Th2 cells can regulate Th1 responses. To evaluate whether transplantation responses might be regulated by Th2-like cells or their cytokines, we treated mice which had received foreign skin grafts of varying antigenic disparities with IL-4 in combination with IL-2 (which we had found augments the generation of Th2-like cells in mice treated with IL-4). In mice receiving skin grafts which were mismatched for MHC and minor transplantation antigens (C57BL/6 host, C3H/HeJ donor), rIL4 plus rIL2 treatment prolonged primary skin graft survival from 9 days (median survival time: MST) to 14 days ($P<0.001$). In mice receiving skins grafts which were mismatched for multiple minor transplantation antigens ((C3H/HeJ x DBA/2)F1 host, B10.BR donor), rIL4 plus rIL2 treatment prolonged primary skin graft survival from 14 days to 27 days MST ($P<0.001$). Sensitization with reprocessed minor antigens ((C3H/HeJ x DBA/2)F1 host, B10.BR as sensitization skin graft donor, B10.D2 as secondary skin graft donor), was not prevented by rIL4 plus rIL2 treatment. These results indicate that rIL4 plus rIL2 treatment can modulate the host rejection response, and that Th2-like responses may therefore be important in the regulation of transplantation responses in vivo.

G 213 KINETIC PATTERN OF CYTOKINE EXPRESSION IN ACUTE GRAFT VS. HOST DISEASE (GVHD), K. Scott Baker, Ruth D. Allen, Charles L. Sidman, Departments of Pediatrics and Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati, Cincinnati, OH 45267.

This study compares the expression over time of a wide range of cytokines as GVHD develops after bone marrow transplant (BMT). A murine model of acute GVHD (B10.BR->CBA) in which donor and recipient mice differ at several minor histocompatibility loci was utilized. CBA mice were given 1000 rads of radiation followed by the transfer of B10.BR bone marrow to which additional quantities of T-cells were added in order to elicit GVHD of a predictable nature as determined by previous experiments. As controls, CBA mice were reconstituted with CBA bone marrow. Animals were sacrificed at 4, 7, 14, 21 and 28 days post-transfer. Cytokine mRNA expression in the spleen was determined by reverse transcriptase-PCR amplification for IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, TNF-α, TNF-β, INF-γ, TGF-β and MIP-1α. All samples were screened for beta-actin expression and were uniformly positive. With the PCR for each cytokine, two-fold dilutions of a known positive cDNA were included to produce standards for comparison between various samples. Photographs of ethidium bromide stained gels were optically scanned for analysis with NIH Image software. The density of each band was determined and converted to a scale of arbitrary units by interpolation into the scanned titration series. Using this approach 3 basic patterns of cytokine mRNA expression were seen. The first was a different expression in GVHD mice vs. controls which began after the first week post-BMT and persisted at all subsequent time points. This pattern was seen with IL-10 (30-fold peak ↑), and MIP-1α (5-fold peak ↑) in the GVHD animals vs. controls. This same pattern was noted for IL-1β, except that a maximum 10 to12 fold decrease was found in the GVHD animals compared to controls. The second pattern showed a temporary increase in expression in the GVHD animals during the first two weeks post-BMT, and a decrease to control levels thereafter. This was seen with IL-4, IL-6, TNF-α and INF-γ (all 2 to 4-fold ↑). IL-2 displayed this same pattern but decreased to control levels sooner, after d7. The third pattern seen was that of no significant difference at any time point and was found for IL-1α, TNF-β and TGF-β. This preliminary study of the kinetics and scope of cytokine expression in GVHD suggests that the induction of GVHD involves the interactions of different cytokines at different time points post-BMT. Further studies are underway to perform similar kinetic analysis on other tissues affected by GVHD such as liver, gut and skin.

G 214 T CELL SUBSETS MEDIATING LETHAL GRAFT-VS-HOST DISEASE (GVHD) IN MICE DIRECTED TO IMMUNODOMINANT MINOR HISTOCOMPATIBILITY ANTIGENS, Marc A. Berger and Robert Korngold, Jefferson Cancer Institute, Jefferson Medical College, Philadelphia, PA 19107

T cell responses to minor histocompatibility antigens (HA) are governed by the phenomenon of immunodominance, as demonstrated by B6 anti-BALB.B in vitro CTL studies. Immunodominance is found in lethal GVHD responses directed to BALB.B minor HA, following transplant of B6 T cells bone marrow to irradiated (825 cGy) recipients of either the BALB.B or CXB recombinant inbred strains. The hierarchy of immunodominance in GVHD differs from the in vitro CTL hierarchy. Lethal GVHD is observed in BALB.B, CXBE, CXBI, and CXBJ strains, but not in CXBG and CXBK strains, which express immunodominant antigens for in vitro CTL generation. A major hypothesis explaining this discrepancy is that GVHD reflects the activity of CD4+, but not $CD8^+$ T cell subsets, in contrast to only the in vitro cytolytic potential of $CD8^+$ T cells. The current study assesses the GVHD potential of both T cell subsets in the B6-> BALB.B, CXBE, CXBI, and CXBJ strain combinations and analyzes an early T cell response in the B6->CXBG and CXBK strains. Results indicate that CD8+ T cells can mediate lethal GVHD in all of the strains tested, CD4+ T cells do not elicit a GVHD response in the CXBE recipients, and the failure to obtain clinical disease in the CXBG and CXBK strains is not due to a lack of induction of a GVH response involving both $CD4^+$ and $CD8^+$ T cells.

G 215 DONOR CELLS OF TH2 CYTOKINE PHENOTYPE REGULATE MURINE GVHD, Daniel H. Fowler, Kazuhiro Kurasawa, and Ronald E. Gress, Experimental Immunology Branch, NCI, NIH, Bethesda, MD 20892

While $CD8^+$ T cells are a key effector of GVHD, $CD4^+$ cells are especially important for the initiation and amplification of GVHD. Regulation of this alloreactive $CD4^+$, T-helper response might therefore modulate the induction of GVHD. Differential cytokine production by T-helper functional subsets ("Th1" secretes IL-2 and IFN-γ, while "Th2" secretes IL-4 and IL-10) can influence *in vivo* responses in murine infectious disease models (cell-mediated immunity is enhanced by Th1 states, and inhibited by Th2 states). Because GVHD is a Th1-driven, cell-mediated transplantation response, we hypothesized that enrichment of donor inocula with cells of Th2 cytokine phenotype might regulate GVHD.

To generate donor cells of Th2-type, B6 mice were treated *in vivo* with combination IL-2/IL-4 for five days. The CD4-enriched splenic population from these mice had a Th2 cytokine phenotype, as defined by increased IL-4 and IL-10, with concomitantly decreased IL-2 and IFN-γ. In a parent=> F1 model of lethal GVHD (Day 7 administration of LPS endotoxin results in TNF-α mediated death), these donor cells of Th2-type consistently protected recipients from otherwise lethal donor cell inocula. Compared to mice undergoing lethal GVHD, Th2-protected mice had lower levels of IFN-γ and TNF-α, decreased $CD8^+$ donor engraftment, and a reduction in the anti-host CTL response *in vivo*. Thus, the Th2-type donor cells regulated both cytokine and cellular events associated with GVHD.

We have also demonstrated the regulatory role of donor Th2-type cells in other models of murine GVHD. In a P1=> P2 model utilizing fully allogeneic donor spleen cell inocula (B6=> C3H, recipient sublethally irradiated with 500 cGy), the administration of additional donor cells of Th2-type reduced the incidence of lethal GVHD; these Th2-protected mice had stable alloengraftment, with >98% donor lymphoid chimerism.

Because of these examples of the ability of donor Th2-type cells to regulate transplantation responses *in vivo*, we propose that the enrichment of marrow inoculum with Th2-type cells may represent a novel strategy for reducing the incidence and severity of GVHD.

G 216 THE USE OF ALLOGENEIC LYMPHOCYTES FOR CELLULAR IMMUNOTHERAPY AFTER BONE MARROW TRANSPLANTATION: STUDIES IN A MURINE MODEL, Bertram Glass, Lutz. Uharek, Tobias Gaska, Mathias Zeis, Winfried Gassmann, Helmut Loeffler, Wolfgang Mueller-Ruchholtz, Institute of Immunology and Department of Internal Medicine II, Univ. of Kiel, Germany

The graft-versus-leukemia effect exerts a substantial part of the antileukemic activity of allogeneic BMT. We investigated (1) whether the additional transfer of allogeneic lymphocytes at time of BMT can provide additional antileukemic activity and (2) whether the incubation of the these cells with IL-2 enhances their graft-versus-leukemia (GVL) activity. *Methods:* Balb/c mice were injected with $5x10^5$ A20 (B cell leukemia, Balb/c origine) cells 2 days prior to lethal (7.5 Gy) total body irradiation (TBI) and transplantation of either syngeneic Balb/c or allogeneic, MHC-matched (H-2^d) DBA bone marrow cells ($2x10^7$ cells). In this experimental system chronic but no lethal acute GVHD occurs. In different experimental groups donor-derived spleen cells with or without IL-2 preincubation (24 hrs) were added. Leukemia-free survival (LFS) was monitored until day 120 post BMT. *Results:* Following syngeneic transplantation LFS was 11% and median survival time (MST) was 39 days. Allogeneic MHC-identical transplantation did not improve these results and resulted in a LFS of 10% and a MST of 39 days. The addition of spleen cells to the allogeneic BM graft reduced the relapse rate significantly and a LFS of 32 % and a MST of 62 days was achieved. Activation of spleen cells by incubation with IL-2 resulted in a LFS of 63%. *Conclusions:* (1) The experimental model presented allows the investigation of cellular immunotherapy following bone marrow transplantation. (2) In this BMT model, characterized by only marginal GVL activity and without acute GVHR, an improved antileukemic effect could be achieved by the addition of allogeneic MHC-matched spleen cells. Experiments to employ cellular immunotherapy using allogeneic lymphocytes following high dose chemotherapy are under way.

G 217 THE SEQUENTIAL ADMINISTRATION OF TH1 AND TH2 DONOR T CELLS FACILITATES FULLY ALLOGENEIC CELL TRANSFERS IN SUBLETHALLY IRRADIATED MICE, Ronald E. Gress, Kazuhiro Kurasawa, and Daniel H. Fowler, Experimental Immunology Branch, NCI, NIH, Bethesda, MD 20892

T cells mediating GVHD and graft rejection (HVG response) appear to act antagonistically during engraftment of allogeneic marrow: T cell-depleted (TCD) allografts effectively eliminate GVHD, but with an increased incidence of graft rejection. Functional subsets of $CD4^+$ T cells are defined by differential cytokine secretion ("Th1" secretes IL-2 and IFN-γ, while "Th2" secretes IL-4 and IL-10). GVHD is a Th1-driven, cell-mediated alloreaction; in a P=> F1 model of lethal GVHD, we have previously shown that donor cells of Th2-type (generated by treating donors *in vivo* with IL-2/IL-4) protect recipients from otherwise lethal donor inocula. In light of this result, we hypothesized that alteration of the Th1/Th2 balance within marrow allografts during engraftment could be utilized as a strategy to overcome graft rejection without lethal GVHD.

To evaluate both GVHD and graft rejection, C3H recipients were sublethally irradiated (500 cGy); the HVG response of these recipients was preserved, as evidenced by their ability to reject TCD splenic inocula from B6 donors. Allografts containing only CD4-enriched cells of Th2-type were also uniformly rejected, consistent with other studies suggesting that CD8 cells may be necessary to overcome graft rejection. Titration of the donor B6 splenic inoculum found that 9×10^7 spleen cells were necessary to abrogate rejection at this radiation dose; these engrafted recipients uniformly died of GVHD (avg time to death = Day 18; diagnosis as defined by histopathologic criteria). However, the administration of additional donor cells of Th2-type (on Day 1 or Day 3 after initial allotransfer) reduced the incidence of lethal GVHD; such recipients had stable alloengraftment (>98% lymphoid allochimerism; >60 days post-transfer).

Thus, "sequential Th1=> Th2 donor lymphoid transfer" emerges as a potential strategy for successful BMT across fully allogeneic barriers in sublethally irradiated hosts: an initial donor T cell-containing inoculum capable of Th1 function *in vivo* (CD8-mediated abrogation of graft rejection) can be subsequently regulated by $CD4^+$ cells of Th2-type, resulting in engraftment with protection from lethal GVHD.

G 218 ANTIBODIES TO TH1 CYTOKINES MODULATE ACUTE GRAFT-VS-HOST REACTION IN VIVO. Frances T. Hakim and Ricardo Gazzinelli, Medicine Branch, NCI and Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD 20892

When C57BL/6 parental donor spleen cells are injected into unirradiated B6D2F1 hosts, an acute suppressive GVHR results. During the first week after induction of GVHR, we have observed that TH1 cytokines including IL-2, IFN-γ and TNF are produced in unstimulated cultures of GVHR spleen cells. After 2 weeks, donor CD4 and CD8 cells outnumber host, donor-derived macrophages have engrafted and mice become susceptible to lethal LPS-induced septic shock due to TNF and NO release from activated macrophages. In order to evaluate the role of TH1 cytokines in engraftment and the generation of lethal GVHD, mice were injected with anti-cytokine antibodies twice weekly beginning with the day of induction of GVHR, and donor engraftment and host susceptibility to septic shock were assessed. At 2 weeks of GVHR, treatment with anti-IL-2 or anti-IFN-γ produced comparable or even increased engraftment of donor CD4 and CD8 cells, as compared to control antibody treatment; donor macrophages engrafted significantly (5% of total GVHR spleen) in control and anti-IFN-γ treated, but none were observed in anti-IL-2 treated mice; very few donor B cells engrafted under any condition. Correlating with the prevention of donor macrophage engraftment, anti-IL-2 but not anti-IFN-γ treatment protected against LPS-induced mortality. Anti-TNF treatment on the same day as LPS challenge prevented LPS-induced septic shock, as well, consistent with observations of TNF release in LPS-treated GVHR mice. Both anti-IL-2 and anti-IFN-γ treatment also induced a 10-fold increase in the intensity of Class II antigen expression on host B cells, comparable to that observed in chronic, B cell stimulatory GVHR. This increase is consistent with an increase in TH2 cytokine production. Thus neither anti-IL-2 nor anti-IFN-γ blocked engraftment of donor T cells. Both treatments may have altered TH1/TH2 cytokine regulation. But only anti-IL-2 blocked LPS-lethal shock susceptibility, perhaps through altered donor myeloid engraftment.

G 219 MATURE DONOR T CELLS ARE PRESENT IN THE THYMUS OF MICE UNDERGOING THE GRAFT-VERSUS-HOST REACTION IN RESPONSE TO MINOR HISTOCOMPATIBILITY ANTIGENS, Brian L. Hamilton and *Rebecca D. Adkins, Department of Immunology, Children's Hospital Oakland, Oakland, CA 94609 and *Department of Pathology, University of Miami, Miami, FL 33101.

Thymic involution and epithelial damage is a feature of the GvHR. The mechanism of this damage is not known. Studies in P→F_1 mice transplanted across MHC barriers have shown that donor T cells are not detectable in the host thymus in the absence of a concomitant inflammatory process such as infection with murine cytomegalovirus. We studied a radiation model of the GvHR in response to minor histocompatibility antigens to better define the role of donor T cells in thymic damage. Irradiated C3H.SW (H-2^b, Thy-1.2, Ly-5.1) mice were transplanted with a mixture of T-depleted bone marrow and lymph node T cells from Thy-1 and Ly-5 congenic C57BL/6 (H-2^b) donors. The marrow donor was Thy-1.1, Ly-5.1 while the T cell donor was Thy-1.2,Ly-5.2. Ten days after transplant, 50% of the T cells in the thymus were derived from the mature T cell donor, while the remainder were of host origin. Over the next 4 weeks, both populations were replaced by T cells derived from the bone marrow donor. The bone marrow derived T cells appeared to mature within the host thymus damaged by the GvHR, but the thymus remained severely atrophic. The peripheral lymphoid tissues (spleen and lymph nodes) were repopulated only by T cells from the mature T cell donor and did not show evidence of repopulation with T cells of bone marrow origin during the first 6 weeks after transplant. In contrast, recipients without GvHR were rapidly repopulated with bone marrow-derived T cells in both the thymus and peripheral tissues. These data demonstrate for the first time that mature donor T cells enter the thymus of radiation chimeras to cause an intrathymic GvHR. The damaged host thymus appears to support a limited maturation of bone marrow derived T cells which is insufficient to reconstitute the peripheral lymphoid organs.

G 220 IFN-γ MEDIATED PREVENTION OF ACUTE GRAFT-VERSUS-HOST DISEASE IN MICE AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

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One of the major complications of allogeneic bone marrow transplantation (BMT) is Graft-versus-Host Disease (GvHD), which is the result of an immunological reaction from donor-type T-lymphocytes, directed against the tissues of the recipient.
Using a H-2 mismatched murine BMT-model, which normally results in a mortality rate due to GvHD of 70-90 % within 100 days after BMT, a strong mitigating effect on GvHD was observed after treatment of the recipients with IFN-γ (2 x 50,000 U/mouse/wk during 6 weeks after BMT, or 1 x 500,000 U/mouse on the day of BMT). A (less) mitigating effect of treatment with an anti-IFN-γ monoclonal antibody (DB-1; 1x 1.4 mg/mouse/wk during 6 weeks after BMT) was also observed. The donors were C57BL/Rij (H-2^b) SPF-mice; C3H/Law (H-2^k) gnotobiotic (Houston-flora) mice were used as recipients. The recipients were conditioned with 9 Gy Rö-irradiation before they were grafted with 10^7 donor bone marrow cells.
The observations were confirmed by histopathological examination of different parts of the gastro-intestinal tract, a major target organ of GvHD. GvHD-scores for colon and cecum in IFN-γ treated animals differed significantly from those of saline (control) or DB-1 treated animals ($p<0.05$). Induction by IFN-γ of non-specific changes in the colon and the cecum resembling GvHD lesions were ruled out by performing histopathology on tissues of C3H recipeints of syngeneic bone marrow which were treated with IFN-γ (50,000 U/mouse, twice weekly).
Determination of the endogenous IFN-γ producing cells in the spleen (spontaneously as well after stimulation with concanavalin-A) showed a significant decrease in IFN-γ treated animals in comparison to the control animals during the first two weeks after BMT. These results suggest that endogenous IFN-γ production in the spleen at the time of hematopoetic reconstitution after BMT is down-regulated by exogenously administered IFN-γ. GvHD development is reflected by an increased production of IFN-γ between day 7 and 10 after BMT. Together with the observations by others that IFN-γ increases natural suppressor activity and that this cell population reaches a peak value in activity at day 6-10 after BMT, our findings indicate that suppression of GvHD is related to the stimulation of natural suppressor activity. Further studies, using a pre-clinical haplo-MHC-mismatched BMT model in rhesus-monkeys, should validate whether this treatment regimen can be extended to the clinical situation.

G 221 DELAYED INFUSION OF DONOR T CELLS AFTER MHC-MATCHED AND HAPLOTYPE-MISMATCHED ALLOGENEIC BMT PROVIDES ANTILEUKEMIC REACTIVITY WITHOUT GRAFT-VS-HOST DISEASE (GVHD), Bryon D. Johnson, Michael B. Weiler and Robert L. Truitt, Medical College of Wisconsin, Milwaukee, WI 53226

GVHD still remains a major problem of allogeneic bone marrow transplantation (BMT). GVHD can be eliminated by depleting mature donor T cells from the BM inoculum. However, T cell depletion often results in an increased incidence of graft rejection and increased frequency of leukemia relapse. We have used MHC-matched, allogeneic [B10.BR into AKR] and haplotype-mismatched [SJL into (SJLxAKR)F1] murine BMT models to develop strategies for avoiding GVHD while still providing an antileukemia or graft-vs-leukemia (GVL) effect, which is primarily mediated by donor T cells. In both BMT models, when spleen cells as a source of T cells were administered to recipient mice at the time of BMT in sufficient numbers, the mice developed lethal GVHD. When infusion of the spleen cells was delayed until 21 days after BMT, few mice exhibited clinical signs of GVHD. Similar results were obtained whether a single or multiple infusions of spleen cells were administered starting on day 21 after BMT. Importantly, the infused spleen cells were able to mount an in vivo antileukemia effect. In the B10.BR/AKR BMT model, infusion on day 21 of spleen cells from donors previously sensitized to AKR antigens induced GVHD. Thus, the ability of previously activated cells to induce GVHD was not inhibited in the same manner as normal cells. These results suggest that a suppressive mechanism present in chimeras at 21 days post-BMT may help to prevent normal spleen cells from becoming activated. Results from limiting dilution assays to determine the frequencies of alloreactive cytotoxic T cells and IL-2-secreting T-helper cells support this hypothesis. We are currently investigating the possibility that the suppressive mechanism is primarily directed at $CD4^+$ T cells. In conclusion, post-transplant immunotherapy with normal mononuclear cells from the transplant donor may be an effective way of eliminating residual disease or treating leukemia relapse without significant GVHD. Determining the mechanism(s) involved in the suppression of GVHD while allowing for an antileukemic effect may provide important information for optimizing future clinical use of this form of therapy.

G 222 MCMV INFECTION POST ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) BETWEEN H-2 MATCHED DONORS AND RECIPIENTS RESULTS IN AUGMENTED ANTI-HOST CYTOTOXIC ACTIVITY, *Monica Jones, ‡Carolyn Cray, and *Robert B. Levy, Departments of *Microbiology and Immunology and ‡Pathology, University of Miami School of Medicine, Miami, FL 33101

Clinical reports have noted that DNA virus presence is frequently associated with an increased incidence of severe graft vs. host disease (GvHD) and subsequent complications resulting in increased morbidity and mortality in bone marrow transplant recipients. The present studies examined the affect of a DNA virus on the resulting anti-host cytotoxic activity which arises following an allogeneic MHC matched BMT. MCMV was introduced to lethally irradiated recipient mice after a marrow transplant from an H-2 matched donor strain which subsequently results in GvHD and mortality. BALB/c recipients of B10.D2 TCD marrow plus $5x10^6$ T-cells (allogeneic) were infected with MCMV 7 days post-transplant. At day 14-15 post-transplant, pooled spleen cells from groups of allogeneic, allogeneic plus MCMV and syngeneic (BALB/c-->BALB/c) BMT recipients were assayed for cytotoxic activity against normal BALB/c ConA blast target cells. Spleen cells from recipients of allogeneic marrow plus T-cells contained a low, but reproducible, level of cytotoxic activity. In contrast, recipients of an allogeneic transplant and MCMV inoculum demonstrated sharply increased levels of anti-host (i.e.BALB/c) activity (3-5x). Spleen cells from syngeneic transplant recipients infected with virus did not lyse B10.D2 target cells indicating that MCMV infection of these BMT recipients did not induce detectable polyclonal cytotoxic activity. Depletion of CD8+, but not CD4+ cells virtually abolished all detectable anti-host acitivity. In total, the findings demonstrate that cytotoxic T-cell activity measured directly from the spleens of BMT recipients several weeks post-transplant is clearly augmented if the recipient is concurrently exposed to MCMV after transplant. We therefore conclude that the presence of a viral pathogen can result in augmented anti-host specific activity early after allogeneic bone marrow transplantation.

G 223 MECHANISM OF THE VETO EFFECT: P-CTL TRIGGER THE RELEASE OF CYTOTOXIC GRANULES FROM VETO CELLS, RESULTING IN P-CTL DELETION Kazuhiro Kurasawa, Hiroo Nakajima, Pierre A. Henkart, Kiyoshi Hiruma, and Ronald E. Gress, Experimental Immunology Branch, NCI, NIH, Bethesda, MD 20892

Veto cells, by suppressing p-CTL with specificity for antigens expressed on the surface of veto cells, may play an important role in the maintenance of tolerance. We have previously reported that T cell depleted BM cells cultured with high dose IL- 2 (activated bone marrow cells, ABM) delete p-CTL that recognize ABM *in vitro*; this p-CTL deletion was associated with enhancement of allogeneic T cell-depleted marrow engraftment *in vivo*. We have been investigating the mechanism whereby ABM delete p-CTL. Using anti-L^d TCR transgenic spleen cells as p-CTL, we found that ABM treated with chloroquine (which reduces the activity of cytotoxic granules) or paraformaldehyde (which fixes cell membranes, but preserves antigenicity) failed to delete p-CTLs. These results suggested that p-CTL were deleted not by a suicide mechanism, but by granule release from the ABM. Furthermore, treatment of p-CTL by cyclosporine resulted in inhibition of veto cell mediated suppression indicating an active role for the p-CTL. We hypothesized that p-CTL derived cytolysin (perforin) might damage the ABM cell membrane, resulting in an influx of Ca^{++} and triggering. To test this hypothesis, we utilized cytolysin gene transfected RBL cells (RBL-cy) as p-CTL. RBL-cy were incubated with IgE anti-DNP Ab and cultured with DNP coated ABM. We found that RBL-cy, but not control RBL, were effectively killed by the ABM. These experiments demonstrate that target cells which recognize ABM and release cytolysin, can trigger ABM with granule release and target cell deletion.

G 224 *Abstract Withdrawn*

G 225 GRAFT-vs-MYELOID LEUKEMIA RESPONSES IN MICE FOLLOWING SYNGENEIC AND ALLOGENEIC BONE MARROW TRANSPLANTATION, Robert Korngold, Jefferson Cancer Institute, Jefferson Medical College, Philadelphia, PA 19107

Allogeneic bone marrow transplantation (BMT) is a highly effective therapeutic approach for patients with myelogenous leukemia. Two major complications are the development of graft-vs-host disease (GVHD) and leukemic relapse, with the incidence of the former inversely affecting the incidence of the latter. Donor T cells responsible for GVHD may also be directed to host histocompatibility (H) antigens present on residual leukemia cells, thereby reducing the risk of relapse. In addition, donor T cells may be able to recognize myeloid leukemia-specific antigens. The optimum therapy would be to avoid GVHD while preserving graft-vs-leukemia (GVL) responses. In this regard, we have developed a model for investigating GVL activity following syngeneic and MHC-compatible allogeneic BMT in B6 mice challenged with the B6 derived MMB3.19 myeloid leukemia line. A dosage of 10^5 MMB3.19 cells was completely lethal in irradiated (850 cGy) B6 recipients, 1d after syngeneic T cell-depleted BMT. The addition of T cells to the donor inoculum prolonged survival, and both $CD4^+$ and $CD8^+$ subsets were found to be capable of mediating this GVL activity. In the C3H.SW->B6 minor H allogeneic model, both $CD4^+$ and $CD8^+$ T cells were found to be capable of mediating GVL activity to MMB3.19 challenge, particularly if donor mice were presensitized with the leukemia cells. However, only the donor $CD4^+$ T cells mediated a GVL effect without the apparent induction of GVHD.

G 226 IL-7 DRIVES DONOR T-CELL PROFIFERATION AND CAN CO-STIMULATE IFN-γ SECRETION AFTER MHC MATCHED ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT). *Robert B. Levy, *Monica Jones, ‡Brian Hamilton, *John Paupe and *Richard Riley, *University of Miami, Dept of Microbiology & Immunology, Miami, FL 33101 and ‡Div. of Immunology, Children's Hospital, Oakland CA 94609.

Transplantation of MHC matched, allogeneic B10.D2 bone marrow + T-cells into BALB/c (B10.D2--> BALB/c) recipients has been shown to result in graft vs. host disease (GvHD) and greater than 80% mortality 8-12 weeks post-transplant. To date, the precise cellular interactions and signals occurring during the acute period following allogeneic BMT have not been clearly defined. A number of cytokines including IL-1, IFN-γ and TNF have been proposed to play important roles during the initiation and effector stages of GvHD. The present studies examined the response by cells from B10.D2--> BALB/c BMT recipients to stimulation with IL-7 *in vitro* during the early period following transplant. The findings indicate that within the first week post-transplant, spleen cells removed from recipients injected with allogeneic - but not syngeneic - T-cells proliferated vigorously in response to rIL-7. Both IL-2 dependent and independent components were identified. Depletion of T-cells prior to culture virtually eliminated this response. Additionally, phenotypic examination of cultures *maintained with* exogenous rIL-7 identified a predominance of T-cells. We conclude that transplant of allogeneic donor T-cells is required for the observed response and moreover, these cells proliferate following exposure to IL-7 *in vitro*. IL-7 specific mRNA was identified in the spleens of all BALB/c BMT recipients during the first week post-transplant. Subsequently, it was found that IL-7 can augment the production of IFN-γ by T-cells from allogeneic BMT recipients stimulated with anti-T-cell receptor (i.e. anti-Vβ) antibody. Thus, IL-7 possesses the capability to functionally enhance the response of donor T-cells post-allogeneic BMT. We therefore hypothesize that IL-7 may play an important role during the early events following allogeneic BMT. This occurs through the expansion of donor T-cells which possess the capacity to produce at least one cytokine (IFN-γ) proposed to participate in the afferent stage of graft vs. host reactions and disease.

G 227 IL-6 INDUCES A SHIFT IN THE PATTERN OF T CELL LYMPHOPOIESIS WHEN ADMINISTERED TO MICE FOLLOWING BMT, Crystal L. Mackall, Anne Galbraith, and Ronald E. Gress, Experimental Immunology Branch, National Cancer Institute, Bethesda, MD 20892

In an effort to devise new methods to enhance T cell regeneration after bone marrow transplantation (BMT), we have used congenic mice to study lymphopoiesis after BMT. Our previous work using this model has shown two primary pathways of lymphopoiesis after BMT as distinguished using Thy-1 congenic markers: a thymic-dependent bone marrow (BM) derived pathway and a thymic-independent peripheral T cell derived pathway. In addition, we have observed that the addition of peripheral $CD4^+$ T cells to T cell depleted BM inocula increases the number of BM derived thymic progeny at 6 weeks post-BMT. Semi-quantitative reverse PCR analysis of thymocytes from animals treated in this manner has shown increased IL-6r RNA levels between 2 and 6 weeks post-BMT compared to control non-BMT thymocytes. Because it was possible that the enhancement of thymopoiesis observed was mediated by IL-6, we analyzed the effects of exogenously administered IL-6 on lymphopoiesis in this model. C57BL/6 mice were given lethal irradiation followed by syngeneic T cell depleted BM with or without congenic lymph node (LN) cells. The mice were treated with 20 μg/day of rhIL-6 given subcutaneously via an osmotic pump from day -1 to day 27 post-BMT. Treated animals showed increased numbers of peripheral blood leukocytes and $MAC\text{-}1^+$ splenocytes compared to sham-treated controls. Analysis of regenerated T cell populations however, showed decreased numbers of BM derived T cells in IL-6 treated animals compared to controls. Further, IL-6 treated animals displayed decreased thymic size, and in animals which received LN inocula, there was both an increased percentage and increased number of LN derived T cells when compared to controls. This is similar to the pattern of lymphopoiesis seen in thymectomized hosts wherein LN cell expansion is increased in comparison to thymus-bearing hosts. Therefore, despite increased IL-6r levels on thymocytes post-BMT and evidence that IL-6 induces thymocyte proliferation in vitro, treatment with IL-6 in vivo resulted in thymic atrophy, and a shift in the pattern of T cell regeneration toward a peripheral T cell derived thymic-independent pathway of lymphopoiesis.

G 228 ASSESSMENT OF CHIMERISM FOLLOWING TRANSPLANTATION OF ULTRAVIOLET(UV) B IRRADIATED PRECURSORS IN RODENTS. Shaun R McCann, Anne O Neill, Mark Lawler, John Hudson, Ted Kloosterman, Rogier Pullens, Hazel Gowing, Anton Martens, Derwood Pamphilon and Anton Hagenbeek. Departments of Genetics/Haematology St James's Hospital and Trinity College Dublin, Ireland; Regional Transfusion Centre Bristol UK; Department of Hemato-Oncology, Erasmus University, Rotterdam, The Netherlands.

Graft versus host disease (GvHD) and graft rejection(GR) following BMT remain serious problems resulting from allo- interactions between the graft and the host. While methods such as T cell depletion may abolish GvHD, they can also result in a higher degree of graft rejection. An alternative approach to reduce graft immunogenecity may be to inactivate immunostimulatory cells such as the dendritic cell(DC) population. Previous work has indicated that ultraviolet B light(UV B) can inhibit lymphocyte reactivity and prevent GvHD. In order to assess the efficacy of UV B in abolishing GvHD while preserving stem cell viability, we have assessed the chimeric status of mice who have received UV B treated marrow in semi allogeneic or allogeneic BMT. 12 microsatellite markers were assessed for strain specific polymorphisms between three strains of mouse(CBA/J, Balb/c and C57/Bl6). Initially polymorphic markers were used to assess the degree of chimerism at a single timepoint in F1 mice(CBA/J X Balb C) receiving marrow from CBA donors which had been treated with different doses of UV B(0-240 J/m^2). Mice receiving untreated marrow remained donor chimeras while mice receiving UV B treated marrow showed low levels of recipient cells. In the allogeneic model, serial sampling was possible from the tail vein at one week intervals. 20 mice received unirradiated marrow and 25% were alive with complete donor chimerism at week 5 post BMT. Mice who died all were predominantly recipient chimeras. In the UV B treated group, 50% of mice receiving 50J/ m^2 were predominantly donor chimeras. In order to assess the efficacy of UV B in eliminating GvHD while maintaining a GvL effect, microsatellite markers have been characterised for the Brown Norway and PVG donor strains for use in the assessment of chimerism in this acute nonlymphocytic leukemia model. Thus microsatellite markers are useful in assessing chimerism post BMT in rodents in a serial fashion and initial results indicate that UV B treatment can influence the degree of chimerism post BMT.

G 229 DONOR Vβ RESTRICTED T CELL EXPANSION CORRELATES WITH DELAYED RECONSTITUTION OF BOTH CONVENTIONAL AND B1 B CELLS AND THEIR PRECURSORS DURING THE MINOR H ANTIGEN GRAFT-VS-HOST REACTION, Richard L. Riley, Beth A. Garvy, Jeanne M. Elia, Melinda S. Merchant, and *Brian L. Hamilton, Dept. Microbiology & Immunology, University of Miami, Miami, FL 33101 and *Oakland Children's Hospital Research Institute, Oakland, CA 94609.

In order to study the affects of graft-vs-host reactions (GVHR) on reconstitution of B lymphocytes during bone marrow transplantation, we employed a murine model of GVHR in which donor (B10.D2) and recipient (BALB/c) mice were MHC identical (H-2^d), but mismatched at multiple non-MHC loci, including Mls-2,3. Recipients of T-cell depleted allogeneic bone marrow regained significant numbers of bone marrow pro- and pre-B cells during the initial 3 weeks post-transplant. In contrast, recipients receiving allogeneic bone marrow plus T cells (GVHR mice) demonstrated only partial recovery of pro- and pre-B cells. Diminished reconstitution of bone marrow B cell precursors coincided with expansion and/or presence of donor V$\beta3^+$ T cells in the spleen, peritoneal cavity, and bone marrow of GVHR mice. In vitro, T cells from GVHR bone marrow and spleen inhibited IL-7 mediated pre-B cell growth, in part through production of γ-interferon. In the periphery, donor derived B cells were delayed in their appearance in both spleen and peritoneal cavity of GVHR mice. Both reconstitution of donor (B10.D2) derived conventional (IgM^+ $Ly1^-$ $Mac1^-$) and B1b (IgM^+ $Ly1^-$ $Mac1^+$) B cells were adversely affected in GVHR mice. Radioresistant B cells of recipient (BALB/c) origin were also detected in GVHR mice. In contrast to allogeneic control transplants, these BALB/c derived B cells had virtually disappeared from both peritoneal cavity and spleen by day 14 post-transplant. These data suggest that the immunodeficiency associated with graft-vs-host responses may result, in part, from inadequate reconstitution of both conventional and B1 B cells in the early stages after bone marrow transplantation.

G 230 HEMATOPROTECTIVE ACTIVITY BY ACETYL-SER-ASP-LYS-PRO AGAINST SUBLETHAL AND LETHAL IRRADIATION: MECHANISTIC STUDIES. James E. Talmadge[1], Linda Kelsey[1], Cynthia Ewel[2], Yun Yan[1], and John Jackson[1]. [1]Department of Pathology/Microbiology, University of Nebraska Medical Center, Omaha, NE 68198 and [2]Henri Beaufour Institute, Inc. Washington D.C.

The tetrapeptide N-Acetyl-Ser-Asp-Lys-Pro (AcSDKP) has radioprotective (sublethal irradiation) activity for early hematopoietic precursors and can prolong the survival of mice receiving lethal irradiation. The administration of AcSDKP by continuous infusion (Alzet pumps) has radioprotection properties for mice receiving lethal irradiation in a dose, timing, and duration dependent manner. AcSDKP has optimal activity when administered at one ng per day per animal by continuous infusion and significantly prolongs the survival of mice (C57BL/6) following lethal irradiation (950 rads). Higher and lower doses of AcSDKP demonstrated significantly less biological activity suggesting a bell shaped dose response curve. Similar strategies of prophylactic treatment with AcSDKP have demonstrated myeloprotective effects against sublethal irradiation. The optimal dose of AcSDKP (one ng per animal per day) when administered by continuous infusion results in a significant acceleration of leukocytic reconstitution in the peripheral blood. The increase in peripheral blood cellularity is a general phenomenon with an increase in the absolute number of not only neutrophils but also lymphocytes and monocytes. In addition there is an accelerated reconstitution of spleen but not bone marrow cellularity. A 60% increase in spleen cellularity was observed as compared to the cellularity of irradiated control mice. There is also an increase in the absolute number of hematopoietic precursors with a significant increase in the total number of high proliferation potential precursors (HPP) in the femur and spleen (>2 fold increase) but only a slight (and not significant) increase in colony forming unit-granulocyte-monocyte (CFU-GMs). These results confirm and extend the in vitro observation that AcSDKP has hematoregulatory properties with an apparent specificity for early progenitors i.e., colony forming unit-spleen. This observation has been extended to the demonstration of biological activity in vivo and suggests a clinical potential as a myeloid protective agent for AcSDKP. This research was supported by a contract with Henri Beaufour, Inc.

G 231 THE EFFECT OF HUMAN ACTIVATED T AND NK CELLS ON ACUTE GRAFT VS HOST DISEASE (aGVHD) AND GRAFT VS LEUKEMIA (GVL) IN SCID MICE. Thompson JS, Xun CQ, Brown SA and Jennings CD, Dept of Medicine and Pathology, University of Kentucky and Veterans Administration Medical Center, Lexington, KY 40536.

A xenogeneic aGVHD and GVL murine model has been developed by transplantation of human activated T, NK or human leukemia cells into SCID mice. Human T or NK cells were isolated from peripheral blood by Ficoll gradient centrifugation and antibody (CD6+TCR alpha/beta or CD16+CD56) complement mediated negative selection. The two groups of cells were pulsed with 10nM Phorbol dibutyrate and 500 nM Ionomycin for 16 hrs and then cultured and activated in rIL-2 (500U/ml) medium for 2-8 weeks. Five x10^7 cells of each group were injected iv into 8-12 week old IgG-free SCID mice conditioned with 4Gy TBI. Clinical and histological signs of aGVHD occured in 100% of the mice injected with 70% NK plus 30% T cells (70NK30T) and 15 of 18 injected mice died by day 14. These findings were not observed in pure T cell (98% T) injected mice. The mice injected with pure NK cells (95% NK) developed mild aGVHD and none of them died by day 14. Cytokine studies showed that TNF-a was produced by NK but not T cells in culture supernatant. IFN-r was produced by either T or NK cells and IL-6 only by T cells. Immunohistology revealed that human TNF-a and IFN-r producing cells in the colon tissue of aGVHD mice treated with NK70T30 but not T or pure NK cells treated mice. Human-SCID leukemia (hu-SCID) were induced by iv injection of 2x10^7 human U937 monocytic or K562 erythrocytic leukemia cells into SCID mice pre-conditioned with Cyclophosphamide 100mg/kg i.p. for 5 days. On day 10 after leukemia cell injection, the hu-SCID mice were irradiated with 4Gy TBI and transplanted with 5x10^7activated human effector cells as follow: (1) pure T cells; (2) pure NK; (3) 70NK30T; (4) saline. Eight of 8 hu-SCID mice treated with 70NK30T developed aGVHD and died by 2 weeks. Ten of 10 hu-SCID mice treated with saline without effector cells died of leukemia infiltration (liver, kidney, bone marrow and CNS) in 3 weeks (U937) and 4 weeks (K562). Seven of 10 and 4 of 5 hu-SCID mice treated respectively with pure T and pure NK effector cells survived over 60 days after transplantation. We conclude that aGVHD lesions can be induced by transplantation of xenogeneic human activated NK and T combined cells into SCID mice. Although T cells are essential to initiate GVHD, final pathogeneic lesions are related to other cells and cytokine dysregulation. T cells appeared to potentiate NK and cytokine mediated aGVHD. On the other hand both T and NK cells alone were able to mediate GVL, suggesting that ex vivo depletion or in vivo prophylactic depletion of NK cells could decrease aGVHD without loss of GVL.

G 232 EFFECT OF ANTI-CD3 F(ab')$_2$ MoAb ON GVH and GVL REACTIVITY WHEN GIVEN IN VIVO AFTER MHC-MATCHED BMT, Robert L. Truitt, Bryon D. Johnson, and Cathleen McCabe, Department of Pediatrics, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

The effect of intact and F(ab')$_2$ fragments of anti-CD3 MoAb (clone 145 2C11) on graft-vs-host (GVH) and graft-vs-leukemia (GVL) reactivity when given *in vivo* after MHC-matched BMT was studied in a murine model of MHC-matched BMT (B10.BR→AKRx). In this model, the severity of GVHD is T-cell dose dependent and correlated with the number of CD4$^+$ T-cells injected. Whole MoAb induced a lethal "cytokine-syndrome" in recipients of BM + spleen cells (BMS) but not BM alone. Anti-CD3 F(ab')$_2$ (6.25-50 μg/dose) administered as 5 doses qe2d between days 0 and 8 after BMT prevented all clinical symptoms of GVHD in BMS recipients (10^7 BM + $2x10^7$ spleen cells). F(ab')$_2$ reversed clinically evident GVHD when started on day 8 or 10, but only stabilized GVH symptoms when delayed for 14-18 days. F(ab')$_2$ treated BMS recipients became complete donor T-cell chimeras. Anti-CD3 F(ab')$_2$ depleted both donor and residual host T-cells and reduced the frequency of IL-2-secreting T-helper cells. F(ab')$_2$ was most effective if given in multiple injections qe2d rather than a single bolus. For GVL analysis, AKRx mice were given 1,000 AKR leukemia cells (30X LD_{100}) i.v. on day -1, then B10.BR BM or BMS cells on day 0. BM controls treated with anti-CD3 F(ab')$_2$ all relapsed; untreated BMS controls died leukemia-free from GVHD. Most BMS chimeras (8/10) given anti-CD3 F(ab')$_2$ qe2dx5 (d0-8) relapsed (GVH-free). Leukemia relapse was avoided by reducing the regimen from 5 to 3 injections (qe2d d0-4), by using a single bolus (same total Ab dose) or by delaying the start of the treatment until day 8. All mice (10/10) given 10 μg anti-CD3 F(ab')$_2$ qe2dx5 between days 8 and 16 survived leukemia-free (>60d) with no significant clinical symptoms of GVHD. Together with our earlier studies, these results indicate that MoAbs administered *in vivo* post-BMT can be as effective as pre-BMT T-cell depletion *ex vivo* in preventing GVHD. However, the beneficial GVL reaction may be abrogated if GVH-prophylaxis is excessive or ill-timed.

G 233 IL-2-INDUCED GVHD PROTECTION IS ASSOCIATED WITH REDUCED IFN-γ PRODUCTION. Min-Guang Wang, Janos Szebeni, and Megan Sykes. Transplantation Biology Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02129.

In a fully mismatched murine bone marrow transplantation (BMT) model, we have recently demonstrated that a short (2.5 day) course of high dose IL-2 (100,000 U/day), begun on the day of BMT, protects against graft-versus-host disease (GVHD). We have studied the possible effect of IL-2 administration on cytokine production. Acute GVHD was induced in B10 mice by BMT with A/J bone marrow cells (BMC) and splenocytes. T cell-depleted B10 marrow was also given in the same inoculum. Cytokine levels in sera and in spleen cell culture supernatants were compared in recipients of these inocula with or without a protective course of IL-2 treatment and in recipients of syngeneic marrow in the first week following BMT. On days 3 and 4 post-BMT, a marked increase in serum IFN-γ levels was noted in GVHD mice compared to syngeneic controls. This increase was blunted, developed later, peaking at day 5, and was of shorter duration in IL-2-protected mice. Serum IFN-γ levels returned to baseline by day 6 or 7 in both groups. When spleen cells isolated from these animals were cultured with host-type stimulators (irradiated B10 spleen cells) in vitro for 24 h, parallel differences in IFN-γ production were observed. This IFN-γ response reflected in vivo priming to host antigens, as much lower IFN-γ levels were detected in supernatants of spleen cells stimulated with third party or donor cells, and naive A/J spleen cells did not produce detectable IFN-γ when stimulated with B10 cells. No consistent difference was observed in IL-2, IL-10, IL-1α, or TNFα production between GVHD controls and IL-2-treated mice, either in sera or spleen cell culture supernatants. Although the timing of the increase is somewhat variable (day 2 to day 5), preliminary data suggest that IL-4 production may be greater in IL-2-treated than in GVHD control mice. Thus, high dose exogenous IL-2 decreases IFN-γ production early post-BMT. Increased IL-4 production may reflect the removal of down-regulation exerted by IFN-γ. The importance of these cytokine changes is currently being investigated in vivo with cytokine-specific mAb treatments.

G 234 EFFECT OF TBI, BUCY2 OR CY5 OF CONDITIONING ON INFLAMMATORY CYTOKINE RELEASE AND DEVELOPMENT OF ACUTE AND CHRONIC GRAFT-VERSUS-HOST DISEASE (GVHD) IN H-2 INCOMPATIBLE TRANSPLANTED SCID MICE. Xun CQ, Thompson JS, Jennings CD, Brown SA and Widmer MB, Dept. of Medicine and Pathology, University of Kentucky and Veterans Administration Medical Center, Lexington, KY 40536 and Immunex Corp., Seattle, WA 98101.

In our previous study, we found that total body irradiation (TBI) was essential to induce acute GVHD post allogeneic H-2 incompatible splenocyte (SP) transplantation in SCID mice. SCID mice (H-2d) conditioned with Cytoxan (Cy) and transplanted iv with $5x10^7$ C57Bl/6 (H-2b) SP developed only chronic GVHD within 3 months rather than acute GVHD. In this study, SCID mice were conditioned with 4Gy TBI or non-TBI regimens, either BuCy2 (Busulfan 4mg/kg/d+Cy 100mg/kg/d for 2 days) or Cy5 (Cy 100mg/kg/d for 5 days) and then transplanted iv with $5x10^7$ SP. The TBI conditioned mice were further divided into 3 transplant groups: 1) TBI and SP were given the same day (TBI+D0 SP); 2) SP given 4 days post TBI (TBI+D4 SP); 3) SP given 7 days post TBI (TBI+D7 SP). GVHD was compared for clinical and histological grades after these different conditioning and transplant protocols. Twenty eight of 28 mice treated with TBI+D0 SP died of grade IV acute GVHD by day 15 post transplantation. Sixteen mice treated with either TBI+D4 SP or TBI+D7 SP developed grade II acute GVHD but none of them died of acute GVHD during 30 days post transplantation. The mice conditioned with non-TBI regimens developed chronic GVHD within 3 months without showing any detectable acute GVHD signs. Serum and in situ colonic cytokines were measured by ELISA and immunohistology. TBI itself significantly increased both serum and colonic TNF-a, IL-1a and IL-6 when compared to non-TBI regimens and normal controls. TNF-a appeared in the serum and colon 4 hr post TBI and peaked in 24 hr and decreased by 72 hrs followed by increasing of IL-1a by 72 hrs and IL-6 by day 5 post TBI. Histoincompatible transplantation augmented these cytokine release. The group treated with TBI+D0 SP showed the most severe acute GVHD and had the highest levels of TNF-a, IL-1a and IL-6. The BuCy2 conditioned group had the lowest levels of cytokine and developed no acute GVHD. When the mice were treated with either soluble TNF receptor or IL-1 receptor antagnist from -day 2 to +day 6 and transplanted with TBI+D0 SP, acute GVHD mortality was reduced from 100% to 0%. These mice developed mild acute GVHD during first month and later developed subacute or chronic GVHD. We conclude that the interaction of inflammatory cytokines released by conditioning regimen and histoincompatibility is a critical factor in the pathogenesis of acute GVHD.

G 235 AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) FOR ADVANCED NEUROBLASTOMA (Nb): CYTOREDUCTION WITH CYCLOPHOSPHAMIDE (Cy), CARBOPLATIN (Cb), MELPHALAN AND FRACTIONATED TOTAL BODY IRRADIATION (fTBI). R. Duerst, L. Constine, P. Savina and A. Guaspari. Univ Rochester Med Ctr, Rochester, NY and Burroughs-Wellcome, Research Triangle Park, NC

To improve the event-free-survival (EFS) of children with poor-prognosis Nb, trials with various myeloablative regimens (with or without fTBI) and ABMT have led to 2-year EFS of 20-50%. In an effort to further improve EFS, we have treated 13 children (2-11 yo) with recurrent metastatic (n=2), Stage D (n=10) or Stage C with elevated N-myc (n=1) Nb according to the following schedule: Cy 1800 mg/m^2 days -6 & -5, Cb 400-700 mg/m^2 days -6 & -5, Melphalan 140 mg/m^2 day -4, and fTBI (6x 200 cGy). This was followed by infusion of immunomagnetic bead-purged autologous bone marrow. Granulocyte-colony stimulating factor (5-10 μg/kgdy) was used post-marrow infusion in 11 of the patients. The interval from initial diagnosis to ABMT was 5-61 mo (median, 9). Serum measurement revealed peak melphalan levels (15 min following a 1 hr infusion) of 5 ± 1 μg/ml and first-order elimination with a half-life of 53 ± 12 min. The regimen has been well tolerated with no toxic deaths. Additional focal radiotherapy (RT, 1500 cGy) is planned after recovery from ABMT (≤~6mo) to the primary tumor and known sites of metastasis (e.g. bones, nodes). 9 of 13 patients have EFS for 9-31 mo (median, 14). Four patients have recurred at 4 to 12 mo post ABMT. 3 of 4 patients who eventually relapsed were in complete remission at the time of ABMT. Focal RT was not administered to these 3 patients but recurrence included metastatic sites in all 4 patients that relapsed. Relapse developed in 1 of 8 patients receiving focal RT and 3 of 5 that did not receive focal RT. Kaplan-Meier estimate of EFS at 30 mo is 66%. Cb will be escalated to 800 mg/m^{2}dy as tolerated in future patients. Further follow-up is required, but ABMT combined with delayed focal RT may further improve EFS for Nb patients.

G 236 THIO-TEPA/CYCLOPHOSPHAMIDE WITH AUTOLOGOUS MARROW RESCUE FOR ADVANCED STAGE NEUROBLASTOMA: PRELIMINARY ANALYSIS FAILS TO DEMONSTRATE EFFICACY OF INVOLVED FIELD RADIATION THERAPY OR PURGING. M. Kletzel, S. Abella, E. Sandler, A. Ogden, K. Stine, S. Shenoy, D. A. Wall, Children's Memorial Hospital/Northwestern University School of Medicine; St. Louis Children's/Washington Univ. School of Medicine; and the Mid-American Pediatric BMT Consortium, Chicago, Illinois 60614.

36 children from 7 institutes received autologous BMT from 6/89-8/93 with advanced stage neuroblastoma (VGPR/CR1-22, PR1-5, CR2/PR2-5; stage D-27, stage C with N-myc amplification-6) as consolidation therapy. All received 4-7 cycles of platinum containing regimens followed by secondary surgery to decrease tumor burden. Children then received local radiation therapy and/or marrow purging (IMMB) based on institutional preference. Five children received peripheral stem cells instead of marrow. All were prepared thiotepa (300 mg/m2 qd x 3), followed by cyclophosphamide (50 mg/kg qd x 4d). G-CSF (10 mcg/kg/d) was used post BMT.

Early toxicity was acceptable. No CNS toxicity. Two patients had grade 4 skin toxicity. There was 1 toxic death secondary to Aspergillosis with 13 documented sepsis episodes. The mean time to ANC > 500 was 19.2 d. and mean time of platelets > 20K was 44.3 d. Overall disease-free survival is 72% by Kaplan-Meyer with 26/36 remaining disease free (16 months median, range 2-45 months). At this analysis, there is no difference if involved field radiation therapy (PFS with XRT 3/11 vs no XRT 5/25) or marrow purging (PFS purged 6/16 vs nonpurged 4/20) were used.

Thus thio-tepa/cyclophosphamide is well tolerated at these doses in pediatric patients. Early evaluation of this cohort fails to demonstrate improved efficacy with either local radiation therapy or purging.

G 237 REVERSIBLE MULTI-ORGAN SYSTEM FAILURE AFTER A NON-TBI BONE MARROW TRANSPLANT REGIMEN FOR PATIENTS WITH NEUROBLASTOMA, Lesley Myers, John Graham-Pole, Curtis Turner, Joseph Wiley, Lisa Diller, Rebecca Murgatroyd, Bone Marrow Transplant Unit, University of Florida (also University of North Carolina and Dana Farber Cancer Institute), Gainesville, FL 32610

Although myeloablative treatment with stem cell support is often used to treat patients (pts) with advanced neuroblastoma (NBL), the best regimen (reg) is unknown. In an attempt to reduce toxicity (tox) while maintaining efficacy, a non-total body irradiation (RT) reg has been piloted at several Pediatric Oncology Group (POG) BMT units. Unexpectedly severe but reversible multi-organ tox has been encountered. The index case is a 3 year old white female with metastatic (met) NBL. She received cisplatinum, etoposide, cytoxan, adriamycin, vincristine, ifosfamide, plus involved field RT before autologous BMT. Her myeloablative reg consisted of etoposide 800mg/m^2/day (day -6 thru -4), carboplatin 667mg/m^2/day (day -6 thru -4), cytoxan 60mg/kg/day (day -3 and -2), and mesna 12mg/kg at 0, 3, and 6 hours/following cytoxan, plus G-CSF until ANC ≥1000. Immediately following chemotherapy (chemo) (day 0) the pt suffered acute hepatorenal followed by cardio-pulmonary failure. The following table shows tox:

	BUN	CR	SGOT	SGPT	BILI	EF	K+	CA	PHOS	U.A.
BASELINE:	10	0.6	10	12	0.1	63%	3.6	8.7	4.6	3cc/kg/hr
MAXIMUM LEVELS:	128	4.6	1719	500	4.0	20%	5.9	12.0	11.9	<0.4cc/kg/hr

DAYS ON: DIALYSIS-28 | VENTILATOR-14 | INOTROPES-21

With intensive supportive care, as indicated in the table, her liver, kidneys, heart and lungs returned to normal and she was discharged from the hospital 42 days post-BMT.

Review of data collected on 25 pts at our and other POG institutions shows a high incidence of similar but lesser tox. We hypothesize the cause of this multi-organ failure to be multifactorial, including (a) direct tox from the ablative reg superimposed on (b) prior chemo tox; (c) abdominal RT; (d) young age. Pts with met NBL may recover with early intensive medical care. Baseline eligibility parameters for proceeding to myeloablation with this reg must be strictly defined and adhered to.

G 238 MULTIPLE MYELOMA CLONES ARE DERIVED FROM A CELL LATE IN B LYMPHOID DEVELOPMENT. J.R. Berenson, R. Vescio, J. Cao, C. Hong, G. Schiller, A. Lichtenstein, and R.J. Berenson. UCLA School of Medicine and DVA WLA, LA, CA; CellPro, Bothell, WA.
Since autologous stem cell infusion may become an important therapy in multiple myeloma (MM), we determined the earliest malignant progenitor. The sequence of the Ig heavy chain variable region (V_H) gene used by the MM clone was obtained on 26 patients after PCR on bone marrow (BM) RNA using V_H family specific and C_H primers. To show whether early cells are involved, we obtained CD34+ BM cells by using Ceprate-separated cells exposed to FAC sorting. Using patient specific complementarity determining region 2 (CDR2) and CDR3 primers, we performed PCR on DNA from the CD34+ cells. Despite a sensitivity of 1 MM cell/10^5 normal cells, none of the samples demonstrated product although β-actin controls were amplified by PCR. To determine whether Cμ-expressing cells are part of the clone, PCR was performed with a CDR1 primer and a Cμ primer. The product was cloned, and colony hybridization was performed with a probe complementary to the framework region 3 (FR3). The colonies were then transferred to a second filter, probed with a patient specific CDR3 probe, and positive colonies were sequenced. Out of > 200 FR3-positive colonies (which, thus, contain the same germline VH gene expressed by the malignant clone), none showed a CDR3 even close to the MM clone. Finally, we investigated somatic hypermutation and intraclonal diversity. Since germline V_H genes may differ in MM, the germline V_H gene was also sequenced from granulocyte DNA. The germline sequences matched previously identified V_H genes but there was marked somatic mutation of the malignant clone. Multiple clones were sequenced and no intraclonal diversity was found. The lack of CD34+ and Cμ-containing malignant cells but presence of somatic hypermutation and lack of intraclonal diversity suggests that this malignancy derives from a cell late in B cell development.

G 239 **A RECIPROCAL BONE MARROW TRANSPLANTATION (BMT) BETWEEN BROTHERS DEMONSTRATING THAT MULTIPLE MYELOMA (MM) IS A TRANSPLANTABLE DISORDER WITH A POTENTIALLY LONG LATENCY**, L. Huebsch, D. Stewart, R. van der Jagt, S. Markman, J. Bormanis, H. Griesser, H.A. Messner. Bone Marrow Transplant Programs of the University of Ottawa (Ottawa General Hospital) and the University of Toronto (Princess Margaret Hospital), Ontario, Canada.
The diversity and frequency of expression of both myeloid and early lymphoid antigens in MM cells, combined with a substantial risk of developing acute myelogenous leukemia or myelodysplastic syndromes suggest that a transformation of a pluripotent stem cell is central to the pathogenesis of MM. Our report lends support to this hypothesis.
In September 1981 an 18 year old man with chronic-phase chronic myelogenous leukemia [Patient A] underwent an allogeneic BMT from his HLA-identical 22 year old brother [Patient B]. Examination of the donor's marrow at that time prior to BMT revealed no abnormalities. Routine yearly bone marrow examinations over the ensuing 10 years revealed no cytologic, cytogenetic or in-vitro growth abnormalities in the patient A. He was a complete chimera by restriction fragment length polymorphism studies. In July 1991 Patient B, the original marrow donor, was found to have a stage III IgG kappa chain MM. In February 1992 patient B underwent an uneventful "autologous" BMT from patient A, with no GVHD prophylaxis. Engraftment, while slow was eventually nearly complete, although the patient failed to achieve a complete remission. In June 1992 patient A's yearly bone marrow morphology was highly suspicious of a plasma cell disorder as well, showing clusters of plasmablasts. Patient A also had a serum protein electrophoresis in August 1992 showing the same monoclonal IgG kappa protein as his brother. Molecular and genetic analysis will be presented that demonstrate the identity of the two myeloma clones.
We conclude that Patient B already harbored MM stem cells in 1981. These cells were transferred in the original marrow allograft and have subsequently engrafted and demonstrated in both patients a very long latency. These observations not only support the hypothesis that the defect in MM is present in pluripotent cells capable of long term engraftment, but also have implications for the ultimate success of autologous BMT in MM.

G 240 **AUTOGRAFT WITH CIRCULATING STEM CELLS IN MULTIPLE MYELOMA.** I.Majolino, S.Vasta, R.Marcenò, R.Scimè, A.Indovina, G.Liberti, F.Buscemi, M.Pampinella, A.Santoro, S.Gentile, F.Caronia. Department of Hematology and BMT Unit, Ospedale Cervello, 90146 Palermo, Italy.
Seventeen patients with multiple myeloma (MM) were autografted using circulating stem cells (CSC) ± bone marrow (BM). They were 41 to 61 (median 53) years of age. Ten were IgG, 3 IgA, 1 IgM, 2 BJ and 1 non-secreting MM. Fourthen patients were stage III and only 3 stage II. When admitted to the program they had already completed at least one full standard chemotherapy program. Fifteen were in partial remission (≥ 50% reduction of monoclonal component, MC), one in complete remission (disappearance of MC and/or BM plasmacytosis <5%) and one in progression. The collections of CSC were run after mobilization chemotherapy (CY 7g/m^2, 6 pts; VCAD 11 pts), followed by GM-CSF or G-CSF in 11 patients. The autografts were done 3 to 73 (median 13) months from diagnosis, following myeloablation with BCNU-etoposide-melphalan (11 pts), melphalan alone (4pts), or busulfan-melphalan (2pts). All patients received the product of aphereses while BM was infused only in four, on the basis of poor peripheral CFU-GM yields. Six patients received also G-CSF or GM-CSF. The recovery of ANC ≥ 0.5 x 10^9/L occurred 8 to 17 days (median 12) and that of platelets ≥ 50 x 10^9/L 9 to 56 days (median 12) following graft. Engraftment was sustained in all cases, and no transplant-related deaths occurred. At the stable hematological reconstitution the patients were started on α-IFN, 1-3MU thrice a week. With the autograft, twelve patients achieved the complete remission, and in six this was accompanied by the disappearance of the MC even on immune-fixation. Eleven patients are still free from progression 2-35 months (median 19) from autograft, and 8 of them are in CR. The PFS estimate does not show a stable plateau, but median survival is not reached with a follow-up of 13-96 months (median 35) from diagnosis. To better define the role of CSC autograft in MM, more patients and a longer follow-up are needed.

G 241 CIRCULATING PROGENITOR CELL COLLECTION WITH CHEMOTHERAPY ± G-CSF IN PATIENTS WITH MULTIPLE MYELOMA. R.Marcenò, S.Vasta, I.Majolino, R.Scimè, A.Indovina, F.Buscemi, M.Pampinella, G.Liberti, S.Gentile, A.Santoro, F.Caronia. Department of Hematology and BMT Unit, Ospedale Cervello, 90146 Palermo, Italy.
The circulating progenitor cell (CPC) mobilizing effect of VCAD (vincristine 2 mg, cyclophosphamide 4 x 0.5g/m^2, adryamicin 2 x 50 mg/m^2 and dexamethasone 4 x 40 mg) was tested in 14 myeloma patients. Their median age was 53.5 years (40 to 60). Nine were IgG, 3 IgA, 1 BJ and 1 IgM. Eleven had high tumor mass and 3 intermediate. Time from diagnosis to mobilization was 12.5 months (3 to 72). At this time, 10 were responsive and four were primarily resistant. Seven patients received G-CSF, 5 mcg/kg/die, continuous iv infusion, starting the day following VCAD. The apheresis program was started at the time of rapid WBC count increase. The G-CSF was discontinued the day of the last apheresis or when WBC increased to > 40 x 10^9/L. Toxicity was milder in patients receiving G-CSF, with shorter duration of neutropenia. Circulating CFU-GM peak values fell within a wide range (96 to 4352/ml), with a median (853/ml) corresponding to 9.2 fold the basal level. The highest values of CPC were observed in patients with low bone marrow plasmacell contamination. G-CSF administration did not heightened significantly the circulating CFU-GM level but the CFU-GM peak coincided temporally to the day of the first apheresis. Also the peak of circulating CD34+ve cells coincided temporally to that of CFU-GM. Patients receiving G-CSF needed a lesser number of aphereses (3 vs 6, p=.002), with better single apheresis yields (4.9 vs 1.2 CFU-GMx10^4/kg). They underwent the first apheresis earlier and completed the full course of apheretic procedures in a shorter time.

G 242 TRANSPLANTATION OF AUTOLOGOUS CD34-POSITIVE PERIPHERAL BLOOD STEM CELLS AS TREATMENT FOR MYELOMA. Gary Schiller, Robert Vescio, Myung Lee, Scott Bearman, Gary Spitzer, Cesar Freytes, Michael Lill, Ronald Berenson, James Berenson. UCLA; Universities of Colorado; St. Louis; Texas; and CellPro, Inc.

In multiple myeloma, transplantation of autologous bone marrow has produced 3y progression-free survival of 40-60%. A major potential problem of autologous BMT is evidence of clonal plasma cells in the bone marrow product. In this report we present 9 pts with advanced, chemotherapy-responsive myeloma age 42-65y (median 56y) who have been enrolled on a trial of progenitor cell procurement followed by high-dose chemotherapy and transplantation of CD34+ peripheral blood progenitor cells. The median time from diagnosis to Tx was 8.5 mos (range 4-45 mos). At Tx, paraproteinemia ± marrow plasmacytosis and extensive bone lesions were identified in all pts. All pts received induction therapy with alkylator-based (4 pts) or VAD chemotherapy (4 pts), or decadron alone (1 pt). Progenitor cells were harvested 14d after cyclophosphamide (2.5 gm/m^2 IV), prednisone (2 mg/kg qd x4), and G-CSF (10 μg/kg SC qd x14). Leukapheresis was performed processing 10 L of whole blood on a Cobe spectra. CD34+ progenitor cells were purified using a cellular immunoadsorption method with biotin-conjugated anti-CD34 Ab12-8 after passage over a column of avidin-coated beads (CellPro, Bothell, WA). CD34+-selected cells infused contained a median of 6.6 x 10^6 cells/kg (range 1.8-9.6) and purity of collection ranged from 50-91%. PCR analysis using patient-specific Ig gene primers showed no evidence of tumor contamination of the CD34+ autograft. Cells were infused one day after completing preparative conditioning with busulfan (.875 mg/kg q 6h x16 doses) and cyclophosphamide (60 mg/kg/d x2). Following infusion, GM-CSF (500 μg IVPB) was given daily. Nine pts have completed the trial; the number of days to granulocyte count >500/μl and untransfused platelet > 20,000/μl were 13.5 d and 12 d respectively. In conclusion, CD34+ stem cells are an effective form of purified hematopoietic support for pts with myeloma undergoing myeloablative chemotherapy.

Lymphoma, Hodgkin's; Preparative Regimens - Etoposide; T-Cell Depletion; Cord Blood; Cytokines and Stem Cells

G 300 Long-Term Follow-Up Proves that Autologous Marrow Transplant(ABMT) For Advanced Hodgkin's Disease (HD) Yields Stable Remissions. **B.S. ANDERSSON, P.L. HUGHES, B.I. SAMUELS, F. HAGEMEISTER, F. SWAN, K. van BESIEN, F. CABANILLAS, R.C. CHAMPLIN. University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030**

In HD patients failing conventional chemotherapy(CT) and XRT, high dose chemotherapy with ABMT has induced remissions, but the durability of response has been uncertain. Since 1978 we have used a combination of cyclophosphamide (6 g/m^2), BCNU (300 mg/m^2), and Etoposide (750-900 mg/m^2) (CBV) followed by ABMT in 111 advanced HD patients with minimum follow-up of 12 mos (12-150 mos). There were 69 men and 42 women; median age was 29 years (16-55). 60 (54%) achieved a complete or clinical complete remission (CR), and 16 (14%) had a partial remission (PR), for a total response rate of 68%. The patients who achieved CR have been followed for a median of 42 mos (4-144+). 70% of the CR's are stable, generating a plateau in progression-free survival (PFS) of 49% among the responders (CR + PR). The five-year DFS for the whole group is 33%, which is higher than expected for advanced HD patients failing conventional CT. Nine patients died early, prior to hematologic recovery (8%), from hemorrhagic episodes and neutropenic infections, and 7 patients have died in CR at 5 to 30 months post transplantation from infections (3), respiratory failure of unknown etiology, and acute leukemia (3), respectively. This therapy was well tolerated and safe in the dose range used. We conclude that CBV with ABMT should be regarded as standard-of-care for patients with recurrent, advanced HD in the following categories:

1. Patients with induction-refractory HD, clinical stages III and IV.
2. HD relapsing after a short first remission, i.e. a duration of less than 12 months.
3. HD relapsing after more than 12 months, and which is refractory to reinduction attempt with a conventional salvage regimen.
4. Patients who experience ≥ second relapse, regardless of duration of previous CR.

G 301 HIGH DOSE CHEMOTHERAPY WITH STEM CELL TRANSPLANT (HDCSCT) IN OLDER PATIENTS WITH LYMPHOID MALIGNANCIES, César O. Freytes, Donna E. Salzman, Carlos Bachier, David Boldt, G. David Roodman, Fiona Craig, Justiniano Castro and C. Frederick LeMaistre, University of Texas Health Science Center, San Antonio, TX 78284

Although the incidence of malignancies increases with age, older patients (pts.) are seldom included in clinical studies of HDCSCT. The purpose of this study was to define the peri-transplant morbidity, mortality and effectiveness of HDCSCT in older pts. We analyzed transplants performed at our institution for lymphoid malignancies in pts. ≥50 years. Twenty-seven pts.(22 males, 5 females) were treated. Ages ranged from 50 to 65 (median 59). Eighteen pts. were treated for non-Hodgkin's lymphoma, 10 low grade, 6 intermediate and 1 high grade, 1 for mycosis fungoides. Four pts. had myeloma, 4 lymphocytic leukemia (3 acute, 1 chronic) and 1 Hodgkin's disease. Twelve pts. were transplanted in sensitive relapse or complete remission, 2 in untested relapse. Thirteen were transplanted in resistant relapse or were primary refractory. Fourteen pts. underwent autologous bone marrow transplant (BMT), 10 peripheral blood stem cell transplant, 2 allogeneic and 1 syngeneic BMT. Twenty-two pts were conditioned with cyclophosphamide, BCNU and VP-16 (CBV)± DTIC. Twenty-two pts. received growth factor support. Fourteen pts (52%) experienced Grade III or IV non-hematologic toxicities according to the SWOG Toxicity Criteria, although 4 represented transient hypotension secondary to DTIC. Peri-transplant mortality (death directly related to a transplant complication within 7 weeks of transplant) was 15%. Seventeen pts (63%) remain alive; 41% (11/27) remain free from progression 4 to 22 months post-transplant (median 11). We conclude that HDCSCT can be performed in older pts. with acceptable morbidity and mortality.

G 302 PHENOTYPIC VARIABILITY AMONG CLONES DERIVED FROM THE U937 CELL LINE: SPONTANEOUS AND INDUCED DIFFERENTIATION, CYTOCHEMISTRY AND ONCOGENE EXPRESSION. M. I. Gaspar Elsas, M. S. Salerno, L. A. A. Silva, I. F. Manhães, G. Pimenta and P. Xavier Elsas. Institute of Microbiology and University Hospital (UFRJ) and Instituto Fernandes Figueira (FIOCRUZ), Rio de Janeiro, Brazil, 21941-590

Previous studies (Gaspar Elsas et al., **Blood** 75, 2427) had shown that individual cells in cultures of the U937 cell line, derived from a histiocytic lymphoma, and widely used as a model of phorbol ester(PMA)-induced differentiation, differed greatly in their ability to respond to this agent, as well as in the expression of the novel surface marker detected by monoclonal antibodies (mAbs) to the Eosinophil Cytotoxicity Enhancing Factor (ECEF). In this study, we evaluated the extent of phenotypic variability in the cells regarding a broad spectrum of biochemical and functional markers. U937 clones were derived by limiting dilution and assessed with respect to growth rates, spontaneous adherence to the substratum, spontaneous morphological differentiation and differentiation induced by PMA or by PMA plus bacterial LPS. We have also assessed: 1) the ability of the clones to secrete ECEF and the surface expression of the corresponding epitopes, with the help of specific mAbs, 2) the cytokine secretion patterns of these clones, in assays for antibody-dependent, eosinophil-mediated killing of schistosomula, as well as in assays for eosinophil differentiation in liquid cultures of human bone marrow cells, and 3) the presence of the marker enzymes Acid Phosphatase and Alpha-naphtyl Acetate Esterase, the pattern of PAS staining and the expression of the c-myc and ets-1 protooncogenes. The clones were highly heterogeneous with respect to all of the above parameters, suggesting that the U937 cell line displays extensive clonal variability, which is not taken in consideration in the many studies using this cell line. Novel phenotypes included clones that differentiate without PMA, as well as clones that had lost responsiveness to PMA, both potentially useful tools for elucidating the molecular mechanisms of PMA-induced differentiation.

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G 303 AUTOLOGOUS BONE MARROW TRANSPLANTATION WITH ANTI-B4-BR DEPLETED MARROW FOR PATIENTS WITH B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA AND NON HODGKIN'S LYMPHOMA. M. Gyger, R. Bélanger, C. Perreault, Y. Bonny, R. Soiffer, D. Esseltine, J. Ritz and D.C. Roy. Unité de Transplantation de Moelle Osseuse, Hôpital Maisonneuve-Rosemont, Montréal, Canada; Div. of Tumor Immunology, Dana-Farber Cancer Institute, Boston; and ImmunoGen Inc., Cambridge, MA.

A number studies have shown that relapse following autologous bone marrow transplantation (ABMT) can originate from neoplastic cells present in the bone marrow (BM) graft. In addition, clinical trials suggest that elimination of these contaminating cells prior to ABMT may result in prolonged disease-free-survival (DFS) for patients with acute lymphoblastic leukemia (ALL) and non Hodgkin's lymphoma (NHL) with poor prognosis. In the present study, we present results of a Phase I/II clinical trial of ABMT and marrow purging with an immunotoxin, Anti-B4-bR, in 30 patients with high risk B-lineage NHL and ALL. Anti-B4-bR consists of an IgG1 monoclonal antibody directed against CD19, an antigen found on >90% of clonogenic ALL cells and also NHL cells, conjugated to whole ricin in which galactose-binding sites of the B-chain have been chemically blocked. We have previously shown that Anti-B4-bR can eliminate >3 logs of clonogenic CD19+ Nalm-6 or Namalwa target cells. Twenty patients (pts) with NHL and poor prognostic features (including failure to achieve complete remission (CR) or relapse after conventional therapy, histologic transformation or extranodal dissemination) achieving a minimal disease (MD) state, with lymph nodes $\leq$2 cm and BM involvement $\leq$5%; and 10 pts with ALL, 5 pts in CR2, 4 pts in CR1 and 1 pt in CR3 underwent ABMT. BM was harvested during last CR or MD just prior to BMT. Median age at ABMT was 39 years. Median time from last CR to BMT was 2.1 mo. Treatment of the marrow graft with 10 nM Anti-B4-bR depleted CFU-GM day 7 and day 14 and BFU-E progenitors by 22.9%, 27.7% and 31.1% respectively. All patients evaluable demonstrated engraftment with purged marrow: median time to >0.5x10^9/l granulocytes: 25 days (14-51) and to >20x10^9/l platelets without transfusion for 4 weeks: 31 d (15-262). One patient, refractory to platelet transfusions, died of a cerebral hemorrhage early post-BMT. Five pts (1 NHL, 4 ALL) relapsed during the first 6 months following BMT. Eighteen of 20 pts with NHL are alive with a Kaplan-Meier 2 year DFS of 80%, median follow-up of 6 months. Six of 10 pts with ALL remain alive and in continuous CR at 25+, 75+, 344+, 642+, 787+ and 804+ days post-BMT. Our results show that marrow purging with Anti-B4-bR allows hematopoietic engraftment and adds no significant toxicity to the BMT procedure. Thus, autologous BMT with Anti-B4-bR purging is a promising alternative for patients with ALL and NHL. Further studies will be designed to determine the efficacy of this approach and the role of marrow purging.

G 304 AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR CHRONIC LYMPHOCYTIC LEUKEMIA (CLL). I. Khouri, M.J. Keating, C. Reading, Y.O. Huh, M. Thomas, A.B. Deisseroth, R.E. Champlin. The University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030

Patients with advanced CLL relapsing post fludarabine usually have poor prognosis. This study was undertaken to evaluate the feasibility and effectiveness of high dose chemotherapy and total body irradiation (TBI) with autologous bone marrow transplantation (BMT) in patients with advanced CLL who failed fludarabine. Eleven patients who had their marrow collected during a prior remission were studied. Eight were male. Their median age was 59 years (range 37-66). The median time from diagnosis to BMT was 48 months (mos) (range 15-198). The median number of prior chemotherapeutic agents was three. At BMT, three were Rai stage 3, one stage 2 and seven had regression of their recurrent progressive disease to stage 1 or 0. Two of the latter had a Richter's transformation, one was at his fourth and three at their second relapse. Seven patients had their marrow purged with anti-CD19 monoclonal antibodies and immunomagnetic separation. This resulted in a median 1.3 log reduction of leukemic cells. The preparative regimen consisted of cyclophosphamide (CY) 60 mg/kg/d x 2 and fractionated TBI (10.2 -12 Gy). All received G-CSF 5-10 mcg/kg. All patients achieved engraftment with a median time to recovery of ANC of 0.5 x 10/L of 16 days (range 10 to 28) and platelets >25k of 34.5 days (14-53). toxicity was minimal and the average hospital stay was 32 days. Six achieved a CR, 4 a nodular Cr, and 1 PR. Residual disease was analyzed with CD5+ CD20+ dual color cytometry and gene rearrangement by Southern blot. Two patients had detected clonal cells by both methods and two by molecular studies only. Patients in CR had correction of their IgM and IgA levels post BMT. Two patients expired in CR, one due to CMV pneumonia. Three with Richter's transformation at 4,6 and 8 mos and two with CLL at 12 and 15 mos post BMT. Two patients had reoccurrence of autoimmune anemia and thrombocytopenia without any evidence of CLL. Six are alive with a median follow-up of 10 mos (range 2-29). We conclude that autologous BMT is feasible and can induce remission in patients with advanced CLL. An earlier transplant at a time when the leukemia is still sensitive to conventional chemotherapeutic agents might improve future results.

G 305 A TRIAL OF AUTOLOGOUS HEMOPOIETIC STEM CELL TRANSPLANTATION FOR RELAPSED OR REFRACTORY NON-HODGKIN LYMPHOMA. Yuju Ohno, Shuichi Taniguchi, Hisashi Gondo, Tetsuya Etoh, Shin Hayashi, Koichi Akashi, Takanori Teshima, Mine Harada, Yoshiyuki Niho. First Department of Internal Medicine, Kyushu University.

Since April in 1988, autologous hemopoietic stem cell transplantation(AHSCT) has been done for 15 patients of relapsed or refractory Non-Hodgkin lymphoma(NHL). The efficacy of conditioning chemotherapy combining Busulfan, Etoposide(VP-16) and Cyclophosphamide(CY) was unsatisfactory because 7 of 8 patients remained refractory or relapsed after AHSCT within 1 year. Therefore, we use Ranimustine(MCNU.250mg/sqm and 200mg/sqm.day-7 and -3.respectively), VP-16(20mg/Kg/day from day -6 to -4), Carboplatin(10mg/Kg/day from day-7 to -4) and CY (40-60mg/Kg/day on day-3, -2)(MECC regimen) as conditioning chemotherapy on 7 relapsed or refractory patients, and examined the efficacy and safety. Although a term of observation is short, the efficacy of MECC regimen is now satisfactory because all 7 patients were successfully induced into complete remission(CR) and maintaining a state of CR for 5-25 months(mean CR duration, 13 months). Stomatitis and dermatitis over Grade 2 according to WHO Toxicity Scale were seen in 3 cases and 2 cases respectively. These were managed with conventional supportive care. MECC regimen is considered to be safe and to have better anti-lymphoma effect than the former regimen, however, increased numbers and longer follow-ups of patients are required.

G 306 A PHASE I STUDY OF CYCLOPHOSPHAMIDE (CY), VP-16, CARBOPLATIN (CBDCA), AND TOTAL BODY IRRADIATION (TBI) WITH AUTOLOGOUS STEM CELL TRANSPLANTATION FOR HEMATOLOGIC TUMORS. T Shea, J Wiley, S Sailer, E. Powell, J Serody, J Mason, AM Storniolo, S Seagren, S Bentley and M Brecher. Departments of Medicine, Pathology, and Radiation Oncology, UNC at Chapel Hill, NC, 27514 and Depts of Medicine and Radiation Therapy, UC San Diego, San Diego, CA, 92123.

23 patients (pts) were treated with fixed-dose CY (2 gm/m^2/day x 3 days), VP-16 (600 mg/m^2/day x 3 days), and CBDCA (1 gm/m^2 by 96 hour infusion) with a dose escalation of BID TBI in 200 cGy fractions (3 pts at 800 cGy, 5 pts at 1000 cGy, 14 pts at 1200 cGy and 2 pts at 1300 cGy). Histologies included: 3 AML (1 CR1, 2 CR2): 2 ALL (1 CR4, 1 CR3): 12 Int/High-grade NHL (2 PR/CR1, 10 sensitive relapse): low-grade lymphoma, 5 (all > 1st sensitive relapse): 1 myeloma (relapse). Reinfusion products included bone marrow (BM) only, 2 pts; 4HC purged BM, 2 pts; peripheral blood stem cells (PBSC) plus BM, 5 pts; and PBSC only, 14 patients. All PBSC only pts had active or prior marrow involvement. A median of 9 x 10^8 mononuclear cells/kg (range 3-27 x 10^8 MNC/kg) were infused per pt.

There were no treatment related deaths. Severe toxicities included delayed platelet recovery (4 pts), transient renal failure (1 pt), grade 3 (severe, not life threatening) mucositis, grade 2 acute and chronic autologous GVHD (1 pt) and a transient 20% drop in one pt's RNEF. All but 1 pt with ALL, 1 pt with myeloma, and 4 pts with high-grade NHL remain progression free 1 to 32 months following BMT.

Aside from substantial but reversible mucositis, this regimen appears to be tolerabe and effective. Additional patients will be treated with 1300 and 1400 cGy of TBI to define the MTD of this regimen. Phase II studies in patients with leukemias and lymphomas are underway.

G 307 ETOPOSIDE (VP) AND CYCLOPHOSPHAMIDE (CY) WITH TBI OR BUSULFAN (BU) FOR ALLO-BMT IN ADVANCED HEMATOLOGIC DISEASES. Alessandrino E.P., Bosi A., Bandini G., Rosti G., Bernasconi P., Guidi S., Lombardini L. Calori E. and Bernasconi C. for GITMO. BMT Unit, Chair of hematology, Policlinico S. Matteo IRCCS, 27100 Pavia (I)

The possibility of enhancing the cytoreductive effect of the conventional BMT preparative regimens by addition of VP has prompt us to investigate this drug with either TBI or BU-based conditioning regimens. Thirty two pts at high risk of relapse underwent allo-BMT using either TBI/VP/CY (11 pts) or BU/VP/CY (21 pts). The BU/VP/CY regimen included BU at a total dose of 16 mg/mq, CY at a dose of 120 mg/Kg, VP given at a dose of 30mg/Kg by continuous iv. infusion over 24 hrs (10 pts), or at a dose of 50 mg/kg over two days by 2 hrs iv. infusion (11 pts). The TBI-based preparation consisted of CY (120 mg/Kg) followed by VP and TBI, VP (50 mg/kg) was given over two consecutive days by 2 hrs iv. infusion. Criteria for pts to be considered at high risk of relapse were: AL other than first CR (15 pts), CGL in advanced phase (5 pts), MDS in transformation (6 pts), AL with resistant disease (4 pts). Time to engraftment, incidence of acute-GvHD and gut toxicity were similar with both the BU/CY/VP and TBI/CY/VP regimens. At present time 5/21 pts in the BU/CY/VP group have relapsed, 10 are alive and disease free(DF). Five pts died before day + 100. In the TBI/CY/VP group 5/11 pts have relapsed, 3 died before day +100, 5 are still alive and DF. Survival probability is 38 % at 4 yrs. in the BU/VP/CY group compared with 0 at 3 yrs. in TBI/CY/VP group. Due to the small number of cases and diversity of diagnosis we can not draw definite conclusions. The BU-based regimen however, seems to be better and these results make tantalising further evaluations of the BU/VP/CY conditioning regimen

G 308 MELPHALAN/BUSULFAN AS CONDITIONING REGIMEN FOR ALLOGENEIC BONE MARROW TRANSPLANTATION. Koen van Besien MD, Raymond Alexanian MD, Charles F. LeMaistre and Richard Champlin MD. The University of Texas MD Anderson Cancer Center, Houston Texas 77030

Conditioning regimens for allogeneic bone marrow transplantation are designed to eradicate malignant cells and cause profound immunosuppression in the host, thus allowing engraftment of donor cells across major and minor histocompatibility barriers. Total body irradiation or high-dose cyclophosphamide are considered necessary to provide sufficient immunosuppression. We report on a patient undergoing allogeneic bone marrow transplantation for refractory multiple myeloma. She could not undergo total body irradiation because of extensive prior radiation to the spine. She was conditioned with busulfan 16 mg/kg po over 4 days (day -6 to day -3) and melphalan 140 mg/m^2 iv day -1. She received a non T-cell depleted marrow infusion from an HLA-compatible sibling on day 0. Prophylaxis for graft-vs-host disease consisted of Cyclosporin A and Methotrexate. She engrafted by day 16 (absolute granulcyte count >500). The marrow on day 21 revealed a left shifted myeloid series; megakaryocytes and erythroid progenitors were present. No residual myeloma was identified. The patient succumbed to a syndrome of multi-organ failure and acute graft-versus-host disease on day 25 after BMT. This report demonstrates that melphalan may potentially serve as an alternative immunosuppressive agent to cyclophosphamide or total body-irradiation for allogeneic bone marrow transplantation.

G 309 INCREASED INCIDENCE OF GRAFT FAILURE IN RECIPIENTS OF LYMPHOCYTE DEPLETED MATCHED UNRELATED AND MISMATCHED FAMILIAL BONE MARROW ALLOGRAFTS. Albert D. Donnenberg, Witold B. Rybka, Steven Neudorf, Steven M. Pincus, Elana J. Bloom, John Lister, Margarida deMagalhaes-Silverman, Edward D. Ball. Bone Marrow Transplantation Program, Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA 15213

In the setting of fully MHC matched familial bone marrow transplantation (BMT), elutriation (CCE) has been successfully employed to prepare bone marrow allografts with defined lymphocyte content. Clinically significant GVHD has been markedly reduced with acceptable engraftment rates (90-95%). We report our experience in BMT of a small series of unrelated (8), and partially matched familial (5) lymphocyte depleted grafts for hematologic malignancies. Apheresed marrow was fractionated by CCE; recipient T-cell dose was adjusted to $0.5x10^6$ T cells/kg recipient ideal body weight. The lymphocyte rich fractions which were not infused were cryopreserved as a source of backup stem cells. BMT cytoreduction was cyclophosphamide (CY) 120 mg/kg and busulfan 16 mg/kg (12) or CY plus TBI 1380 cGy (1). Only 3 of 13 recipients engrafted, all of whom developed GVHD (1 grade I, 1 grade II, 1 grade III). Three patients died too early to fully assess engraftment. Seven experienced immunological rejection, 3 of whom engrafted after receiving a second preparative regimen and the backup fractions; one died of GVHD, two died of sepsis. In contrast, engraftment was seen in all 4 recipients of matched sibling CCE lymphocyte depleted grafts performed concurrent to this series. Graft preparations were indistinguishable among patients who did and did not engraft with respect to nucleated cell, CFU-GM, and CD34 dose. CD34 recovery in the graft fraction was 34%. These results indicate that the present lymphocyte dose was insufficient, in combination with these preparative and immunosuppressive regimens, to assure engraftment in settings other than matched sibling BMT.

G 310 IDENTIFICATION AND SEPARATION OF $CD3^+CD4^-CD8^-$ T CELLS FROM HUMAN BONE MARROW FOR USE IN BONE MARROW TRANSPLANTATION. A.Hinkle, B. Taylor, R. Quinones. Children's National Medical Center and George Washington University, Washington, D.C. 20010. We hypothesize that bone marrow transplantation (BMT) may be improved by manipulating the cellular composition of the infused marrow to improve engraftment and provide graft versus leukemia (GVL). Elutriation separates T cells from a stem cell enriched fraction and provides BM fractions which can be engineered to select specific T cell subpopulations for infusion. T cell depletion prevents GVHD but may eliminate potential mediators of engraftment and GVL. Our goal is to define and isolate a subset of T cells which, when added to the infused marrow, would enhance engraftment and GVL while not causing GVHD. Several murine models have identified the $CD3^+CD4^-CD8^-$ T cells (double negative T cells, DNT) which express TCRαß as such effectors. We demonstrate in human marrow that DNT are 5.5% ± 3.6% (range 1.5-10.8%) of the $CD3^+$ cells and 38% ± 9.2% (range 28-53%) of these express TCRαß. In peripheral blood, DNT constitute 4.8% ± 2.5% (range 1.9-10.7%) of $CD3^+$ cells with a lower percentage, 28% ± 6.4% (range 23-32%), expressing TCRαß. In preclinical studies using elutriated BM fractions, we have separated DNT from $CD3^+CD4^+$ and $CD3^+CD8^+$ cells using AIS MicroCellector flasks. By depleting $CD4^+$ and $CD8^+$ cells, we have enriched DNT to 90% ± 8.5% (range 81-98%) of $CD3^+$ cells. This method is reproducible and results in good purity, viability, and immediate availability of cells. AIS selection systems have demonstrated scale up capability for clinical use. We intend to utilize this separation procedure in clinical bone marrow transplants.

G 311 UNRELATED SELECTIVE CD8+ T-CELL DEPLETED ALLOGENEIC BONE MARROW TRANSPLANTATION AFTER BUSULFAN AND CYCLOPHOSPHAMIDE, Matt E. Kalaycioglu, Andrew Fishleder, and Brian J. Bolwell, Departments of Hematology and Medical Oncology and Laboratory Hematology, Cleveland Clinic Foundation, Cleveland, OH 44195

Selective CD8+ T-cell depletion (TCD) has been shown to reduce the incidence of graft-versus-host disease (GVHD) without an apparent increase in leukemic relapse after a total body irradiation containing preparative regimen (Blood 76: 418-423, 1990). We have treated 8 patients (pts) for hematologic malignancies with busulfan (1mg/kg orally every 6 hours for 16 doses) and cyclophosphamide (60mg/kg IV daily for two doses) (BuCy2) followed by HLA-matched, unrelated bone marrow transplant following selective CD8+ TCD. CD8+ T-cells were depleted using M450 microspheres conjugated with CD8 monoclonal antibodies (Dynal, Oslo, Norway). The average depletion of CD8+ cells was 95.3% (89-98). Additional GVHD prohylaxis was provided with cyclosporin and methylprednisolone. G-CSF 16 mcg/kg/day was given until the absolute neutrophil count (ANC) was $>1000/ml^3$. 7 pts engrafted to an ANC $>500/ml^3$ at an average of 12 days (9-24). 1 of these pts subsequently rejected the donor marrow and failed to engraft after a autologus transplant of stored marrow. 1 patient failed inital engraftment but died of sepsis 15 days after a second transplant. These 2 pts died of complications related to pancytopenia. 1 patient died of fulminant relapse and 1 died of venocclusive disease. 4 pts are alive in complete remission at 53, 83, 293, and 460 days post-transplant. Of these 4, 2 had Grade I-II acute GVHD of the skin. No other GVHD has been noted. We conclude that BuCy2 followed by unrelated allogeneic BMT with CD8+ TCD is effective in preventing GVHD .

G 312 **BONE MARROW "BOOST" FOLLOWING T CELL DEPLETED BMT**

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Eight patients transplanted for non-malignant conditions (7 SCIDS, 1 AA) received lectin treated T cell depleted BMT's from haplo-compatible donors. Median age at the time of initial transplant was 0.7 yrs (range: 30 d - 12.3 yrs). Median initial cell dose was 1.6 x 10^8 cells/kg (range: 0.28 - 3.5). Conditioning therapy for the initial transplants ranged from no conditioning (1 pt), Cy/ATG (3 pts), Cy/ATG/TBI (2 pts), Bu/Cy/ATG (1 pt), and Ara C/Cy/TBI/ATG (1 pt). Each of these patients subsequently received a second T cell depleted transplant ("boost") from the same donor because of evidence for engraftment in conjuncton with a delay in the recovery of T cell immunity. Median age at boost was 2.0 yrs (range: 0.6 - 12.8 yrs) with a median time to boost after initial BMT of 0.7 yrs (range: 77 d - 4.7 yrs). Median cell dose was 1.5 x 10^8 cells/kg (range: 0.18 - 2.9). No conditioning therapy was used prior to the boost (except 1 pt who received ATG) and no GVHD prophylaxis was used during either the initial or subsequent BMTs.

Seven of the eight patients are surviving at a median follow up of 2.0 yrs (range: 0.4 - 5.2 yrs). One pt died of Aspergillus pneumonia. Following BMT boost, T cell function improved (PHA +1232%, MLC +2911%, PWM +2914%; p< 0.1, 0.2, 0.4 respectively) Lymphocyte phenotype also changed with increases in the percentage of CD3+ cells (250% p<0.05), CD3+DR+ (162% p<0.1), CD4+ (158% p<0.05), CD4+Leu8- (128% p<0.1), CD4+Leu8+ (216% p<0.05), and CD8 (141% p<0.1). There was also a 37% decrease in the CD16+/CD56+ natural killer cell population (p<0.05).

These results suggest that bone marrow "boosts" are an effective means for improving T cell immunity in patients who fail to recover adequate immune function after T cell depleted bone marrow transplantation.

G 313 USE OF CD34+ POSITIVELY-SELECTED STEM CELLS FOR ALLOGENEIC BONE MARROW TRANSPLANT FROM HLA MISMATCHED RELATIVES, Mark J. Mogul, Finn B. Petersen, Linda L. Meaux, Patrick G. Beatty, Bone Marrow Transplant Program, University of Utah Health Sciences Center, Salt Lake City, UT 84132

Allogeneic bone marrow transplant is the therapy of choice for numerous life-threatening disorders. However, the lack of a suitable match limits the availability of this procedure. We describe a process which allows selection of CD34+ marrow and peripheral blood stem cells from HLA-mismatched relatives as a possible solution to this dilemma. This process would presumably provide sufficient numbers of hematopoietic precursors to repopulate the patient's marrow, would remove mature T-cells capable of causing significant graft-versus-host disease, and also remove antigen presenting cells which may be otherwise capable of stimulating the remaining donor immune system to reject the graft.

We have successfully used such CD34+ selected marrow and peripheral blood stem cells from a haplo-identical parent to engraft a patient with recurrent T-cell lymphoma/leukemia. Patient UUPN #85 was initially diagnosed with Stage III T-cell lymphoma confined to his mediastinum, and was induced into a complete remission with standard-dose therapy, but had a rapid on-therapy systemic relapse. He was reinduced into a second remission and then underwent a haplo-identical bone marrow transplant using CD34+ selected marrow and peripheral blood stem cells from his father. His preparative regimen included total body irradiation (225 cGy x6), and Cytoxan 120 mg/kg. Graft-versus-host disease prophylaxis consisted of Cyclosporine and Methotrexate. His donor received G-CSF for six consecutive days (day -13 to day -8), and on the last two days underwent pheresis for CD34+ stem cells. On day zero, bone marrow was harvested from the donor as well. 5.5 x 10^6 CD34+ cells/kg of patient were selected from the peripheral blood, and 6.5 x 10^6 CD34+ cells/kg were selected from the bone marrow. There was a 3-log depletion of T-cells from the peripheral blood, and no T-cells detectable by FACS after depletion from the bone marrow. The patient engrafted with an absolute neutrophil count of >500 on day +26 and had continuous trilineage marrow engraftment. Engraftment was further documented by DNA typing on three separate occasions. Unfortunately, the patient expired on day +105 of irreversible pulmonary regimen-related toxicity. An autopsy confirmed trilineage engraftment, no sign of graft-versus-host disease, and no sign of malignancy.

G 314 THE HUMAN HEMATOPOIETIC STEM CELL RESULTING IN DURABLE ENGRAFTMENT MAY BE A LARGE CELL. R.Quinones, P.Dinndorf, A.Hinkle, B. Taylor. Children's National Medical Center and George Washington University, Washington, D.C. 20010. We have used extended cycle elutriation (ECE) in 17 refractory leukemia patients (pts) to control the infused T cell content in HLA disparate bone marrow transplants (BMT) to promote graft vs leukemia (GVL) without severe GVHD. ECE resulted in a stem cell enriched fraction extensively depleted of >3 logs of lymphocyte-sized cells (LSC), as quantified by assessing T cells by flow cytometry and limiting dilution analysis. Following ECE, pts received 1.4 $\pm$0.7 x 10^8 BM nucleated cells/kg, 2.5 $\pm$0.9 x 10^5 CFU-GM/kg, 3.1 $\pm$1.5 x 10^6 $CD34^+$ cells/kg, with 4.2 $\pm$4 x 10^4 T cells/kg added-back. The maximum number of ^{6} LSC given with the T cell add-back was 1.2 x 10^6/kg ($\leq$7.5% $CD34^+$). After intensive cytoreduction, 16 pts achieved engraftment (median time to ANC >500, 22 days and to platelet independence, 54 days). One pt with extensive myelofibrosis failed to engraft. Actuarial disease free survival is 46% at 2 years (median 29 months, range 5 to 52). These pts have durable allografts with T and B cell immune recovery. Murine models show that, following elutriation, LSC provide durable engraftment, while larger cells provide committed progenitors producing short-term engraftment. Elutriated human BM studies have shown the in vitro long-term BM culture initiating cells to be in the LSC fraction. Our data show that the larger sized cell ECE fraction extensively depleted of LSC, containing only 1/3 of the $CD34^+$ cells, provides both committed progenitors producing short-term engraftment and sufficient long-term pluripotent progenitors to result in durable engraftment across HLA barriers. Our data do not address a potential role in hematopoietic recovery of the $CD34^+$ LSC. These data have implications for future marrow manipulation strategies.

G 315 CHIMERISM ANALYSIS BY FLUORESCENT IN SITU HYBRIDIZATION IN ALLOGENEIC BONE MARROW TRANSPLANTATION: PARTIAL T-CELL DEPLETION VS UNMANIPULATED GRAFTS, G. Rondón, S. Giralt, M. Leahy, K. van Besien, R. Mehra, M. Andreeff and R. Champlin, M.D. Anderson Cancer Center, Houston, TX 77030

Selective T-cell depletion from donor marrow reduces acute GVHD, but mixed chimerism, graft failure and relapse may occur. Chimerism was studied in 43 patients that received sex mismatched partial T-cell depleted or unmanipulated allografts from an HLA-compatible sibling or matched unrelated donor. 24 patients (10 AL, 2 CML CP, 8 lymphoma, 3 CLL, 1 MM) received unmanipulated marrow, 12 patients in CML chronic phase received CD3 depleted marrows. Patients were prepared with a variety of regimens, and in all of them cyclosporine vs administered as GVHD prophylaxis. The percentage of host and donor cells in bone marrow aspirate was determined using probes for the X and Y chromosome (Imagentics, Spectrum CEP®, Farmington, MA) at >60 days, between 60 and 180, 180 to 360 and >360 after BMT.

Type Depletion	<D60 %Donor Cells	D60-D180 %Donor Cells	D180-D360 %Donor Cells	>D360 %Donor Cells*
None	98.9, (83.2-100) n=10	98.4 (86.3-100) n=14	99.25 (84.9-100) n=6	99.0 (91.8-100) n=7
CD8	98.9 (97.0-100) n=8	98.4 (95.0-100) n=7	99.0 (97.0-100) n=5	97.5 (92.0-100) n=5
CD3	87.9 (79.4-96.7) n=2	99.4 (97.1-100) n=4		99.4 (80.7-99.7) n=3

*95% CI=±3.4% for X and ±3.9% for Y

Conclusions: Mixed chimerism was a rare occurrence after allogeneic BMT in the three categories studied.

G 316 REMOVAL OF CD3+ T-CELLS FROM UNRELATED ALLOGENEIC BONE MARROW GRAFTS FOR TRANSPLANTATION USING MAGNETIC FERROFLUIDS AND HIGH GRADIENT MAGNETIC CELL SEPARATION, Terry E. Thomas, Sara J.R. Abraham, Gordon L. Phillips and Peter M. Lansdorp, Terry Fox Laboratory, B.C. Agency, 601 West 10th Ave., Vancouver B.C., Canada.

We have developed a technique for removal of CD3+ T-cells from clinical sized suspensions of peripheral blood or bone marrow using specific labelling of target cells with antibody complexes and magnetic colloidal dextran iron followed by High Gradient Magnetic Separation. Colloidal particles act as a true solution facilitating rapid and quantitative cell labelling. Cell suspensions are efficiently labelled in blood bags and washes are not required prior to magnetic separation. Cells are cross-linked to unmodified colloidal dextran iron by purely immunological means avoiding labour intensive, costly and inefficient techniques (with inevitable batch to batch variation) for the covalent labelling of colloidal particles. In our method tetrameric antibody complexes are used to cross-link magnetic particles to selected target cells. These complexes consist of two mouse monoclonal antibody molecules bound in a complex by the $F(ab')_2$ fragments of two rat antibody molecules which recognize the Fc portion of the mouse IgG1 molecule. One mouse antibody (DX1) specifically binds dextran (on colloidal iron- dextran particles) and the other CD3 (UCHT1). After labelling cells are pumped through a HGMS wire filter which is placed in a magnetic field (0.5Tesla). A 200ml sample (marrow buffy coat or peripheral blood leukapheresis collection) can be processed in 15min. Pre-clinical separations of human peripheral blood have achieved 2.73±0.18 Log depletion (mean±1SE) of CD3+cells while recovering 80.2±4.8% of the negative cells. A large scale pre-clinical separation of human bone marrow recovered 84% of the C34+ cells with 2.75 log depletion of CD3+ cells. This method is now available for the removal CD3+ cells from allogenic bone marrow and seems to have significant advantages over other T-cell depletion methods.

G 317 RECOMBINANT IL2 AFTER AUTOLOGOUS BMT IN 69 PATIENTS TREATED FOR HEMATOLOGICAL MALIGNANCIES, Blaise D., Stoppa A.M., Attal M., Pico J.L., Michallet M., Reiffers J., Gisselbrecht C., Tabilio A., Vey N., Gabus R., Olive D., Brandely M., Maraninchi D. Institut Paoli Calmettes, Marseille, France.

Over a 4 year period we have treated more than 120 patients in different phases of an approach to assess the association of non purged auto BMT followed with rIL2. We have previously reported both feasability as well as high immunological stimulation. In order to assess impact on outcome we studied 44 pts, treated for AL in CR1 (AML=16 ; ALL=28) and 25 for malignant lymphomas (ML) (HG NHL=14 ; HD=11) at different stages. All patients received the same rIL2 regimen (provided by Roussel Uclaf) consisting in a continuous infusion over 5 cycles (C1=5 days ; C2-C5=2 days) every other week, starting in average on day 63 post BMT and a scheduled dose varying from 12 to 24 M IU/m^2/day. rIL2 was in fact delivered during 8 days (3-13) over a period of 1.6 mths (0.1-2) for an average of 125 M IU/m^2 (39-353). Discontinuation of treatment was due mainly to capillary leak syndrom related toxicities (60%) or poor plts count (10%). No patients died directly in relation to rIL2 toxicities. Very high stimulation of both NK and T cells were see (all $p<0.05$).

- The 44 pts with AL were prepared with CyTBI. Median follow up is 24 months. 30 months probabilities for relapse, survival and DFS are 27%, 87% and 73% for AML and 52%, 63% and 48% for ALL. Although follow-up is short these results compares favorably with historical groups in term of survival. Impact on relapse prevention seems to be effective in AML. Definitive impact should be demonstrated in the analysis on the on going european randomized trial where 170 pts with CR1 AL have been so far randomized in the same schedule.

- Among the 25 pts with ML 12 were in CR or sensitive relapse (NHL=9 ; HD=3), and 13 were refractory (NHL=5 ; HD=8). All pts were prepared with BEAM With a median follow up of 24 mths DFS for NHL and HD is 63% and 51%. This results compares favorably with historical controls especially for no refractory NHL.

These results are in favour of positive impact of rIL2 in adjuvant setting and invite further comparative studies to optimize the treatment.

G 318 ESTABLISHMENT OF A CORD BLOOD BANK, Jeffrey McCullough, John Wagner, Elizabeth Perry, Susan Fautsch, Mary Clay, Harriet Noreen, Miriam Segall, and David Stroncek, University of Minnesota Hospital, Minneapolis, MN 55455

Successful hematopoietic reconstitution has been accomplished using human umbilical cord blood (UCB). To date, this reconstitution has been done using matched and partially mismatched sibling donor UCB; however, UCB might have advantages over bone marrow for transplants between unrelated individuals. In order to evaluate this possibility, it is necessary to establish a "bank" of stored UCB so that a larger number of transplants can be carried out. However, there are many issues which must be resolved, specific procedures developed, and requirements defined in order for such a "bank" to be established. In this study, the requirements and procedures to operate an UCB bank are described. These include: 1) Procedures for health history screening of UCB donors (mothers and infants); 2) Procedures for collection of UCB; 3) Short-term storage conditions for UCB prior to freezing for long-term storage; 4) Procedures for laboratory testing of UCB to minimize the risks of disease transmission; 5) conditions for and duration of long term storage; 6) Testing of the UCB if indicated for genetic diseases; 7) Strategies and methods for testing UCB for maternal cells; 8) An algorithm or strategy for HLA typing of the UCB collected in order to freeze UCB in the most cost-effective manner; 9) Methods for removing red blood cells from UCB so as to minimize the problems of ABO incompatible transplants; 10) Procedures for freezing UCB; 11) Data elements and computer software necessary to establish a "bank" inventory; 12) General strategy for searching the bank inventory for patient matches; 13) Procedures and conditions for shipping frozen UCB to distant sites for transplantation; 14) FDA regulatory issues involved in establishing a UCB bank and interstate shipping of UCB for transplantation; and 15) Quality assurance program to be used in the operation of the UCB bank.

The availability of these procedures and standard requirements will make possible the establishment of unrelated cord blood banks and facilitate the use of cord blood stem cells for transplantation.

G 319 COLLECTION AND SHORT-TERM STORAGE CONDITIONS FOR HEMATOPOIETIC PROGENITOR CELLS FROM CORD BLOOD. Witold B. Rybka, Patricia J. Hamilton, Rochelle Roscoe, Joseph Mierski, Judith Keane, Alan Winkelstein, Elizabeth Curry Johnson, Robert A. Dracker, Edward D. Ball. Bone Marrow Transplant Program, Pittsburgh Cancer Institute, and The Central Blood Bank, Pittsburgh, PA 15213, and The Biocyte Corp., Stamford, CT 06902.

Cord Blood (CB) cells provide hematopoietic stem cells (HSC) capable of engrafting bone marrow. Long-term banking will be required for clinical utilization. Hematopoeitic progenitor cells (HPC), clonal assay (CFU) and CD34+ cells, are used as surrogate markers for HSC. We have used HPC to assess optimal conditions for harvesting and banking CB collections. 22 CB collections were assessed under various conditions comparing ACD to Heparin as an anticoagulant and storage at 22°C vs. 4°C assessing samples at 3, 24, 48, and 72 hrs after collection. (CB cells were obtained from the umbilical cord and placenta following placental delivery by sterile venous puncture and gravity flow into a collection kit containing anticoagulant. HPC were quantified by CFU using standardized reagents (Terry Fox Laboratories) measuring CFU-GM, BFU-E, CFU-GEMM and by flow cytometry for CD34 antigen (8G12 and QBEND-10 epitopes) excluding markers for mature lineages (CD3, CD11b, CD14, CD19). At 22°C, both Heparin and ACD showed stable recoveries of mononuclear cells and CFU to 72 hrs after collection (63-100%). CD34+ cell measurments remained unchanged in Heparin, but increased in ACD suggesting increased non-specific binding over time. In ACD, but not Heparin, there was a progressive loss of granulocytes over time paralleled by a decrease in total cell viability as measured by acridine orange and ethidium bromide dyes. In ACD, storage at 4°C was accompanied by a greater granulocyte loss and wider variability in CD34+ cell measurements than at 22°C. Holding CB collections up to 72 hrs in either ACD or Heparin at 22°C results in remarkable stability of HPC. An increased holding time prior to cryopreservation would improve the feasibility of CB banking.

G 320 EFFECT OF SEQUENTIAL IMMUNOTHERAPY WITH IL-2 AND BUFFYCOAT ON THE ANTILEUKAEMIC ACTIVITY OF DONOR LYMPHOCYTES IN A PATIENT WITH RELAPSED AML AFTER BMT, Renate Arnold, Donald Bunjes, Matthias Theobald, Bernd Hertenstein, Markus Wiesneth, and Hermann Heimpel, Depts Internal Medicine III/Transfusion Medicine, University of Ulm, Robert-Koch-Str. 8, D-89070 Ulm, FRG.

A 26 year old female received a bmt from an HLA identical brother for treatment of AML, FAB M4 in first CR. Gvhd prophylaxis consisted of CSA/MTX. Neither acute nor chronic gvhd were observed. 17 months post bmt the patient (pt) relapsed. After reinduction chemotherapy the pt reached a second CR. Consecutively, 3 cycles of IL-2 were given from 12/1992 to 4/1993. In May 1993 bone marrow evaluation revealed an incipient 2. relapse with 10% blasts. IL-2 therapy was stopped and buffy coat transfusions (BC) from the original bone marrow donor on 4 consecutive days were given. After BC the pt developed no gvhd, no cytopenia and remains in CR with the last bone marrow evaluation on day 63 after BC. The bone marrow revealed an infiltration with small lymphocytes. T-cell and LAK responses to leukaemia or host minor antigens were analysed by using limiting dilution assays before and after BC. After IL-2 and before BC no LAKp or CTLp reactive with patient leukaemia could be detected. In contrast a high frequency of leukaemia-reactive HTLp (1/35000) was found. After BC no LAKp, CTLp or HTLp against leukaemia or host could be found. We speculate that the favourable clinical response of the pt was the result of antileukaemic T cell reactivity stimulated by IL-2 and that this response was boostered by the transfer of naive, non tolerant donor T cells with the BC. The fact that we were unable to detect this reactivity after BC therapy may be due to the limited expansion of reactive T cells induced by the small number of residual leukaemic blast or could be the result of redistribution of reactive T cells to the marrow.

G 321 EFFECTS OF RECOMBINANT HUMAN CYTOKINES ON *EX VIVO* EXPANSION OF ISOLATED CD34$^+$ HEMATOPOIETIC PROGENITOR CELLS IN SERUM-FREE CULTURE CONDITIONS, William Biddle, Michelle Wysocki, Barbara Dadey, Jean Donovan and James Pancook, Life Technologies, Inc., Grand Island, NY 14072

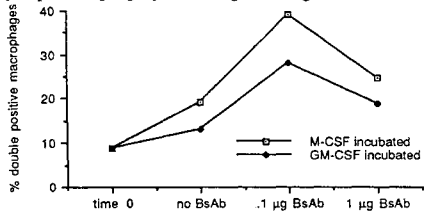
The *ex vivo* culture of human hematopoietic progenitor cells is a newly-emerging technology which may ultimately prove useful as an alternative or adjunct biological therapy for malignant or aplastic states and genetic therapies. As *ex vivo* expansion of hematopoietic cells approaches clinical reality, it necessitates the need for more defined culture systems. An enriched population of CD34$^+$ cells was obtained from human bone marrow aspirates using a positive selection process (AIS MicroCellector™). These cells were cultured under serum-free conditions utilizing a newly developed cell culture medium (Life Technologies, Inc.). This medium is composed entirely of components of either synthetic, plant or defined human origin. CD34$^+$ cells were cultured for one to several weeks in the presence of various combinations of cytokines including IL-1, 3, 6, stem cell factor (SCF), G-CSF, GM-CSF and erythroprotein. Stem cell factor was demonstrated to be important for optimal cell expansion levels. A combination of IL-3, SCF and GM-CSF resulted in upwards of 100 fold expansion within two weeks of culture. These expansion levels were routinely similar or greater then could be achieved utilizing standard culture conditions (Iscove's medium with 20% FBS). The resulting cells were shown to consist of a population enriched in early committed myeloid progenitor cells and highly expressed CD33 and CD15. The total number of CD34$^+$ cells although diminishing in total percentage, actually was increased during the culture period indicating preservation and amplification of this stem cell compartment. CD34$^+$ cells cultured in serum-free medium in the presence of various cytokine combinations retained the ability to form multilineage colonies in semi-solid medium. This newly developed culture system should allow investigators to more thoroughly characterize the regulatory factors required for hematopoiesis without interference of the confounding substances contained in fetal bovine serum.

G 322 HUMAN INTERLEUKIN-3 (RHIL-3) FOR GM-CSF AND G-CSF UNRESPONSIVE GRAFT FAILURE AFTER BONE MARROW TRANSPLANTATION. Hilde Demuynck, Marc A. Boogaerts, Gregor E.G. Verhoef, Pierre Zachée, Department of Haematology, University Hospital Gasthuisberg, Leuven, Belgium

Delayed engraftment and graft failure after autologous or allogeneic bone marrow transplantation (BMT) are serious complications characterised by a high morbidity and mortality. Therefore rhIL-3 was administered to 5 patients with delayed engraftment after autologous BMT (2 Hodgkin's disease, 3 NHL) and one patient with secondary graft failure after matched unrelated allogeneic BMT for chronic myelogenous leukemia. Their mean age was 31 years (range : 17-43). All patients responded only temporarily or failed on previous treatment with myeloid growth factors (rhG-CSF and/or rh GM-CSF). The mean time from BMT to the start rhIL-3 was 216 days (range : 46-393). rhIL-3 was administered subcutaneously or by a 6 hour intravenous infusion at a starting dose of 5 µg/kg/d. Doseescalation to a maximum of 15 µg/kg/d was allowed in case of insufficient response. The median treatment duration was 56 days (20+-77 days). rhIL-3 therapy resulted in an increase in the absolute neutrophil count (ANC) in 5/6 patients. Their mean level of ANC rose from 0.86 10^9/l (range : 0.16-0.19 10^9/l) to a mean peak value of 3.29 10^9/l (range : 0.44-5.55 10^9/l). Dose escalatation was effective in 1/3 patients. The median time to response (2-fold increase of ANC) was 23 days (range : 3-28 days). The mean ANC at the end of rhIL-3 administration was 2.23 10^9/l (range : 0.27-3.60 10^9/l). Loss of neutrophil response despite continued rhIL-3 administration was noted in 1 patient. The ANC dropped only modestly after permanent cessation of the rhIL-3 therapy. Parallel to the rise of ANC, an increase in eosinophil counts was noted from a mean pretreatment level of 0.008 10^9/l (range : 0-0.04 10^9/l) to a maximum of 1.11 10^9/l (range : 0.78-2.08 10^9/l). The levels decreased rapidly to pretreatment values after interruption of rhIL-3. Despite a rise of reticulocytes in 4/6 patients, no changes in red cell transfusion requirements were noticed. All patients were severely thrombocytopenic at the start of the therapy. No platelet rises were observed. Further decreases in platelet counts and increased platelet transfusion requirements were noticed in 3/6 patients. Severe bleeding diathesis was the reason of premature treatment cessation in 1 patient. Adverse effects were common including flu-like illness (6), fever (5), myalgia (3) and fluid retention (2). Severe adverse reactions were the cause of premature treatment interruption (bronchospasm (1), increased bleeding tendency (1)). Two patients are still alive. One patient is still on growth factor therapy for persistent pancytopenia 517 days after BMT. Causes of death were : refractory infection (2), intracranial hemorrhage (1), relapse of Hodgkin's disease(1). We conclude that rhIL-3 can be successfully applied for delayed neutrophil engraftment after BMT. However, no major reduction in red cell nor platelet transfusion requirements may be expected. Severe adverse reactions are common.

G 323 BISPECIFIC ANTIBODY MEDIATED PHAGOCYTOSIS OF LYMPHOMA-LIKE CELL LINE BY MONOCYTE DERIVED MACROPHAGES IS ENHANCED BY IFNγ AND M-CSF. Pamela Ely, Letha E. Mills, Solveig G. Ericson, Paul M. Guyre, Michael W. Fanger. Dartmouth Medical School, Lebanon, NH 03756.

Immunologic approaches to cancer therapy following bone marrow transplantation (BMT) using antibodies, cytokines, and activated effector cells offer the possibility of enhancing radio-chemotherapeutic responses. Macrophages which are functional throughout BMT are able to mediate tumour phagocytosis and extracellular lysis but may not be efficient enough in a non-targeted setting to result in clinically significant anti-tumour responses. We have shown that the thioether-linked F(ab')2 construct linking the F(ab')2 moiety of the anti-FcγRI mAb 22, binding outside the ligand binding site, and the F(ab')2 moiety of an anti-CD37 antibody can mediate antibody-dependent phagocytosis of lymphoma-like cells by monocyte-derived macrophages (MDM). The flow cytometry-based phagocytosis assay, adapted from Munn *et al.*(JEM 172:231, 1990), measures double positive effector cells indicating ingestion of the fluorochrome-labeled target cell. Using target cells labeled with PKH-26 and MDM effectors stained with FITC-anti-CD14, we found increased antibody-dependent phagocytosis in the presence of BsAb compared to monoclonal antibody- dependent or antibody-independent controls. Further, the IFNγ-treated macrophages were more phagocytic than untreated macrophages consistent with the fact that the expression of the targeted antigen, FcγRI, increases following IFNγ incubation. Finally, when the influence of growth factors was compared, M-CSF enhanced antibody-dependent phagocytosis to a greater degree than GM-CSF.



These studies suggest that clinical trials using bispecific antibodies with cytokine priming in lymphoma following BMT are warranted.

G 324 Cytokine Therapy for Patients Relapsing After Allogeneic Bone Marrow Transplantation. Giralt S, Hester J, Talpaz M, Kantarjian H, Van Besien K, Andersson B, Chan KW, Gajewski J, Mehra R, Przepiorka D, Deisseroth A, Champlin R. University of Texas, M.D. Anderson Cancer Center, 1515 Holcombe Blvd Houston, TX 77031.

Purpose: To determine toxicity and efficacy of the use of different cytokines in patients who have relapsed with hematologic malignancies after an allogeneic bone marrow transplant (BMT)

Patients & Methods: 19 patients (8 AML, 7 CML, 3 MDS, 1 CLL) who relapsed between 0 and 360 days after allogeneic BMT received 5 mcg/kg of G-CSF subcutaneosly to reinduce remission. 10 patients (5 CML, 5 AML) received Interferon 2.5-5.0 x 10^6 u/m^2 subq TIW and interleukin 2 1.8x10^6 IU/m^2 daily or bid 5 x a week until progression or response.

Results: 6 of 19 patients responded to G-CSF therapy (1 CML, 4 AML, 1 CLL), 3 patients remain in remission at 18+, 1+ and 4+ months, 1 patients relapsed at 1 month while 2 others had remissions lasting 12 months. Toxicity was minimal. We did not see any responses in patients with extramedullary disease or peripheral blood blasts. 3 of 10 patients treated with IFN/IL2 have responded. 2 patients with CML in CP achieved hematologic remissions of which one had significant cytogenetic improvement (50% diploid metaphases). 1 patient with AML had clearing of her peripheral blood blasts and decrease in her bone marrow blasts to 5%. Toxicity was significant at the dose of IFN:5 x 10^6 u/m^2 TIW and IL2:1.8 x 10^6 u/m^2 BID, 5 x a week, with 7 of 9 patients requiring 50% dose reduction due to Grade 3 toxicity (2 CNS somnolence, 2 fever>40, 2 PS>2, 1 capillary leak). One patient redeveloped grade 3 toxicity after a 50% dose reduction, one patient developed Grade 2 GVHD, while 6 other patients have tolerated IFN:2.5 x 10^6 u/m^2 TIW and IL2:1.8 x 10^6 IU/m^2 5 x a week without grade 3 or 4 toxicities.4 patients developed $\geq$ Grade 2 GVHD that responded to steroid therapy.

Conclusions: Cytokine therapy can be effective in reinducing remissions in patients who have relapsed after allogeneic BMT. Further exploration of these strategies and combination with chemotherapy is warranted.

G 325 ISOLATION OF HEMATOPOIETIC STEM CELLS IN MICE WITH LONG-TERM RECONSTITUTION USING VERY FEW CELLS, Robert A. Good*, Guy W. Bradley*, Muneo Inaba+, Nahoko Ogata, Susumu Ikehara+ and Hajime Ogata*, *Department of Pediatrics, All Children's Hospital, University of South Florida, St. Petersburg, FL 33701. +First Department of Pathology, Kansai Medical University, Osaka, Japan.

We have developed a method for isolating and defining a population of primitive hematopoietic stem cell (PHSC) that fits the best definition of these cells. This is a dormant (non-dividing) population of cells with impressively uniform characteristics. They are characterized as a very small component of marrow cells and are insensitive to treatment of mice with 5-FU (*in vivo*). Shortly following 5-FU injection of the mice, a fraction of PHSC respond to the decline in number of BMC by entering a cycling phase. Precise timing of BMC harvesting after 5-FU treatment and methods to distinguish PHSC from cycling cells in the marrow are essential to the application of the 5-FU method of enrichment of PHSC. The number of cells in the low density PHSC enriched population after injection of 150 mg/kg 5-FU i.v. and removal of cells with lineage markers reached a minimum 3 days after 5-FU injection. PHSC resistant to 5-FU are enriched 1000 fold in this fraction. 98% of the resultant cells in the blast gate are cells which are Thy 1^{lo}, lack IL-3 receptors and of course are of low density. The markers of these cells are Thy-1^{lo}, lineage specific surface marker negative, WGA, Sca-1 and MHC class I antigen highly positive and c-kit and transferrin receptor negative. In numerous experiments, it has been possible by injection of 100, 20, or even only 4 such cells to induce impressive lymphohematopoiesis reflected in the peripheral blood of lethally irradiated syngeneic recipient mice.

G 326 HEMATOPOIETIC ACTIVITY OF ACETYL-SER-ASP-LYS-PRO (AcSDKP) ON BONE MARROW CELLS. John D. Jackson[1], Yun Yan[1], Cynthia Ewel[2], Linda Kelsey[1] and James E. Talmadge[1]. [1]University of Nebraska Medical Center, Omaha, Nebraska and [2]Henri Beaufour Institute, Inc., Washington, D.C.

The tetrapeptide AcSDKP is a potent inhibitor of hematopoietic stem cell proliferation. The effect of AcSDKP was examined in long-term bone marrow (LTBM) cultures and in short-term liquid cultures in the absence/presence of cytokines. The murine bone marrow short-term cultures, were incubated with AcSDKP in the presence or absence of IL-3, SCF, G-CSF or GM-CSF for various time periods. The percent of CFU-GM in cell cycle was inhibited following 48 hours in culture; however, cultures containing exogenously added cytokines required 72 hours in culture to inhibit the number of progenitors in cell cycle. Dose response studies showed effects at 10^{-12} to 10^{-14} M AcSDKP with decreased activity at higher or lower concentrations. In the LTBM culture studies, we investigated the effects of AcSDKP on the production of granulocyte/macrophage colony forming cells (CFU-GM) and high proliferative potential colony forming cells (HPP). AcSDKP was added daily to LTBM cultures at various concentrations (10^{-8} M to 10^{-16} M) for up to five weeks. Cultures were assayed at one, three or five weeks of culture. AcSDKP was active at 10^{-12} M with less activity seen at higher or lower concentrations. The number of non-adherent CFU-GM per LTBM culture was not changed at one week of treatment with 10^{-12} M AcSDKP but decreased at weeks three and five. HPP progenitors were decreased throughout the treatment period. The number of CFU-GM and HPP in cell cycle was significantly decreased from week one to the end of the study. AcSDKP had no effect on the number of adherent CFU-GM, HPP or adherent cellularity per culture or percent in cell cycle. These studies suggest that the concentration of AcSDKP and the timing of exposure to the tetrapeptide is critical in inducing an effect on hematopoietic progenitors. The presence of cytokine or stromal cells can also affect the response of progenitor cells to AcSDKP. This research was supported by a contract with Henri Beaufour Institute, Inc.

G 327 FUNCTIONAL CAPACITY OF GRANULOCYTE PRECURSORS GENERATED *IN VITRO* FROM MARROW PROGENITOR CELLS, Jane L. Liesveld, Dina L. Plekavich, Abigail W. Harbol, Maureen C. Kempski, and Camille N. Abboud, Department of Medicine, Hematology Unit, University of Rochester School of Medicine, Rochester, NY 14642

Many investigators have now reported that enriched CD34+ cells from marrow or blood or light density marrow cells can be successfully stimulated with hematopoietic growth factor combinations to result in expansion of both myeloid progenitors and granulocyte precursors. It is postulated that infusion of such expanded populations could further decrease times to neutrophil engraftment. In this work, we have begun to examine the functional capacity of granulocytes generated in such *in vitro* culture systems. Such cells have been compared to normal blood-derived neutrophils in terms of morphology, ability to polymerize G actin to F actin, phagocytosis capability, and NBT reduction capacity. CD34+ cells isolated from marrow or light density marrow cells were grown in IMDM + 10% FBS in the presence or absence of 10 ng/ml SCF, 20 ng/ml IL-6, 20 ng/ml IL-3, 10 ng/ml G-CSF and/or 10 ng/ml GM-CSF. Cultures were maintained for 14 days with one feeding and factor repletion at 7 days. In such preparations, 15%-50% of the cells differentiated to or beyond the "band" neutrophil stage. Complete segmentation of granulocytes was rarely observed, and hyposegmentation was often aberrant with nuclear budding noted. Granulocytes derived from such cultures were able to phagocytose latex beads as assessed by light microscopy and by light microscopy analysis of cell pellet sections. Expanded neutrophils were also able to polymerize G to F actin in response to 10^{-9}M FMLP or 100 ng/ml IL-8, but the F-actin content as assessed by FACS analysis of NBD phallacidin stained cells was generally less than that of blood PMNs analyzed in parallel. These data suggest that granulocytic cells derived from factor-treated light density or CD34+ cells have functional capabilities although they appear to lack complete capacity for normal differentiation and function. Whether this reflects *in vitro* culture conditions or may be intrinsic to these "expanded" cells remains undetermined.

G 328 Ex Vivo Expansion of Functional Myeloid Cells Using Simultaneous Addition of IL3, G-CSF and SCF.

Michael C. Lill, Maureen Lynch, John K Fraser,Grace Y Chung, Gary Schiller, John A. Glaspy, Lawrence Souza*, Gayle C. Baldwin, Judith C. Gasson. Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA; *AMGEN, 1840 DeHavilland Drive, Thousand Oaks, CA.

Current hematopoietic stem cell transplantation protocols subject the recipient to at least 8 to 10 days of neutropenia with associated morbidity, mortality, and expense. In this study we have exploited the proliferative and differentiative capacity of the hematopoietic stem and progenitor cell compartment in vitro to generate the mature cells required to abrogate this neutropenia. In order to determine the optimal culture conditions necessary for this expansion CD34-positive cells were purified from either bone marrow or from peripheral blood progenitor cells mobilized with G-CSF±Stem cell factor(SCF) using the Ceprate CD34 biotin kit(CellPro#LC34-2). The CD34 cells were then expanded for two weeks in various combinations of IL-3, IL-6, G-CSF, and SCF.

The optimal combination for expansion of myeloid cells (simultaneous addition of 50ng/ml IL-3, G-CSF, and SCF) resulted in an average 773-fold expansion (range 493-1068). 75±10% of these cells were $CD11b^+$ and 86-91% were morphologically recognizable myelocytic cells. These cells exhibited normal phagocytosis and intracellular killing of *Staphylococcus aureus*. Substituting IL-6 for G-CSF in this combination resulted in considerably lower fold expansion (254±55-fold, range 125-431) and generated a population that was slightly less mature (i.e., 55±6% $CD11b^+$). This population also exhibited increased frequency of CFU-GM (6.3±0.2 per 1,000 cells; versus 1.1±0.1 per 1,000 cells with G-CSF). However, because of the difference in fold expansion the G-CSF, IL-3, SCF population contained a higher total number of progenitors. Similarly, sequential addition of late-acting factors to early-acting factors generated a less mature population with considerably lower fold expansion. These studies demonstrate that it is possible to use a relatively simple combination of growth factors to generate sufficient expansion of functionally effective myeloid cells for potential clinical use in abrogation of post transplant neutropenia.

G 329 ADHERENT NATURAL KILLER (A-NK) CELL INFUSION WITH IL-2 TWO DAYS AFTER PBSC TRANSPLANTATION DOES NOT ADVERSELY AFFECT ENGRAFTMENT AND RESULTS IN AMPLIFIED KILLER CELL FUNCTION POST TRANSPLANT. J. Lister, SM Pincus, WB Rybka, AD Donnenberg, TL Whiteside, ED Ball. University of Pittsburgh, Pittsburgh, PA.

In a phase I/II trial combining adoptive immunotherapy (AI) with high dose chemotherapy and peripheral blood stem cell transplantation (HDC-PSCT) we observed no adverse effect on engraftment, no toxicity directly attributable to AI and measureable peripheral blood NK activity before hematologic recovery. PBSC were collected on G-CSF accelerated recovery from chemotherapy induced nadir. Subsequently, four daily leukaphereses, for generation of adherent natural killer cells (A-NK), starting 2 days after cessation of a six day constant intravenous infusion of low dose IL-2 at 3 $X10^5$ i.u./m^2 (LD-IL-2), were processed and expanded in the presence of IL-2 for 2 to 3 weeks. Flow cytometry on the peripheral blood during A-NK leukapheresis revealed a minor population of cells with phenotype $CD3^+16^+56^+34^+$. We postulate that this population of cells resulted from the LD-IL-2 and may be a LAK precursor. On day 0 after myeloablative chemotherapy PBSC were given, on day 2 A-NK cells were infused and a four day continuous intravenous infusion of IL-2 (2 X 10^6 i.u./m^2/day) began. On day 6 the dose was decreased to LD-IL-2 which was continued for 90 days. Patients received 0.8 - 39 X 10^6 CD34+ lin- cells /kg and 0.4 - 49 $X10^4$ CFU-GEMM / kg. Days to platelets self-supporting > 20,000 ranged 9 - 45 and to granulocytes > 500, 11 - 16. No adverse effect on engraftment was noted as compared to historical data. A-NK cell numbers transfused ranged 0.8 - 4.0 X 10^{10}, with NK phenotype (CD3-56+) accounting for 64 to 98 % of cells. Lytic units versus K562 and Daudi per 10^7 cells ranged between 3168-33968 and 3363-23435 respectively. A lymphocytosis preceded by 2 days neutrophil engraftment and lytic activity versus K562 and/or Daudi was observed in the peripheral blood (peak LU 772-1673, normal [median (80% midrange]): 132 (50,300) before day 12. Cells with NK phenotype accounted for 19-47% of leukocytes before day 12.

G 330 IN VITRO ACTIVATION OF PERIPHERAL BLOOD PROGENITOR CELLS WITH INTERLEUKIN-2, Amitabha Mazumder, Udit N. Verma, Ellen Areman, Bone Marrow Transplantation Program, Georgetown University Medical Center, Washington DC.

Based on our previous studies demonstrating marked anti-tumor activity of interleukin-2 (IL-2) activated bone marrow in vitro and in vivo, we studied generation of anti-tumor cytotoxic effector cells from PBSC mobilized by cytoreductive chemotherapy and myeloid growth factors. We studied fresh and frozen/thawed PBSC from 12 patients, 8 with breast cancer and 4 with lymphoma. The cells were placed in culture at varying cell densities in either serum-containing (IMDM with 10% fetal calf serum (FCS) or serum free (X-Vivo 10) culture medium, supplemented with IL-2 in a final concentration of 600 or 6000 I.U./ml at 37°C for 24 hours in flasks or in culture bags. Anti-tumor cytotoxicity was tested against A375 (melanoma), K562 (CML) and Daudi (Burkitt's lymphoma) cell lines in standard 4 hour ^{51}Cr release assay. Chemotherapy and growth factor priming could lead to important qualitative and quantitative alteration of lymphoid cells, therefore we also looked at distribution of lymphoid cells contained in primed PBSC by FACS and at cytotoxicity of primed vs. unprimed PBSC was compared in a murine model. Marked cytotoxicity was seen against all cell lines tested (A375: 37.5% ± 5.5; K562: 54.6% ± 5.4; Daudi: 51.7% ± 9.2). Cytotoxicity was comparable in serum-containing and serum-free culture conditions (68.7% vs 69.4%) and in tissue culture flasks and bags (68.7% vs 72.7%). Cell density up to 10 x 10^6/ml was not associated with any significant decline in cytotoxicity (1 x 10^6 vs 10 x 10^6/ml: [A375: 39.0% vs 23.5%; K562: 66.1% vs 66.0%]). IL-2 activation of PBSC after thawing led to generation of cytotoxicity comparable to that obtained with fresh PBSC (57.6% vs 60.6%). On flow cytometric analysis proportion of $CD3^+$ was found to be variable (1.42% to 31.19%) but in majority it was lower than (median 9.6%) normal PBMNC. In the murine model primed PBSC were found to be having higher cytotoxicity than unprimed (C1498; 35.3% vs 23.2%). The results of this study indicate that it may be feasible to activate large numbers of previously frozen PBSC with IL-2 and serum-free medium in tissue culture bags at concentrations of up to 10 x 10^6 cells/ml for clinical autologous transplantation.

G 331 CHARACTERIZATION OF QUIESCENT $CD34^+$ CELLS THAT ARE HLA- $DR^{+/-}LIN^-$, C-KIT^{br} AND NON-PROLIFERATIVE IN LONG TERM MARROW CULTURE. J.E. Wagner, D. Collins, S. Fuller, L. Shain and J. Lebkowski. University of Minnesota School of Medicine, Minneapolis, MN; R&D Systems, Minneapolis, MN; Applied Immune Sciences, Inc., Menlo Park, CA.

We investigated the possibility that human pluripotential hematopoietic stem cells (PHSC) could be separated from committed progenitors by counterflow elutriation (CE) and then enriched on the basis of CD34 expression, using methods similar to those for isolating murine PHSC. In 27 individual experiments, 2.7-7.8 x 10^8 marrow mononuclear cells were injected into a Beckman JE5.0 elutriator at 15 mL/min (FR 15), rotor speed of 3000 RPM and T of 25°C. Fractions (FR) of cells were collected at 25, 29, 33, and 37 mL/min with the remaining cells captured by turning the rotor off (R/O). Cells in FR 25/29, FR 33/37 and R/O were sequentially loaded into AIS MicroCELLector devices coated with soybean agglutinin (step 1) and devices coated with anti-CD34 (step 2). In contrast to the $CD34^+$ cells in FR 33/37 and R/O, $CD34^+$ cells in FR 25/29 were $CD3^-$, $CD4^-$, $CD8^-$, $CD19^-$, $CD33^-$ and $CD38^-$ with 10-28% expressing HLA-DR. In short-term methylcellulose and long-term bone marrow culture (LTBMC) with irradiated allogeneic stroma, FR 25/29 $CD34^+/Lin^-$ cells failed to proliferate unlike FR 33/37 and R/O $CD34^+$ cells. In order to determine which cytokines might induce proliferation of these cells, FR 25/29 $CD34^+$ cells were evaluated for expression of growth factor receptors. FR 25/29 $CD34^+$ expressed receptors for c-kit (67.9±19.2%), IL-6 (48.9±20.7%), IL-3 (41.0±14.0%), IL-1 (74.3±14.0%), GM-CSF (43.9±19.0%) and G-CSF (23.9±16.7%). Demonstrable differences in fluorescence intensity were observed between FRs of $CD34^+$ cells with quiescent FR 25/29 $CD34^+/Lin^-$ cells being c-kit^{bright}, IL-$6r^{dull}$, IL-$3r^{dull}$, IL-$1r^{bright}$, GM-$CSFr^{bright/dull}$ and G-$CSFr^{dull}$. Notably, 76.4±25.9% of FR 25/29 $CD34^+$ also expressed receptors for MIP1α, a negative regulator of hematopoiesis. In summary, we have identified a subpopulation of CD34+ cells that is non-proliferative *in vitro* but has growth factor receptors. The effect of cytokines, anti-MIP1αr and TGFβr, as well as the effect of adding back this population to the lymphocyte depleted graft in clinical transplantation will be reviewed.

GVHD; CML; Breast Cancer; Marrow Processing

G 400 INDIVIDUALIZED PROPHYLAXIS AGAINST GRAFT-VERSUS-HOST DISEASE, Johan Aschan, Olle Ringdén, Eva Andström, Per Ljungman and Mats Remberger, Departments of Clinical Immunology, Transplantation Surgery and Medicine, Huddinge Hospital, S-141 86 Huddinge, Sweden.

Seventy-three leukemic patients undergoing BMT with HLA-identical donors were given individualized prophylaxis against GVHD in order to reduce the immunosuppression, provoke mild GVHD and decrease relapse incidence by the graft-versus-leukemia (GVL) effect. Post grafting immunosuppression were given depending on the estimated risk of developing GVHD. Patients > 30 years of age, seropositive for > 2 herpes viruses, splenectomized or male recipients of marrow from female donors were regarded as high risk patients. If one of these criteria was fulfilled, treatment consisted of a short course MTX combined with CSA. CSA was at the earliest possible time after engraftment changed to weekly MTX for 3 months post BMT. Patients without any high-risk criteria for GVHD were treated with MTX alone for 3 months. Conditioning consisted of BuCy in 35 patients and CyTBI in 38 patients. Thirty-nine patients conditioned with CyTBI and given 4 doses of MTX plus CSA for one year served as retrospective controls. Disease status and risk-factors for GVHD were comparable in the three groups. CSA was discontinued at day 69 (mean) and had to be reinstituted in 16/56 (29%). With the individualized prevention of GVHD the incidence of grade II-IV acute GVHD was 16 and 10% among BuCy and CyTBI treated patients compared to 8% in the control group (ns). Also overall acute GVHD was unchanged, 53 and 71% vs 58%. The incidence of chronic GVHD increased to 59 and 40% from 25% in the individualized treated patients given BuCy and CyTBI and the control group, respectively (p=0.03 BuCy vs control group). Only increase of limited chronic GVHD was seen. The probability of death due to GVHD did not increase with the new prevention, 7 and 0% vs 8% in the MTX+CSA group. The incidence of relapse at two years was 6% among BuCy treated patients and 35% among CyTBI treated patients in the individualized group (p=0.01) compared to 36% in the control group (p=0.01 BuCy vs control group). Two-year relapse-free survival was 76, 58 and 51%, respectively (p=0.06 BuCy vs control group).

Conclusion: This way of individualizing the prophylaxis against GVHD did not increase the incidence of, or mortality from, acute GVHD. The incidence of limited chronic GVHD increased as intended. The relapse incidence decreased among BuCy treated patients in the individualized group, who also had a tendency towards better relapse-free survival.

G 401 USE OF FK-506 FOR GRAFT-VERSUS-HOST DISEASE (GVHD) FOLLOWING BONE MARROW TRANSPLANTATION (BMT).

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FK-506 is an effective immunosuppressant that has been shown to reverse or stabilize GVHD in animal models. We now update our experience using FK-506 following allogeneic BMT in patients who have failed or are intolerant to prednisone and/or cyclosporine for GVHD.

Thirty-three adult patients were treated: CML 12, AML 11, ALL 4, AA 3, Others 3. Donors included HLA identical sibs, mismatched family members and unrelated donors. All patients had failed high dose steroids and/or cyclosporine. Indication for FK-506: Chronic GVHD 24, acute GVHD 6, cyclosporine toxicity 3. Major GVHD disease site was: liver 10, skin 6, lung 4, muscle 5, gut 5. Multiple organs were involved in 18 patients.

Patients received FK-506 0.1mg/kg/day IV continuous infusion or orally. Dose was adjusted to maintain trough level 1.0-2.0 ng/ml and also adjusted according to clinical or biochemical parameters.

Patients treated	33	
Responders	14	(42%)
Alive, continuing FK-506	12	(36%)
Nonresponders	7	(21%)
Inevaluable(< 1 month on FK-506)	12	(36%)

Responders have been on FK-506 from 3 months-3 years. Response has allowed taper of steroids (mean prednisone dose has been decreased from 40 mg qd to 10 mg qd). Deaths on FK-506 have been due to progressive GVHD (5), infection (4), multiorgan failure (6), or respiratory arrest (1). Of these, 10 patients had been on FK-506 less than 1 month.

In summary, 12/33 patients have responded and are alive on FK-506 with significant decreased requirement for steroids. FK-506 is a promising agent to be further explored in the treatment of GVHD.

G 402 CYTOKINE INVOLVEMENT IN PREDICTING CLINICAL GRAFT VERSUS HOST DISEASE, Anne M. Dickinson, Lisbet Sviland*, Graham Jackson, Philip Usher*, Penny Taylor, Janice Dunn, Xaio Nong Wang, Peter J. Hamilton and Stephen J. Proctor, Departments of Haematology and Pathology*, Royal Victoria Infirmary, Newcastle upon Tyne.

An *in vitro* skin explant model has been used to predict the severity of acute graft versus host disease (GVHD) in 34 HLA identical bone marrow transplant recipients (correlation coefficient 0.6 $P<0.001$). Supernatants from HLA matched patient/donor mixed lymphocyte cultures (MLC's) were analyzed for levels of tumour necrosis factor alpha (TNFα) and interferon gamma (IFNγ). High levels of both cytokines correlated with the development of GVHD Grades II or above ($P<0.05$). The supernatants were also tested for induction of Class II MHC antigen expression on third party skin and results correlated with clinical outcome in 17/22 cases (77%) (correlation coefficient 0.65 $P<0.001$). Helper T lymphocyte precursor (HTLp) assays correlate well with skin explant assays for predicting GVHD. The results suggest that measurement of cytokines (IL2, TNFα and IFNγ) in HLA-matched MLC supernatants is of predictive value and that the skin-explant model is a useful model for studying the aetiology of GVHD in man.

G 403 HAEMOPOIESIS & DENDRITIC CELL DIFFERENTIATION. Derek N.J. Hart and William Egner. Haematology/Immunology Research Group, Christchurch School of Medicine, Christchurch, New Zealand.

Dendritic cells (DC) are widely distributed tissue-resident cells which are powerful stimulators of primary T lymphocyte responses. Expression of the leucocyte common (CD45) antigen and irradiation-reconstitution studies clearly indicate a bone marrow (BM) origin for DC. Further studies on the growth and differentiation of these cells have been hampered by a lack of well defined DC lineage specific markers and the fact that activation and/or differentiation of DC may be necessary for maximal co-stimulatory activity. Early studies indicated that GM-CSF induced phenotypic and functional changes in murine Langerhans cells (LC). Latterly GM-CSF has been used to grow cells with DC-like characteristics from murine blood and BM. Limited in vitro growth of human blood DC-like cells and putative cord blood DC have been described using GM-CSF + TNFα. The relationship of these "DC precursors" and their progeny to the monocytic-macrophage differentiation pathway is contentious in the absence of selection against the latter. However DC do not appear to respond to M-CSF and M-CSF and macrophage deficient mice have normal numbers of LC. Human BM, unlike rodent BM, contains potent antigen presenting cells which can be distinguished from strongly CD14 positive monocytes. The CD1 marker does not predict for the costimulatory cell in human BM culture. Morphological assessment of cells with dendritiform morphology is difficult. Careful analysis of high purity blood and BM preparations using new DC markers will be an essential preliminary to define the growth conditions which favour DC haemopoiesis., differentiation and functional maturation.

G 404 HDL-CHOLESTEROL (HDL-C)/TRIGLYCERIDE (TG) LEVELS MAY BE IMPORTANT MARKERS FOR ACUTE GRAFT VS HOST DISEASE (AGVHD) COMPLICATING BONE MARROW TRANSPLANTATION (BMT), Kathryn Klopfenstein, Rajni Agarwal, Sarah Jenkins, James Sambrano, Kathleen Fender and Judith Harmony, Children's Hospital Medical Center, Hematology/Oncology Division, Cincinnati, OH 45229. Department of Pathology and Lab Medicine, University of Cincinnati School of Medicine, Cincinnati, OH 45267.

AGVHD is a major cause of morbidity and mortality following BMT. We had observed, in a retrospective study, that a statistically significant rise in TG levels correlates with the onset and severity of AGVHD. We report here the prospective analysis of a group of 22 pediatric BMT patients. Seven patients had autologous BMT and 15 received allogenic BMT. Of these, 11 patients developed AGVHD. Lipid profiles for all patients were monitored weekly from pre-transplant to discharge, with additional twice-weekly observations for patients with AGVHD from the day of diagnosis.

The lipid levels in patients with AGVHD obtained on the day of diagnosis were compared to control values. Control values were obtained by using the mean day of diagnosis (day +22) of AGVHD as a reference. TG levels for all patients developing AGVHD increased from baseline. TG levels were statistically significant as compared to controls ($t=3.3$, $p=0.014$). TG levels peaked at the time of diagnosis in 6 of 11 patients. In 5 patients, TG levels peaked later in the course when GVHD became more severe. HDL-C levels decreased rapidly in all patients post transplant, however, patients with AGVHD had significantly lower levels of HDL-C ($t=5$, $p=.001$) with a nadir at approximately 10 days prior to diagnosis. An interesting correlation in AGVHD patients was that HDL-C trough preceded the TG elevation, an observation not seen in other conditions associated with hypertriglyceridemia.

This data suggests that the significant fall in HDL followed by a rise in TG levels at the onset of AGVHD may be an important marker in the diagnosis and monitoring of AGVHD.

G 405 ADJUSTED DOSE CONTINUOUS INFUSION CYCLOSPORIN A TO PREVENT GRAFT VERSUS HOST DISEASE (GVHD). K.Miller, D. Schenkein, R. Comenzo, D. Weckstein, J. Erban, T. Fogaren,* E. Berkman, and A. Rabson *. We administered Cyclosporin A (CsA) by continuous intravenous infusion for prophylaxis against GVHD and adjusted the dose to maintain a constant whole blood level. Fifty patients, ranging in age from 16-56, mean 39.6 years, undergoing allogeneic transplantation for various hematological malignancies received CsA as a continuous intravenous infusion. CsA was started on day -1, and continued until day +22 when oral CsA was initiated. The whole blood level of CsA was determined and the dose adjusted to maintain a fixed level. The dose of CsA was adjusted to maintain the whole blood level between 325-375 ng/ml by RIA or 450-500 ng/ml by the fluorescence polarization immunoassay. All patients received the necessary dose to maintain the targeted whole blood CsA level. All patients required multiple adjustments (mean 6:range 4-12) of the CsA dose to maintain the targeted whole blood level. No patient had the dose of CsA held or modified for toxicity or an adverse reaction. Methotrexacte 15mg/m^2 I.V. was given on days +1, followed by 10mg/m^2 on days +3 and +6. The overall mean daily administered CsA dose/kg +/-S.D was 2.9mg +/-.78mg, with a range of 2.1 to 4.8mg/kg. The weight of the patient did not correlate well with the required CsA dose ($r=.54$). There was no correlation with the body surface area, preparative regimen or underlying disease and the dose of CsA administered. All patients received the required dose of CsA to maintain the targeted level. The mean weekly dose of CsA administered (mg/kg +/-S.E.M.) was 2.84 +/-.10 range (1.9-4.5),3.15 +/-.12 (range 1.7-4.8),3.0 +/-.09 (range 2.1-4.7), and 2.87 +/-.10 range (2.2-4.2) for weeks 1-4 respectively following the transplant. The dose of CsA administered for week 2 was significantly greater than for weeks 1 and 4 ($P<.05$). CsA administered as a continuous infusion was well tolerated. The mean rise in creatinine was 0.89mg/dl. There was an association between the concomitant administration of amphotericin B and CsA and the development of nephrotoxicity. Hypertension developed in 69% of the patients and all responded to oral nifedipine. None of the patients developed serious neurologic side effects. Increased bilirubin occurred in 86% of the patients, mean rise 3.8mg/dl. Greater than grade I acute GVHD developed in only 13% of the patients and 29% developed chronic GVHD. We conclude that administering CsA as an adjusted dose by continuous intravenous infusion is well tolerated, and is effective in preventing acute GVHD in patients undergoing allogeneic bone marrow transplantation.

G 406 SERUM LEVELS OF SOLUBLE INTERLEUKIN-2 RECEPTOR IN ALLOGENEIC BONE MARROW TRANSPLANTATION

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In order to evaluate the usefulness of soluble interleukin-2 receptor (sIL-2R) as a predictor of acute graft-versus-host disease (GVHD), we serially measured serum levels of sIL-2R in 28 consecutive patients who received allogeneic bone marrow transplantation (BMT). In 15 patients who developed acute GVHD, peak levels of sIL-2R were significantly higher than those in 12 patients who did not develop acute GVHD. Furthermore, the peak levels of sIL-2R were significantly higher in the patients with grade II or more acute GVHD, compared with those in the patients with grade I acute GVHD. These observations suggest that the serial measurement of serum sIL-2R may be a useful indicator for the diagnosis and/or the evaluation of acute GVHD and that sIL-2R may be actively involved in the development of acute GVHD.

G 407 CYCLOSPORINE COMBINED WITH METHYLPREDNISOLONE OR METHOTREXATE IN PROPHYLAXIS OF MODERATE TO SEVERE ACUTE GRAFT-VERSUS-HOST DISEASES,

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To evaluate the efficacy of cyclosporine(CYA) regimens in preventing moderate to severe acute graft-versus-host disease(GVHD), 25 patients received immunosuppressive therapy consisting of either CYA and methylprednisolone or CYA and methotrexate(MTX) and the incidence and severity of acute GVHD was compared. These patients had leukemia or myelodysplastic syndrome(MDS) and received bone marrow transplants(BMT) from genotypically HLA-identical siblings. The incidence of grade I-IV acute GVHD in patients on the CYA/methylprednisolone regimen was 64% (7 of 11) compared with 50% (7 of 14) in those on the CYA/MTX regimen. Five of 11 patients with the CYA/methylprednisolone regimen developed moderate to severe acute GVHD (grade II-IV), fatal in 3 cases. No patient on the CYA/MTX regimen developed moderate to severe acute GVHD. Engraftment was faster in the CYA/methylprednisolone group than in the CYA/MTX group. The incidence of toxicity observed soon after BMT was comparable between groups. The CYA/MTX regimen may be superior to the CYA/methylprednisolone regimen for preventing moderate to severe acute GVHD.

G 408 CUTANEOUS CYTOKINE EXPRESSION FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION. L Ochs, B Blazar, J Roy & D Weisdorf. Univ of Minnesota Medical School, Minneapolis, MN.

Chronic graft-versus-host disease (GVHD) is a frequent complication of allogeneic bone marrow transplant (BMT), but the pathogenesis remains unclear. Earlier studies suggest that GVHD may be initiated by major histocompatibility complex-restricted cytotoxic T-lymphocytes. However, the ongoing tissue injury and fibrosis characteristic of chronic GVHD suggests an autoimmune syndrome where pro-inflammatory cells and cytokines may perpetuate both the tissue damage and the fibrosis. The inflammatory tissue injury may be mediated by tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin-1 (IL-1) and IL-2, while platelet derived growth factor (PDGF) and transforming growth factor-β (TGF-β) may perpetuate the fibrosis characteristic of the syndrome. Using reverse transcription–polymerase chain reaction (RT-PCR), we studied punch biopsies of the skin from 4 patients with extensive chronic GVHD (median 274 days after BMT) and 6 post-BMT controls (median 200 days) with skin biopsies not diagnostic of chronic GVHD (although 2 of the 6 had extracutaneous chronic GVHD). Total RNA was isolated from the skin specimens and RT-PCR (35 cycles) was performed to examine expression in the skin biopsies of IL-1, IL-2, TNF-α, IFN-γ, TGF-β, PDGF B chain, and β-actin expression to verify RNA integrity. In post-BMT controls and patients with cutaneous chronic GVHD, expression in the skin of IL-1 (67% vs. 75% of specimens, respectively), TNF-α (100% vs. 75%), TGF-β (100% vs. 75%) and PDGF (both 100%) were frequent. In contrast, IL-2 expression was present in 50% of those biopsies without chronic GVHD, while none of the skin samples from patients with chronic GVHD expressed IL-2. In addition, only 17% of samples without chronic GVHD expressed IFN-γ, compared to 50% of chronic GVHD samples. Although these results are preliminary, they suggest that IFN-γ may potentiate the tissue injury seen in active cutaneous chronic GVHD. Cutaneous transcription of IL-1, TNF-α, TGF-β and PDGF is common in post-BMT controls and with chronic GVHD. Finally, IL-2 expression is uncommon in cutaneous chronic GVHD. Although IL-2 may be essential in supporting cytotoxic T-lymphocyte proliferation, it may not be a necessary component of the inflammatory response in cutaneous chronic GVHD. While the cellular sources of these cytokines are uncertain, their expression and secretion *in situ* may propagate the cytotoxic cascade and perpetuate the injury. Better understanding of the inflammatory sequence may allow design of more specific therapy.

G 409 CYCLOSPORINE-A AND GRAFT-VERSUS-HOST DISEASE IN CHILDREN: THE AIEOP-BMT GROUP EXPERIENCE.

Paolo Paolucci, Franco Locatelli, Cornelio Uderzo, Giorgio Dini, Marco Zecca, William Arcese, Chiara Messina, Marino Andolina, Roberto Miniero, Fulvio Porta, Attilio Rovelli, Arcangelo Prete, Andrea Pession. From the AIEOP-BMT Group.

We retrospectively analyzed the data base of the Italian Association of Pediatric Hematology/Oncology (AIEOP) BMT Group on the incidence and severity of GvHD in children given allogeneic BMT from HLA-identical sibling and receiving Cyclosporine-A (CsA) alone as GvHD prophylaxis. The study population included 145 patients (pts) for acute GvHD (A-GvHD) and 114 children at risk for chronic GvHD (C-GvHD) (surviving >100 days after BMT). Twelve pts had non-malignant diseases and 133 pts were affected by malignant disorders. Among the 145 pts (50 Females, 95 Males), 107 (74%) presented A-GvHD and 38 (26%) had no sign of disease. In the group of pts with A-GvHD, 38 children (26% of the whole study population) were found to have grade II disease, 9 (6%) grade III, 4 (3%) grade IV. Donor-recipient sex pairs had no statistically significant influence on incidence of A-GvHD neither did donor-recipient age class stratification. Of 114 pts evaluated for C-GvHD, 86 (76%) developed no disease while 23 pts (20%) presented secondary C-GvHD and 5 (4%) had *de novo* C-GvHD. The incidence of C-GvHD was higher in F-M than M-M donor-recipient sex pairs (33% vs 11%, $p < 0.05$), with no difference between F-F and M-F. In pts over 10 years, a higher incidence of C-GvHD was observed in both female donors and recipients compared with male donors and recipients (48% vs 20% and 47% vs 19% respectively, $p < 0.05$). Disease status at BMT was the most important factor determining outcome of pts with acute leukemia, disease-free survival (DFS) being 63%, 28% and 7%, respectively, in children transplanted at an early phase, at a more advanced stage and in relapse ($p < 0.005$). Pts with acute leukemia presenting moderate-severe A-GvHD had a better although not statistically significant DFS compared with both pts presenting no GvHD or grade I A-GvHD and children developing *de-novo* or secondary C-GvHD (62% vs 49% vs 44%, respectively). The anti-leukemic effect of A-GvHD was clearly evident only in pts at a less advanced stage of disease. In fact, in the group of children with early leukemia, pts with grade II-IV A-GvHD showed a better disease-free interval (DFI) than those without or with grade I A-GvHD or C-GvHD (100% vs 70% vs 64%, respectively, $p < 0.05$). We conclude that in pediatric pts CsA is effective in preventing and controlling severe acute and chronic GvHD without substantially eliminating the protective role played by donor immunocompetent cells against leukemia relapse.

G 410 SHORT-COURSE INTERLEUKIN-2, CYCLOSPORINE AND STEROIDS FOR PREVENTION OF GRAFT-VS-HOST DISEASE AFTER HAPLOIDENTICAL MARROW TRANSPLANTATION.

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In the Sykes model, administration of IL-2 early ameliorated GVHD after transplantation of mismatched marrow and spleen cells. To determine the feasibility of this approach, we tested a short course of IL-2 early posttransplant in 5 patients with leukemia in relapse undergoing haploidentical marrow transplantation. The preparative regimen consisted of thiotepa 10 mg/kg IV, cyclophosphamide 60 mg/kg IV X 2 and TBI 3 Gy X 4. GHVD prophylaxis consisted of IL-2 at 3 MIU/m^2/day CI for 4 days beginning 4 hours after infusion of the marrow, cyclosporine 3 mg/kg IV CI from Day -2 and methylprednisolone 0.5 mg/kg IV BID from 12 hours after the end of the IL-2 infusion. Methotrexate 10 mg/m^2 was given IV on Days 3, 6 and 11 if the infusion of IL-2 could not be completed. The marrow harvested for infusion contained 2.6-6.5 X 10^8 nucleated cells/kg, 2.1-4.8 X 10^6 CD34+ cells/kg, 1.8-4.7 X 10^7 CD3+ cells/kg and 0.8-8.5 X 10^6 CD3-CD56+ cells/kg. All patients discontinued IL-2 at 3 days of infusion; 3 for fever, 1 for headache and 1 for liver dysfunction. There was no post IL-2 lymphocytosis seen. The absolute lymphocyte counts postinfusion were all less then 0.1 X 10^9/L. Three-color flow demonstrated a predominance of CD3+CD4+CD8- cells amongst these lymphocytes postinfusion. All patients engrafted. By in situ hybridization or cytogenetics, responding patients had >90% donor cells in peripheral blood or marrow at Day 30. One patient failed to achieve remission. The remaining patients had no evidence of residual leukemia by cytogenetics or in situ hybridization for t(9;22) where applicable. The two 3-antigen mismatched marrow recipients developed Grade 4 GVHD that was fatal. Two of three 2-antigen mismatched marrow recipients developed Grade 3 GVHD that responded to steroids, and the last patient had no GVHD. One died with progressive leukemia, one died with VOD and centrolobular necrosis, and the third is alive and in remission without GVHD 5 months posttransplant. Adjunctive use of IL-2 posttransplant is feasible and results in mild-to-moderate GVHD in 2-antigen mismatched marrow recipients, but this approach is not sufficient for 3-antigen mismatched marrow transplantation.

G 411 IN VITRO ANALYSIS OF THE EFFECTOR CELLS INVOLVED IN THE GRAFT-VS-LEUKAEMIA EFFECT AFTER DONOR BUFFYCOAT TRANSFUSIONS IN PATIENTS WITH RELAPSED CML, Donald Bunjes, Matthias Theobald, Bernd Hertenstein, Jürgen Novotny, Markus Wiesneth, Renate Arnold and Hermann Heimpel, BMT Unit, Department of Internal Medicine III, Ulm University Hospital, Robert-Koch-Str. 8, 89070 Ulm, FRG

4 pts who achieved a complete response (PCR negative) after treatment of their relapsed CML with donor buffycoat were studied. T cell and NK/LAK responses to leukaemia or host minor antigens were analysed by using appropriate limiting dilution technology. No CML-specific HTL-p or CTL-p could be detected at any time point after BC therapy and no NK/LAK cells reactive with patient leukaemia could be found after stimulation with high-dose IL-2 (100 U/ml). In contrast high frequencies of host-reactive HTL-p (1/7000 - 1/30000) were present in all 4 pts. However this strong antihost response was restricted to HTL-p since no host-reactive CTL-p could be elicited. The appearance of host-reactive HTL-p was associated with the development of aGvHD/cGvHD and bone marrow hypoplasia/aplasia in all 4 pts. We then applied the same technology to the study of the antileukaemic and antihost response prior to BMT, post BMT and immediately prior to BC therapy in 3 pts. No CML-specific T cell or NK/LAK response was detectable in any of these patients. Host-reactive HTL-p were present in 3/4 patients pretransplant and in 2 pts with a GvHD post BMT but no host-specific CTL-p were found. The frequencies of host-reactive HTL-p were 2-3 fold lower after BMT than after BC therapy. Donor HTL-p and CTL-p were areactive with both CML and host minor antigens prior to BC therapy. From these findings we conclude that: 1) host-reactive donor HTL-p are primarily responsible for initiating the antileukaemic response after BC therapy. 2) in most patients BC treatment is simply a form of transfusion GvHD with leukaemic haemopoiesis as its primary target.

G 412 RISK OF RELAPSE IN CYCLOSPORIN (CSA) TREATED PATIENTS RECEIVING AN ALLOGENEIC BONE MARROW TRANSPLANT (ALLO BMT) FOR CHRONIC MYELOID LEUKEMIA: J. M. Meharchand, G. M. Fyles, J. H. Lipton, M. D. Minden, I. Tejpar, H. L. Atkins, H. A. Messner.

70 patients in 1st chronic phase, receiving the same preparative regimen for matched related allo BMT were seguentially treated with 3 different regimens for prophylaxis of GvHD. All patients received a short course of Methotrexate. Patients in group one (grp1) received Prednisone (N=22) those in group 2 (grp2) received CsA for a minimum of 6 months (n=16) and those in group 3 (grp3) received CsA for a minimum of two months (n=31). The three groups were similar in age, duration of disease and stage of disease at transplant. The minimum follow-up was 15 months. Significant acute GvHD was significantly lower in group 2 (31%) compared to group 1 (63%) and group 3 (51%). The incidence of grade 3 and 4 GvHD was considerably higher in group 1 (50%) compared to group 2 (20%) and group 3 (13%). The relapse rate was highest in the group receiving long-term CsA prophylaxis (group 2=82%) and lowest in group 1 (13%). The shorter course of CsA prophylaxis with early discontinuation in the absence of GvHD lead to a reduced relapse rate in group 3 (19%) with improved survival (77%) compared to group one (27%). A significant number of patients relapsing after first bone marrow transplant could be rendered cytogenetically disease free by discontinuation of CsA where appropriate, interferon therapy or second bone marrow transplant. Thus, the disease free survival at present in group 2 and 3 is 73% (69% and 74% respectively) which is significantly different from the 27% disease free survival in group 1 ($p<0.05$).

G 413 MINIMAL RESIDUAL DISEASE IN PATIENTS WITH CML FOLLOWING BONE MARROW ALLOGRAFTING: H. A. Messner, W. M. XU, X. H. Piao, L. Addy, N. Jamal and M. D. Minden, Ontario Cancer Institute, Institute of Medical Science, University of Toronto.

Bone marrow samples of 70 transplant recipients with chronic myeloid leukemia were studied by Southern blot analysis and RT-PCR using a two step procedure with nested primers. Twenty-two patients were studied once, 48 were assessed on multiple occasions. All patients remained in a hematological remission during the study. The time of follow-up after the transplant ranged from 2 to 144 months with a median of 42 months. Thirty-nine patients (56%) were negative by RT-PCR and Southern blot studies at the time of their last evaluation. The proportion of RT-PCR negative patients increased with the duration of follow-up after the transplant. 36% of patients studied after one year were RT-PCR negative compared to 60% after 2 years and 78% after 5 years or more. In addition to the length of follow-up after transplantation the continued requirement for immunosuppression was found to be of prognostic value. Patients maintained on immunosuppression had a higher probability to remain RT-PCR positive. Clinical parameters such as age, gender, time from diagnosis to BMT, as well as acute and chronic graft-versus-host disease did not influence the RT-PCR data. The majority of patients studied on multiple occasions demonstrated a stable RT-PCR and Southern blot pattern. However, some uni or multi directional transitions were observed. In spite of differences in the RT-PCR pattern none of the studied patients progressed to a hematological relapse. RT-PCR studies performed on colonies grown from patients that were RT-PCR positive, Southern blot negative confirmed that some of the clonogenic progenitors are able to produce bcr/abl transcripts and are therefore potentially capable to extend the malignant clone.

G 414 CYCLOPHOSPHAMIDE (CY), CYTOSINE ARABINOSIDE (Ara-C), SPLENIC IRRADIATION (RT), AND LOW DOSE TOTAL BODY IRRADIATION (TBI) AS A PREPARATIVE REGIMEN FOR ALLOGENEIC BONE MARROW TRANSPLANTATION (AlloBMT) FOR ADULT PATIENTS (PTS) WITH CHRONIC PHASE CHRONIC MYELOGENOUS LEUKEMIA (CML-CP), DAVID J. OBLON, TOM JOHNSON, LESLEY MYERS, GERALD ELFENBEIN, ALAN MILLER, ROY WEINER, DEPARTMENTS OF MEDICINE, BROWN UNIVERSITY, PROVIDENCE, R.I. 02908, UNIVERSITY OF FLORIDA, GAINESVILLE, FL. H. LEE MOFFITT CANCER CENTER, TAMPA, FL., TULANE UNIVERSITY, NEW ORLEANS, LA.

Regimen related toxicity (RRT) remains a major barrier to successful outcome after AlloBMT for pts with CML-CP. We evaluated the toxicity and efficacy of Cy/Ara-C/Splenic RT/TBI as a preparative regimen in 28 adult pts with CML-CP. The regimen was Ara-C, 100mg/m^2/day (D) IV continuous infusion on D -9 to -5; Cy 60mg/kg IV over 1hour on D -4 and -3; splenic RT, 550 cGy in 5 fractions on D -10 to -1; and TBI, 550 cGy in one fraction on D zero. Minimum follow-up is 15 months (range 15-103). The patient characteristics were: median age 34 (range 22-50), with 10 pts > 40 years; male:female 17:11; and 16/28 were within 1 year of diagnosis at time of BMT. Median time to engraftment was: absolute granulocyte count >500/mm^3, D 26; unsupported platelet count >50,000/mm^3, D 29. RRT occurred in 7/28 pts. Each suffered interstitial pneumonitis which was fatal in all 7 pts. Two of the 10 pts > 40 years developed RRT, i.e. interstitial pneumonitis. The 4 deaths that were not RRT included: staphlococcal pneumonia (1), chronic graft versus host disease (2), sarcoma (1). One pt developed a hematologic relapse of CML at day +473. 17/28 (61%) are alive in continuous clinical remission. We conclude that 1) Cy/Ara-C/Splenic RT/TBI is an effective preparative regimen for AlloBMT for pts with CML; 2) RRT was confined to interstitial pneumonitis; 3) RRT in pts > 40 years is similar to younger pts; 4) veno-occlusive disease is rare; 5) the rate of hematologic relapse is low, but further follow-up is needed.

G 415 INDUCTION OF GRAFT-VS-LEUKEMIA (GVL) REACTION AS THERAPY FOR RELAPSED LEUKEMIA AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT). D Porter, M Roth, C McGarigle, J Ferrara, J Antin. Brigham and Women's Hospital, Boston MA, and University of Michigan Medical Center, Ann Arbor, MI. Patients who relapse after allogeneic BMT may be cured with a second BMT, but the survival rate is poor. Donor mononuclear cell infusions may be used to induce GVL and re-establish remission with less morbidity than a second BMT. We have treated 18 patients with relapsed leukemia after allogeneic BMT with donor MNC infusions. 17/18 marrow grafts were from HLA-identical siblings. Fifteen patients had CML and 3 had AML. All patients with CML and 1 patient with AML received interferon-α (IFN-α) for 6-12 weeks prior to infusions and for 4 weeks after the final infusion. Donor MNC were collected by leukapheresis once weekly for 4 weeks and freshly administered to patients (median 3.64 [range 0.9-8.45] x 10^8 cells/kg). Patients were monitored for graft-vs-host disease (GVHD), hematologic and cytogenetic responses, and all patients were followed with serial PCR analysis to detect cells containing bcr/abl mRNA transcripts. Patient responses are summarized as follows:

Dx	n	molecular remission	aGvHD ≥ grade 2	cGvHD
CML sp	10	7/10	3	7
CML ap/bc	5	0/5	2	0
AML	3	n.a.	1	n.a.

Response did not correlate with MNC dose or grade of acute GVHD. Toxicity from GVHD was mild-moderate and generally responsive to immuno-suppression. Other complications included opportunistic infections (n=3), pancytopenia (n=6) and marrow aplasia (n=2). Marrow aplasia was reversed with additional donor marrow in 1 patient. Chronic phase CML was predictive for response (p=0.037). Donor MNC infusions with IFN-α is effective anti-leukemic therapy for patients with relapsed CML in chronic phase. The efficacy of this therapy for patients with more advanced stages of CML and with acute leukemia remains to be determined.

G 416 HIGH-DOSE ADJUVANT TREATMENT WITH AUTOLOGOUS BONE MARROW TRANSPLANT FOR HIGH-RISK BREAST CANCER. Tauseef Ahmed, Evi Razis, Perry Cook, Mario Beer, Diana E. Lake, Robert A. Preti, Mohsin Ali, Abraham Mittelman, New York Medical College, Valhalla, NY 10595

Patients (pts) with breast cancer and >10 involved axillary nodes and/or inflammatory tumor are at high risk of relapse following surgery. Standard-dose adjuvant chemotherapy is not associated with prolonged disease-free survival. High-dose chemotherapy with autologous bone marrow transplant (ABMT) is reported to have encouraging results. However, several of these studies used carmustine and cisplatin, with significant and sometimes lethal non-hematologic toxicity. We decided to explore a combination of thiotepa, mitoxantrone and carboplatin, which has already been extensively tried and found active and relatively well-tolerated in lymphoma. Of the 52 pts entered, 29 had Stage II and 13 Stage IIIA disease, with >10 involved axillary nodes (median 15 nodes, range 10-29), and 13 had Stage IIIB disease. The tumor was hormone receptor negative in 25. The median greatest diameter was 4.5 cm (range, 1-14). The pts' median age was 43 (range, 21-60) and 6 pts were postmenopausal. One pt refused high-dose chemotherapy. Treatment consisted of 3-4 cycles of doxorubicin-containing standard-dose chemotherapy (FAC), followed by thiotepa, 250 mg/m^2/day on days 1-3, mitoxantrone, 40 mg/m^2 on day 1 and carboplatin, 330 mg/m^2/day on days 1-3 (TMJ). Hematologic support consisted of ABMT and peripheral blood cells -which in 31 pts had been previously mobilized by GM-CSF given daily for a week- followed by post-transplant GM-CSF, daily until blood count recovery. The time to recover to 1 x10^9/l WBC was 17±6 days (mean ± s.d.) and 13±8 days were needed to reach 20x10^9/l platelets. A total of 37±30 units of platelets and 12±5 units of packed RBC had to be transfused. Two pts had delayed recovery of their blood counts, at 3 and 6 months, respectively. Treatment morbidity was relatively low, with none of the pts needing ICU, hemodialysis or respiratory support; sepsis was present in one case, lethargy was observed in 3 and colitis in 8 pts. No other non-hematologic toxicity rated grade 3 or higher was observed. Median follow-up to date is 15 months (range, 3-38) from initial surgery. So far, 7 pts have relapsed and 5 of them are deceased. The regimen used is well tolerated and safe in the adjuvant setting.

G 417 TANDEM HIGH DOSE CHEMOTHERAPY WITH AUTOLOGOUS HEMATOPOIETIC PROGENITOR SUPPORT FOR TREATMENT OF STAGE IV BREAST CANCER, J. D. Bitran, L. White, S. Hanauer, B. Samuels, L. Klein, Lutheran General Hospital, University of Chicago Pritzker School of Medicine, Park Ridge, IL 60068

We initiated a phase II study of tandem (double) high dose chemotherapy (HDCT) with autologous hematopoietic progenitor support (ABMT) in women with Stage IV breast cancer in CR or PR. HDCT-1 consisted of cyclophosphamide 2.5gm/m^2 and thiotepa 225 mg/m^2 days -6, -4, -2 supported by ABMT (marrow or peripheral blood hematopoietic progenitors [pbhpc]). HDCT-2 consisted of melphalan 140mg/m^2 with pbhpc's on an ambulatory basis. Since 4/92, 14 women have entered and performed on study (median age, 44 yrs [range 37-58]; median PS of 1 [range 0-1]). Dominant metastatic sites were: Liver-3 patients (pts), Lung-3 pts, Bone + Bone Marrow-5 pts, Skin + Nodes -3 pts. All 14 pts underwent HDCT-1, the response rates were 6CR 8PR; three PR pts had no additional improvement and were ineligible for HDCT-2. Grade 3 and 4 toxicities included: diarrhea, mucousitis and one death at day 45 from sepsis. Median time to ANC > 500 and plts > 20K were 13 and 17 days, respectively. Of the 10 pts who were eligible for HDCT-2, seven pts have completed, two pts are too early (TE). One patient developed Grade 2 cardiac toxicity from cyclophosphamide and is ineligible. Response rates following HCDT-2 were 7CR with 3 additional PR pts achieving a CR. Median time to ANC > 500 and plt > 20K were 21 and 28 days, respectively. Grade 3 toxicity was mucositis. Survival and freedom from treatment failure or progression at 1 year is 93%. These data demonstrate successive conversion rates with tandem HDCT and ABMT in Stage IV breast cancer.

G 418 MITOXANTRONE, THIOTEPA, AND CYCLOPHOSPHAMIDE AS PREPARATION FOR AUTOLOGOUS BONE MARROW TRANSPLANT FOR HIGH-RISK ADJUVANT AND ADVANCED BREAST CANCER, **Damon LE, Rugo HS, Ries CA, Linker CA, Tracy MA, Ceechi G and Wolf JL, University of California, San Francisco and the Alta Bates Comprehensive Cancer Center, Berkeley, CA.**

Eighty breast cancer patients were treated with autologous bone marrow transplant (ABMT) following 3-4 cycles of standard doxorubicin-based chemotherapy. Eligibility criteria were: Stage IV (n=33), partial (PR) or complete response (CR) to standard chemotherapy; Stage IIIB (n=5), PR or CR to pre-operative standard chemotherapy; Stage II/IIIA (n=42), ≥ 10 axillary lymph nodes involved with cancer, or T3 primary tumor with ≥ 1 positive axillary lymph node/hormone-receptor negative/S-phase > 5%/aneuploidy. Eighty-one percent of all patients were hormone-receptor positive. The median age of all patients was 46 years (24-62). The median number of axillary lymph nodes involved was 14 in adjuvant patients and 10 in inflammatory patients. In metastatic patients, the sites of disease were viscera (50%), soft tissue (38%) and bone-only (12%). ABMT preparation was given on days -6 through -3 and consisted of cyclophosphamide 1.5 g/m^2/d (6 g/m^2 total dose), thiotepa 150 mg/m^2/d (600 mg/m^2 total dose), and escalating doses of mitoxantrone at 6-15 mg/m^2/d (total doses 24, 30, 40, 50, or 60 mg/m^2). Stage IIIB and IV patients were treated one dose-level of mitoxantrone higher than adjuvant patients in order to define toxicity. Patients received bone marrow (n=48), peripheral blood stem cells (PSC) (n=24), or both (n=8) on day zero. There were 4 ABMT-related deaths (5%): alveolar hemorrhage (2), pulmonary capillary leak syndrome with congestive heart failure (1), and myocardial infarction (1). Other major complications included congestive heart failure (2), atrial fibrillation (1), CMV esophago-gastro-duodenitis (4) and viral/interstitial pneumonitis (4). There was one case of non-fatal veno-occlusive disease of the liver. Mucositis was moderate at all mitoxantrone levels requiring narcotics for 0-53 days (mean 12 days) and parenteral nutrition for 0-67 days (mean 19 days). Patients receiving PSC or PSC plus marrow were discharged from hospital a median of day 25 compared to median day 33 with marrow only. With a median follow-up of 12 months, 37 adjuvant patients (88%) are alive and relapse-free as are 3 of 5 inflammatory patients. The progression-free survival for metastatic patients at 18 months is 45%. To date, the maximum tolerated dose of mitoxantrone in this regimen has not been exceeded. This preparative regimen for breast cancer ABMT is well tolerated with a low regimen-related death rate.

G 419 METASTATIC BREAST CANCER: SUGGESTION OF A DOSE-RESPONSE RELATIONSHIP IN THE SETTING OF HIGH DOSE CHEMOTHERAPY AND STEM CELL RESCUE. Karen K. Fields, Gerald J. Elfenbein, Janelle B. Perkins, Oscar F. Ballester, John W. Hiemenz, William E. Janssen, and Paul E Zorsky. Division of Bone Marrow Transplantation; H. Lee Moffitt Cancer Center; University of South Florida; Tampa, Florida 33612.

Between October of 1989 and September of 1993, we have enrolled 104 patients with metastatic breast cancer on 2 phase I/II protocols consisting of high doses of ifosfamide (I), carboplatin (C), and etoposide (E) [ICE] or mitoxantrone (MI) and thiotepa (TT) [MITT] followed by hematopoietic stem cell rescue. Patients achieving at least a partial response following standard therapy or a 2 day course of ICE (miniICE) received ICE; patients failing both standard therapy and miniICE received MITT. Maximally tolerated doses were observed at dose level 16 for ICE (I-20.1g/m^2, C-1.8g/m^2, E-3.0g/m^2) and dose level 5 for MITT (MI-90mg/m^2 and TT-1.2g/m^2). Actuarial data for progression-free survival (PFS) at 12 months for each regimen are listed below according to dose level (DL). Ranges of total doses are given. Numbers (n) of patients are in parentheses. Maximum follow-up (MAX F/U) is also shown.

ICE DL (n)	I (g/m^2)	C (g/m^2)	E (g/m^2)	PFS	MAX F/U
1-10 (22)	6-14.4	1.2-2.1	1.8-2.1	25%	42.3 mo
11-16 (36)	17.1-24	1.8	2.1-3.0	45%	24.9 mo

MITT DL (n)	MI (mg/m^2)	TT (mg/m^2)	PFS	MAX F/U
1-4 (14)	45-75	900-1200	5%	36.5 mo
5-7 (32)	90-105	1200-1350	15%	26.3 mo

Statistical analyses performed by the log rank (Mantel-Haenszel) test show a significant difference (p=0.048) in the PFS curves for ICE DL 1-10 and DL 11-16. Because of the small sample size in DL 1-4, the difference in the MITT data did not achieve statistical significance, but a trend was noted. Although these were not randomized trials and follow-up is still short, the data suggest a role for dose intensity within the realm of high dose therapy for metastatic breast cancer, especially for patients with chemo-responsive disease.

G 420 HIGH-DOSE CYCLOPHOSPHAMIDE, ETOPOSIDE, AND CARBOPLATIN (CEC) FOLLOWED BY AUTOLOGOUS BONE MARROW AND/OR STEM CELL SUPPORT FOR HIGH-RISK BREAST CANCER. Stuart L Goldberg, Thomas R Klumpp, Kenneth F Mangan, John S Macdonald, Temple University Bone Marrow Transplant Program Philadelphia, PA 19140

Twenty patients with stage IV (n=13), stage III (n=6), or high-risk stage II (n=1) breast cancer were treated with cyclophosphamide (6,000 mg/M^2), etoposide (2,400 mg/M^2), and carboplatin (1,200 mg/M^2) followed by autologous bone marrow (n=2), peripheral blood stem cells (n=4), or both (n=14). Patients received a median of 2 (range 0-3) courses of conventional-dose chemotherapy prior to transplant. Patients received G-CSF (n=15), GM-CSF (n=1), or no growth factor (n=4) following transplant. All achieved neutrophil and platelet engraftment. The median time to neutrophil engraftment (defined as absolute neutrophil count 500 polys/μL or greater) was 11 days (range 9-22 days). The median time to platelet engraftment (unsupported platelet count 20,000/μL or greater) was 12 days (range 5-40 days). The median duration of hospitalization was 20 days following BMT (range 15-53 days). One patient (5%) suffered toxic death at day +53 due to hepatic veno-occlusive disease. To date 14 patients are evaluable for response, of whom 8 (57%) achieved a complete response. The 6 remaining evaluable patients had no response. After a median follow-up of 6.8 months, 17 patients (85%) remain alive and 9 patients (45%) remain alive and free of progression. The median progression-free survival among patients with stage IV disease was 283 days whereas the median progression-free survival among patients with high-risk stage II or stage III disease has not yet been reached (p=0.16 logrank). This CEC ABMT regimen is active in breast cancer, yielding complete responses in the majority of patients. Patient accrual is continuing.

G 421 A PHASE I/II TRIAL OF HIGH-DOSE ETOPOSIDE (VP-16), CARBOPLATIN (Cb) AND MELPHALAN (L-PAM) CONDITIONING REGIMEN FOR AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN STAGE IV BREAST CANCER. P. Kouides, C. Abboud, J. DiPersio, P. Savina, A. Guaspari and R. Duerst. Univ Rochester Med Ctr, Rochester, NY and Burroughs-Wellcome, Research Triangle Park, NC

The relative refractoriness of breast cancer to even myeloablative doses of chemotherapy prompted a Phase I/II trial of a combination of potentially non-cross resistant agents. Since October, 1991, over 40 patients (pts.) with Stage IV breast cancer were treated with a conditioning regimen of: VP-16 300 mg/m^{2}d (days -6 to -3), Cb 400-700 mg/m^{2}d (days -4 & -3) and L-PAM, 140 mg/m^2 (day -2). 7 pts received Cb at 800 mg/m^2 total, 4 at 1000 mg/m^2, 6 at 1200 mg/m^2 and the remaining pts. at 1400 mg/m^2. Predominant sites of metastatic disease at the time of ABMT included bone, lungs, and liver. Almost all pts. received at least one salvage chemotherapy regimen prior to ABMT. All but one of the pts. first underwent peripheral blood stem cell harvesting after Cyclophosphamide (3 gm/m^2) followed by GM- or G-CSF priming. GM-CSF was begun after reinfusion on Day 0. Engraftment was relatively prompt with median onset of ANC >500 x 2 days and Platelet count >20,000 without platelet transfusion on days +11 (8-23) and +13 (10-33) after marrow/stem cell infusion with length-of-stay 24 days (17-40). Pharmacokinetic measurement of L-PAM revealed first-order elimination with a T 1/2 of 62 ± 12 min. Toxicity (FDA ABMT grading system) was minimal in all but 2 pts. 1 pt. developed reversible Gr 3 nephrotoxicity and the other fatal venocclusive disease. Both received 1400 mg/m^2 Cb. Estimated (Kaplan-Meier) median progression-free-survival (PFS) is 8 mo with estimated PFS at 1 year of 29%. Further follow-up will determine the efficacy of this regimen in the treatment of advanced breast cancer.

G 422 IMMUNE RESPONSES OF RECIPIENTS WHO RECEIVE PERIPHERAL BLOOD STEM CELL TRANSPLANTS (PBSCT) AND AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT), Feroze A. Momin, Lawrence G. Lum, Ornella Bitonti, Sandra Galoforo, and BMT TEAM. BMT Program, Detroit Medical Center, Department of Medicine and Pediatrics, Wayne State University, Detroit, MI.

Although the immunodeficiency after ABMT is thought to be less severe than allogeneic BMT, there is a paucity of data on immune reconstitution (IR) after ABMT. The kinetics of IR were determined after ABMT + PBSC in 6 ABMT recipients studied sequentially 3 times at 14 day intervals beginning after engraftment. Proliferation, cytokine mRNA expression or cytokine secretion by PBL were induced by anti-CD3 or anti-CD3 + anti-CD28 coactivation. Constitutive or stimulated mRNA expression for IL-2, IL-2r_α, and GM-CSF was assessed by reverse transcriptase and polymerase chain reaction; constitutive or stimulated IL-1β, IL-2, GM-CSF, and TNFα secretion were measured by ELISA. PBL from 6 of 6 responded to anti-CD3 stimulation, 4 of 6 responded to anti-CD3 + anti-CD8 coactivation. Constitutive IL-2, GM-CSF, and IL-1_β secretion by PBL post ABMT was comparable to PBL from normals and increased after anti-CD3 or anti-CD3 + anti-CD28 costimulation. PBL post ABMT secreted GM-CSF constitutively and after costimulation with anti-CD3+ anti-CD28. PBL from 1 of 6 recipients were severely deficient secretion post ABMT which correlated with delayed marrow engraftment. In summary, *in vitro* immune function tests of PBL from recipients of autologous bone marrow and PBSCT show early recovery of immune functions leading to cytokine synthesis and secretion. This data suggest that T cells in PBSCT play a significant role in accelerating immunohematopoietic reconstitutuion.

G 423 ISOLATED CENTRAL NERVOUS SYSTEM (CNS) RELAPSE AFTER HIGH DOSE CHEMOTHERAPY (HDC) IN PATIENTS WITH ADVANCED BREAST CANCER, T.M. Zimmerman, and S.F. Williams.

Relapse after achieving a complete remission (CR) from HDC in advanced breast cancer commonly occurs in sites of previous bulk disease. In response, many centers now give consolidative radiotherapy to patients who achieve a CR. We recently have noted the emergence of a relapse pattern of isolated CNS disease. Since 1990, 101 women have received HDC (cyclophosphamide and thiotepa) with stem cell support for locally advanced or metastatic breast cancer. Of these, 6 developed an isolated CNS relapse as detailed below.

Stage	Age	Sites of Prior Disease	Response to HDC	Time to Relapse
IV	43	Supraclavicular(SC) & Mediastinal Lymph Node (LN)	CR	3 mos
IV	27	SC LN	CR	4 mos
IV	38	Solitary rib lesion	CR	12mos
IV	28	SC LN, Liver, rib	PR (persistent faint rib uptake)	4 mos
IV	48	SC and Inguinal LN	CR	12 mos
IV	46	SC LN and Pulmonary	CR	9mos

Our results suggest that in patients who have good tumor cytoreduction, there appears to be a significant risk of CNS relapse. While high dose thiotepa readily crosses the blood brain barrier, it clearly does not sterilize subclinical disease in the CNS. As such, a response to this relapse pattern is indicated. Should screening for asymptomatic CNS disease be performed in patients who achieve a CR or should prophylactic cranial irradiation be given to all these patients routinely? Could immune modulation play a role in maintaining a remission in breast cancer? Regardless, an effort needs to be made to prolong the remission in patients with metastatic breast cancer after high dose chemotherapy through consolidative radiotherapy to sites of bulk disease and some form of cranial prophylaxis.

G 424 CD34+ PROGENITOR CELL SELECTION FOR CLINICAL TRANSPLANTATION. Heimfeld S, Shpall EJ, Jones RB, Berenson RJ. CellPro, Inc., Bothell, WA and UCHSC, USA.

CD34 antigen expression is limited to hematopoietic progenitor and stem cells, and is not found on mature blood cells nor on most types of malignant cells. CellPro has designed a unique avidin-biotin immunoadsorption system (CEPRATE™ SC) for clinical scale purification of CD34+ cells. This system has now been used in a wide variety of clinical settings. A Phase II study evaluated the ability of CD34+ cells isolated from bone marrow or G-CSF mobilized peripheral blood (PBPC) to reconstitute hematopoiesis in breast cancer patients undergoing high-dose chemotherapy. Four cohorts were treated, receiving CD34+ selected marrow and/or PBPC ± G-CSF post-transplant as indicated in the table below:

Patient Cohort	N	CD34+ Marrow	CD34+ PBPC	Growth Factor	Days to ANC>500	Days to Plat. Ind.
1	7	+++		None	23	23
2	10	+++		G-CSF	13	16
3	12	+++	+++	G-CSF	9	9
4	11		+++	G-CSF	9	13

These results indicate that CD34+ bone marrow cells can engraft, that G-CSF given post-transplant accelerates neutrophil recovery, and that CD34+ PBPC give even more rapid recovery of both ANC and platelets. A sensitive immunohistochemical technique has shown that CD34+ selection results in 2->5 logs depletion of contaminating breast cancer cells. Additional data will be presented on other clinical trials utilizing CD34 positive selection for autologous transplantation in breast cancer, lymphoma, and myeloma. CD34 selection for allogeneic transplantation as an alternative to other T-cell depletion methods to reduce GVHD, and its application for ex-vivo expansion and gene therapy will also be discussed.

G 425 Closed System Percoll-processing of bone marrow for transplantation (BMT): A rapid, efficient method for mononuclear cell enrichment. **K. Keller, Y.O. Huh, L. Huynh, K. Nellis, D. Thandi, D. Cecil, J.P. Hester, K. van Besien, R.E. Champlin, and B.S. Andersson. Bone Marrow Transplantation Clinical Lab, U.T. M.D. Anderson Cancer Ctr, Houston, Tx 77030**

To control graft-versus-host-disease after allogeneic BMT and to eliminate malignant cells after autologous BMT, various purging techniques are utilized, all of which have in common a first step to obtain an enriched mononuclear cell (MNC) suspension for further separation. The use of a blood cell separator allows a swift recovery of the majority of the MNC, and also allows for the sterile recovery of erythrocytes, to be transfused back to the donor/patient, resulting in a low blood loss from the marrow harvest procedure. Percoll, which can be mixed with physiologically acceptable solutions for the maintenance of a physiological pH, ionic strength, and osmolarity is optimal as a density gradient material in marrow processing. Further, Percoll batches can be selected for low endotoxin content, increasing the safety of its use in marrow processing for clinical BMT. We report on our utilization of Percoll together with a COBE 2991 blood cell separator to obtain MNC suspensions, to be used directly for clinical transplantation or for use in marrow purging. The advantages with this improved technique for marrow processing are: 1. Speed; total processing time is only about 2 hours. 2. Efficiency; no significant loss of mononuclear cells or loss of CD34+ progenitor cells were experienced between the buffy coat and the Percoll gradient steps. Further, no delayed engraftments were experienced in patients who received the processed marrow after myeloablative therapy. 3. Sterility of the technique; there is minimal risk for bacterial or fungal contamination during the procedure using disposable tubing sets. Thus, the erythrocytes in the "waste line" can be safely transfused back to the donor/patient after the marrow harvest. The technical details of the procedure, detailed recovery data of MNC, platelets, hematocrit, GM-CFC, CD34+ progenitor cells, and engraftment data from patients transplanted with marrow processed with this technique after receiving myeloablative therapy will be presented.

G 426 LARGE VOLUME EX VIVO EXPANSION OF CD34 POSITIVE(+) AUTOLOGOUS HEMATOPOIETIC PROGENITOR CELLS (AHPC) FOR TRANSPLANTATION

Elizabeth J. Shpall*, Malcolm Purdy*, Christopher J. Hogan*, Roy B. Jones*, Scott I. Bearman*, Ronald J. Berenson, Ian McNiece**, Shelley Heimfeld.
From the Bone Marrow Transplant Program· Univ of Colo, Denver, CO*, CellPro Inc., Bothell, WA, & Amgen Inc., Thousand Oaks, CA**.

In a previous clinical study of 56 patients receiving CD34+ AHPCS, adequate engraftment was demonstrated in patients who received an average of $2.8x10^6$ colony forming units Granulocyte-Macrophage (CFU-GM). Our goal was to produce a similar quantity of CFU-GM, in an ex vivo culture system. Progenitor cells from 17 breast cancer patients undergoing high dose chemotherapy with autologous HPC support were used for the pre-clinical studies. The optimal culture conditions defined in our pre-clinical studies include: 1) culture initiation with CD34+ marrow (M) or peripheral blood (PB) progenitors as opposed to unselected progenitor cell fractions, where minimal CFU-GM expansion was demonstrated 2) media containing 10 ng/ml each of stem cell factor (S), interleukin-3 (IL3), IL6, and granulocyte colony stimulating factor (G) 3) with 10% autologous plasma (AP) 4) incubation in sterile teflon-coated gas perfusable bags (BAGS) 5) culture at 37°C, 5% CO_2 for 7 days without perturbation. The CFU-GM and fold-expansion (the mean value from a minimum of three replicates) are:

PARAMETER	INPUT CFUGM	MAXIMAL CFUGM	FOLD
M UNSELECTED	250	3300	17
M CD34+	3300	580000	184
PB 10%AP	12000	1091000	91
PB 0%AP	12000	540000	45
PB FLASK	5200	650000	100
PB BAGS	5200	563000	94
M S,IL3,6	2750	126000	43
M S,IL3,6,G	2300	420000	183
PB S,IL3,6	2300	206000	90
PB S,IL3,6,G	7150	845000	174

The maximum CFU-GM production was demonstrated on day 7 with marrow and PBPC, and on day 7 for BM. The persistence of long term culture initiating cell, erythroid, and megakaryocyte colonies within the cultures was demonstrated. Using this culture technique, one CD34+ PBPC fraction (approximately $60x10^6$ cells) will produce a minimum of $5x10^7$ CFU-GM, which should be capable of restoring hematopoiesis following high-dose chemotherapy. A clincal trial to evaluate this system will be performed.

G 427 COLLECTING TO A TARGET MARROW NUCLEATED CELL COUNT ALONE IS SUFFICIENT TO ASSURE RAPID ENGRAFTMENT OF UNPURGED CRYOPRESERVED AUTOLOGOUS MARROW, Arabella B. Tilden, Gretchen A. Cloud and William P. Vaughan, UAB, Birmingham, AL 35294

Marrow was collected for ABMT from 30 pt, 19 with breast ca, 7 with NHL and 4 with HD. Based on mid-harvest cell counts we obtained a volume of marrow expected to yield 2 x 10^8/kg nucleated cells (NC) for cryopreservation. The marrow collected was processed using stainless steel screens and buffy coat preparation on a COBE 2991, followed by controlled rate freezing and storage in liquid nitrogen. We analyzed the marrow for mononuclear cell count (MNC) CFU-GM and BFU-E as well as CD34+/CD33- cell count after processing but before cryopreservation. One pt's processed yield was only 1.1 x 10^8 NC/kg but the NC yield of the remainder ranged from 1.5 x 10^8/kg to 3.7 x 10^8/kg (med 2.2 x 10^8/kg, N=30). CFU-GM ranged from 0.2 to 8.8 (med 0.97 x 10^4/kg, N=30), BFU-E ranged from 1.2 to 10.1 (med 4.4 x 10^4/kg, N=30), total CD34+ cell dose ranged from $<1.0 \times 10^6$ to 14.0 x 10^6 (med 3.0 x 10^6/kg, N=22) and the CD34+/CD33- cell dose ranged from $<1.0 \times 10^6$ to 7.0 x 10^6 (med 2.0 x 10^6/kg, N=20). All pt received high dose thiotepa or BCNU in their preparative regimen and G-CSF from d +3 until an AGC > 500/uL. Days to ANC $\geq$ 500 ranged from 8-23 (median 12) and days to platelets $\geq$ 25,000 ranged from 10-32 (median 17). By Pearson's correlation analysis, we found no correlation between doses of NC, MNC, CFU-GM, BFU-E, CD34+ or CD34+/33- and days to neutrophil or platelet recovery. Thus collecting approximately 2 x 10^8 NC/kg for unpurged ABMT insured rapid engraftment regardless of other parameters. Doses of CFU or CD34+ cells may be predictive of time to engraftment only when quantities of total NC reinfused are limited as a result of inadequate collection, improper processing technique or purging.

G 428 ENRICHMENT OF CD 34+ BONE MARROW CELLS BY AN IMMUNOMAGNETIC METHOD, Regina Wieland, *Klaus Lennartz, Bernhard Kremens, Werner Havers, Division of Hematology and Oncology, Dept. of Pediatrics, *Dept. of Cell Biology, University of Essen, 45122 Essen, Germany

1-4% of human mononucleated bone marrow (BM) cells express CD34, a stem cell antigen of hematopoiesis. CD34+ cells form CFU-GEMM in culture and reconstitute hematopoiesis in patients after myeloablative therapy. The intention of our experiments was to enrich CD34+ BM cells using the Magnetic Activated Cell Sorter MACS (Miltenyi Biotec, Germany) to shorten fluorescence activated cell sorting time and to facilitate experimental and diagnostic studies of human BM stem cells and therapeutic procedures like hematopoietic rescue by peripheral stem cell transplantation.

BM mononucleated cells of ten donors were successively labelled with the monoclonal antibody ANTI-HPCA-1, Goat-anti-Mouse-Biotin-IgG1, Streptavidin-FITC and superparamagnetic biotinylated beads. Applying a high gradient magnetic field supplied by MACS , the cells were separated into a magnetic and a non-magnetic fraction. After exclusion of dead cells we analysed by FACS the cells before separation, the magnetic and the non-magnetic fraction. The enrichment rate E was defined as

$$E = \frac{\%\text{pos. cells after sort} \;/\; \%\text{neg. cells after sort}}{\%\text{pos. cells before sort} \;/\; \%\text{neg. cells before sort}}$$

Before separation 4.1% (median; range 1.8-12.2) of the cells were positive. After MACS separation the magnetic fraction contained 43.6% positive cells (15.4-66.4). The enrichment rate E was 14.9 (7.9-38.6). The recovery of vital cells was 78.6% (59.5-91.3). The recovery of positive cells was 76.5% (23.4-130.0), 83.5% (56.1-99.0) of them were found in the magnetic fraction.

Non-Malignant Hematologic Diseases; Blood Stem Cell Transplants; Gene Therapy; Immune Reconstitution Post BMT

G 500 PCR-VNTR ANALYSIS OF DIFFERENT HEMATOLOGIC LINEAGES IN STABLE MIXED CHIMERAS AFTER BMT IN THALASSEMIA, **Andreani M., Manna M., Nesci S., Tonucci P., Talevi N., Londei M., and Lucarelli G., Divisione di Ematologia e Centro Trapianto Midollo Osseo. Ospedale di Muraglia, USL 3. Pesaro, Italia. • The Kennedy Institute of Rheumatology, London, UK**

Marrow engraftment was determined by RFLP analysis of VNTR loci in 141 non consecutive thalassemic patients transplanted from an HLA identical sibling. The incidence of MC observed was 31.9%, 25.7%, 18.3% and 12% at 2, 6, 12 and 24 months after the transplant respectively. Different pretransplant conditioning regimens were responsible for variations in the incidence of MC. The frequence of MC was statistically significant higher in the group of patients treated with protocol 12 (BU14, CY120, ALG from -5 to +5) respect to the group receiving protocol 6 (BU14, CY200) (p = 0.05). The origin of different cell subset populations in patients with stable mixed chimerism, transfusions independent for more than two years after the transplant, was investigated. PCR analysis of VNTR loci was carried out on CD2+ and CD19+ dynabeads purified mononuclear peripheral blood cells and on CD33+ and Glycoforin A+ mononuclear bone marrow cells. Moreover the donor/recipient origin of eritroid lineage was investigated by PCR analysis of single and /or 10-15 pooled BFU-E. Preliminary data showed that in the patients with stable M.C. the proportion of donor/recipient DNA detected in CD2+ and CD19+ cells was equivalent to that observed in the whole peripheral blood. A mixed origin of the cells was also found when the different bone marrow cellular lineages were investigated. Supported by AIRC, Milano, Italy.

G 501 Y-FISH IN APLASTIC ANEMIA (AA) PATIENTS SUBMITTED TO SEX-MISMATCHED ALLO-BMT, Bernasconi P., Boni M., Cavigliano P.M., Troletti D., Passamonti F., Alessandrino E.P., Caldera D., Bonfichi M., Bernasconi C., Cattedra di Ematologia Universita' di Pavia, Divisione di Ematologia Policlinico San Matteo Pavia, Italy.

Y-FISH was applied in three males suffering from AA, transplanted with a female donor. In all of them the technique has succeeded in determining engraftment within 10 days from allo-BMT. Now all the pts have achieved CR with different clinical outcomes.Three months post-allo-BMT two of them showed 0.8% and 1% respectively of y spot positive cells. At the last examination, on day +848 and on day +547, all their cells are of female sex. The last pt was studied with Y-FISH and with cytogenetics. Two months after BMT 10% of his marrow cells were Y-spot positive, while all of them were of female sex on chromosome analysis. At that time peripheral blood values were in the normal range with a normocellular marrow. Three months later the percentage of male cells reached 72% and 47% on Y-FISH and on chromosome study respectively. A marrow biopsy documented relapse. The dosage of immunosuppressive therapy was changed. In the following months the percentage of Y spot positive cells remained 48-54%, while no mitosis were obtained. On day +424 Y-FISH positive cells are 38%. A male Karyotype is detected in 30% of marrow cells. A new marrow biopsy has shown CR, despite the persistence of recipient cells. Our data show that Y-FISH determines engraftment and chimerism earlier and more precisely than conventional cytogenetics, helping the clinician in adjusting the dosage of immunosuppressive therapy in AA pts submitted to allo-BMT.

G 502 FANCONI ANEMIA: PRELIMINARY REPORT OF THE FANCONI ANEMIA TRANSPLANT REGISTRY ON 278 TRANSPLANTS, Richard E. Harris, Eliane Gluckman, Mudra Kumar, H. Joachim Deeg, Lynn Frohnmayer, Arleen D. Auerbach, Jose Zanis Neto, Mary Flowers and Mary Horowitz, **Fanconi Anemia Transplant Registry (FATR),** BMT Program, Children's Hospital Medical Center, Cincinnati, OH 45229

Data on 278 patients who have undergone marrow transplant for Fanconi anemia were obtained from the International Bone Marrow Transplant Registry (IBMTR), the National Marrow Donor Program (NMDP), the Fanconi Anemia Research Fund (FARF), the International Fanconi Anemia Registry (IFAR), the Fred Hutchinson Cancer Research Center, and the transplant teams in Paris and Cincinnati, as well as additional transplant centers. Patients were uniquely identified by their date of birth and date of transplant to avoid duplication. Data are currently available on 199 matched sibling donor (MSD) transplants, 46 related haploidentical (HAP) transplants, and 33 unrelated donor (URD) transplants. The 2-year disease-free survival (DFS) was 62% for MSD, 35% for HAP, and 30% for URD transplants. The DFS for fully matched (HLA-A, B and DR identical) HAP (N = 19) and URD (N = 27) transplants were 50% and 38% respectively (NS). The DFS for all fully matched alternative donor transplants (N = 46) was 43% (p = 0.05 vs MSD). The DFS for fully matched URD transplants (N = 27, DFS 38%) appears to be higher than that of partially matched HAP transplants (N = 27, DFS 25%, p = 0.08). Of six 1-antigen mismatched URD transplants, none survive. With MSD transplants, the best DFS was seen among patients receiving a preparative regimen of low-dose cyclophosphamide (15-25 mg/kg) combined with low-dose thoracoabdominal or total lymphoid irradiation (400-600 cGy) and GVHD prophylaxis utilizing cyclosporine and ATG or ALS (N = 22, 2 yr DFS = 90%); with the same conditioning but without ATG/ALS, 2-yr DFS was 69% (N = 92; p = 0.05 vs group receiving ATG/ALS). A preliminary risk factor analysis shows the best survival among patients transplanted before the age of 10 yrs, before the development of leukemia, or before receiving more than 10 transfusions. Current cautious recommendations are as follows: 1) Patients who have a MSD should undergo a transplant as soon as hematologic manifestations dictate a need for therapy; 2) Patients without a MSD but with a fully matched alternative donor could be considered for transplant if they have shown resistance to androgen therapy; 3) Patients without either a MSD or a fully matched HAP or URD should be evaluated individually. Future options might include gene therapy, particularly for these high risk patients.

G 503 BONE MARROW AND CORD BLOOD STEM CELL TRANSPLANTATION FOR THALASSEMIA IN THAILAND, S. Issaragrisil, S. Visuthisakchai, V. Suvatte, D. Chandanayingyong and A. Piankijagum, Department of Medicine, Pediatrics and Transfusion Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

In Thailand, thalassemias and hemoglobinopathies are prevalent. The frequencies of α-thalassemia are 20-30%, β-thalassemia 3-9%, hemoglobin (Hb) E 13% and Hb Constant Spring at least 4%. These abnormal genes lead to over 60 different thalassemic syndromes with varying in severity. Among these, homozygous β-thalassemia and β-thalassemia/Hb E disease are the most common syndromes in which the patients can be born alive.

Bone marrow transplantation was first used to cure thalassemic patients in July 1988. Up to present 16 transplants were performed in 15 patients. Three were homozygous β-thalassemia; 12 were β-thalassemia/Hb E disease. Eight were males and 7 females. The age ranged from 1.3 - 14 years. The conditioning regimen comprised busulfan 14 -16 mg/kg and cyclophosphamide 200 mg/kg and post transplant prophylaxis consisted of short methotrexate and cyclosporine. Two patients received busulfan 600 mg/Sq.m and cyclophosphamide 200 mg/kg for conditioning.

Nine patients had complete engraftment as evidence by DNA analysis and sex chromosome study. Three patients had partial engraftment, loss their graft with autologous recovery 3 months after transplant. Second transplant was performed by using Bu 600 mg/Sq.m plus Cy 200 mg/kg for conditioning in one patient with success. One patient who received one HLA-antigen mismatched marrow had no engraftment. One patient died early due to CNS complication. One patient died after engraftment due to acute GVHD and fungal infection. Those who had graft failure had severe manifestations as evidence by marked hepatosplenomegaly and previously multiply transfusion.

A 2.5 year old patient with β-thalassemia/Hb E disease underwent cord blood stem cell transplantation. Complete engraftment was evident by sex chromosome study. At present the patient survives 80 days post transplant without evidence of disease.

Based on these results hematopoiesis of donor in origin can be maintained after transplantation and the patients can be cured, Graft failure may be a problem in those with severe manifestations. However Bu 600 mg/Sq.m plus Cy 200 mg/kg is effective in eradicating thalassemic stem cells in those cases. Cord blood is an alternative source for transplantation. Cord blood transplantation is first demonstrated to cure thalassemic patients in this report.

G 504 MIXED CHIMERISM MAY PREDICT GRAFT REJECTION IN PATIENTS TRANSPLANTED FOR SEVERE APLASTIC ANEMIA(SAA). Mark Lawler, Shaun R McCann, Per Ljungman, Judith Marsh, Gerard Socie, Anna Locasciulli, Eliane Gluckman, Peter Humphries, Jill Hows, Andrea Bacigalupo. Department of Genetics/Haematology St James's Hospital/Trinity College Dublin, Ireland for the EBMT working party on SAA.

While BMT is a viable treatment for SAA, the problem of graft rejection(GR) remains and may reflect several factors including low donor cell infusion, conditioning therapy and type of GvHD prophylaxis. In an effort to predict which groups of patients might be more likely to reject their grafts, we have studied the incidence and significance of mixed chimerism following BMT for SAA. 63 patients have been entered into the study from 13 European institutions. 57 patients have been analysed and as 4 patients have received a second transplant, 61 transplant events are currently being monitored. Samples have been assessed as early as 17 days and as late as 4,987 days post BMT. Mixed chimerism(MC) has been detected in 24 patients. In 9 patients MC was of a transient nature and was only detected within the first 100 days post transplant. Recipient cells persisted in 15 patients beyond six months post BMT. 5 patients showed levels of recipient cells less than 10% with a follow up of 1-4 years. All these patients are alive and well. The remaining 10 patients showed evidence of recipient cells at higher levels(15-60%) within the first year post BMT and this was followed by graft rejection in 6 cases. In 3 patients GR has occurred during cyclosporin withdrawal and this has prompted routine monitoring of patients during withdrawal of immunosuppressive therapy. In two patients leukemic transformation of donor cells has been documented leading to AML M5 and M0 respectively. If MC is an early indicator of GR, then assessment of chimerism may allow subsequent modulation of post transplant therapy and indicate if GvHD prophylaxis has an influence on GR . 20 patients have now been entered into a prospective randomised study of CSA V CSA + MTX GvHD prophylaxis to attempt to address this issue.

G 505 Aplastic anemia and fulminant liver failure from non-A, B, C hepatitis: Successful outcome following orthotopic liver transplant (OLT) and allogeneic BMT. S. Neudorf, G. Reyes, A. Tzakis, J. Mirro, and T. Starzl, Departments of Pediatrics and Surgery, University of Pittsburgh, Pittsburgh, PA 15213.

Although non-A, B, C hepatitis is usually self-limited, it has been associated with fulminant liver failure and severe aplastic anemia. Occasionally both may occur in the same patient, with the aplasia identified after successful liver transplant. Treatment of such cases using immuno-suppression has largely been unsuccessful but BMT using an HLA-identical, MLC-nonreactive sibling donor has been effective treatment. We report a 13 year old boy who received a cadaveric OLT for acute hepatic failure due to non-A, B, C hepatitis. FK-506 was successfully used to prevent liver rejection. Approximately two weeks post-OLT, the patient developed pancytopenia and a bone marrow biopsy showed <10% cellularity. The patient was treated with GM-CSF and erythropoietin and had a transient improvement in neutrophil counts but remained RBC and platelet transfusion dependent with no change in reticulocyte counts. Allogeneic BMT was performed 6 months following OLT using cyclophosphamide (50 mg/kg/day) for 4 days followed by 750 cGy total lymphoid irradiation. The patient received 2 x 10^8 nucleated donor bone marrow cells (non-T-cell depleted) from his HLA identical sibling. Following BMT, the patient received a 3-week course of GM-CSF beginning on day +2 post BMT. FK-506 was discontinued 1 day pre-transplant and restarted day +2 as a continuous infusion of 0.1 mg/kg/day as prophylaxis for graft versus host disease (GVHD) and to prevent liver rejection. Serum FK-506 levels were maintained at 1 ng/ml and he was converted to oral FK-506 (3 mg PO bid) when his mild mucositis resolved. Engraftment (ANC > 500/mm^3) was documented by day +12 post BMT and the patient has not required platelet transfusions since day +25. There was no evidence of hepatic veno-occlusive disease (VOD), hepatotoxicity, and no GVHD. The patient is currently alive and well (Karnofsky score = 100) 2 years post BMT. This case suggests that allogeneic BMT may be effective therapy for patients who develop severe aplastic anemia following OLT. The absence of acute GVHD, particularly in the HLA-unrelated liver, suggests that FK-506 may be a particularly effective drug for the prevention of GVHD.

G 506 INFUSED CFU-GM PREDICTS ENGRAFTMENT KINETICS IN PATIENTS RECEIVING G-CSF-MOBILIZED PERIPHERAL STEM CELLS AFTER MARROW ABLATION. S.A. Bentley, M.E. Brecher, D.E. Killian, E. Powell, S.B. Schoultz, J.M. Wiley, T.C. Shea. Departments of Pathology, Internal Medicine and Pediatrics, University of North Carolina, Chapel Hill, NC 27514

The validity of clonal stem cell (SC) assays as predictors of marrow engraftment remains controversial. In general, SC assays correlate well with engraftment kinetics after autologous transplantion but less so after allogeneic transplantation. There is relative little data for peripheral SC recipients. In the past year we transplanted 13 patients with peripheral SC (5 males and 8 females, with various malignant marrow disorders), including 4 who also received autologous marrow. All patients were conditioned with an ablative regime consisting of 6 g/m^2 cyclophosphamide, 1800 mg/m^2 VP-16, 1000 mg/m^2 carboplatin and 8-12 Gy TBI. Peripheral SC were mobilized with 7-12 days of 5 μg/kg/day SQ G-CSF. Aphereses, processing at least 10 L whole blood per procedure, were begun on day 5 and continued qd or qod till at least $8x10^8$ TNC/kg were obtained (an average of 5 collections). The median number of CFU-GM infused was $10.8x10^4$/kg (range 2.6-79.$6x10^4$/kg) and the median interval to peripheral neutrophils > $500x10^9$/L was 11 days (range 9-16) days. There was a linear relationship between log CFU-GM infused and days to peripheral neutrophils > $500x10^9$/L ($r=-0.79$, $p<0.01$). Exclusion of those patients receiving marrow along with peripheral SC did not alter the correlation coefficient. There was also a significant correlation between infused CFU-GM and the period of thrombocytopenia and red cell transfusion dependence. Four patients remain red cell and platelet transfusion dependent >50 days post transplant, despite early and sustained neutrophil recovery. These patients ranked in the lowest 6 for infused CFU-GM, receiving a mean of $6.6x10^4$ CFU-GM/kg (range 2.6-9.$1x10^4$ CFU-GM/kg). We conclude that the number of infused CFU-GM is a major determinant of short-term engraftment kinetics after marrow ablation and infusion with G-CSF-mobilized peripheral SC. A low number of infused CFU-GM also appears to predict poor or delayed platelet recovery.

G 507 MOBILIZATION OF PERIPHERAL BLOOD PROGENITOR CELLS WITH LOW DOSES OF GM-CSF (LEUCOMAX®). Bo Björkstrand, Junji Suwata, Per Ljungman and Gösta Gahrton. Dpt of Medicine, Karolinska Institute & Huddinge Hospital, Stockholm, Sweden.

The feasibility of low doses of GM-CSF (Leucomax®) for mobilization of peripheral blood progenitor cells for autologous transplantation, was investigated in a pilot dose escalation study. GM-CSF was administered at steady-state hematopoiesis as one daily s.c. bolus injection for eight consecutive days. Three patients were treated on each of the following dose levels: Group I: 1.0 µg/kg/d.; II: 2.5 µg/kg/d.; III: 5.0 µg/kg/d. and IV: 7.5 µg/kg/d. Nine patients had multiple myeloma, one had NHL and two had CLL. Five patients (one in gr. II, one in gr. III and all three in gr. IV) were previously treated with stem cell toxic drugs (melphalan, chlorambucil or CdA), while the remaining seven had received other types of stem cell non-toxic chemotherapy. Cells were harvested by daily leukapheresis on days 5, 6, 7 and 8 of GM-CSF-administration, using a Fenwal CS-3000 cell processor.

WBC rise, with a maximum on day 4, was greater in groups III-IV compared to groups I-II (138% and 72%, respectively, compared to basal levels). Elevation of ANC counts was greater in gr. IV (206%) compared to gr. I-III (117%). Mean CFU-GM levels for gr. I-III were $3.05x10^4$/kg b.w. (range, 1.09-4.80), and did not differ between these groups. For gr. IV, the mean CFU-GM count was lower ($0.93x10^4$/kg b.w.; range, 0.38-1.38), which was considered to be due to factors related to prior chemotherapy, as stated above. There was a clear difference in CFU-GM counts between the group of patients previously treated with stem cell toxic drugs as compared to the patients not receiving such treatment, with mean CFU-GM levels of $1.09x10^4$/kg b.w. (range, 0.38-1.59) and $3.53x10^4$/kg b.w. (range, 2.46-4.80), respectively. Four patients (two in gr. I, one in gr. III and one i gr. IV) have to date been transplanted with the cells obtained from this harvest exclusively. For the patients in gr. I and III, ANC>$0.5x10^9$/l was achieved on days 13, 12 and 11 post-transplant. Platelet counts of >$50x10^9$/l were achieved on days 32 and 63 for the patients in gr. I and on day 11 for the patient in gr. III. The patient in gr. IV has a chronic graft failure five months post-transplant.

We conclude that treatment with low doses of GM-CSF at steady-state hematopoiesis can yield sufficient numbers of hematopoietic progenitor cells to enable fast granulocyte- and platelet engraftment after myeloablative chemo-radiotherapy. However, there was a trend for faster platelet recovery at the 5µg-dose level. Prior treatment with stem cell-toxic drugs was predictive for a bad progenitor cell yield, despite the use of higher doses of GM-CSF. Further studies of larger and more homogenous patient groups should be performed.

G 508 REPERTOIRE OF T LYMPHOCYTES INFILTRATING GVHR-DAMAGED ORGANS AFTER MURINE BONE MARROW TRANSPLANTION. Martine Bruley-Rosset*, Isabelle Miconnet*, Thierry Roger$, Veronique de La Salle* and Michel Seman$ *INSERM 267, Villejuif 94800 France; $Jussieu, Paris 75005 France.

A lethal GVHR develops after the graft of bone marrow and spleen cells from B10.D2 donors to lethally irradiated (DBA/2 x B10.D2)F1 recipients which are H-2 compatible but differ for multiple minor Histocompatibility Antigens (mHAg) and Mls^a. By using CD4$^+$ T cell clones specific for mHAg involved in GVHR we previously demonstrated an individuality in the pattern of cell and tissue expression for each mHAg. In this study we addressed the question of a possible selection of T cells through their expression of particular Vβ element during the pathogenesis of GVHR. Since some organs are predominantly affected during the disease, we performed a kinetic analysis of TCR Vβ usage directly in T lymphocytes infiltrating lymphoid organs, gut and liver. CD4$^+$ $\alpha\beta^+$ T cells expanded strongly in spleen, lymph nodes and intra-epithelial intestinal lymphocytes (iIEL) early after the graft. The over-expression of Vβ6$^+$ and Vβ3$^+$ T cells within the CD4$^+$ compartment probably reflects the response to Mls-1^a and Mls-2^a, respectively. Lymphocytes infiltrating the liver are mainly composed of Vβ3+ and Vβ4+ Tcells beginning 2 weeks after grafting. As these cells are found mainly within CD8$^+$ or CD4$^-$ CD8$^-$ but not within CD4$^+$ T cell compartments, we postulate that they recognize mHAg.

In conclusion our data demonstrate that the T cell repertoire emerging during GVHR is restricted at the level of TCR Vβ usage, suggesting that among the numerous antigen disparities, some behave as immunodominant.

G 509 MOBILIZATION OF PERIPHERAL BLOOD STEM CELLS (PBSC): COMPARING CYCLOPHOSPHAMIDE AND GROWTH FACTOR BASED REGIMENS. G. Elfenbein, W. Janssen, R. Smilee, R. Carter, D. Rahn, M. Cairo, J. Hiemenz, P. Zorsky, K. Fields, O. Ballester, J. Perkins, L. Kronish. Moffitt Cancer Ctr, Div of Marrow Transplant, Univ. South FL Tampa, FL

We have studied several regimens for mobilizing PBSC. PBSC were collected during WBC recovery following treatment with: cyclophosphamide (CYCLO); CYCLO with etoposide (CYVP16); or CYCLO or CYVP16 followed by G-CSF (CYGCSF; CYVPG). Alternately, PBSC were collected without prior chemotherapy in the sixth through eleventh days of GM-CSF administration (GMCSF). Nucleated cells (Nucl), CD34 positive cells (CD34), and CFU-GM colony forming cells (CFUGM) were enumerated. For those patients who were transplanted following high dose therapy with ifosfamide + carboplatin + etoposide (ICE) or mitoxantrone + thio-tepa (MITT), the number of days to reach ANC>500/μL was recorded.

		Cells Collected (avg ± std)			Median Days ANC<500 (n)	
Regimen	n	Nucl-E9	CD34-E7	CFUGM-E5	ICE	MITT
CYCLO	6	11± 5	4± 3	10± 6		
CYVP16	4	23±13	**39±10**	15± 9	12	24
CYGCSF	14	20± 7	12±10	10±10	(6)	(14)
CYVPG	8	**41±12**	**49±29**	**67±41**		
GMCSF	39	16± 8	16±11	**3± 3**	20 (10)	43 (9)
ANOVA		p=0.003	p=0.002	p<0.001	NA	NA

The median days to reach ANC>500 that we have observed for standard autologous bone marrow transplant are 16 and 28 days for ICE and MITT respectively. We conclude that: (1) different mobilization regimens produce different numbers of circulating stem cells, in total and by type; and (2) the rate of engraftment after stem cell transplant may be faster when PBSC are employed, but only with certain mobilization and conditioning regimens.

G 510 RESOURCE CONSUMPTION IN THE TREATMENT OF PATIENTS WITH METASTATIC BREAST CANCER RECEIVING BONE MARROW TRANSPLANT (BMT) WITH OR WITHOUT PERIPHERAL BLOOD PROGENITOR CELLS (PBPC). R. Ghalie, A. Kucharsky, R. Aiello, S. Greenstein, K. Matuszewski, H. Kaizer. Rush University, Chicago, IL 60612.

PBPC are increasingly used for transplant because they accelerate hematologic recovery after myeloablative therapy. Since a significant number of patients may be eligible for PBPC transplants, analysis of resource consumption and the impact on treatment cost is needed. To investigate the clinical and financial consequences of PBPC mobilization and use, we studied 3 consecutive groups of patients with metastatic breast cancer treated in our center on a uniform BMT protocol. All patients received thiotepa, cyclophosphamide, cisplatin or carboplatin, and BMT with or without G-CSF. The most recent patients also received PBPC. PBPC were mobilized with cyclophosphamide (4 gm/^{2}), VP16 (1 g/m^2) and G-CSF. Disease extent and prior therapies were comparable in the 3 groups:

	Group A (n=16)	Group B (n=11)	Group C (n=15)
Treatment dates	5/89-2/91	3/91-7/91	8/91-8/92
G-CSF	No	Yes	Yes
PBPC transplant	No	No	Yes

Clinical variables studied included length of hospitalization (LOH), and number of red cells (RBC) and platelets (SDP) transfused. Patient charges were adjusted to 1992-dollars and included PBPC mobilization and the required aphereses (median=2). Charges were computed for 60 days after BMT and included blood products (TRX), pharmacy (PHAR), laboratory (LAB), and room (ROOM) charges. As seen below, there was a significant decrease in resource consumption in patients receiving PBPC (median values):

	Group A	Group B	Group C	p (A *vs* B *vs* C)
LOH	39	42	29	0.0001
RBC	20	12	5	0.0001
SDP	19	11	4	0.0001
TRX ($)	20,361	12,934	5,691	0.001
PHAR ($)	74,422	95,702	71,346	0.05
LAB ($)	40,150	31,384	18,844	0.007
ROOM ($)	40,150	46,647	32,075	0.03
TOTAL ($)	215,830	211,425	139,008	0.05

In conclusion, PBPC significantly decreased treatment toxicity (data not shown), transfusion requirements, hospitalization duration, and treatment charges. Further decrease in cost could be possibly obtained by shifting patient care to the outpatient setting, thereby reducing rooms and pharmacy charges.

G 511 IMMUNE FUNCTION AND PHENOTYPE OF PERIPHERAL BLOOD AND BONE MARROW STEM CELL PRODUCTS AND PERIPHERAL BLOOD RECONSTITUTION, Cathy Gordy[1], Greg Perry[2], Michelle Thomas[1], Elizabeth Reed[3], Mark Arneson[3] and James E. Talmadge[1]. [1]Departments of Pathology/Microbiology, [2]Cell Biology/Anatomy, and [3]Internal Medicine, University of Nebraska Medical Center Omaha, NE 68198

We examined the immune function and membrane phenotype expression of peripheral blood stem cell (PBSC) and bone marrow transplant (BMT) products of patients with NHL and breast cancer. In addition, we examined these parameters on peripheral blood leukocytes (PBL) prior to and following transplant. We observed that the mitogen response of PBL from patients prior to mobilization was depressed approximately 50% compared to normal samples. Further, the mitogenic response of cells from GM-CSF mobilized PBSC products, BM cells and PBLs of patients post transplant were significantly depressed to levels ~80% below that of normal samples. A comparison of frozen to fresh PBSC demonstrated that freezing did not depress immune function. Additional studies revealed that the mitogenic response of PBSC compared to PBL obtained by venipuncture at the time of aphoresis was significantly reduced suggesting that aphoresis was in part responsible for the loss of the mitogen response. PBL at 15, 30 and 100 days post transplant also had a depressed mitogenic function. In contrast natural killer cell activity returned to normal levels by 30 days post transplantation. Mechanistic studies revealed that prior to mobilization with GM-CSF and aphoresis, the PBL had little natural suppressor (NS) activity. However, there was an increase in NS activity in PBL's during mobilization, as well as, in PBSC and BMT products. High levels of NS activity were observed in the PBL for up to 100 days post transplant. It appears that the GM-CSF mobilization and aphoresis may upregulate NS activity and thus have a role in the immune dysfunction that occurs post transplantation. In addition to functional abnormalities in the stem cell products and PBL's post transplantation there are substantial phenotypic abnormalities. Both the stem cell products and the PBL's following transplant have a CD4:CD8 inversion. Parallel with the increase in NS activity we noted an increase in TCRα/β+CD4-CD8- cells which are the morphologic homologue of NS activity. These data suggest that NS cells selected for and or increased by GM-CSF mobilization and aphoresis may play a role in the immune dysfunction following induction chemotherapy and transplantation. Supported in part by the Nebraska Cancer and Smoking Disease Research Program.

G 512 OPTIMAL HARVESTS OF PERIPHERAL STEM CELLS DEPEND ON WHEN AND HOW THE LEUKAPHERESIS IS PERFORMED. M.Hansson, A.Svensson, P.Engervall and T.Söderström. Karolinska Hospital, Dept Clin Immunol and Transfusion med., Immunhemotherapy Unit, 171 76 Stockholm, Sweden

Peripheral Stem Cells (PSC) harvested by leukapheresis after mobilization with chemotherapy ± CSF, provide a useful source of progenitor cells for autologous transplantation. In this study, 42 timed leukapheresis periods based on numbers of CD34+ cells in peripheral blood were performed on 30 patients with HD, NHL, ALL or AML (mean: 3 harvests/period). Patients were monitored daily by analyzing whole blood with flow cytometry using moAb's to CD14, CD45, CD33 and CD34. PSC collections were performed by leukapheresis (Cobe Spectra) with settings to collect the mononuclear cell (MNC) fraction and by processing 10-12 L of blood. Mobilization with G-CSF resulted in stem cells and progenitor cells (CD34+ cells and GM-CFC) appearing in the circulation with a peak for 1-2 days whereafter they declined, while MNC counts continued to increase. The yields of CD34+ cells, GM-CFC and MNC in the harvest were compared to the levels detected in blood. Occasionally, we noted a discrepancy between the yields of harvested MNC and CD34+ cells, in that the latter corresponded to only 2-3x the level in one liter of blood while MNC yield was 10x the level in one liter of blood.

To maintain optimal yields of MNC without "loss" of CD34+ cells, small samples were drawn from the collection line during the leukapheresis procedure and the cells were evaluated for forward and side scatter properties by flow cytometry. This is a rapid procedure that provides feed back information to the leukapheresis operator regarding the relative proportion of different cell populations in the collection line and a possibility to adjust for better recovery of progenitor cells when needed. We conclude that both **when** to perform PSCC, and **how** to perform it, varies between patients, depending on the use of CSF for mobilization as well as individual variations. The use of flow cytometry to monitor each patient for leukocyte populations prior to and during leukapheresis may improve the quality of each harvest, thus reducing the number of leukapheresis procedures needed for transplantation.

G 513 MOBILIZATION OF PRIMITIVE HEMATOPOIETIC PROGENITORS BY GRANULOCYTE COLONY-STIMULATING FACTOR IN NORMAL SUBJECTS.

Mine Harada, Tomoaki Fujisaki, Teruhisa Otsuka, Yuujyu Ohno and Yoshiyuki Niho, First Department of Internal Midicine, Kyushu University, Fukuoka 812, Japan

In order to know the quality of peripheral blood stem cells (PBSC), we performed granulocyte corony-stimulating factor(G-CSF) induced mobilization of PBSC in normal subjects, and studied their hematopoietic activity by using semisolid and long-term culture (LTC) systems. Five mormal volunteers received 2- or 3-days sequential subcutaneous injections of G-CSF (Filgrastim, 1.5 μ g/kg). After this stimulation, committed progenitor cells such as CFU-GM, BFU-E and CFU-Mix were increased along with leukocytes. Furthermore, LTC initiating cells (LTC-IC) were also mobilized into circulation. Replating capacity of the primary colonies formed before and 5 weeks after the LTC was also increased with the G-CSF mobilization. These observations suggest that G-CSF can mobilize not only committed progenitors but also very primitive progenitors (LTC-IC) into peripheral blood, and that PBSC mobilized by G-CSF may be an alternative of bone marrow cells for an allogeneic trasnplantation setting.

G 514 TRANSPLANTATION OF CD34 POSITIVE(+) MARROW AND/OR PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) INTO BREAST CANCER PATIENTS FOLLOWING HIGH-DOSE CHEMOTHERAPY (HDC)

Roy B. Jones*, Elizabeth J. Shpall*, Scott I. Bearman*, Solomon Stemmer*, Malcolm Purdy*, Shelly Heimfeld, Ronald J. Berenson. From the Bone Marrow Transplant Program- Univ of Colo, Denver, CO*, & CellPro Inc., Bothell, WA.

Fifty-six breast cancer patients received high-dose cyclophosphamide, cisplatin, and BCNU followed by infusion of autologous CD34+ hematopoitic progenitor cell support. The study included 5 sequentially treated patient cohorts. Marrow buffy coat cells (cohorts 1-4) or G-CSF-mobilized PBPCs from 3 leukaphereses (cohorts 4,5), were incubated with the biotinylated anti-CD34 antibody 12-8. The cells were then applied to a column of avidin coated beads (CellPro Inc., Bothell WA) for purification. The positively selected fractions contained 1.3-1.5 x10^8 CD34+ marrow cells and 1.1-2.6 x10^8 CD34+ PBPCs/3 phereses. The cohort description, as well as the number of days to granulocyte count (ANC) ≥500/ul and a platelet count (PLAT) >20,000/ul are summarized in the following table with data expressed as "mean (range)":

PATIENT COHORT	N	CD34+ MARROW	CD34+ PBPCs	GROWTH FACTOR	#DAYS TO ANC > 500	#DAYS TO PLAT > 20K
1	7	***		NONE	22 (12-34)	22 (16-33)
2	10	***		G-CSF	11 (9-13)	20 (11-31)
3	8	***		GM-CSF	17 (11-27)	33 (20-150)
4	11	***	***	G-CSF	11 (10-12)	14 (9-26)
5	20		***	G-CSF	10 (9-12)	15 (9-156)

Using a sensitive, quantitative immunohistochemical technique, 1.0 to >4 logs of breast cancer cell depletion was documented in the positively-selected fractions of marrow and PBPCs where tumor was initially detected. The data demonstrate that CD34+ progenitor cells produces consistent engraftment following HDC. Granulocyte and platelet recovery were comparable for patients who received G-CSF-stimulated CD34+ PBPCs with (cohort 4) or without (cohort 5) CD34+ marrow. Longer follow-up will be required to assess the ultimate therapeutic effect of the entire treatment program.

G 515 SEQUENTIAL PERIPHERAL BLOOD PROGENITOR CELL (PBPC) TRANSPLANTS FOR PATIENTS WITH METASTATIC BREAST CANCER. H. Kaizer, R. Ghalie, L. Valentino, J. Feingold, S. Manson, J. Pruett, M. Cobleigh, J. Wolter, S. Lincoln, A. Korenblit, S. Adler, B. McLeod, C. Richman. Rush Medical Center, Chicago, IL 60612.

We investigated a dose-intensive protocol using tandem courses of high-dose chemotherapy and PBPC transplant in patients with metastatic breast cancer. Patients were not eligible if they had hormone-responsive disease, progressive CNS metastases, or more than 20% marrow involvement. From 8/91 to 7/93, 36 patients were enrolled. All patients had measurable or evaluable disease: 22 had visceral metastases, 9 had soft tissue disease only, and 5 had bone disease only. To minimize the risk of selecting drug-resistant tumor cells, the protocol did not involve the use of standard-dose induction chemotherapy, although 7 patients had received such therapy. PBPC were mobilized with cyclophosphamide (4 g/m^2), VP16 (1 g/m^2), and G-CSF (median = 4 apheresces). The regimen for the first transplant consisted of thiotepa (600 mg/m^2), carboplatin (800 mg/m^2), and cyclophosphamide (6,000 mg/m^2). Responding patients received another course of chemotherapy consisting of busulfan (16 mg/kg) and VP16 (60 mg/kg). Patients with localized metastases were also eligible for consolidative irradiation. Hematologic recovery was comparable in both transplant courses, with a median of 8 days to neutrophils > 0.5x10^9/L and platelets > 20x10^9/L. Engraftment rates were similar in patients receiving PBPC only (n=17) compared to the rates in patients receiving bone marrow in addition to PBPC (n=16). The median length of hospitalization was 3 weeks and the median interval between the two courses was 8 weeks. The toxicities of the second course were more severe than that of the first course. Three patients died after the second transplant from respiratory failure associated with veno-occlusive disease of the liver. Three patients were not transplanted because they progressed after mobilization. Eight patients received only one of the two scheduled transplants: 5 progressed after the first course and 3 achieved a complete remission after the first course then refused the second course. Of the 25 patients completing both transplants, 16 achieved an objective response including 8 complete remissions. Only 2 of these complete responses were obtained following the first transplant course. Of the 36 patients enrolled, 8 remain in remission with a median follow-up of 14 months (range, 3-24 months). Only one of these 8 patients had visceral metastases. We conclude from this pilot study that tandem PBPC transplants can be given over a short interval because of rapid hematologic recovery. The complete remission rates and event-free survival of this particular protocol do not appear to be significantly better than the results reported with single transplants.

G 516 ANALYSIS OF SURFACE IMMUNOPHENOTYPE OF THE HARVESTED MONONUCLEAR CELLS FROM PERIPHERAL BLOOD STEM CELL HARVEST, Akira Kubota, Koji Nagafuji, Shoichi Inaba, Naoki Harada, Tetsuya Eto, Mine Harada, Teruhisa Otsuka, Seiichi Okamura and Yoshiyuki Niho, The First Department of Internal Medicine and Transfusion Service, Kyushu University Hospital, Fukuoka, Japan.

We studied the surface immunophenotype of the harvested mononuclear cells (MNC) from peripheral blood stem cell (PBSC) harvest. Twenty seven patients with hematological malignancies (11 AML, 8 ALL, 8 NHL) received consolidation chemotherapy of complete remission. Peripheral blood MNC were harvested by leukapheresis during the phase of recovery from intensive chemotherapy (Ara-C+MIT or Ara-C+VP16). A blood cell separator (COBE Spectra) was used for continuous flow leukapheresis. The volume of blood processed amounted to 150 ml/kg of the patient's body weight. G-CSF was used for mobilization of PBSC in ALL and NHL cases. Surface immunophenotyping of harvested MNC was carried out by flow cytometry using FACScan.

The positivities of monocytic marker CD11b, CD14 and CD33 in total MNC were 63.9%, 59.5% and 64.0% respectively. The positivities of T lymphoid marker CD2 and CD3 were 33.1% and 33.0% respectively. The positivity of B lymphoid marker CD19 was 0.41%. The CD34-positive cells were 2.87% of MNC.

The CD4-positive cells were 47.6% of gated lymphocyte, whereas the CD8-positive cells were 38.1% of gated lymhocyte. The CD4/CD8 ratio was below one in 11 of 27 cases. The NK population, CD16+/CD56+ was 3.75% of lymphocyte. CD4+/CD45RA population was 29.4% of the CD4-positive lymphocyte. CD4+/CD45RO population was increased to 62.5% of the CD4-positive lymphocyte. T-cell activation marker HLA-DR was positive in 26.9% of CD4-positive lymphocyte and 46.9% of CD8-positive lymphocyte.

The majority of the harvested MNC was monocyte and T cell. B cell was a small population of MNC. T cells were activated with high expression of CD45RO and HLA-DR. The monocyte and T cells may be actively involved in the hematological recovery by the production of hematopoietic cytokines. CD8-positive cytotoxic/suppressor T cells were increased in some cases, which may play some role in immunoreaction after PBSC transplantation.

G 517 CHEMOTHERAPY AND G-CSF MOBILIZED PERIPHERAL BLOOD LEUKOCYTES POSSESS A HETEROGENEOUS POPULATION OF CD34$^+$ STEM CELLS SUFFICIENT FOR AUTOLOGOUS HEMATOPOIETIC ENGRAFTMENT, D. Maharaj, R. Riley*, G.J. O'Neill, J. Phillips*, and J. Elia*, Bone Marrow Transplant Program, Div. Hematology/Oncology and *Dept. Microbiology & Immunology, University of Miami School of Medicine, Miami, FL 33101.

The efficacy of hematopoietic reconstitution using peripheral blood stem cells (PBSC) without bone marrow following marrow-ablative chemotherapy has not been clearly determined. Twelve patients with various cancers (non-Hodgkins lymphoma, Hodgkins lymphoma, breast cancer, seminoma) were given standard induction chemotherapy followed by G-CSF treatment (5ug/kg/day). Leukopheresis was initiated at WBC of 1.0 x 10^9/L and continued daily until a total of 1 x 10^9 mononuclear cells/kg were collected for transplant (mean phereses = 7.2 $\pm$ 2.4). CD34$^+$ cells were observed with values of up to 5% depending on the patient and day of leukopheresis. Peak CD34$^+$ cells were generally observed in early pheresis collections (days 1-4) and then decreased. Preliminary results indicated that most CD34$^+$ cells ($\geq$70%) were CD38$^+$ HLA-DR$^+$; however, limited proportions of less mature CD38$^-$ HLA-DR$^-$ cells were also evident. CD34$^+$ cells in the leukopheresis products did not correlate with the hematopoietic colony forming capacity (CFU-GM, CFU-GEMM, BFU-E) of the products. Eight of the above patients have been transplanted successfully with autologous peripheral blood progenitor cells without need for additional bone marrow. The median day to neutrophil recovery ($\geq$0.5 x 10^9/L) was 9 (range 7 to 12 days) and to platelet recovery ($\geq$20 x 10^9/L) was 8 (range 5 to 13 days). There was no correlation between rapid engraftment following transplant with either the total colony forming units (CFUs) or the total percentage of CD34$^+$ mononuclear cells. Although these results suggest that chemotherapy/G-CSF mobilized PBSC alone are sufficient for rapid, long term engraftment, factors other than colony forming capacity and proportions of CD34$^+$ cells are important in defining the engraftment capacity of leukopheresis products.

G 518 FACTORS AFFECTING THE RATE OF NEUTROPHIL AND PLATELET ENGRAFTMENT FOLLOWING AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION. Kenneth F Mangan, Thomas R Klumpp, Stuart L Goldberg, and John S Macdonald. Temple University Bone Marrow Transplantation Program, Philadelphia, PA 19140

Fifty two consecutive patients undergoing autologous peripheral blood stem cell infusion with (n=22) or without (n=30) concomitant autologous bone marrow infusion were retrospectively analyzed to determine the effect of various clinical and laboratory factors on the rate of engraftment. Patient diagnoses included breast cancer (n=24), non-Hodgkin lymphoma (n=20), myeloma (n=5), and Hodgkin's disease (n=3). High-dose conditioning regimens included cyclophosphamide(CTX)-etoposide-carboplatin(CEC) (n=22), CTX-etoposide-cisplatin(CEP) (n=13), CTX-total body irradiation (n=6), and other high-dose regimens (n=11). Thirty-five of the 52 patients (67%) received hematopoietic growth factor (HGF). Mobilization regimens were GM-CSF (n=20), CTX plus G-CSF (n=14), G-CSF alone (n=9), or no mobilization (n=9). For the entire group, the median time to neutrophil engraftment (defined as 500 polys/uL) was 12 days (range, 8-32 days), whereas the median time to platelet engraftment (defined as an unsupported platelet count of 20,000/uL) was 15 days. Multivariate regression analysis utilizing the Newton-Raphson algorithm in an accelerated time-failure model revealed that the primary factors independently associated with accelerated neutrophil engraftment included the administration of post-transplant HGF and the use of the G-CSF or CTX-G-CSF mobilization regimens. The factors independently associated with accelerated platelet engraftment included the use of the CTX-G-CSF mobilization regimen and the C.E.P. conditioning regimen.

G 519 G-CSF MOBILIZED PERIPHERAL BLOOD PROGENITOR CELLS (PSC) WITH HIGH-DOSE THERAPY FOR RESPONDING PATIENTS (PTs) WITH METASTATIC BREAST CANCER, E. Stadtmauer, D.Biggs, C. Sickles, P.Mangan, D. Magee, D. Frazier , J. Moore, G. Buzby and L. Silberstein, Bone Marrow Transplant Program, University of Pennsylvania Medical Center, Philadelphia, PA 19104

Between September 1991 and August 1993 24 women with responding metastatic breast cancer underwent G-CSF mobilized PSC harvest prior to PSC infusion and G-CSF augmented marrow recovery as the sole source of hematopoietic support after CTCb (cyclophosphamide 6 g/m^2, thiotepa 500 mg/m^2, and carboplatin 800 mg/m^2) . Pts age 32-58 (median 42) received G-CSF 10 ug/d sc and were in a clinical CR (4) or PR (20) after recent conventional dose chemotherapy. Pts had unfavorable characteristics including: most in PR (83%), 13 (54%) with bone involvement, and 19 with at least 2 prior chemotherapy courses. Thirteen pts were excluded from traditional bone marrow transplant (9 positive bone marrow, 2 pelvic irradiation, 2 hypocellular bone marrow biopsy). PSCs were collected via a double-lumen pheresis catheter by 10-15 liter leukaphereses daily or every other day from day 5 after start of G-CSF until a minimum 6 x 10^8 mononuclear cells/kg collected (6.2-11.8, median 9.2). A median 22.9 x 10^4 (range 8.8-75) CFU-GM/kg and and a median 8.79 x 10^6 (range 3.5-39.1) CD34+ cells/kg were harvested per pt. A median of 4 (3-9) collections were required to obtain these cells. 11 of these pts are evaluable for hematopoietic engraftment. One pt died day 17 of ARDS with peripheral granulocytes present. For the 10 evaluable pts all reestablished normal hematopoiesis with a median day to ANC>500/ul 11 (9-28) and platelet>20,000/ul 14 (10-37). No clear relationship to number of PSC, CFU-GM or CD-34+ cells infused was observed. Median hospital stay was 27 days (20-52). G-CSF alone stimulates adequate numbers of PSC for use with CTCb to result in reliable hematopoietic engraftment in pts with responding metastatic breast cancer without exposing pts to the added risk and time delay of chemotherapy mobilization.

G 520 IN VITRO EXPANSION OF PERIPHERAL STEM AND PROGENITOR CELLS. A. Svensson, M. Hansson and T. Söderström Karolinska Hospital, Dept Clin Immunology and Transfusion med., Immunhemotherapy Unit, 171 76 Stockholm, Sweden

Peripheral Stem Cells (PSC) may be used alone or in combination with bone marrow for Autologous Bone Marrow Transplantation (ABMT). Analysis of CD34 expression and colony assays (GM-CFC) are useful methods to perform quantitative and qualitative evaluation of the PSC harvest. Occasionally, a discrepancy may occur between the level of CD34+ cells and the GM-CFC growth in a given leukapheresis harvest. This may reflect a qualitative difference between different harvests and depend on whether the leukapheresis was performed "early" (before day12) or "late" (after day12) in mobilization period after chemotherapy ± G-CSF treatment. We have initiated *in vitro* long term cultures, using frozen PSC, from 2 different harvests from each patient in parallel with identical growth conditions (different combinations of SCF, GM-CSF, IL-3 and IL-6). Once a week the cultures were monitored by the following parameters: expansion of total cells, sub populations identified by moAb's to CD45, CD14, CD34, CD33, HLA-DR, CD45RA, CD3 and the GM-CFC (14-day) assay.

Our results demonstrate that both committed progenitors (GM-CFC) and the more immature stem cells (CD34+, CD33- or CD34+CD45RA-) can expand in these cultures. However, the level of expansion and the capacity to maintain hematopoietic progenitor cells over a long period *in vitro* (up to 6 weeks) differ between different harvests within the same individual.

G 521 Peripheral Blood Stem Cell Collection Analysis - Quality Control and Correlation with Clinical Outcome

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Over the past 18 months, the Ottawa Regional Autologous Bone Marrow Program has collected data with a view towards developing a system of quality control of bone marrow and peripheral blood harvest (PBSC) specimens. Twenty patients underwent peripheral blood and marrow harvesting in the past eighteen months (Non-Hodgkin's lymphoma n=15, Hodgkin's n=5). Each patient had stem cell collection done after chemotherapy (DHAP n=16 ; CHOP n=4) and G-CSF infusion with 3 PBSC collections done over days 7-10. Quality control of the stem cell and marrow collections included triple analyses of CD34/38/DR, methylcellulose cultures, and DNA rearrangements using Southern blotting techniques. Friedman's non-parametric ANOVA and Spearman's rank correlation test were used for statistical analysis. Methylcellulose cultures show growth of CFU's was optimal on day 8 of G-CSF infusion ($p<.0001$). Flow cytometry indicated the best increment of $CD34^+/38^+$ and $CD34^+/lin$ negative cells on day 8 and CD34 +/DR+ on day 9. CD34+/38+ count correlated best with CFU-GM's, CFU-mega and to days to patient recovery of ANC≥500. CD34+DR+ correlated best with CFU-GM's and to days of patient recovery to 20 x 10^9/l platelets and numbers of transfused packed red cells but not to CFU-mega, although this is only a trend and more patients will be required to confirm this.

In conclusion, it would appear that triple analysis CD34/38/ DR is the best predictor of clinical outcome and should become part of quality control analysis.

G 522 CYCLOPHOSPHAMIDE AND G-CSF MOBILIZED PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) TO SUPPORT INTERMEDIATE DOSE IFOSFAMIDE (I), CARBOPLATIN (C), ETOPOSIDE (E), AND DEXAMETHASONE (SPICE) INDUCTION THERAPY IN RELAPSED LYMPHOMA: TIMING OF PROGENITOR CELL COLLECTION. C Wheeler, J Axelrod, C Sieff, LN Shulman, A Elias, WH Churchill, K Antman, L Ayash, R Mazanet, I Tepler, L Schnipper, S. Wegner, G Schwartz, JH Antin. Harvard Medical School, Boston, Mass. 330 Brookline Ave, Boston, Mass 02215

We employ PBPC collected after cyclophosphamide, 4 grams/m2 in four divided doses over 12 hours with MESNA followed by G-CSF, 5 mcg/m2 sq daily through pheresis. Collections begin when total WBC reaches 1000 (day 1) and continues (excluding weekends), for six consecutive phereses (i.e. through day 8). CFU-GM and CD 34 were measured on each pheresis product. Median CFU-GM and CD 34 for the first six patients are:

Collection	CFU-GM$x10^4$/kg (median)	CD $34x10^6$/kg
1	0.3 (.07-7)	3.0(1-4.2)
2	0.7 (.7-1.4)	7.6(2-14.6)
3	1.6 (.14-3.0)	11.6(1.9-25.2)
4	1.9 (.8-5.3)	10.5(5-89.9)
5	2.3 (1.3-3.2)	18.5(6-34)
6	2.5 (.9-4.4)	21.1(1.9-30)

Pts received I(10 g/2) C(400 mg/m2) and E(600 mg/m2) supported by two collections (early and late) of PBPC/cycle 48-72 hrs after chemo, and G-CSF. ANC>500 15 d from start of chemotherapy (12-18), plt>100,000 22 d (20-42). The data suggest that progenitors mobilized by cyclophosphamide/G-CSF continue to rise after WBC recovery, remain elevated up to eight days later, and are associated with hematopoetic recovery with an intermediate dose regimen.

G 523 MULTICOMPARTMENT, NUMERICAL COMPUTER MODEL OF THE PHARMACOKINETICS OF GENE MEDICINES Tamara A.S. Ledley and Fred D. Ledley, GeneMedicine, Inc., Houston, TX 77054; Departments of Cell Biology and Pediatrics, Baylor College of Medicine, Houston TX, 77030; Department of Space Physics and Astronomy, Rice University, Houston TX.

The development of gene therapies which can be administered like conventional medicines to have a finite and controlled duration of action within the body requires an understanding of the pharmacokinetics of both the administered gene and the expressed gene product. Various kinetic processes can affect the level and persistence of the gene product including the: i) distribution and fate of the DNA vector; ii) dynamics of DNA uptake and trafficking through cells; iii) half life of DNA in the cell; iv) rate of transcription; v) half life of mRNA; vi) rate of translation; vii) distribution and fate of the gene product. We have constructed a computer model to investigate how the kinetics of these processes can affect the observed level of the therapeutic product over time. The model has five compartments: MILIEU, ENDOSOME, CELL, RNA, and PRODUCT. Kinetic constants for the half life (first order clearance, Ke) of DNA in the MILIEU, ENDOSOME, and CELL; the Ke of mRNA; the Ke of PRODUCT; the time for endosomal transit of DNA; the time for post-translational processing of the gene product (i.e. secretion); and the rates of transcription and translation were varied and the level and persistence of the gene product was calculated. These studies demonstrate how first order kinetics results from the summation of complex kinetic processes and illustrate what levels of gene products might be obtained using gene therapies. The results emphasize that the half lives of DNA, RNA, and gene product have a major effect on the maximal level, total production, persistence, and observed half life of the therapeutic product and highlight molecular and cellular strategies for enhancing the therapeutic profile of gene medicines.

G 524 RECOVERY OF CYTOTOXIC FUNCTION OF MYELOID CELLS IN THE IMMMEDIATE PERIOD AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) Solveig G. Ericson, Cheryl Guyre, Pamela Ely, Michael W. Fanger, Letha E. Mills. Dartmouth Medical School, Lebanon, NH 03756

Recovery of myeloid cell function is critical in patients post bone marrow transplantation in order to avoid the complications of serious bacterial and fungal infections. Even with cytokine stimulation post-ABMT and the use of cytokine primed peripheral blood stem cell harvests, there is a 5-10 day period in patients post-BMT when the absolute neutrophil count (ANC) is less than 500/µl. We undertook to study if (1) the few circulating myeloid cells in the early post-transplant were functional, and (2) if the administration of exogenous rhG-CSF affected functional recovery of myeloid cells post-ABMT. Cytotoxic ability of myeloid cells post-ABMT was determined in 24 patients undergoing ABMT for the underlying diagnoses of: breast cancer (n=10), acute myeloid leukemia (AML; n=9), Hodgkin's Disease (HD; n=2), non-Hodgkin's lymphoma (NHL; n=1), small cell lung cancer (n=1), and ovarian cancer (n=1). The patients with AML received autologous marrow alone, the remainder of the patients received autologous marrow, rhG-CSF primed peripheral blood stem cells (PBSCs) and rhG-CSF post ABMT. At different points in the ABMT course antibody-dependent cellular cytotoxicity (ADCC) was determined at a single-cell level in a modified plaque assay (Connor *et al*, J. Immunol. 145:1483, 1990) using monolayers of ox erythrocyte (oxE) target cells. PMNs and monocytes were isolated from heparinized blood samples by using discontinuous density gradient centrifugation with Ficoll-Hypaque (densities of 1.077 and 1.119 g/dl). The percent plaque-forming cells (%PFC) was determined in wells containing rabbit anti-oxE antibody-sensitized oxE cell layers. We found that both PMN and monocyte ADCC function returned prior to the ANC≥ 500/µl, and that despite the varied underlying diagnosis and pretreatment therapies, the 24 patients showed very similar patterns of recovery of cytotoxic function, with a decreased or absent functional ability in the immediate post-ABMT period. From these studies it appeared that even with exogenous rhG-CSF and PBSC support, the patients have several days of absolute neutropenia and that the myeloid cells have decreased functional ability during this time period. We are now attempting to determine if these cells are residual cells post-BMT or if they are reinfused functionally immature myeloid cells from the autologous graft. One possible avenue to further decrease periods of absolute neutropenia post-BMT would be to infuse *ex vivo* expanded progenitors induced to granulocytic maturation.

G 525 IMMUNOPHENOTYPE OF REGENERATING NATURAL KILLER CELLS IN PERIPHERAL BLOOD AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION. Marianne Hokland, Hanne Jørgensen and Peter Hokland, Institute of Medical Microbiology and Department of Hematology, University of Aarhus, 8000 Aarhus, DK-Denmark.

Natural Killer cells (NK cells) are generally detectable in peripheral blood very shortly after autologous bone marrow transplantation (ABMT) and are in addition most often fully functional. In contrast, NK cells from a minority of few ABMT patients show a severely depressed cytotoxic capability several months after ABMT. By using three-colour immuno-flourescence and flow cytometry we wanted 1) to describe the expression of certain adhesion molecules and activation markers on the surface membrane of CD56+/CD3- NK cells during the first six weeks of bone marrow regeneration following ABMT, and 2) by comparing these results to the functional capacity of the NK cells to delineate a NK cell phenotype, which correlates with high NK cell activity. Our preliminary results indicate that post-ABMT NK cells differ among patients with regard to their expression of the IL-2 receptor (CD25), MHC class II antigen and the b2-integrin CD11c. Furthermore, it seems as though the patients with high expression of these antigens on their NK cells can be identified as those with highest *in vitro* NK cell activity. In contrast, no difference was found with regard to the expression of CD45RA, CD45RO, CD11a and CD2 on post-ABMT NK cells between groups of patients with high and low *in vitro* NK cell activity. Additional post-ABMT patients should be included in the hope that this study will provide a phenotypical description of the NK cells with high activity, thus making it possible to pin-point the patients which will benefit from immuno-therapy with NK/LAK stimulating agents and/or at which time point after ABMT that such therapy should optimally be commenced.

G 526 ACTIVATION OF THE CD28 PATHWAY UPREGULATES CYTOKINE MRNA EXPRESSION AND SECRETION IN T CELLS FROM ALLOGENEIC BONE MARROW TRANSPLANT RECIPIENTS, Lawrence G. Lum, Indira D. Joshi, Sandra Galoforo, Mark Wing, and Mitchell R. Smith, Departments of Medicine and Pediatrics, Wayne State University, Detroit, MI 48201.

Immunodeficiency in bone marrow transplant (BMT) recipients limits the success of BMT due to fatal infections. Binding of anti-CD28 monoclonal antibody (anti-CD28, 9.3) to CD28 antigen, a 44 kDa glycoprotein expressed as a homodimer on T cells, enhances anti-CD3 monoclonal antibody (anti-CD3, OKT3)-induced cytokine mRNA expression and secretion in normal T cells. In an earlier study, peripheral blood mononuclear cells (PBMC) from short and long-term autologous and allogeneic BMT recipients were tested for the effects of CD28 pathway activation. Depressed anti-CD3 induced DNA synthesis in PBMC from a number of autologous BMT recipients could be partially restored by coactivation of the CD28 pathway (Lum et al, in press). In this study, we examined in detail the effects of anti-CD28 coactivation on the functions of anti-CD3 activated T cells from allogeneic BMT recipients who received HLA-identical marrow grafts: a healthy short-term recipient (3 mos after BMT), a recipient with chronic graft-vs-host disease (6 mos after BMT), and a healthy long-term recipient of an unrelated marrow graft (1 yr after BMT). We examined the effects of coactivation on RNA and DNA synthesis, mRNA expression for IL-2, IL-$2r_\alpha$, and GM-CSF, and secretion of IL-2 and GM-CSF. Coactivation of T cells increased anti-CD3-induced DNA and RNA synthesis and mRNA expression for IL-2 and IL-$2r_\alpha$ in all 3 recipients. Coactivation increased mRNA for GM-CSF in 1 of 3 recipients, IL-2 secretion in 2 of 3 recipients, and GM-CSF secretion in 1 of 3 recipients. Coactivation of recipient T cells may be a useful immuno-modulatory strategy to accelerate immunohematopoietic recovery after BMT.

G 527 HEMATOPOIETIC, LYMPHOPOIETIC AND IMMUNO-THERAPEUTIC PROPERTIES OF RECOMBINANT HUMAN INTERLEUKIN-7 FOLLOWING SPLIT DOSE POLYCHEMOTHERAPY. Greg Perry[2], Linda Kelsey[2], Connie Faltynek[3], John Jackson[2] and James E. Talmadge[2], Departments of [1]Pathology/Microbiology, [2]Cell Biology/Anatomy, University of Nebraska Medical Center, Omaha, NE 68198 and [3]Sterling Winthrop, Collegeville, PA.

The activity of recombinant human interleukin-7 (r Hu IL-7) for hematopoietic and lymphopoietic reconstitution was examined following the administration of a maximum tolerated, split dose, polychemotherapy protocol of cyclophosphamide (60 mg/kg; qdx3), cisplatinum (2.05 mg/kg; qdx3), and BCNU (20 mg/kg; 1x on the third day) by phenotypic and functional analysis. These studies focused on subpopulations in the blood, thymus, bone marrow (BM) and spleen. The injection of r Hu IL-7 (1 ug/A/day bid for 20 days) was initiated 24 hours following split dose polychemotherapy introduced a significant margination of stem cells (CFU-GM and HPP) to the spleen and a reduction in BM stem cell activity. R Hu IL-7 also induced a significant increase in the T cell mitogenic response (Con-A) of cells in the bone marrow and thymus, but not the spleen. FACS analysis revealed an accelerated reconstitution of $CD\text{-}4^+$ and $CD\text{-}8^+$ cells in the thymus and a significant increase in B220 cells in the BM and spleen. R Hu IL-7 also had significant therapeutic activity for moderate to large metastatic mammary tumor burdens when used in combination with the polychemotherapy protocol. In these studies tumor bearing mice received a BM transplant (1×10^6 syngeneic cells) and r Hu IL-7 therapy was initiated 24 hrs following completion of polychemotherapy. The greatest therapeutic activity was observed in mice with moderate tumor burdens where r Hu IL-7 therapy was initiated on day nine following the iv injection of 100,000 tumor cells from the clone 66 mammary tumor cell line. In these studies 90% of mice were cured (verses a 40% cure with chemotherapy and BMT alone). Significant, but lesser therapeutic activity was observed in animals treated with r Hu IL-7 beginning on day 13 following the iv injection of a similar number of clone 66 tumor cells. Preliminary studies suggest that mice with heavy tumor burdens needed a higher dose of r Hu IL-7 (optimal dose of 10 ug/A/day) compared to mice (1 ug/A/day) with moderate tumor burdens). We conclude that r Hu IL-7 has significant hematopoietic mobilization properties and immunorestorative and therapeutic activity following aggressive chemotherapy. Research supported in part by the Nebraska Cancer & Smoking Disease Research Program.

G 528 LAK ACTIVITY FROM THE SPLEEN OF CANCER PATIENTS. D.N.Rangel Pestana*, A.B. Pereira Lima*, R.P.Falcão*, J.C. Voltarelli*
Dept. of Clinical Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, BRAZIL.

LAK cells are generated from peripheral blood lymphocytes incubated with large doses of recombinant interleukin-2 (IL-2). They have been used in several clinical trials as adoptive immunotherapy for refractory solid or lymphohematopoietic cancer. LAK cells can also be generated from bone marrow or various lymphoid organs and we have previously demonstrated comparable activies in the peripheral blood and spleen from normal donors (J Biol Response Mod 9: 103-107, 1990). In this study we generated LAK activity from splenic cell suspensions of 9 patients with cancer (5 solid tumors, 4 lymphomas) and 8 controls splenectomized for non-immunological causes. Splenocytes were incubated with 1,500 U/ml of recombinant IL-2 for 7 days and tested in a chromium release assay against K562, Jurkat and P815 cell lines. Cytotoxic activity was similar in the patient and control groups for K562 (43 vs. 45%, median), P815 (27 vs. 19%) and Jurkat (47 vs. 31%). There was a positive correlation between cytotoxicity and the expression of the p75 chain of IL-2 receptor, but not of the p55 chain (CD25). These results demonstrate the immunocompetence of splenocytes from cancer patients to generate LAK function. Thus, spleen cells could be used for autologous adoptive immunotherapy, as previously accomplished in one case of unresectable hepatoma (Cancer 58: 1001, 1986).

Late Abstracts

VIRUS AND HOST FACTORS INFLUENCE THE OUTCOME OF CMV INFECTION FOLLOWING BMT

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In spite of new developments in the diagnosis and therapy of CMV infection CMV disease is still affected by a high mortality in patients after BMT. But only a minority of patients with CMV infection after BMT develop CMV disease. Different detection methods were assessed for their positive and negative predictive values for the development of CMV disease in patients with CMV infection after BMT.

PCR technique was found to provide an optimal sensitivity and negative predictive value, but the positive predictive value for the onset of CMV disease was only 61%. Unfortunately positive culture from urine samples or throat washings (64%) as well as from blood samples (69%) did not provide significantly higher positive predictive values.

Thus, other parameters were looked for which might influence the onset of CMV disease in a patient with culture-proven CMV infection. The immune reconstitution was found to have a major impact on the development of CMV disease in patients after allogeneic BMT. A significant drop in the lymphocyte count, especially in the CD4+ T-cell count, was found to be significantly associated with the onset of CMV disease. Apart from host-derived also virus-derived factors were found to influence the virus-host interaction. By amplifying specific functionally relevant regions of the CMV genome, the association of various virus strains and the clinical course of the virus infection were analysed. An association of mutations in the major transactivating domain of the immediate early gene and the development of CMV disease could be demonstrated.

LEUKEMIA/LYMPHOMA (LL) CELL ADHERENCE TO MARROW STROMAL CELL IS BIPHASIC: VLA-4 MEDIATES ONLY THE EARLY PHASE OF LL/MSC INTERACTION, H.S. Juneja, C. Patrick, Jr., S. Lee, F.C. Schmalsteig, and L.V. McIntire. Dept Int Medicine, Univ of Texas Medical School, Houston, TX, Cox Laboratory for Biomedical Engineering, Rice Univ, Houston, TX and Dept of Pediatrics, UTMB at Galveston, TX.

Adhesion between human T and B-lymphoblastic cell lines and marrow stromal cells (MSC) is partially mediated by VLA-4 on the LL cells and its ligand VCAM-1 on the MSC (Exp. Hematol. 21:444, 1993). A temporal study on the heterotypic adherence between a human B-lymphoblastic cell line (UTMB-460) (Leuk. Res. 10:1209, 1986) and MSC monolayers grown in the absence of hydrocortisone was done by using ^{51}Cr-labelled UTMB-460 cells or by utilizing a Parallel Flow Detachment Assay (PFDA) (Blood 75:227, 1990). A liquid shear stress of 14 dynes/cm^2 was used to dislodge the UTMB-460 cells at variable time periods. Under no-flow conditions heterotypic adhesion between UTMB-460 and MSC monolayers occurs very rapidly ($\leq$1 minute) after the two cells come into contact. Percent (mean±SD; n=4 to 19) UTMB-460 cells adherent to MSC at 1, 3, 5, 15, 30, and 60 min is 26 ± 4, 43 ± 3, 37 ± 2, 47 ± 4, 62 ± 2 and 52 ± 3% respectively. No further increase occurs between 1 and 4 hrs. Percent of UTMB-460 cells adhering to MSC at 30-60 min. is significantly different from percent adherence at 1-15 min (59 ± 2% vs. 41± 2%; n=59 & 29, p<.0005). MoAb HP2/1 (anti-CD49d) inhibits UTMB-460 cell/MSC adherence in the early (1-15 min, n=40) and late phase (30-60 min, n=24) by 90% and 60% respectively (p<.0005). In contrast, the percentage of UTMB-460 cells whose adherence to MSC is unaffected by anti-CD49d increases from 10% in the early phase to 40% at 30-60 min. These data indicate that adherence of LL cells to MSC is biphasic. The early phase of LL cell/MSC interaction is mediated entirely by VLA-4. The late phase of this interaction does not involve the ß2 integrins (ICAM-1 & LFA-1), CD44 or LAM-1.

HLA-DP MATCHING IN BONE MARROW TRANSPLANTATION, H. Schmidt, M. Bitzer, S. Fenchel, G. Ehninger, G. Pawelec and C.A. Müller, Med. Universitätsklinik u. Poliklinik, Abt. II, D72076 Tübingen, Germany

Long lasting remissions can be achieved in leukemia as well as in severe aplastic anemia by allogenic bone marrow transplantation (BMT). One of the main problems after BMT remains severe acute graft versus host disease (GvHD) (> grade II) occurring in 30 % of the patients after BMT with marrow from HLA identical siblings and in up to 50 % after BMT with marrow from unrelated donors or not completely HLA-identical family donors. Besides intensification of the immunosuppressive therapy during BMT improving HLA matching of donor and recipient might diminish the incidence of GvHD. In a retrospective study we examined HLA-DP matching of donor and recipient in allogeneic bone marrow transplantation. The HLA-DPA and HLA-DPB genotype of 91 patients (44 with CML, 6 with SAA, 38 with acute leukemia, 1 with myelodysplasia and 2 with lymphoma) and their bone marrow donors were determined by oligotyping. 62 times donor and recipient were HLA identical siblings, in 7 transplants the donor was a not completely HLA-A,-B,-DR matched relative and in 29 transplants a HLA identical unrelated person. In 10 of the 62 (16 %) HLA-A,-B,-C,-DR identical siblings differences in HLA-DP genotype could be detected (once only HLA-DPA, 3 x HLA-DPB and 6 x in both chains). In the other pairs HLA-DP differences were detected in 4 out of 6 (66.7 %) when transplants with not completely HLA-A,-B,-DR matched relatives were done and in 20 out 29 (68 %) when matched unrelated donors were used. Mixed lymphocyte cultures had been performed in all patients. Only when considering a GvHD index < 0.1 % HLA-A,-B,-DR and -DP matched donor recipient pairs could be clearly differentiated from pairs with an HLA-DP mismatch. Preliminary results indicate that patients with completely matched family or even unrelated donors might suffer from less severe GvHD. Therefore it seems advisable to do HLA-DP geno typing especially since this difference could not be detected by mixed lymphocyte culture.

A SERIES OF MATCHED UNRELATED DONOR (MUD) BONE MARROW TRANSPLANTS (BMTs) AT A SINGLE PAEDIATRIC INSTITUTION. P. Veys, S. Calderwood, J. Doyle, M. Freedman, F. Saunders. Department of Haematology and Oncology, The Hospital for Sick Children (HSC), Toronto, Canada.

Between 1990 and 1993, 17 MUD BMTs have been performed at HSC. In all, 108 searches were initiated and 26 (24%) donors were found who were HLA antigen 6/6 serological, and class II molecular matches. Average time from search initiation to BMT was 5.5 months and the chance of finding a matched donor increased from 9% during the period 1988-1990 to 31% between 1991-93. The average age of the patients was 4.2 years (range 11 mo - 13 years) and diagnoses included SCID (3), osteopetrosis (2), MPS type II (1), WAS (2), SAA (3), relapsed ALL (3), JCML (1), RAEB (1), congenital amegakaryocytic thrombocytopenia (1). Engraftment was achieved in 14/17 patients with median time to reach ANC=0.5 of 21 days, ANC=1.0 of 26 days and platelets ≥ 30 of 23 days. 7/9 patients receiving BU/CY, 3/4 receiving CY/TBI, and 3/3 receiving VP-16/TBI conditioning engrafted fully. 2 patients, one with osteopetrosis the other with MPS type II, failed to sustain engraftment with BU/CY; a further patient with SAA failed to engraft following CY/TBI. Event-free survival with a median follow-up of 12 months was 71%. Three patients have died, cause including: graft failure (1), pneumonitis (1), and relapse (1). Reversible toxicity included mucositis (4), renal failure (6), VOD (1). In all transplants, the marrow was non T-cell depleted, and GvHD prophylaxis consisted of CyA + MTX (14) and CyA + Pred (SCID x3). Acute GvHD occurred in 11/17 patients. Grade I (2), Grade II (5), Grade III (3), Grade IV (1). Chronic GvHD occurred in 4 patients and has resolved in 2.

In conclusion, non-T-cell depleted MUD BMT is a feasible therapeutic option in 1/3 children at HSC with favourable outcome and acceptable toxicity. Successful engraftment can be achieved with BU/CY conditioning, avoiding TBI in this patient group.