BONE MARROW TRANSPLANTATION

Organizers: Robert Peter Gale and Richard E. Champlin

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Poster Sessions

Tolerance and Immune Modulation

D 001 NEW APPROACHES TO TRANSPLANTATION TOLERANCE, David H. Sachs[1], David M. Bodine[2], Arthur W. Nienhuis[2], Christian LeGuern[1], and Megan Sykes[1]. [1]Massachusetts General Hospital/Harvard Medical School, Boston and [2]NHLBI, NIH, Bethesda, MD.

Induction of bone marrow chimerism is a reliable means of inducing lasting donor-specific tolerance across major histocompatibility (MHC) barriers while avoiding the need for chronic immunosuppressive drugs. Mixed chimerism is preferable to full allogeneic chimerism when completely MHC-mismatched BMT is performed, as host bone marrow cells (BMC) provide a continuous source of antigen-presenting cells (APC) which are needed to maintain normal immunocompetence. However, the use of lethal irradiation prior to BMT is associated with marked toxicity and other complications. We have therefore developed rodent models utilizing relatively non-toxic conditioning regimens to permit the induction of mixed chimerism across allogeneic and xenogeneic barriers. The allogeneic model involves recipient pre-treatment with anti-CD4 and anti-CD8 mAbs followed by a low dose (3 Gy) of whole body irradiation (WBI) and 7 Gy of local thymic irradiation prior to BMT. This regimen permits the induction of mixed chimerism and skin graft tolerance across MHC barriers in mice [1], but is insufficient to permit BMC engraftment in the xenogeneic rat→mouse combination. However, the addition of anti-Thy1.2 and anti-NK1 mAbs to the regimen induced mixed xenogeneic chimerism and donor-specific skin graft tolerance in this xenogeneic combination [2].

A recent approach which uses BMT for the induction of donor-specific tolerance involves transplantation of autologous BMC which have been modified so as to express alloantigens. This approach minimizes the risks of graft-versus-host disease (GVHD) and of engraftment failure associated with allogeneic BMT. We have utilized retroviral gene transduction for this purpose. B10.AKM mice were pre-treated with anti-CD8 mAb, received a lethal dose of WBI, and were then reconstituted with autologous BMC which had been transduced with a recombinant retrovirus containing the K^b gene linked to a B19 parvovirus promoter and a neomycin resistance gene. Twelve weeks post-BMT, quantitative PCR was used to demonstrate that proviral sequences were present in 5-30% of PBL in all recipients. These animals demonstrated specific prolongation of B10.MBR skin grafts, which express K^b. These grafts survived 90 to 130 days, while third party class I disparate B10.BR skin grafts were rejected within 20 to 40 days. A similar approach is now being tested in our pre-clinical miniature swine model.

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D 002 NEW APPROACHES TO TOLERANCE, Samuel Strober, Varghese Palathumpat, Tamotsu Niki, Ingo Schmidt-Wolf, and Sussan Dejbakhsh-Jones, Stanford University, Stanford.

Cloned lines of murine CD4$^-$ CD8$^-$ $\alpha\beta^+$ T cells, as well as CD4$^-$ CD8$^-$ $\alpha\beta^+$ T cells in the fresh bone marrow appear to play a critical role in regulating graft versus host disease (GVHD), allogeneic bone marrow engraftment, and immune tolerance. In the case of allogeneic bone marrow transplantation, co-injection of enriched or highly purified donor CD4$^-$ CD8$^-$ $\alpha\beta^+$ cells blocks acute lethal GVHD induced by typical donor CD4$^+$ and CD8$^+$ T cells in sublethally irradiated (400 rads, whole body irradiation) H-2 incompatible recipients. The enriched donor CD4$^-$ CD8$^-$ $\alpha\beta^+$ T cells also promote the engraftment of donor marrow cells which ordinarily would be rejected by the recipients. The chimeric recipients are tolerant of donor tissue, and will reject third-party, but not donor skin grafts.

The cloned lines and fresh marrow CD4$^-$ CD8$^-$ $\alpha\beta^+$ cells show a limited diversity of the β chain genes of the TcR which preferentially use the Vβ15 and Vβ7 genes associated with the Jβ2.6 segment. These receptors recognize antigens on the surface of autologous T and B cells. The cloned lines of CD4$^-$ CD8$^-$ $\alpha\beta^+$ cells secrete a 20 Kd immunosuppressive cytokine with a unique N-terminal amino acid sequence. The latter cytokine inhibits IL-2 secretion in the mixed leukocyte reaction (MLR). Thus, the CD4$^-$ CD8$^-$ $\alpha\beta^+$ cells may promote tolerance induction after allogeneic marrow transplantation by regulation of IL-2 secretion by both host and donor immunocompetent cells.

D 003 MIXED AUTOLOGOUS/ALLOGENEIC BONE MARROW TRANSPLANTATION FOR THE INHIBITION OF GVHD. Megan Sykes, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02129.

Prevention of graft-versus-host disease (GVHD) by T cell-depletion of allogeneic marrow is associated with an increased incidence of failure of alloengraftment and increased leukemic relapse rates. Increased relapse rates have also been observed in patients receiving potent immunosuppressive therapy following bone marrow transplantation (BMT). New approaches to avoiding GVHD while preserving the beneficial effects of allogeneic T cells are therefore needed. The addition of T cell-depleted (TCD) autologous bone marrow cells (BMC) to the inoculum can delay GVHD mortality in murine allogeneic BMT[1]. This protective effect can be augmented by instituting an eight day delay between administration of irradiation plus TCD syngeneic BMC and the time of allogeneic BMT[2]. Complete allogeneic lymphoid reconstitution is achieved in such animals.

In an attempt to identify the element in syngeneic BMC which confers GVHD protection, we expanded host-type BMC in vitro and co-administered these cultured cell populations with allogeneic GVHD-producing inocula to lethally irradiated recipients. Culture of BMC in IL-2 generated CD3$^+$, NK1$^+$, CD4$^-$, CD8$^-$ cell lines with the capacity to delay GVHD mortality[3]. However, much greater and more lasting GVHD protection could be produced by administering high doses of IL-2 along with TCD syngeneic BMC at the time of allogeneic BMT. While treatment with IL-2 alone decreases both acute and chronic GVHD mortality, maximal early protection is observed when TCD syngeneic BMC are co-administered[4]. Surprisingly, NK1$^+$ precursor or effector cells do not appear to be required for this protective effect. All surviving animals demonstrate allogeneic lymphohematopoietic reconstitution. Timing of IL-2 administration is critical in determining whether or not protection is observed; IL-2 is protective if begun on the day of allogeneic BMT, but accelerates GVHD if begun instead on day 7 following BMT.

In addition to permitting normal alloengraftment, GVHD prophylaxis with IL-2 plus TCD syngeneic BMC preserved the graft-vs-leukemia (GVL) effect of allogeneic T cells against the host-type leukemia/lymphoma EL4[5]. Allogeneic CD8$^+$ T cells are the sole mediators of this GVL effect. The mechanism of IL-2-induced suppression of GVHD appears to involve inhibition of donor CD4 T cell activity, and not inhibition of CD8 cells, thus explaining the preservation of GVL against EL4. The mechanism of this CD4 inactivation and of the additive protective role played by TCD syngeneic BMC is currently being investigated.

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Immunology

D 004 ADVANCES IN GVHD: CYTOKINE DYSREGULATION AND NOVEL LYMPHOCYTE SUBSETS

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Graft versus host disease (GVHD) is a complex immunopathologic process with at least two phases. During the first or afferent phase, mature T lymphocytes in the donor bone marrow recognize recipient alloantigens. Elimination of all mature T cells from the bone marrow decreases clinical GVHD, but rates of graft rejection and leukemic relapse have increased; more precise understanding of the pathophysiologic mechanisms of GVHD is therefore required. Recent research in murine models has focussed on subclasses of donor T cells bearing specific T cell receptor-beta (TCR-beta) chains. Phenotypic analyses of T cells expanding early during GVHD have shown a predominance of certain TCR-beta families. This suggests that elimination of some, but not all of the TCR-beta families could reduce GVHD. This prediction is supported by studies with lymphocytes from TCR-beta transgenic mice, in which endogenous beta and gamma TCR gene rearrangements are restricted. Lymphocytes from these mice fail to initiate GVHD, despite their ability to recognize alloantigens *in vitro*.

The second or efferent phase of GVHD consists of dysregulated cytokine secretion, the subsequent recruitment of secondary effector cells and target organ destruction. The nature of effector cells during the efferent phase of GVHD has been investigated in a murine BMT model to minor histocompatibilty antigens (B10.BR -> CBA). Examination of the skin by two color immunofluorescence has revealed an effector lymphocyte of donor origin with a novel phenotype: CD4+CD8-CD3-. Laser cytometry demonstrates that these cells are negative for cytoplasmic and cell surface CD3. Isolation and transfer of these cells to secondary recipients reproduce GVHD epidermal damage, implicating them as effector cells in GVHD of the skin.

Dysregulated cyokine secretion is of pivotal importance in GVHD pathophysiology. Current studies of inflammatory cytokines active during GVHD have focused on Interleukin 1 (IL 1). In the (B10.BR -> CBA) murine model, both IL 1 and tumor necrosis factor have been demonstrated in the skin of mice with GVHD using PCR. The systemic administration of an IL 1 receptor antagonist during the first ten days after transplant reduced the mortality and the immunosuppression of GVHD. IL 1 is also an important hematopoietic growth factor, but engraftment of donor bone marrow was not impeded by use of the receptor antagonist; in fact, hematopoietic stem cells were spared from GVHD damage. IL 1 is thus a critical mediator of the systemic inflammation of GVHD and the data focus attention on the role of monokines during this process. An IL 1 receptor antagonist offers new opportunities to understand and to control GVHD.

D 005 LYMPHOCYTES FUNCTIONS AND ONTOGENY IN GENE-TARGETTED MUTANT MICE

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T lymphocytes recognize their antigen peptides and Major Histocompatibility Complex products with the use of their T cell antigen receptors (TcR). In addition to the α and β chains of TcR, the interaction between T cells and their target cells or antigen presenting cells is also assisted by a series of other cell surface polypeptides. Most notable of these are CD4 and CD8, which are selectively expressed on mature helper/inducer and killer/suppressor T cells, respectively. Upon engagement of their ligands, a series signals are being transduced intracytoplasmically via some of these molecules and their associated proteins. Perhaps the most important enzyme in this signal transduction process is the lymphocytes specific tyrosine kinase lck. In an attempt to gain better understanding on the roles of these molecules in T lymphocyte functions and ontogeny, we generated a series of mutant mice with disruptions in these genes. These mutant mice are being analysed in order that we can evaluate the importances of these genes in T cell development.

In addition to studying development, the roles of these molecules in autoimmune diseases, transplant rejection and tumor injection can also be analysed in the appropriate experimental mouse strains carrying mutations of these genes.

D 006 HOW DO B CELLS DEVELOP?

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The B cell system in humans and mice develops as a result of two major waves of cellular proliferation and selection. A primary genesis of B cells occurs continuously from stem cells in the bone marrow. In vivo immunolabeling reveals many early B lineage precursors in microenvironments near the surrounding bone, adhering to resident stromal cells which provide obligatory stimuli for B cell development. In subsequent stages of differentiation, pro-B cells (pre-pre-B cells) transiently express nuclear terminal deoxynucleotidyl transferase (TdT) during the rearrangement of IgM heavy chain genes and develop B lineage-associated cell surface antigens before expressing cytoplasmic μ chains to become pre-B cells; these finally rearrange light chain genes and divide to give rise to B cells bearing surface IgM molecules. Studies of cell population dynamics in mice reveal a series of mitoses and an expansion of cell production during successive stages of B cell genesis. This is accompanied by considerable cell loss. Many precursor B cells with defective gene rearrangements or autoreactive specificities appear to undergo apoptotic cell death and are eliminated by resident macrophages, selecting out dysregulated or unacceptable B cell clones. In systemic graft-versus-host disease, B cell genesis in the bone marrow is ablated from the earliest detectable pro-B cells onwards. B cell development is regulated by both local and systemic factors. Cell adhesion molecules (including VLA4, VCAM1, CD44 and hyaluronate) maintain precursor B cells in close association with stromal cells which release short range cytokines. Some stromal cell products are localized solely at the interface with B precursor cells, forming restricted molecular microenvironments. Interleukin (IL)-7, a cytokine produced by B cell-supportive stromal cells, stimulates proliferation of early precursor B cells both in vitro and when administered systemically in recombinant form in vivo. Products of macrophage activation in vivo also elevate pro-B cell proliferation and, if prolonged, may predispose to dysregulation of B cell clones. Terminal maturation of newly-formed B cells is hastened by inflammatory mediators (IL-1, IL-4, IFN). Newly formed virgin B lymphocytes migrate to the spleen, lymph nodes and mucosal lymphoid tissues where most die after a pre-programmed short lifespan. Activation of virgin B cell clones by antigen, however, initiates the second wave of proliferation and differentiation to form antibody-producing cells and B memory cells. Germinal centers, arising from oligoclonal B cell proliferation, are sites of isotype switching, hypermutation of immunoglobulin genes, massive cell loss selecting for enhanced antigen-binding affinities, and the production of memory B cells; these can exhibit a long life span in the continuing presence of antigen and they recirculate repeatedly between lymphoid organs and the blood. Memory B small lymphocytes traffic through the bone marrow, forming a small fraction of the intramyeloid B lineage cells. Some memory B cells activated by antigens in the spleen also migrate to the bone marrow which becomes the major site of long-lived plasma cells. Bone marrow inocula can thus contain some secondary B cells capable of adoptively transferring some antibody production and responsiveness to recall antigens. In addition, however, bone marrow transplantation offers opportunities to define the reconstitution of de novo genesis of B cells in recipient bone marrow and to assess regulatory factors which may therapeutically modify this process.

D 007 THE ROLE OF NATURAL KILLER (NK) CELLS IN HEMATOPOIETIC ENGRAFTMENT AND IMMUNE RECONSTITUTION FOLLOWING BONE MARROW TRANSPLANTATION. Craig W. Reynolds[1], Pierre Tiberghien[2], William J. Murphy[1] and Dan L. Longo[1]. [1]Biological Response Modifiers Program, NCI-FCRDC, Frederick, MD 21702 and [2]Hospital Jean Minjoz, Besancon, France.

Natural Killer (NK) cells are reported to have an important role in the resistance of recipients to bone marrow transplantation (BMT). However, these same cells have also been shown to produce a variety of cytokines which could enhance the engraftment and growth of hematopoietic stem cells, as well as the functional activity of newly produced effector cells. The present series of studies in rats and mice were undertaken to examine these potential positive and negative regulatory roles for NK cells following syngeneic and allogeneic BMT. Consistent with a negative regulatory role for NK cells, isolated large granular lymphocytes (LGL), with high levels of NK activity, were shown to effectively lyse allogeneic but not syngeneic stem cell-enriched BM preparations. Depletion of NK activity in recipients receiving allogenic BMT resulted in: (1) an increase in spleen colony-forming units (CFU-s) and extramedullary hematopoiesis; (2) an acceleration in the appearance of donor-derived cells, especially platelets, red blood cells and granulocytes; and (3) a reduction in the number of transfused BM cells needed for survival. This enhanced survival was independent of the recipient's genetic susceptibility to transplant using allogeneic Balb/C BM. Furthermore, the addition of activated NK cells to in vitro BM cultures, in the presence of exogenous cytokines, resulted in the inhibition of hematopoietic colony formation. Antibody to IFN-γ partially reversed this inhibitory effect, suggesting a role for this cytokine in the negative regulation of BM stem cells. In contrast to the negative regulatory effects of NK cells, we also observed in vitro that, in the absence of added hematopoietic growth factors, activated NK cells could support the hematopoietic growth of syngeneic BM stem cells. Furthermore, the addition of syngeneic NK cells to the BM used for reconstitution of lethally irradiated hosts resulted in greater hematopoietic engraftment. These data are consistent with both a positive and negative regulatory role for NK cells in hematopoiesis. Although NK cells are most commonly associated with a role in the rejection of allogeneic BMT, the data here suggest that the transfer of activated NK cells, syngeneic with the donor BM cells, could provide a significant positive effect on hematopoiesis and subsequent immune reconstitution following BMT.

Hematopoiesis

D 008 IN VIVO INFUSIONS OF CYTOKINES IN MURINE RECIPIENTS OF T-CELL DEPLETED BONE MARROW ALLOGRAFTS, Bruce R. Blazar[1], Patricia Taylor[1], Daniel A. Vallera[2], Departments of [1]Pediatrics and [2]Therapeutic Radiology, University of Minnesota, Mpls., MN 55455

Our laboratory has tested the effect of the in vivo administration of recombinant cytokines on survival, hematopoiesis, and chimerism in the context of irradiated murine C57BL/6 (H-2^b) recipients of T-cell depleted fully allogeneic BALB/c (H-2^d) bone marrow grafts. In previous studies, we have observed that a 14 day continuous subcutaneous administration of granulocyte/macrophage colony-stimulating factor (GM-CSF) at 1 μg/day significantly increased survival, resulted in a variable effect on hematopoietic recovery, and significantly increased the predilection toward recovery of host cells. To further our understanding of these data, we have separately studied 2 lineage-restricted cytokines, granulocyte colony-stimulating factor (G-CSF) and macrophage colony-stimulating factor (M-CSF). G-CSF (1 μg/day) significantly improved survival, accelerated granulocyte recovery, and had no impact on engraftment. M-CSF (20 μg/day) had no impact on survival, decreased leukocyte and erythroid recovery, and decreased alloengraftment. Taken together, these data suggest that cytokine induced stimulation of granulocytes is associated with improved survival, while stimulation of monocytes is associated with a propensity toward host cell repopulation. In order to evaluate the effects of a cytokine which can also stimulate earlier prgenitor cells, we have tested interleukin-1 (IL-1) alone and in combination with GM-CSF as a means of stimulating early highly proliferative colony forming cells. Recipients of IL-1 had significantly improved survival, a striking neutrophilia (24-fold), and significantly higher levels of donor engraftment. Recipients of IL-1 (1 μg/day) had a higher proportion of day 7 splenic-localized donor granulocytes and a 7-fold increase in day 11 splenic-localized donor T-cells, without changes in the number of host T-cells. Despite the donor T-cell expansion, graft-versus-host disease did not occur suggesting that donor T-cells had been educated in the thymus. IL-1 (0.3 μg/day) was synergistic in combination with GM-CSF (0.3 μg/day) for acceleration of leukocyte recovery and promotion of alloengraftment. Sequential adminstration of IL-1 followed by GM-CSF was not beneficial. Syngeneic experiments revealed that IL-1 administration could improve the survival of recipient's limited numbers of bone marrow cells. Secondary transfer experiments using marrow from controls of IL-1 treated recipients showed that IL-1 did not alter the number of day 12 CFU-S cells suggesting that IL-1 stimulated more committed progenitor cells. These data demonstrate the potent hematopoietic and engraftment promoting effects of IL-1 which is most evident in stimulation of donor committed myeloid lineage and T-lineage populations. In addition to these studies, a review of the current status and future directions of cytokine studies in irradiated mice and in irradiated and non-irradiated recipients of allogeneic or syngeneic bone marrow will be presented.

Concepts Behind Transplants

D 009 HOW DO TRANSPLANTS CURE CANCER. R.P. Gale, UCLA School of Medicine, Los Angeles, CA.

Bone marrow transplants are frequently used to treat cancer. The underlying notion is that the intensive pretransplant chemotherapy and/or radiation will be more effective than conventional chemotherapy in eradicating cancer. Here I consider three issues central to this approach: (1) is necessary to eradicate all malignant cells to cure cancer; (2) is more intensive treatment better; and (3) is a transplant necessary.

Considerable data suggest that complete eradication is of cancer may not be necessary for cure. For example, clonal remissions are reported in AML indicating persistence of cells related to the leukemia clone. Analyses of leukemia-related gene markers in ALL and lymphomas also indicate persistence of cells related to the malignant clone in persons in remission for prolonged intervals. Similar conclusions apply to persons receiving transplants for CML. In summary, these data suggest that complete eradication of cancer cells may be unnecessary for cure.

A second issue is whether more intensive therapy increases remissions and/or cures. These data are controversial and may differ for different cancers. For example, data from twin receiving transplants for AML suggest no further dose with increased doses. In contrast, twin transplants in ALL show fewer relapses with higher doses. Data in lymphoma, breast cancer and myeloma are contradictory; these will be discussed.

Is a transplant needed after the doses required for successful transplants. This is unknown since dose-exploration studies of conditioning regimens are not reported. Also, most transplant data show no dose-response relationship between conditioning with relapse or cure. Finally, there is little doubt that stem cells survive currently used conditioning regimens and that bone marrow recovery is accelerated by giving hematopoietic growth factors posttransplant. Whether the degree of acceleration achieved will be sufficient without a transplant requires prospective studies. Preliminary data suggest this approach may be possible. This strategy may be especially applicable if the trend towards multiple courses of less intensive pretransplant conditioning continues.

D 010 WHAT IS GRAFT FAILURE?, Paul J. Martin, Fred Hutchinson Cancer Research Center, Seattle, WA.

The term "graft failure" encompasses a variety of pathophysiologic states characterized by inadequate hematopoietic function after marrow transplantation. Implicit in the definition are concepts of a minimally acceptable level of marrow function for each lineage and time intervals after transplantation when such threshold levels should initially be reached and thereafter surpassed. The term "primary" graft failure indicates a lack of adequate marrow function at any time after transplantation, whereas the term "secondary" graft failure indicates the development of inadequate marrow function after initial engraftment has already been achieved. Arbitrary thresholds of marrow function and time after transplantation cannot accurately measure the incidence of primary graft failure because some patients show successful engraftment later than expected, while others die before engraftment can be achieved. In particular, Kaplan-Meier plots of time to engraftment underestimate the true incidence of primary graft failure because of censoring. As an alternative approach, time to initial engraftment can be modeled as a function of the number of hematopoietic stem cells in the graft, their doubling time, and the time required for maturation. Data will be presented to analyze the utility of such models in understanding parameters of initial engraftment in different marrow transplant situations. Unlike primary graft failure, secondary graft failure can be precisely defined, although assessment of whether inadequate marrow function is due to reversible or irreversible causes often remains extremely difficult. Data will be presented to analyze the frequency of secondary graft failure and to describe the subsequent clinical outcome in different marrow transplant situations. Mechanisms of primary and secondary graft failure remain elusive in many cases. Causes to be considered include stem cell damage mediated by *ex vivo* manipulation, drugs, viral infection or rejection, inadequate cellular microenvironment, deficiency of growth factors necessary for proliferation and maturation of hematopoietic stem cells, or excessive levels of cytokines that inhibit hematopoiesis.

Leukemia

D 011 AUTOTRANSPLANTS (Tx) FOR MULTIPLE MYELOMA (MM) - A PROGRESS REPORT. Bart Barlogie and Sundar Jagannath, University of Arkansas for Medical Sciences, Arkansas Cancer Research Center, Little Rock, Arkansas 72205

Since 1983, 204 patients with MM have received high dose therapy, 83 without and 121 with Tx. Among persons with refractory MM, melphalan at doses $\leq$ 100 mg/M^2 (MEL-100) without Tx was as effective as the CBV regimen with peripheral blood stem cells (PBSC) and superior to HD-CTX at MTD. Because of limited extramedullary toxicity, this justified further dose escalation of MEL and addition of TBI. With TBI 850 cGy, MEL-140 was superior to thiotepa (750 mg/M^2) with significantly higher CR rates, progression-free and overall survival for VAD-responsive MM. MEL-TBI (the superior regimen) was more beneficial for remission consolidation than for salvage treatment. To further increase tumor cytoreduction, a double transplant (DTx) was developed with 2 cycles of MEL-200 3 to 6 months apart, with bone marrow autografts collected prior to the first Tx and usually supported in addition by PBSC mobilized with HD-CTX and GM-CSF. Post-HD-CTX platelet recovery and PBSC mobilization both were reliable indicators of hemopoietic stem cell reserve, as both parameters correlated with post-transplant engraftment kinetics. Compared with MEL-TBI, MEL-200 caused less stomatitis and no mortality (0 out of 40) even when applied twice to 20 patients. Engraftment kinetics were similar after 1st and 2nd Tx. DTx is now used as consolidation therapy after intensive remission induction in newly diagnosed MM (median age, 55 years; range 29-68 years). Median tumor cytoreduction increased from 85% after VAD x 3 (range 12-99%) to >99.9% after one Tx (range 80-99.9%); the corresponding cumulative response rates using $\geq$ 75% tumor regression ($\geq$ 90%) were 53% (32%) for the 34 patients completing VAD x 2-3; 63% (53%) for the 19 patients having completed induction therapy; and 100% (90%) for the 10 patients completing one transplant including 4 true CR's. There was one steroid-related suicide but no other treatment-related mortality. This "total therapy" effects progressive cytoreduction so that tumor regression by >90% was obtained in virtually all patients after one Tx. Thus, Tx permits the safe pursuit of high dose therapy for elderly patients often initially with impaired performance. It is hoped that DTx will effect CR rates > 50% and durable disease control not achieved previously with standard melphalan-prednisone.

D 012 BMT FOR ACUTE LYMPHOBLASTIC LEUKAEMIA, John Barrett , RPMS, Hammersmith Hospital, London, U.K.

The management of poor risk ALL is beset with problems of treatment selection related to the nature of the leukaemia itself:

1) ALL is heterogeneous both in its ontogeny, and in its potential for cure.

The small number of patients in individual subgroups means that there is little reliable data on outcome for uncommon disease subtypes.

2) Chemotherapy cures the majority of children and some adults. Only a few poor risk patients are therefore considered sultable BMT candidates.

3) Poor risk diagnostic criteria for chemotherapy outcome apply equally to BMT.

4) Although BMT may be more efficient at curing ALL than chemotherapy, the advantage is offset by a progressive increase in transplant related mortality with age.

5) In relapsed patients achieving second remission the same risk factors for further relapse apply to chemotherapy and BMT.

Because of this disease free survival results for chemotherapy and BMT tend to overlap in any ALL disease category. Possible solutions to the impasse are:

1) Retrospective comparative studies of large chemotherapy and BMT databases: Several recent studies have done this and others are underway.

2) Decreasing transplant mortality - a 10% improvement in BMT outcome would make BMT clearly more favourable in many currently controversial situations: There is some evidence that BMT outcome has improved over the last decade.

3) Improving the anti-leukaemic effect of BMT in chemoresistant ALL: New developments in understanding the graft-versus-leukaemia effect may be helpful.

D 013 BONE MARROW TRANSPLANTATION FOR CML, Richard E. Champlin, M.D., U.T., M.D. Anderson Cancer Center, Houston, Texas.

Allogeneic bone marrow transplantation from HLA-identical siblings has been extensively evaluated for treatment of CML. Long term disease-free survival has been achieved in 50-70% of in chronic phase and approximately 20% after transformation. Clinical data have demonstrated several important biologic principles. High dose chemotherapy and TBI usually does not eradicate the malignancy since the majority of patients receiving identical twin or T-cell depleted transplants relapse. Patients receiving allogeneic non-T-cell depleted transplants generally do not relapse, although many have Ph^1- positive cells identified by cytogenetics or molecular methods over extended periods. Together, these data indicate that a presumably immune-mediated graft-versus-leukemia effect is critical to prevent relapse. Marrow transplants using marrow depleted of CD8-positive cells are not associated with an increased rate of relapse in indicating that these cells are not required for GVL in this disease.

Alternative forms of BMT have been evaluated for patients lacking an HLA-identical sibling. Marrow transplants from HLA-closely matched unrelated donors are associated with a higher risk of acute GVHD, but possibly a lower rate of relapse compared to matched sibling grafts; overall survival appears somewhat reduced for chronic phase patients but similar in patients with more advanced disease. Autologous transplants using marrow depleted of leukemia cells by long term culture or by "purging" techniques may restore Ph^1-negative hematopoiesis and posttransplant interferon therapy may delay recurrence of the disease. A controversy has emerged regarding the role of alpha interferon versus allogeneic BMT for management of newly diagnosed patients with CML. Alpha interferon can eliminate detectable Ph^1 chromosome-positive cells and restore diploid hematopoiesis in up to 20% of patients with newly diagnosed CML, and some investigators advocate an initial trial of interferon reserving allogeneic transplantation for patients who fail to achieve a complete cytogenetic response within one year. This approach has the potential risk of progression of CML while on interferon therapy and its impact on transplant outcome and overall survival is uncertain.

D 014 TRANSPLANTS IN ACUTE MYELOGENOUS LEUKEMIA. R.P. Gale, UCLA School of Medicine, Los Angeles, CA.

Allogeneic and autotransplants are widely used to treat AML. Their role is controversial. HLA-identical sibling transplants in 1st remission have fewer relapses than chemotherapy and perhaps slightly better leukemia-free survival even after adjusting for selection biases. Results of alternative donor transplants (HLA-matched related or unrelated) are inferior to HLA-identical sibling transplants. Most data suggest that similar LFS is achieved by reserving allotransplants for persons failing chemotherapy. There are no convincing data that adjusted results of autotransplant are superior to chemotherapy in remission or LFS or that in vitro bone marrow treatment is effective. Different conditioning regimens seem to result in similar LFS. Cyclosporine alone or with methotrexate results in comparable relapse and LFS.Risk factors for different treatments are generally similar; high-risk subjects do poorly with transplants and chemotherapy.

Allotransplant in 2nd remission results fewer relapses but comparable 2y LFS to chemotherapy. Results of both strategies are similar to autotransplants. Longer followup is needed to know if there are different outcomes.

Persons never achieving remission and those with advanced leukemia have fewer relapses and better LFS with allotransplants than chemotherapy or autotransplants.

Critical review of these data should help define the role of transplants in AML.

Transplants

D 015 ALLOGENEIC BONE MARROW TRANSPLANTATION IN FANCONI ANEMIA. E. Gluckman[1], A.D. Auerbach[2], M. Horowitz[3], for the IBMTR, 1. Hospital Saint-Louis, Paris France, 2. The Rockfeller University, New-York, 3. IBMTR, Milwaukee, Wisconsin.

Fanconi anemia is a rare autosomal recessive disorder characterized by progressive pancytopenia, diverse congenital abnormalities and predisposition to malignancy. Although the molecular basis of this syndrome remains unknown, occurrence of FA can now be detected routinely by study of induced chromosomal breakage in cultured lymphocytes, fibroblasts, amniocytes or chorionic villus cells after exposure of these cells to low concentration of DNA cross-linking agents. Bone marrow clonal cytogenetic abnormalities have been reported in these patients, as well as in some FA patients with no evidence of leukemia at the time of study. Bone marrow transplantation is the best treatment of this lethal disease. It is known that high dose Cyclophosphamide or high dose chemotherapy is highly toxic due to the cell's sensitivity to DNA cross-linking agents. In a previous analysis of the IBMTR, it was shown that regimens with low dose Cyclophosphamide and limited field irradiation gave a higher survival compared to regimens with high dose Cyclophosphamide with a long term survival of 75 % versus 27 %. The analysis of the registry will focus on several points. The most important is the accuracy of the diagnosis which is based on the cytogenetics, because of the possible absence of phenotypic abnormalities in children or even in young adults presenting as aplasia or leukemia. Other non FA familial aplastic anemia must be differentiated because of difference of conditioning. The date of transplant, the influence of previous androgens and transfusions are significant prognostic factors. BMT with HLA identical siblings gives good results but very little is known about the outcome and the conditioning in myelodysplastic syndrome or leukemia associated with Fanconi anemia. In the absence of HLA identical donor several possibilities can be discussed a new pregnancy is possible at the condition to perform a prenatal diagnosis and an abortion if the foetus is affected. Cord blood can be frozen at birth. Until now, 4 Fanconi anemia patients have been transplanted with HLA matched sibling cord blood, 3 are doing well with a complete engraftment. Matched unrelated transplants are another option, the results seem poorer than with HLA id sib transplants because of the increased risk of rejection and GVH.

D 016 NEW THERAPIES FOR APLASTIC ANEMIA. Edward C. Gordon-Smith, Fran Gibson, Alison Milne and Andrew Laurie, St. George's Hospital Medical School, London, UK.

The pathogenesis of aplastic anemia (AA) remains uncertain though recent in vitro studies suggest that the major defect lies in the haemopoietic stem cell and not in the stroma.[1,2] The role of immune responses whether cellular or humoral in mediating the damage to the stem cell is unknown and in vitro studies produce comflicting results. Imbalances of growth factor and inhibitor production have been implicated in the pathogenesis of AA[3,4] but proof of their importance in vivo is lacking. It is thus not surprising that most therapies for AA are empirical or are based on rather shaky premises. The two most recent approaches have been the addition of cyclosporin to treatment schedules[5,6] and the use of ic growth factors. The addition of cyclosporin to ALG improved response rates at 3 and 6 months (65% vs 39%) but did not affect long term survival superficially[5]. A randomised study of cyclosporin alone suggests that it is as effective as ALG[6]. These results should not be taken to indicate a major immune mechanism for AA since cyclosporin and ALG each have marked effects on cytokine production. The use of rh-CSF's seems a logical step in the management of AA. A multicentre trial with GM-CSF post ALG shows that GM-CSF to capable of raising the neutrophil and eosinophil counts in the 28 d following ALG, that infection and antibiotic usage are less, that the response is proportional to the initial neutrophil count and disappears once the GM-CSF is discontinued. Bleeding episodes were not increased. In this relatively small trial (13 pts GM-CSF, 14 placebo) long term survival was not significantly different in the two groups though the trend was for the treatment arm to do better. Trials with G-CSF are also under way and confirm the responsiveness of the stem cell in most AA. IL-3 on the other hand, on its own, has been disappointing in its action. These operations confirm that stem cells, or at least committed precursor cells, do remain in AA and are capable of responding at least to later acting CSF's. Whether long term administration will help achieve remission and whether survival will be accompanied by an increase in clonal disorders remains to be seen.

1) Marsh JCW, et al. Blood 76;1748:1990. 2) Gibson FM, et al. Br J Haematol 75;421:1990. 3) Zoumbos N.C. et al. NEJM 312;257:1985. 4) Nakao S. et al. Br J Haematol 71;431:1989. 5) Frickhofen N. et al. NEJM 324;1297:1991. 6) Gluckman E. et al. Blood Suppl 11;A78:1988.

D 017 **BONE MARROW TRANSPLANTATION IN SICKLE CELL DISEASE.** Sergio Piomelli, Columbia University, College of Physicians and Surgeons, New York, N.Y. 10032

It has been known for several years that patients with sickle cell syndromes can be affected by devastating strokes. Recent evidence, however, suggests that neurological damage, evidenced by MRI of the brain, occurs in a significant percentage of asymptomatic patients. Moreover, recent studies suggest that these disorders influence the neuropsychological function of the patient. Thus, from a neurological point of view, all patients with sickle cell syndrome are at risk of some damage. If the neurological adverse effects, obvious or subclinical, are added to the unnumerable complications of sickle cell disease, it appears obvious that, on the basis of our current knowledge, sickle cell syndromes are associated with significant risks to all patients. Therefore, there appears to be a need for active and preventive intervention.

At the current state-of-the-art, prevention can be achieved by chronic transfusion, by pharmacological intervention (hydroxyurea) or by bone marrow transplantation. Several patients with sickle cell syndromes have already been transplanted and in them the disease has been eradicated. Bone marrow transplantation can thus cure sickle cell disease. Current controversy deals, however, with the precise indication and choice of patient. In order to assess the risk/benefit of this procedure, it appears advisable to perform a randomized study, comparing bone marrow transplant with these other available modalities. This should preferably be done in the youngest patient, where the risk of bone marrow transplantation appears to be minimal.

D 018 MARROW TRANSPLANTS IN APLASTIC ANEMIA. R. Storb, Fred Hutchinson Cancer Research Center and the University of Washington School of Medicine, Seattle, Washington.

Allogeneic marrow grafts from HLA-identical siblings have proven to be effective therapy for severe aplastic anemia with restoration of normal hematopoiesis and long-term survival for up to more than 20 years in 70 to 80% of recipients. Younger patients survive better than older patients. Marrow grafts from HLA-nonidentical family and unrelated donors have been less successful and are the focus of ongoing clinical research. Graft rejection and graft-versus-host disease (GVHD) have remained major problems. Many pre- and posttransplant immunosuppressive regimens to prevent these complications have been studied. The risk of graft rejection is increased in patients who have been transfused before transplant. This risk can be reduced by the infusion of larger numbers of transplanted marrow cells. The incidence of graft rejection is 10 to 32% when cyclophosphamide alone is used as the conditioning regimen. The addition of donor buffy coat cells and total body or limited field irradiation have reduced the risk of graft rejection, but increased the incidence of complications including chronic GVHD, retardation of growth and development, and secondary malignancies. More recently, a combination of cyclophosphamide and antithymocyte globulin has been used to reduce the risk of graft rejection further, thus obviating the need for additional donor buffy coat infusions. Accordingly, most recent results of marrow transplantation from HLA-identical siblings show survivals in excess of 90%. GVHD is an immune disorder caused by donor lymphoid cells reacting with host histocompatibility antigens. Moderate to severe GVHD is seen in 18 to 40% of HLA-identical sibling recipients. Previous pregnancy in female donors and increasing age of the patient are factors predictive of its development. Methotrexate combined with cyclosporine has been the most effective prophylactic immunosuppressive agent. Extensive chronic GVHD occurs in approximately one-quarter of recipients. Cyclosporine and prednisone are used to treat this disorder.

Autotransplants

D 019 DOSE INTENSIVE THERAPY IN BREAST CANCER, Karen Antman, Scott I. Bearman, Nancy Davidson, Elisabeth de Vries, A Massimo Gianni, Christian Gisselbrecht, Herb Kaiser, Hillard Lazarus, Robert B Livingston, Dominique Maraninchi, T. J. McElwain, Makoto Ogawa, William Peters, Giovanni Rosti, Robert B Slease, Gary Spitzer, Tomoo Tajima, William P. Vaughan, & Stephanie Williams. Harvard Medical School, Boston

Breast cancer currently develops in one of nine American women. Relapse rates at 10 years correlate with the number of axillary lymph nodes, from ~20% for no+ lymph nodes, 60% for 1-3 nodes & >85% for ≥4 nodes. Metastatic breast cancer is essentially incurable with conventional therapy with a median survival of ~2 years after diagnosis of metastases. The median survival of women with metastatic disease has not changed in the 5 decades for which statistics are available. In laboratory models of breast cancer, delivery of the highest possible doses of chemotherapy is essential to achieve cure. In the laboratory, resistance to alkylating agents can often be overcome using a 5-10 fold higher dose. Because the limiting toxicity of higher chemotherapy doses is myelosuppression, many authors have used autotransplants to ensure prompt marrow recovery.

Metastatic Disease: Theoretically an induction regimen could reduce tumor bulk, decrease the number of resistant cells, & allow the selection of patients with sensitive tumors for high dose therapy. Alternatively, conventional dose therapy could induce multidrug or specific resistance or allow the growth of partially resistant clones. There are at least 4 studies of combination chemotherapy in 53 *previously untreated patients with inflammatory or metastatic breast cancer (ie no induction therapy)*. Twenty-five (47% achieved a complete response & 9 (17%) were disease free at the time of analysis. Multiple regimens have been used in 306 *stage 4 patients responding to induction therapy*. A total of 58% of these patients have achieved complete responses & 28% were in continuous complete response at the time of data analysis. The mortality was 9% overall (range 3-24%).

Primary therapy: Five regimens have been used in 56 women with *stage II or inflammatory breast cancer* responding to conventional dose (*induction therapy*) at the time of transplant. A total of 79% of these patients were in CR after conventional dose therapy prior to the transplant, & 89% were in complete response after high dose therapy. Of the total, 54% were in continuous complete response at the time of data analysis with relatively short follow up times of 1 to 37 months. Four institutions are studying *adjuvant high dose therapy in patients with 10 or more involved lymph nodes* at the time of primary treatment. A total 88% were in continuous complete response at the time of data analysis.

Summary: Thus several regimens designed for breast cancer yield both a high complete response rate & durable remissions in patients responding to conventional dose chemotherapy. Transplant early in the course of the illness, after a good response to conventional dose therapy yields a complete response rate higher than the 10 to 20% reported with conventional dose therapy. With follow-up of 24-60 months from the time of transplant, these *unmaintained* responses appear to be relatively durable (16 to 25% in continuous complete response). Mortality for dose intensive therapy in breast cancer have ranged from 3 to 24% (compared to 3-4% for conventional dose therapy). Randomized trials in stage II (with 10 or more positive nodes), III & IV breast cancer are currently planned or under way.

D 020 AUTOLOGOUS BONE MARROW TRANSPLANTATION IN AGGRESSIVE LYMPHOMA, James O. Armitage, University of Nebraska Medical Center, Omaha, NE.

Autologous bone marrow transplantation has become a widely accepted therapy for certain patients with aggressive non-Hodgkin's lymphoma. Previous studies have documented that patients with aggressive non-Hodgkin's lymphoma can be cured utilizing high doses of chemotherapy and/or radiotherapy followed by reinfusion of autologous bone marrow cells or hemopoietic stem cells derived from the peripheral blood. This treatment can be performed with a fairly low mortality (i.e. less than 10%) in young, healthy patients who have minimal preceding therapy and a low tumor burden. It has also been shown that a major prognostic factor is the responsiveness of the lymphoma to preceding chemotherapy. However, when even the best subgroups of patients with relapsed aggressive lymphoma undergo high dose therapy and autologous bone marrow transplantation, the long term survival rates are only 30%--40%.

A number of trials have utilized high dose therapy and autologous bone marrow transplantation earlier in the course of patients with aggressive non-Hodgkin's lymphoma. These trials have involved transplantation in patients who have failed to achieve complete remission quickly with "traditional" chemotherapy or patients who have achieved remission but had adverse prognostic factors which in historical controls have suggested a high chance for relapse. The results with autologous bone marrow transplantation in these patients have been remarkably similar. Long term disease-free survival has been reported in 60%--80% of patients and the treatment related to the mortality has been low. These results suggest that autologous bone marrow transplantation should be offered to "high risk" patients as part of their primary therapy. However, there are a number of reasons for caution. When autologous bone marrow transplantation is done as part of primary therapy, some of the patients undergoing the transplant process will already be cured or would be able to be cured with further standard therapy. In this setting transplant related mortalities are least acceptable. It is also true that no completed prospective trial has documented the superiority of autologous bone marrow transplantation over traditional chemotherapy in any high risk group of patients. However, two prospective trials are currently underway.

Autologous bone marrow transplantation for patients with aggressive non-Hodgkin's lymphoma has become a standard therapy after relapse. However, this might not be the best strategy and further clinical trials are urgently needed.

D 021 BONE MARROW TRANSPLANTATION (BMT) IN THE MANAGEMENT OF ADVANCED HODGKIN'S DISEASE. Gordon L. Phillips, Donna E. Reece, Joesph M. Connors. Leukemia/Bone Marrow Transplantation Program of British Columbia: Division of Hematology, Division of Medical Oncology, British Columbia Cancer Agency, Vancouver General Hospital, and University of British Columbia, Vancouver, British Columbia V5Z 4E3 Canada.

Since the 1980s, the use of myeloablative therapy and either allogeneic (allo-) or especially autologous (Au) BMT has become an accepted therapy for selected patients with Hodgkin's disease (HD), mainly those who progressed after primary chemotherapy. Results from these AuBMT have been much better than the historical experience using conventional salvage chemotherapy; a limited experience suggests that patient selection is unlikely to be the reason for this result. Consequently, Phase III studies may never be performed. Currently, the main questions in BMT for HD are as follows: 1) Should BMT be performed in some HD patients during CR-1; 2) In others, is the first sign of failure of primary chemotherapy the optimal time to perform BMT; 3) Should patients who fail primary chemotherapy receive chemosensitivity testing prior to BMT; 4) Can lymphablation be improved by newer conditioning regimens -- or novel approaches such as radioimmunoconjugates; 5) Should allo-BMT or Au peripheral blood cells be used in preference to AuBMT; 6) Is post-BMT immunomodulation worthwhile; 7) What is the role of currently-available hematopoietic growth factors in this setting. All of these questions lead to one final question -- how can more HD patients be cured? These and other points will be discussed.

Alternative Donor Transplants

D 022 MARROW TRANSPLANTATION FROM RELATED DONORS OTHER THAN HLA GENOTYPICALLY IDENTICAL SIBLINGS. Claudio Anasetti for the Seattle Marrow Transplant Team, Fred Hutchinson Cancer Research Center, Seattle Washington.

The effect of donor HLA incompatibility on the outcome of marrow transplantation was analyzed in 570 patients with hematologic neoplasms. Each patient received marrow from a family member who shared one HLA haplotype with the patient but differed to a variable degree for the HLA-A, B and D antigens. Of the haplotype not shared: 51 were phenotypically identical, 261 were incompatible for one locus, 196 for two loci, and 62 for three loci. For 229 patients the donor was a sibling, for 270 a parent, for 40 a child, and for 31 other relatives. All but 18 patients were treated with cyclophosphamide and total body irradiation. Conditioning was followed by the infusion of unmodified donor marrow cells. Graft failure occurred in 9% of the patients surviving a minimum of 21 days after transplantation. The risk factors for graft failure were: a positive crossmatch of patient serum against donor lymphocytes, patient homozygosity for the mismatched HLA locus, and posttransplant immunosuppression with methotrexate alone as compared to methotrexate plus cyclosporine. Occurrence of acute GVHD was evaluated in patients who achieved sustained engraftment. The risk factors for acute GVHD of grades III-IV were the degree of donor vs patient incompatibility for HLA and older patient age; posttransplant immunosuppression with methotrexate plus cyclosporine was associated with a lower risk of GVHD when compared to other immunosuppressive regimens. In patients transplanted from phenotypic or one locus mismatched donors, survival was similar to the survival of patients with the same type and stage of leukemia who were transplanted from HLA genotypically identical siblings at our institution. Patients transplanted from donors mismatched for two or three HLA loci have lower survival rates than patients transplanted from matched donors because of complications resulting from acute and chronic GVHD. We conclude that transplantation from a donor mismatched for one single HLA locus should be considered standard therapy for any patient with a malignancy who is a candidate for marrow grafting. Novel and more effective approaches to prevent GVHD are needed for patients whose best available donor is incompatible for two or three HLA loci and the only potential curative treatment is allogeneic marrow transplantation.

D 023 BONE MARROW TRANSPLANTS FROM UNRELATED DONORS FOR LEUKEMIA. M.M. Horowitz, International Bone Marrow Transplant Registry (IBMTR), Medical College of Wisconsin, WI.

Large registries of volunteer donors now permit unrelated donor bone marrow transplants for patients without HLA-identical siblings. We studied 219 transplants from unrelated donors done for leukemia between 1985 and 1990 and reported to the IBMTR by 42 teams. One hundred fifty-nine were serologically matched at six HLA-A, B, and DR loci. Sixty were disparate at one locus: 29 at HLA-A, 15 at HLA-B and 16 at HLA-Dr. Results were compared to 1501 HLA-identical sibling transplants and 172 transplants from related donors, other than HLA-identical siblings, mismatched at zero or one HLA-A, B, or DR locus. Controls were transplanted for the same diseases at the same centers during the same time period. Two-year probabilities (± 95% confidence level) of grade II-IV acute graft-versus-host disease (GVHD), chronic GVHD and leukemia-free survival according to donor type are shown below:

Relationship and Histocompatibility	N	Acute GVHD	Chronic GVHD	LFS*
HLA-identical sib	1501	31 ± 2%	38 ± 3%	50 ± 1%
or 1-locus mismatched relative	172	36 ± 8%	33 ± 13%	27 ± 10%
Matched unrelated donor	159	51 ± 9%	55 ± 13%	33 ± 12%
1-antigen mismatched unrelated donor	60	63 ± 13%	87 ± 19%	21 ± 16%

*For patients with acute leukemia in remission or CML in chronic phase only.

Results of unrelated donor transplants are significantly worse than HLA-identical sibling transplants but similar to transplants from 0 to 1-antigen mismatched relatives other than HLA-identical siblings.

D 024 UNRELATED DONOR TRANSPLANTS FOR SEVERE ACQUIRED APLASTIC ANEMIA (SAA), Jill M. Hows, on behalf of the Severe Aplastic Anaemia Working Party of the EBMTG, and the IMUST Study Group, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK.

Severe acquired aplastic anemia (SAA) as defined by the International Aplastic Anemia Study Group criteria is best treated by BMT when the patient is below 35-40 years old and has a HLA identical sibling. Studies from the SAA Working Party of the EBMT group indicate that in the medium term survival is equivalent for patients treated by immunosuppression (IS) or BMT. An important exception is patients with very severe aplastic anemia (VSAA), defined by $< 0.2 \times 10^9/l$ peripheral blood neutrophils, who are under the age of 20 yrs. This group has a significantly superior medium term survival after BMT compared with IS. Of relevance to all patients with SAA is the fact that patients are not cured by IS. Their intrinsic "stem" cell defect remains and in up to 57% of cases clonal evolution into myelodysplasia and/or acute myelogenous leukemia, leads to late deaths. Unrelated donor BMT (UD-BMT) should therefore be considered in younger patients with SAA, who do not have a suitable family donor. In the past results of UD-BMT for SAA have been poor due to multiple factors: the length of time taken to find unrelated donors, poor patient selection and inadequate pre BMT immunosuppression. Pooled retrospective survival figures kindly provided by Seattle, Wisconsin, Hammersmith and the French Transplant group (GEGMO) for patients receiving UD-BMT between 1982-90 are summarised. For HLA-A, B, DR serologically matched UD-BMT 9/29 (31%) of patients are surviving. For HLA-A, B, DR partially mismatched UD-BMT 6/17 (35%) survive. There were more pediatric cases in the mismatched group. Currently, the International Marrow Unrelated Search and Transplant (IMUST) Study includes patients with SAA receiving UD-BMT. These patients are prospectively compared with a cohort of HLA identical sibling transplants (ID-BMT), matched for trasplant center, recipient age, and presence/absence of infection/sensitization at the time of BMT. Since February 1989, 14 UD-BMT have been carried out by IMUST Study Centers, with 12 cohorted ID-BMT controls. UD-BMT donors were older 33 (24-43) years than ID-BMT donors 23 (10-37) years. Mean recipient age was similar, 18 (12-31) years for UD-BMT and 21 (6-38) years for ID-BMT. 5/12 ID-BMT and 8/14 UD-BMT recipients were infected/sensitized at time of BMT. 50% of UD-BMT, and 27% of ID-BMT recipients received intensive irradiation containing conditioning protocols. 7 patients, 5 UD-BMT and 2 ID-BMT either failed to engraft or died within 28 days of BMT. Of evaluable recipients, 2/11 after UD-BMT and 4/10 after ID-BMT developed grade II-IV acute GVHD. 10/14 UD-BMT and 7/12 ID-BMT recipients survive > 3/12 after BMT. The presence of infection/sensitization at BMT correlated with poor survival at > 3/12 post BMT, both in ID-BMT and UD-BMT groups. From these preliminary results we conclude that when UD-BMT is carried out in non infected, non sensitized patients results are acceptable. In the future better patient selection, more intensive IS protocols and improved donor selection routines will improve the outlook of patients with SAA who lack family donors. Perhaps the use of a ready accessible cord blood bank will be particularly relevant to this group of patients.

Issues in Transplants - I

D 025 CYCLOSPORINE INDUCED SYNGENEIC/AUTOLOGOUS GRAFT-VS-HOST DISEASE: AN IMMUNOTHERAPEUTIC APPROACH AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION, Allan D. Hess, Stephen J. Noga, Richard J. Jones, Andrew M. Yeager, Georgia B. Vogelsang, and George W. Santos, Oncology Center, The Johns Hopkins University, 600 North Wolfe Street, Baltimore, Maryland.

Graft-vs-host disease (GVHD) is an immunologic process which develops in the majority of allogeneic BMT recipients despite HLA identity. Although morbidity and mortality due to GVHD limits BMT, there appears to be a therapeutic anti-tumor effect. Decreased leukemic relapse rates and prolonged disease free survival are apparent in BMT patients who survive a severe episode of GVHD. Conversely, the lack of GVHD in autologous BMT recipients is associated with increased leukemic relapse rates. Therefore, additional therapy is required to reduce relapse. An autoimmune, T cell mediated GVHD-like syndrome (Syngeneic GVHD) can be reproducibly induced in rats and in man if a limited course of CsA is administered following autologous or syngeneic BMT. The induction of syngeneic GVHD appears to correlate with the production of MHC class II specific autocytotoxic T cells. Since many hematopoietic malignancies express class II MHC antigens and the syngeneic GVHD has been shown to be MHC class II restricted, this autoimmune response may provide a therapeutic advantage for elimination of residual leukemia in autologous BMT recipients. A rat (Lou M) model for studying the effects of syngeneic GVHD effector cells on the CRL1662 myeloma (which expresses MHC class II antigen) was developed. A series of studies in this animal model revealed that 1)syngeneic GVHD mediated an anti-tumor effect with an estimated 1-2 log tumor cell kill, 2)administration of γ-interferon potentiated the anti-tumor effect of syngeneic GVHD resulting in cure in 40% of the animals, and 3)the effect of γ-interferon was due to upregulation of class II antigen expression on the tumor cells making them more susceptible to immune recognition. Based on the results in the animal model, clinical trials were initiated in patients with non-Hodgkins's lymphoma (NHL, diffuse intermediate or high grade) or with acute myelogenous leukemia (AML). All patients received marrow purged with 4-hydroperoxycyclophosphamide and I.V. CsA therapy initiated on the day of transplant and continued for 28 days. Overall 19/23 NHL patients and 15/19 AML patients developed cutaneous autologous GVHD confirmed by skin biopsy (grade 2). Autologous GVHD developed at a median of 18 (range 13-48) days for NHL patients and 33 (range 14-49) days for AML patients and was confined to the skin with no patients exhibiting gastrointestinal or hepatic dysfunction due to autologous GVHD. The skin autologous GVHD resolved spontaneously within 2 weeks (range 1-5 weeks) or with a short course of steroids. Further, the onset of autologous GVHD was associated with the appearance of class II specific cytolytic T cells similar to the animal model. Overall 16/23 NHL patients survive disease-free for an actuarial event-free survival at 34 months of 62%, compared to 40% in 34 consecutive historical controls treated similarly, but without CsA, between May 1981 and Nov. 1988 (p=0.05). The relapse rate was 29% in the CsA NHL patients compared to 50% in the historical controls (p=0.03). Comparatively 13 AML patients (10 CRI, 2CR2, 1CR3) are alive without relapse at a median of 12 months (range 3.2+ - 21.6+) after transplant. Although the clinical results appear promising, prospective randomized trials will be needed to confirm a significant anti-tumor effect of autologous/syngeneic GVHD. CsA induced GVHD appears to be a promising immunotherapeutic approach following autologous BMT. Further, this approach may be coupled with cytokine therapy.

D 026 PERIPHERAL BLOOD VERSUS (plus) BONE MARROW CELLS FOR AUTOLOGOUS TRANSPLANTATION, Anne Kessinger, University of Nebraska Medical Center, Omaha, Nebraska 68198.

Throughout the last decade, autologous transplantation of hematopoietic stem cells collected from peripheral blood to restore marrow function damaged with high doses of anti-cancer therapy has been used with increasing frequency. As the indications for peripheral stem cell transplantation (PSCT) evolve, questions regarding the intercalation PSCT in the framework established for autologous bone marrow transplantation (ABMT) have become an issue. To date, PSCT has been used rather than ABMT in two clinical scenarios. The first involves patients who are candidates for high dose therapy and an autologous transplant, but have bone marrow abnormalities that make ABMT undesirable or impossible. These abnormalities have included hypocellular or acellular bone marrow in harvestable sites, usually as a result of prior pelvic irradiation or prior chemotherapy. Histopathologic evidence of bone marrow metastases is another abnormality that can preclude ABMT. The second clinical situation in which PSCT might be preferable to ABMT concerns the expectation that PSCT would result in a shorter period of aplasia following high dose therapy than ABMT. This expectation can be met if the stem cells are collected while their numbers are deliberately expanded in the circulation (mobilized). Even though successful mobilization cannot be achieved in every patient, transplantation of mobilized peripheral stem cells decreases the period of aplasia about one week compared to ABMT. This shortened aplasia effect can also be realized when mobilized peripheral stem cells are added to autologous bone marrow at the time of transplantation. Additional benefits of PSCT have been hypothesized, although supporting data is immature or not yet available. Some of these potential benefits include the possibility that PSCT results in a more rapid immune reconstitution after marrow injury than ABMT. Other possible benefits include a decreased potential of occult tumor cell reinfusion with PSCT. That PSCT might have a therapeutic as well as a restorative capability has also been pondered. Even though the entire potential of peripheral stem cell transplantation has not yet been explored, with the availability of ABMT and PSCT, nearly every patient in need of an autologous hematopoietic stem cell transplant can be accommodated.

D 027 LATE EFFECTS OF RADIATION, Hans-Jochem Kolb [1], Klaus Beisser[1], Mary M.Horowitz[2], Wolfgang Günther[1], Robert P.Gale[2], Thomas Duell[1], Ekkehard Schaeffer[1], Ernst Holler[1], Theodor M.Fliedner[3], International Bone Marrow Transplant Registry (IBMTR), European Late Effect Project Group (EULEP) and European Cooperative Group of Bone Marrow Transplantation (EBMT).[1]GSF-Forschungszentrum für Gesundheit und Umwelt, Hämatologikum, 8000 Muenchen 70, Germany, [2]IBMTR Milwaukee Wi; [3]Inst.fuer Arbeits- u. Sozialmedizin, Universitaet Ulm.

Late effects of radiation include cataracts, infertility and, most seriously new malignancies. The definition of risk factors of the development of new malignancies is important for the future design preparatory treatment. 109 patients with new malignancies of 9732 transplanted patients have been reported to the IBMTR. The spectrum of tumors was similar to that observed following organ transplantation: lymphoma including Hodgkins Disease (57), de novo leukemia or myelodysplastic syndrome (10), skin cancer (13) and cervical cancer including dysplasias (7). 13 patients had other solid tumors and in 9 patients the tumor was not specified. In dogs the effect of radiation could be studied independently of the primary disease. In an attempt to increase the radiation dose by fractionation and protraction to a curative range, doses were escalated up to 30 Gy. Tumor-free survival was not significantly shortened by chemotherapy as compared to that of untreated dogs (p=0.06, log rank). It was shortened by radiation in a dose-dependent way, at doses of 10 Gy, 20 Gy and 25 Gy respectively (p=0.0013, log rank multigroup). Allogeneic transplants shortened the median age of tumor development by 20 months as compared to autotransplants (p=0.09, log rank). The tumor-free survival time after autotransplantation was shorter after a shorter treatment schedule (3 or 5 days) than after a longer schedule (7 days)(p=0.018). Tumor-free survival was not signficantly improved by hyperfractionation as long as the total treatment time was unchanged. The spectrum of tumors in radiated dogs differed from that of untreated dogs. Radiated dogs developed more often sarcomas, particularly hemangiosarcoma. In dogs higher doses of radiation are tolerated at a low dose rate and in prolonged treatment schedules. In man risk factors of radiation remain to be defined from the primary disease, previous treatment and concurrent chemotherapy.

Issues in Transplants -II

D 028 WHO SHOULD GET SECOND TRANSPLANTS? Kerry Atkinson, Department of Haematology, St. Vincent's Hospital, Sydney, NSW, 2010, Australia.

Second transplants need to be considered in two clinical situations after marrow transplantation. Firstly, failure of sustained engraftment; secondly, recurrence of the underlying malignancy. In patients with failure of sustained engraftment a trial of recombinant human GM-CSF is indicated first with a chance of improving blood counts in a significant proportion of patients. If this is unsuccessful a second transplant utilising a preparative regime aimed primarily at further minimising host immune competence such as a combination of anti-thymocyte globulin (ATG) and cyclophosphamide or ATG alone followed by a T-replete marrow infusion should be employed. A second marrow transplant can also be utilised for recurrence of the underlying haematological malignancy after first transplant. However, several large multi-institutional studies have now shown a striking difference in long-term leukemia-free survival rate between those relapsing early (for example, < six months) after first transplant compared to those relapsing later after first transplant. Leukemia-free survival post second transplant for those relapsing within six months of first transplant is < 10%, whereas for those relapsing > six months from first transplant it is approximately 30%. Transplant-related mortality, particularly from complications influenced by chemotherapy, such as interstitial pneumonitis and hepatic veno-occlusive disease, are more common after second than after first transplant. If a T-cell depleted transplant has been utilised for first transplant a T-replete transplant should be utilised for second transplant and is probably worth employing a less stringent graft-versus-host disease prophylactic regime after second than after first transplant in order to harness a greater graft-versus-leukemia effect after second transplant. Additional factors associated with a favourable outcome after second transplant include good risk leukemia (chronic myeloid leukemia in chronic phase, acute leukemia in complete remission) and a high Karnovsky peformance score pre second transplant. The ideal candidate for second transplant should thus be a fit, young person with good risk leukemia, relapsing six months or later after first transplant.

D 029 APPLICATIONS OF GENE MARKING PRIOR TO AUTOLOGOUS BONE MARROW TRANSPLANTATION, Malcolm K. Brenner,[1] Donna R. Rill[1], Robert C. Moen[2], Michael Buschle[1], Chris Bartholomew[1], Nicholas K. Foreman[1], Joseph Mirro, Jr.[3], Robert J. Krance[1], and James N. Ihle[1], [1]St. Jude Children's Research Hospital, Memphis, TN 38101, [2]Genetic Therapy, Inc., Gaithersberg, MD, [3]Children's Hospital of Pittsburgh, Pittsburgh, PA.

Autologous bone marrow transplantation (ABMT) is widely used as treatment for malignant disease. Although the major cause of treatment failure is relapse, it is unknown if this arises entirely because of residual disease in the patient or whether contaminating cells in the rescuing marrow contribute. Attempts to purge marrow of its putative residual malignant cells may delay hemopoietic reconstitution and are of uncertain efficacy. We will discuss how retrovirus mediated gene transfer may be used to elucidate the source of relapse following ABMT for acute myeloid leukemia and for neuroblastoma, to evaluate the efficacy of purging and to analyze the responses of the autograft to exogenous hemopoietic growth factors. Clonogenic neuroblastoma and myeloid leukemic blast cells in patient marrow can be transduced with the Neo^R gene-containing helper-free retroviruses, with median efficacy of >4%. Transduced colonies grow in selective media and the presence of the marker gene can be confirmed in individual malignant colonies by PCR. If such malignant cells remain in harvested "remission" marrow, they will therefore be marked following exposure to retroviruses. Detection of the marker gene in the malignant cells present at any later relapse would be firm evidence that residual disease contributed to disease recurrence. Use of two retroviruses with separate restriction sites to mark a purged and unpurged aliquot would readily allow the assessment of the efficacy of purging in a single patient, so permitting rapid subsequent evaluation of purging techniques. The technique also marks normal marrow progenitors from AML and neuroblastoma patients. These colony forming cells can be detected in long-term marrow cultures at a frequency of 1 to 18% for up to 10 weeks after exposure to the vector. Animal models and analysis of probability tables suggested that these levels of marking in vitro would be sufficient to provide information about the mechanisms of relapse and the biology of marrow regeneration in vivo. Clinical studies began in September 1991, and preliminary results are now available.

D 030 IN UTERO TRANSPLANTATION OF FETAL HEMATOPOIETIC STEM CELLS (HSC), Esmail D. Zanjani[1], Michael R. Harrison[2], and Mehdi Tavassoli[3], [1]VA Medical Center, Reno, NV, [2]University of California, San Francisco, CA, and [3]VA Medical Center, Jackson, MS.

The preimmune status of the early gestational age fetus provides a permissive environment that surmounts the immunological barrier and permits the engraftment and expression of allogeneic or xenogeneic HSC. Previously we used the in utero approach to allogeneic HSC transplantation to achieve hematopoietic chimerism in large animal models. These chimeric animals have shown significant engraftment and multilineage expression of donor lymphoid, myeloid and erythroid cells for 5 years without significant graft loss or GVHD. We now report on a similar in utero approach to establish long term (>2 years) engraftment and expression of human fetal liver HSC in sheep. Engraftment occurred in 40% (13 of 33) of recipients. Of 5 live born sheep, all were chimeric, showing human cells in the marrow, with 3 also exhibiting human cells in the blood. Engraftment was multilineage, involving lymphoid, myeloid and erythroid donor (human) cells. Donor (human) hematopoietic progenitors (CFU-Mix, CFU- GM, BFU-E, CFU-E) capable of forming colonies in vitro were present in marrow of all chimeric lambs. Interestingly, these progenitors have continued to exhibit responsiveness to human specific growth factors both in vitro and in vivo. Thus the administration of rHuIL-3 and GM-CSF to chimeric sheep resulted in a 2.1-3.4 fold increase in the relative expression of donor (human) cells. Therefore, the integration of human HSC into the hematopoietic framework of the host appears to be incomplete, with donor progenitors retaining certain phenotypic characteristics that can be exploited to preferentially manipulate the donor (human) cell population in these animals. In both the allogeneic and xenogeneic in utero transplantations, donor HSC primarily seeded in host bone marrow. Since the donor cells were of liver origin and the host liver at the time of transplantation was the major hematopoietic organ, this near exclusive seeding to the marrow indicates the greater affinity of marrow for the homing HSC. Nonetheless, no cells of donor origin appeared in the circulation of the host until the perinatal period, suggesting that donor HSC that engraft in host marrow, while able to expand with the developing marrow spaces, do not undergo terminal differentiation. The absence of a significant immunological barrier and the availability of expanding marrow homing sites renders the fetus an ideal host (and donor) for HSC transplantation.

Late Abstracts

BONE MARROW TRANSPLANTATION IN CHILDREN AND IN ADULTS WITH THALASSEMIA, Lucarelli G, Galimberti M, Polchi P, Angelucci E, Baronciani D, Giardini C, Donati M, Giorgi C and Filocamo M. Divisione Ematologica e Centro Trapianto di Midollo Osseo, Ospedale di Pesaro, 61100 Pesaro, Italy.

From Dec. 17, 1981 through Sept. 10, 1991 in Pesaro 470 consecutive thalassemic patients have been transplanted from HLA identical donors. Survival and event-free survival (EFS) leveled off about one year post-transplant at 82 and 74 percent, respectively. Moving through different AGVHD prophylaxis from 1981, in January 1983 we adopted a regimen that includes Bu 14, Cy 200 and Cyclosporine alone from day -2. This Protocol, denominated as Protocol 6, appeared as the less toxic and reduced the rejection rate from 12% to 6%. Univariate and multivariate analysis of pre-transplant characteristics done on 172 patients consecutively transplanted with Protocol 6 from January 1983 through March 1989, indicated that hepatomegaly, portal fibrosis and the poor quality of chelation given through the years before transplant significantly affected survival and EFS. Patients have therefore been categorized into three Classes of risk: Class 1 has none, Class 3 has all and Class 2 has one or different association of two of the three risk factors.

Probability of survival were found to be 97%, 86% and 58% respectively for Class 1, Class 2 and Class 3. Probability of EFS were 94%, 83% and 53% while probability of rejection were 3%, 6% and 12% for Class 1, Class 2 and Class 3 . Since March 1989 Class 1 and Class 2 thalassemic patients continued to be transplanted using the Protocol 6. For thalassemic patients belonging to Class 3 a new preparatory regimen was introduced in March 1989 consisting of Bu 14, Cy 120, Anti-lymphocyte Globulin 10 mg/kg form day -5 through day +5, Cyclosporine from day -2, Cyclophosphamide 7.5 mg/kg on day +1, MTX 10 mg/kg on day +3 and on day +7 (Protocol 12). An update of results in 53 patients of Class 1 and in 170 patients of Class 2 shows 98% and 86% probability of survival and 94% and 84% probability of EFS respectively. In 19 Class 3 patients aged less than 17 years transplanted since March 1989 with Protocol 12 survival and EFS were 100% and 70% respectively. After the individuation of the three Classes of risk, thalassemic patients are included in the transplant program independently of the age, but according to the Class to which they belong. Preliminary results in 24 adult patients indicate 80% survival and 75% EFS.

ANTI-VIRAL THERAPY AND ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) FOR HIV-1 RELATED MALIGNANCIES, K. Holland and R. Saral, Emory University, P.O. Drawer AR, Atlanta, GA 30322.

It is postulated that for patients with HIV-1 infection, the use of a marrow ablative chemo-radiation therapy in preparation for allogeneic BMT combined with anti-HIV-1 viral chemotherapy has the potential advantage of destroying the host's hematopoietic-lymphoid-monocyte reservoir for HIV-1 and of inhibiting the proviral infection of transplanted uninfected donor cells. A well suited population to examine this biologic effect is in patients who have hematopoietic malignancy and who are otherwise candidates for allogeneic BMT. Data from a pilot trial evaluating the toxicity of azidothymidine administered to patient who are HIV-1 seropositive with hematopoietic malignancies undergoing allogeneic BMT will be presented. Strategies to enhance retroviral therapy including combinations of anti-viral agents and interferon will be discussed.

Immunology, Tolerance, GvHD

D 100 MIXED XENOGENEIC CHIMERAS (MOUSE + RAT → MOUSE): THE MOUSE THYMUS IS SUFFICIENT TO SUPPORT XENOGENEIC RAT T-CELL MATURATION, Ashraf Y. Abou El Ezz, Mary L. Hronakes, Sherry M. Wren, Sallie S. Boggs, Suzanne T. Ildstad. We recently reported a model for preparation of mixed xenogeneic chimeras in which reconstitution of B10 mouse recipients with T-cell depleted syngeneic mouse plus 40 x 10^6 untreated rat bone marrow cells resulted in stable lymphoid chimerism (2% - 62% rat), excellent survival (86% survive at >180 days), and the induction of donor-specific transplantation tolerance for rat skin and islet cell xenografts. We observed that while platelet and lymphoid chimerism was mixed (both mouse and rat), red blood cell production was exclusively mouse. We have now examined rat T-cell development and maturation in mixed xenogeneic chimeras. For the most part, maturation of T-lymphocytes occurs in the thymus, requires interaction with the thymic stroma, and is reflected by an acquisition of a number of surface markers characteristic for mature T-cells. In the rat, immature rat T-lymphocytes are Thy 1.1^{bright} plus double-positive for both CD4 plus CD8 ($CD4^+CD8^+$). In striking contrast, mature rat T-cells are Thy 1.1 negative and positive for either CD4 ($CD4^+CD8^-$) or CD8 ($CD4^-D8^+$). In all mixed xenogeneic chimeras examined from 8 weeks to 28 weeks following reconstitution, an immature rat T-cell profile was present in the thymus (Thy 1.1^{bright}, $CD4^+$, $CD8^+$). In striking contrast, all rat splenic lymphoid cells were of mature (Thy 1.1^-, $CD4^+$, or $CD8^+$) phenotype. Similarly, mouse T-cell maturation proceeded in a normal phenotypic fashion, with immature mouse T-lymphocytes in thymus (Thy 1.2^{bright}, $CD4^+$, $CD8^+$) and phenotypically mature mouse T-cells in the periphery. Taken together, these data suggest that the xenogeneic mouse thymus is sufficient to support maturation of rat T-lymphocytes and that the presence of developing mouse T-lymphocytes in the thymus does not interfere with xenogeneic rat T-lymphocyte maturation.

D 101 IN-VITRO CLONOGENIC MONITORING OF PERIPHERAL STEM CELL GROWTH BEFORE AND DURING INTERLEUKIN-3 ADMINISTRATION. Roxanne Alter, Lisbeth A.Welniak, John D.Jackson, Julie M.Vose, Leslie Garrison, Dennis D.Weisenberger, and Anne Kessinger, University of Nebraska Medical Center, Omaha, NE. 68198 and Immunex Corporation, Seattle, WA.

As a part of an ongoing phase I study, we are investigating the effect of Interleukin-3 (IL-3) administration on peripheral blood stem cells (PBSC) collected for autologous transplantation following high dose chemotherapy. We report here the results of in vitro clonogenic assays and mononuclear cell (MNC) counts of PBSCs collected with escalating doses of IL-3. IL-3 was administered subcutaneously for 14 days after the first PBSC collection, which served as the baseline control. During IL-3 administration, PBSCs were collected on days 5, 8, 11, 13, and 14. At the present time, eight patients and three dose escalations have been studied. A significant increase of CFU-GM number was seen on both days 11 and 13, with a 2.5 and 3.3 fold increases respectively, as compared to the baseline. Significant increases of CFU-MEG colonies were seen on day five. Changes were seen in the CFU-MIX and BFU-E but did not reach statistical significance. Dose escalation and the effects of IL-3 administration on in vitro cultures will continue. It is apparent from the first three dosage levels (125ug/m^2, 250 ug/m^2, and 500ug/m^2) of IL-3, however, that IL-3 can increase the number of available hematopoietic cells, including progenitor cells. Future studies will determine the appropriate dosage for maximum efficiency.

D 102 STRONG DONOR MONONUCLEAR CELL REACTIVITY FOR HERPES SIMPLEX VIRUS (HSV) ANTIGEN IN HSV IMMUNE DONORS COMBINED WITH HSV IMMUNITY IN PATIENTS IS ASSOCIATED WITH ACUTE GRAFT-VERSUS HOST DISEASE

L. Boström, O. Ringdén, M. Forsgren. Departments of Clinical Immunology and Transplantation Surgery, Karolinska Institute, Huddinge Hospital, and Central Laboratory of Microbiology, Stockholm, Sweden.

Prior to bone marrow transplantation (BMT) the IgG antibody titres for cytomegalovirus (CMV), herpes simplex virus (HSV) and varicella zoster virus (VZV) were analysed in 51 donors and recipients of allogeneic bone marrow. Simultaneously the DNA synthesis of the peripheral blood mononuclear cells and bone marrow cells from the donors were measured after stimulation with antigen prepared from CMV, HSV and VZV. The patient median age was 31 years (1-58 yrs) in the 19 females and 32 males. Eighteen patients had chronic myeloid leukaemia, 11 acute lymphoblastic leukaemia, 7 acute myeloid leukaemia, 5 myeloma and 10 others. Prophylaxis against graft-versus-host disease (GVHD) was: methotrexate (MTX) + cyclosporine (CSA) 32 patients, MTX single therapy 1, CSA single therapy 2, T cell depleted bone marrow 15 and two patients with HLA identical twin donors received no prophylaxis. Forty-one of the donors were HLA identical and 10 were partially HLA identical. Forty-seven of the 51 patients had engraftment and were evaluable for acute GVHD. Ten patients developed grade II-IV acute GVHD (8 grade II, 2 grade III), 21 had grade I and 16 no acute GVHD. In student's t-test it was found that a high IgG-titre for HSV in the recipient prior to BMT was associated with grade II-IV acute GVHD ($p=0.05$). When comparing different groups with Chi-2-test the combination: HSV seropositive patients with HSV seropositive donors, and a strong donor mononuclear cell reactivity for HSV antigen, 7/17 (41%) developed grade II-IV acute GVHD compared to 3/25 (12%) in the remaining patients ($p=0.04$). Conclusion. The reported data indicate that HSV immune donor mononuclear cells in peripheral blood may initiate a graft-versus-host reaction.

D 103 PREVENTION OF GRAFT-VERSUS-HOST DISEASE AND POSSIBLY GRAFT REJECTION IN A MHC INCOMPATIBLE MURINE MODEL BY SPECIFIC DESTRUCTION OF HOST-REACTIVE MATURE T CELLS FROM THE DONOR. M. Cavazzana-Calvo °, C. Fromont *, D. Guy-Grand and A. Fischer *. ° INSERM U 132, Hôpital Necker-Enfants Malades. * CTS, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75743 PARIS CEDEX 15, France.

We have previously shown in a human cell model that anti-IL2 receptor A chain specific antibody (33B3.1) conjugated to the A chain of ricin inhibits primary mixed lymphocyte culture (MLC) and human leucocyte antigen HLA-restricted T cell-specific cytotoxic activity in vitro. In order to investigate the biological effects of such a depletion we have used a murine model in which acute GVHD is induced by injecting parental (C3H/eB) lymphocytes IV into lethally irradiated (C3H/eB x DBA/2)F1 mice. In this GVHD model, we have studied three main types of gut alterations : donor T-cell infiltration of the intestinal crypts, increase in crypt height and accelerated epithelial renewal. Our results have shown that alloreactive T-cell depleted preparation-induced little, if any, GVHD in the gut, contrary to untreated preparations. On the basis of these preliminar results we are testing whether the addition of T cells specifically depleted of alloreactive T cells can prevent graft rejection without inducing GVHD in an haploidentical setting.

D 104 ANTI-CD6-bR:AN ANTI-PAN T CELL IMMUNOTOXIN, Collinson A.R.,Goldmacher V.G.,Rassmussen R,Lambert J.M., ImmunoGen Inc. and Dana-Farber Cancer Institute, Cambridge,MA 02139, Anti-T-cell antibodies used together with complement have been extensively applied for purging T cells from donor marrow in allogeneic BMT for the prevention of GVHD. In this study we report the development of a potent anti-pan T cell immunotoxin capable of killing cells in an antigen dependent manner. The immunotoxin is composed of a high affinity anti-CD6 antibody (IgG2a $Kd=2x10^{-10}$ M) conjugated to the plant toxin ricin which has been chemically modified in a manner which blocks the lectin binding sites of the B-chain (blocked ricin). Conjugation of blocked ricin (bR) to the antibody has minimal effect on the avidity of the antibody and no effect on the ribosome inactivating activity of ricin. Anti-CD6-bR is a specific and highly toxic immunoconjugate killing the antigen positive MOLT4 cell line with an IC_{37} of $4x10^{-12}$ M using a clonogenic assay and 24 h exposure of immunotoxin. Nonspecific toxicity was assessed on the antigen negative namalwa cell line, which had an IC_{37} of $1.6x10^{-9}$ M.Specific toxicity of the immunoconjugate can be completely blocked by addition of excess free anti-CD6 antibody during the exposure period. The toxicity of the immunoconjugate is dose dependent; at $5x10^{-11}$ M conjugate, 2.5 logs of MOLT4 cells are killed by 24 hour exposure to conjugate while greater than 5 logs of cells are killed when continuously exposed (11 days) to conjugate. Anti-CD6-bR is also capable of killing CD6 positive cells in human PBL. Continuous exposure of PBL to $1x10^{-10}$ M immunotoxin killed >3 logs of cells reaching the lower limit of sensitivity of this assay due to outgrowth of $CD6^-$ cells. An application of this immunoconjugate for the prevention and treatment of GVHD is suggested.

D 105 TOTAL SKIN ELECTRON BEAM THERAPY (TSEBT) FOR SEVERE SKIN ACUTE GRAFT VERSUS HOST DISEASE (GVHD): A CASE REPORT, KE Dusenbery, PM Anderson, FC Deibel, DJ Weisdorf, AH Filipovich, Departments of Radiation Oncology, Pediatric Oncology, and the Bone Marrow Transplant Program, University of Minnesota, Minneapolis, MN 55455.

Acute GVHD, mediated by donor T Lymphocytes, occurs with high frequency after unrelated allogeneic bone marrow transplantation. Although lymphocytes are extremely sensitive to irradiation, irradiation has not been systematically investigated for treatment of skin GVHD. We report the use of high dose rate total skin electron beam therapy (TSEBT) in a 13 year-old boy with acute Grade IV skin GVHD developing after unrelated donor transplantation for CML. The skin GVHD in this case was previously unresponsive to topical steroids, intravenous high dose steroids, and two courses of anti-thymocyte globulin in addition to continuous use of cyclosporin A. TSEBT was then initiated, using a 9 MeV electron beam at an average whole skin dose rate of 9 cGy/minute. The technique involved treating 12 partial skin fields arranged to give a total skin dose of 200 cGy over 2 days. Daily treatment and set-up time averaged only 30 minutes. Dramatic improvement occurred after the first 100 cGy fraction. Treatments were discontinued after a total skin dose of 800 cGy over 11 days with steady improvement. Subsequent staphylococcal septicemia 4 days later was associated with a flare of the skin GVHD, and a second course of TSEBT was initiated, again with improvement after 200 cGy. Unfortunately, the patient expired of complications (acute renal failure and pulmonary hemorrhage), so no conclusions regarding the duration of response could be drawn. In addition to its clinical effectiveness, TSEBT was well tolerated by the patient. The use of TSEBT for acute skin GVHD deserves further study, and may provide a selective treatment with minimal toxicity. Diagrams illustrating the technique of high dose rate TSEBT and patient photographs documenting response will be presented.

D 106 RECONSTITUTION OF B CELL FUNCTION IN SCID MICE WITH CELLS FROM BALB/c ADULT BONE MARROW DEPLETED OF LINEAGE COMMITTED CELLS, Meenal Elliott and John F. Kearney, Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294

It has been shown that normal adult bone marrow is capable of long term reconstitution of normal numbers of lymphocytes and cells of other hemopoietic lineages in mice as well as in humans following high dose irradiation. However, human bone marrow transplant recipients are susceptible to serious infections by invasive bacteria such as H. influenzae type b, N. meningitidis, and S. pneumoniae for upto one year or longer following transplantation. This reflects a lack of capacity to respond to bacterial polysaccharide antigens during this period. In inbred strains of mice, it has been observed that the capacity to respond to S. pneumoniae with antibodies expressing the optimally protective T15 idiotype is lost following lethal irradiation and bone marrow reconstitution.

Adult bone marrow cells were stained with anti-CD4, anti-CD8, anti-Mac1, and anti-B220 antibodies and sorted by flow cytometry on the basis of size and light scatter properties as well as lack of staining. Sorted cells were infused into adult SCID mice some of which also received 50 ul of anti-asialoGM1 antibody. The recipient mice were monitored at two week intervals to detect donor derived immunoglobulin in the serum. Four months post transplant, they were immunized with S. pneumoniae vaccine, bacterial levan, group A Streptococcal vaccine or, NP-CG. Reconstitution of responses to (i) the PC determinant of S. pneumoniae with the expression of the T15 idiotype, (ii) the β2,1 linkages of bacterial levan and (iii) the GlucNAC determinants of group A Streptococcus were observed only in the anti-asialoGM1 treated and not in the untreated recipients. The primary IgM response to NP-CG in both groups of mice was normal but they failed to mount an appreciable secondary IgG response to this antigen. It is postulated that depletion of NK cells in the recipient SCID mice allowed the emergence and establishment of B cell specificities that failed to reconstitute in previous experiments. This work was supported by NIH grants AI30879, AI23694 and CA13148.

D 107 BLOCKING OF MIXED LYMPHOCYTE REACTION BY SPLEEN CELLS FROM TOTAL LYMPHOID IRRADIATED MICE INVOLVES INTERRUPTION OF THE IL-2 PATHWAY. Elizabeth H. Field*‡ and Gail C. Becker*. *University of Iowa College of Medicine and ‡Department of Veterans Affairs Medical Center, Iowa City, IA 52242.

Treatment with Total Lymphoid Irradiation (TLI) prior to organ transplantation results in high incidences of donor specific tolerance. However, the exact mechanism of how TLI induces or maintains tolerance is not known. In many experimental systems of tolerance, lack of IL-2 plays a central role in tolerance induction, as stimulation of immunocompetent cells with antigen in an insufficient IL-2 environment results in tolerance. To examine whether tolerance induction by TLI involves the IL-2 pathway, we examined how TLI cells affect the ability of immunocompetent cells to produce IL-2 and express IL-2 receptor in an *in vitro* model of tolerance, the MLR (mixed lymphocyte reaction). Responder cells from MLR cultures in which cells from TLI treated mice were added proliferated 50-70% less and produced 68-94% less IL-2 than responder cells from control cultures. However, co-culture of TLI cells into MLRs did not alter IL-2 receptor expression on responder cells, as measured by two color FACS analysis. We found no evidence that TLI cells deleted MLR responder cells. Interestingly, exogenous IL-2 did not restore proliferation of MLR responder cells. These results suggest that TLI may induce tolerance by interrupting the IL-2 pathway in immunocompetent cells. Moreover, that exogenous IL-2 failed to restore immunocompetence suggests that tolerance in the TLI model may be easy to induce and very stable and provides rationale for the high incidences of donor specific tolerance after TLI treatment.

D 108 **Bone Marrow Veto Cells Mediate Clonal Deletion of Precursor Cytotoxic T Lymphocytes,** Ronald E. Gress and Kiyoshi Hiruma, Experimental Immunology Branch, NCI, NIH Bethesda, MD 20892.

Veto cell mediated suppression of CTL responses has been proposed as one mechanism by which self tolerance is maintained in mature T cell populations. We have reported that murine bone marrow cells cultured in the presence of high-dose IL-2 (activated bone marrow cells, ABM) mediate strong veto suppressor function in vitro and enhance allogeneic T cell-depleted marrow engrafts in vivo. We found that fresh bone marrow (BM) cells from athymic NCr-nu mice (H-2^d), also mediated strong veto cell activity. To examine mechanisms by which these veto cell population in BM suppress precursor CTL (p-CTL) responses, we used as a responding cell population spleen cells of transgenic mice expressing at high frequency TCR specific for H-2 L^d; these cells were stimulated by H-2 L^d antigens in MLC. Flow cytometric analysis was performed by staining the responding cell population in MLC with the mAb 1B2 specific for the transgene-encoded TCR and determined changes of 1B2 positive T cells. Such experiments demonstrated that the anti-H-2^d cytotoxic response by these cell populations was specifically suppressed by H-2^d ABM and NCr-nu BM, and that 1B2 positive p-CTL were in fact deleted from the responding cell population by incubation with veto cells expressing the target antigen. Veto cells therefore exert their effect by clonal deletion of p-CTL.

D 109 REDUCTION IN THE SEVERITY OF GVHD BY SELECTIVE DEPLETION OF DONOR T CELL RECEPTOR V_β POPULATIONS REACTIVE WITH THE HOST. Frances T. Hakim, Susan M. Payne and Gene M. Shearer, Experimental Immunology Branch, NCI, NIH, Bethesda, MD 20892.

One goal of transplantation research has been to selectively remove donor T cell populations reactive with the host, in order to minimize graft-vs-host disease (GVHD), while enhancing immune functional recovery. A murine model of allogeneic transplantation of bone marrow and lymph node cells, matched to the host at major histocompatibility loci but disparate at minor loci, was used to analyze the T cell receptor V_β populations involved in GVHD. In Mls disparate transplants, selective expansion of elements of the splenic T cell receptor V_β repertoire indicated that Mls reactivity might play an important role in early GVHD. One week after transplantation of BALB/c (H-2^d, Mls^c) cells into irradiated DBA/2 (H-2^d, Mls^a) hosts (BALB/c→DBA/2), 65% of the CD4 and 29% of the CD8 splenic T cells expressed the Mls^a-reactive $V_{\beta 6}$ and $V_{\beta 8.1}$ T cell receptors (compared with 16% and 7% respectively in syngeneic BALC/c transplants). T cells expressing V_β subpopulations not reactive with Mls^a ($V_{\beta 14}$ and $V_{\beta 8.2}$), however, were reduced in the BALB/c→DBA/2 population as compared with the syngeneic transplant. To assess the dependence of GVHD upon Mls-reactivity, $V_{\beta 6}$ and $V_{\beta 8.1,2}$ expressing T cells were removed from the BALB/c donor inocula by antibody and magnetic bead treatment prior to injection into DBA/2 hosts. More than 90% of BALB/c→DBA/2 mice died by 3-5 weeks after undepleted transplants, but >70% survived to 15 weeks after V_β depleted transplants. In contrast, BALB/c→C57BL/6 grafts (H-2 disparity) produced a marked expansion of donor T cells, but no selective expansion of $V_{\beta 6}$ or $V_{\beta 8.1}$. Furthermore, C57BL/6 hosts died at 6-10 days, whether or not the donor BALB/c $V_{\beta 6}$ and $V_{\beta 8.1,2}$ subsets had been depleted. These results suggest that it may be possible to reduce the severity of GVHD without removing all donor T cells, thus increasing the rate of post-transplant immune recovery.

D 110 INFLUENCE OF ANTI-INTERFERON-γ MONOCLONAL ANTIBODIES ON GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION IN MICE

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One of the major complications of allogeneic bone marrow transplantation (BMT) is Graft-versus-Host disease (GvHD). This disease is the result of an immunological attack of donor-type T lymphocytes, directed against the recipient tissues. We investigated the influence of inhibition of the cytokine-cascade by an anti-interferon-γ monoclonal antibody (DB-1) on GvHD in a H-2 mismatched murine BMT-model. Recipient mice (C3H/Law; H-2^k) received a bone marrow graft (10^7 BM cells) from C57BL/Rij (H-2^b) donors, after being conditioned with total body irradiation (9 Gy, X-rays). The experimental group was injected i.p. with DB-1 (1.4 mg/mouse) once weekly during the first 6 weeks after BMT, starting on the day of BMT. The control group received a BMT alone. Per group, the survival during the first 80 days after BMT was studied in 10 animals, while an extra number of 8 animals were used for histological confirmation of the results. Of the latter animals, as well as of the animals that died during the experiment, histology was performed on the liver, the spleen, the skin, and on different parts of the gastrointestinal tract.

The 80-day survival of the experimental group was 67% (mean survival: 62 d. ± 6 s.e.m.), versus 30% of the control group (mean survival: 46 d. ± 8 s.e.m.). The difference in survival was significant ($p<0.01$, Kaplan-Meyer). The animals that died during the experiment showed macroscopically signs typical for GvHD. This diagnosis was confirmed by histological examination of the different organs. On histological examination, all animals that were sacrificed during the experiment, showed signs typical for GvHD; being it that some of the lesions observed in the experimental group were less extended and less severe.

From the results it can be concluded that by using the DB-1 antibody, the severity of GvHD is mitigated, but cannot be completely prevented, as was expected. This can be the result of an incomplete inhibition of the T cell response by this monoclonal antibody. *In vitro* experiments seem to confirm this hypothesis. Further studies, using a more potent polyclonal anti-interferon-γ antibody which showed a better *in vitro* activity on the T cell response are underway.

D 111 **Anti-CD3 Monoclonal Antibody Treatment Enhances Engraftment of T cell-depleted Bone Marrow Allografts in Mice,** Kiyoshi Hiruma and Ronald E. Gress, Experimental Immunology Branch, NCI, NIH Bethesda, MD 20892.

In order to investigate the role of CD3 positive T cells in allogeneic marrow rejection in mice and to examine the effects of anti-CD3 monoclonal antibody (mAb) on allogeneic marrow engraftment, a hamster mAb, 145-2C11, with specificity for the CD3ε portion of the murine T cell receptor complex was administered to B6 (H-2^b) mice which had been sublethally irradiated with 626 cGy and injected with $10x10^6$ T cell-depleted B6C3F1 (H-$2^{b/k}$) bone marrow cells. Chimerism status was assessed by flow cytometric analysis of peripheral blood lymphocytes using H-2^k-specific mAb 5-6 weeks after bone marrow transplantation. When hosts were treated with 400μg of anti-CD3 mAb at the time of marrow injection, the percentage of donor-type cells was 75.2±15.0%, while it was 1.9±1.2% in untreated mice. We also found that colony stimulating factor(s) (CSF) was produced in the sera of anti-CD3 mAb-treated mice and that factor-neutralizing antibody inhibited enhancement of engraftment by anti-CD3 mAb stimulated spleen cell supernatants. These results demonstrate that anti-CD3 mAb not only suppressed T cell function, but also induced CSF in host mice, and that enhancement of marrow engraftment in anti-CD3 mAb-treated mice was due to the production of factors as well as suppression of host T cell function.

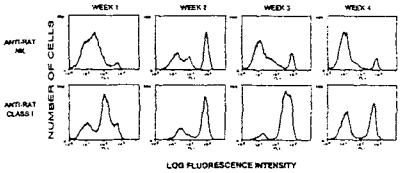
D 112 ACUTE HUMAN VERSUS MOUSE GRAFT-VERSUS-HOST DISEASE IN NORMAL AND IMMUNE-DEFICIENT MICE, W.Huppes, C. Zurcher and D.W. van Bekkum, TNO Institute for Applied Radiobiology and Immunology, Lange Kleiweg 151, P.O. Box 5815, 2288 HV Rijswijk, The Netherlands.

Reports of persistent engraftment of human lymphocytes and myeloid cells in hereditary immune-deficient (h.i.d.) SCID and Bg/Nu/XID mice have raised the question why all attempts to graft human cells into artificially immune-suppressed (i.s.) normal mice have failed so far. We provide evidence that this difference is due to the absence of natural antibodies (Nab) in the mutant mice. We demonstrate that human PBL can be grafted in normal mice i.s. by heavy doses of TBI, provided the transplant is performed when the recipients lack Nab in their serum, eg. as in newborn normal mice, in mice treated with anti-mouse IgM antibody from birth, and in three week old B-cell deficient CBA/N mice. In all cases, large numbers of human PBL were required, i.e. $2x10^7$ cells per gram body weight. Under these conditions acute and fatal GvHD developed in the recipients, regardless whether these were artificially i.s. or h.i.d. The clinical manifestations and the histopathology of this xenogeneic acute GvHD are quite different from those of allogeneic GvHD. The former is primarily confined to the hematolymphoid tissues and locations close to accumulations of proliferating lymphoblasts. The discordant xenogeneic GvHD is induced by T-lymphocytes and can be abrogated by treatment with anti-human T-cell serum. In the latter experiment surviving animals were found to be long term hu-mouse chimaeras.

D 113 THE FULLY XENOGENEIC CHIMERA (RAT→MOUSE) IS AN IN VIVO CULTURE MODEL FOR RAT NATURAL KILLER CELLS EARLY AFTER TRANSPLANTATION. Suzanne T. Ildstad, Traci P. Beck, Marcel Van den Brink, Ashraf Y. Abou El Ezz, Sherry M. Wren, Mary Lynn Hronakes, William H. Chambers. University of Pittsburgh, Department of Surgery, and the Pittsburgh Cancer Institute Pittsburgh, PA 15261.

We have developed a model to induce stable fully xenogeneic chimerism through transplantation of untreated rat bone marrow into lethally irradiated mouse recipients (40 x 10^6 WF rat →B10 mouse). In this model, survival of animals was excellent (85% survive at 120 days), and there was no evidence of GVH disease. We previously characterized production of multiple rat-stem-cell derived lineages and reported that rat-derived NK cells in a xenogeneic environment are present and functional after full repopulation. We have now examined the kinetics of repopulation for rat-derived NK cells. We report here for the first time that the fully xenogeneic chimera is in effect an in vivo culture model for NK development early after bone marrow transplantation. In one color flow cytometric analysis using a species-specific anti-rat NK cell monoclonal antibody (3.2.3), we examined the kinetics of rat NK cell repopulation in mouse recipients early after bone marrow transplantation. Rat-derived NK cells approximated 12.5% - 16.2% of total splenic lymphoid cells at week 1, increased to 48% to 52% at week 2, declined to 21% to 11% by week 3, and normalized to range from 9.7% to 12% by week 6 (Figure 1). The rat NK cells were functional in both YAC tumor lysis and ADCC assays, suggesting that the xenogeneic mouse host environment was sufficient to support the generation, maturation, and function of rat-derived natural killer cells.

KINETICS OF RAT NK CELL REPOPULATION IN SPLEEN OF FULLY XENOGENEIC CHIMERAS



D 114 SELECTIVE DEPLETION OF DONOR NK CELLS IN VIVO DECREASES GVHD WITHOUT LOSS OF GVL REACTIVITY AFTER MHC-MATCHED BONE MARROW TRANSPLANTATION, Bryon D. Johnson and Robert L. Truitt, Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI 53226.

It is thought that natural killer (NK) cells may play a role in graft-vs-host (GVH) reactions after allogeneic bone marrow transplantation (BMT), but the use of NK cell-specific reagents has been limited. An NK allele-specific monoclonal antibody, anti-NK 1.1, was used to study the impact of in vivo donor NK cell depletion on GVH disease (GVHD), graft-vs-leukemia (GVL) reactivity and donor T cell chimerism after allogeneic murine BMT. AKR/J ($H\text{-}2^k$) recipient mice were preconditioned with suboptimal irradiation (9 Gy = LD_{50}) and transplanted with major histocompatibility complex (MHC)-matched B10.BR ($H\text{-}2^k$) BM cells with or without added spleen cells as a source of T cells. The addition of increasing numbers of spleen cells to the BM inoculum produced GVHD of varying intensities. The beneficial effect of NK-depletion on GVHD was dependent on the intensity of the GVH reaction. Donor NK cell-depletion had no effect on the survival of mice with severe GVHD after MHC-matched BMT (B10.BR into AKR) or after MHC-mismatched BMT (B10.BR into DBA/2; $H\text{-}2^k$ into $H\text{-}2^d$). However, donor NK-depletion increased survival of AKR hosts given sufficient B10.BR splenic T cells to induce mild-to-moderate GVHD. Ex vivo depletion of donor $CD8^+$ T cell also reduced GVH-associated mortality, but the use of both CD8 and NK-depletion offered no improvement over either alone, suggesting an interaction between $CD8^+$ and NK 1.1^+ cells. In contrast to CD8-depletion, donor NK-depletion did not compromise the rapid and complete establishment of donor T cell chimerism nor the ability of chimeras to mount an effective GVL reaction. Thus, elimination of donor NK cells may provide an alternate strategy for reducing GVHD without loss of GVL reactivity following MHC-matched allogeneic BMT.

D 115 TUMOR RESISTANCE INDUCED BY SYNGENEIC BONE MARROW TRANSPLANTATION AND ENHANCED BY INTERLEUKIN-2: A MODEL FOR THE GRAFT VERSUS LEUKEMIA/LYMPHOMA REACTION, Larry W.Kwak and Ronald Levy, Division of Oncology, Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305.

Lethally irradiated C3H/HeN mice reconstituted with normal syngeneic bone marrow survived significantly longer than unmanipulated control mice following challenge with a lethal dose of 38C13 lymphoma cells two to three weeks post-bone marrow transplantation (BMT). Although the magnitude of this effect was modest, it was highly reproducible. This resistance producing effect of BMT could be enhanced by interleukin-2 (IL-2) administration and could be abrogated by anti-asialo-GM1 antiserum treatment of recipients. These findings are consistent with the hypothesis that cells with natural killer phenotype are activated by BMT and can mediate tumor resistance. These studies provide a model to dissect the cellular mechanism, independent of donor alloreactivity, of the graft-anti-tumor effect of BMT and, in addition, guide clinical trials exploring IL-2 therapy as a form of immunomodulation post-autologous BMT.

D 116 Selectively Expanded Vβ3+ Donor T-Cells From Acute Bone Marrow Transplant Recipients Can Produce IFN-γ. R.B. Levy, M. Jones, D. Sarapata, D. Perez, B.L. Hamilton, and R. Riley. Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL 33101.

We recently found that a marked and selective expansion of Vβ3+ T-cells occurs within 10-12 days following transplant of allogeneic MHC matched, non-MHC mismatched bone marrow plus T-cells into lethally irradiated Mls1^{b}2*3^a BALB/c, BALB.K and C3H/HeJ recipients when the donor is Mls1^{b}2^{b}3^b. Donor Vβ3+ T-cells rapidly expand (>20 fold) within "GvHR" (allogeneic BM_T plus $3x10^6$ CD3+ allogeneic T-cells) but not "Control" (allogeneic BM_T plus $3x10^6$ CD3+ syngeneic T-cells) recipients to comprise 40-60% of Thy1.2+ spleen and LNC. Examination of IL-2, IL-4, and IFN-γ mRNA following PCR analysis indicated that although IL-2 could be detected in day 4 and 10 GvHR spleen cells using high (i.e. 10^4) cell numbers, IL-4 and IFN-γ mRNA were readily detectable using minimal (10^3) cell numbers at both time points post-transplant in GvHR but not control recipients. Bioassay of these spleen cells for IL-2, IL-4, and IFN-γ at 4 and 10 days indicated little spontaneous production. However, following anti-CD3 stimulation at day 10, although little IL-2 or IL-4 were produced by either "Control" or "GvH" spleen cells, both produced high levels of IFN-γ. In contrast, anti-Vβ3 mAb stimulation failed to stimulate IFN-γ production by "Control" spleen cells but readily stimulated IFN-γ production by "GvH" cells. These results demonstrate: 1) despite the presence of mRNA for IL-2, IL-4, and IFN-γ, there appeared to be little of these cytokine proteins spontaneously secreted by acute GvH spleen cells, and 2) the selectively expanded donor Vβ3+ cells in transplant recipients are clearly functional 10-12 days post transplant as determined by the ability to produce IFN-γ. Thus, despite Mls "superantigen" stimulation of this Vβ TcR family, these cells are neither clonally deleted nor anergic in BMT recipients during the first two weeks post transplant.

D 117 PROCESS-SCALE AND ANALYTICAL CELL SEPARATION WITH BIORECEPTOR FERROFLUIDS AND HIGH-GRADIENT MAGNETIC SEPARATION, P. A. Liberti and Y. Wang, Immunicon Corp., 1310 Masons Mill II, Huntingdon Valley, PA 19006

Recently we reported the development of antibody coated ferrofluids [magnetic liquids which are colloids composed of small ($<0.05\mu$) coated magnetic particles] and novel internal and external high gradient magnetic [HGMS] devices for use in all separations [P. A. Liberti and B. P. Feeley, ACS Symposium Series No. 464, "Cell Separation Science and Technology" Am Chem Soc Wash., DC 1991]. Using microtitre well volumes of cells we demonstrated efficient negative depletion of T-Cell subsets [CD4, CD8+] directly from blood employing a two-step incubation where cells are first incubated with specific MAb followed by incubation with goat anti-mouse Fc ferrofluid [GAMFcFF]. Separation of labelled cells requires three minute HGMS; the entire procedure can be done in ten minutes or less. By comparing this procedure with one employing MAb directly immobilized on ferrofluid, it has been found that the kinetics and efficiency of the two-step incubation procedure are remarkably better. Further, for the latter unbound MAb in the first incubation surprisingly need not be removed prior to addition of GAMFcFF. The economics and convenience of using common capture ferrofluids as well as the kinetics and simplicity make the two-step approach the method of choice. We have employed this approach in scaling up the separation process and have developed batch [15ml] and flow through internal gradient cell separation devices which can remove 10^8 - 10^9 cells and are suitable for negative and positive depletions. As above, separations can be performed in 10-15 minutes or less. Cell recoveries range from 60-85% and viability is excellent. With improvements in ferrofluid chemistry which permits use of lower levels of labelling MAb and capture ferrofluid as well as improvements in device design and methodology it is anticipated that 95% positive cell recovery can be routinely obtained. The use of these methods for analysis of cells and cell surface receptors will also be described.

D 118 LYMPHOID RECONSTITUTION FROM MATURE T CELL POPULATIONS AFTER SYNGENEIC BMT, Crystal L. Mackall and Ronald E. Gress, Experimental Immunology Branch, NCI, NIH, Bethesda, MD 20892.

Previous studies have emphasized the role of marrow (prethymic) T cell precursors in the generation of peripheral T cell populations following BMT. In order to investigate the role of mature T cell populations in immune reconstitution after BMT, the source and phenotypic characteristics of reconstituting T cells were studied in lethally irradiated mice receiving syngeneic T cell depleted (TCD) BM containing Thy congenic lymph node cells. In thymectomized mice given 5 x 10^5 lymph node cells and TCD marrow, the majority of reconstituting T cells were lymph node derived, in contrast to thymus bearing mice in which T cells were nearly entirely bone marrow derived. Lymph node derived cells were exclusively αβ T cell receptor (TCR) positive with normal Vβ family diversity; expansion was not due to a graft-vs-host reaction. TCD bone marrow derived cells in thymectomized mice were both αβ and γδ TCR$^+$ and were predominantly CD4$^+$ cells. During the time of greatest expansion in T cell number, both populations displayed evidence of increased numbers of activated (H1.2F3 positive) cells and loss of CD45RB expression in the CD4$^+$ subset consistent with activation. This work provides evidence for the existence of mature (lymph node derived) progenitor cells that are capable of expanding into a diverse population after BMT and which under certain conditions may be the predominant source for T cell reconstitution. As well, the data suggests that polyclonal activation of T cells may be important in the peripheral expansion of these T cell populations.

D 119 MECHANISMS INVOLVED IN TOLERANCE AFTER HAPLOCOMPATIBLE T CELL DEPLETED BONE MARROW TRANSPLANTATION (TCD-BMT). Anna Merino, Yaara Dror, Mallika Benkerrou, Beth Colombe and Morton J. Cowan. Division of Pediatric Bone Marrow Transplantation, University of California, San Francisco, CA 94143-0105.

We have evaluated the engraftment and the development of tolerance in 14 recipients of haplocompatible parental TCD-BMT, 11 for the treatment of severe combined immunodeficiency (SCID), 2 for leukemia and 1 for Wiskott-Aldrich syndrome. All donor/recipient pairs were at least 2/6 HLA antigen disparate and mixed lymphocyte culture (MLC) reactive pre BMT. No patient had GVHD>grade I and all were more than 1 year post BMT. 10/14 patients developed a split lymphoid chimerism with donor T cells and host B cells and monocytes. Both leukemic and 2 SCID patients were full chimeras with donor T, B and monocytes. We performed MLC using purified responder cell populations: engrafted T cells all of donor origin (T_{eng}) or freshly isolated donor T cells (T_{don}), and recipient irradiated (*) stimulator cell populations which were T depleted (E^-). In the split chimeras the T_{eng} cells had low or no responses in MLC to HLA mismatched E^- recipient cells compared to fresh T_{don} cells. The data are expressed as % of maximum response to a 3rd party stimulator (m±SD):

	$T_{don}xE^-rec^*$	$T_{eng}xE^-rec^*$	p
Full (4)	7±4	6±3	>0.2
Split (10)	158±24	17±6	<.001

However, in 4 of 8 of the split chimera patients the addition of small numbers ($5x10^3$) of freshly isolated T_{don} cells resulted in an average 2 fold increase ($p<.001$) in the T_{eng} cell response to host E^- cells, even when the T_{don} cells were irradiated. $5x10^3$ T_{don} alone had minimal responses to E^- host cells in the MLC. In 3/3 full chimeras the addition of fresh T_{don} cells to the MLC had no effect on the response. Phenotyping results of the responding cells were similar to control MLC and there was no evidence for a suppression mechanism of tolerance. Our results are consistent with either deletion of a T cell subpopulation responsive to recipient alloantigens and/or an anergy model where engrafted T cells do not respond to class II MHC antigens because they fail to produce a necessary cytokine.

D 120 T CELL DEPLETION OF HUMAN BONE MARROW AND HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS WITH THE AIS CELLECTOR™ CD3, AIS CELLECTOR™ CD8, AND THE AIS CELLECTOR™ GOAT ANTI-MOUSE. David Okrongly, Margo Peacock, Marisa Tantoco, Arnon Nagler*, Susan Stone, and Thomas B. Okarma. Applied Immune Sciences, Inc., Menlo Park, CA; *Hadassah University Hospital, Jerusalem, Israel.

The AIS CELLector™ technology incorporates a polystyrene surface which has been chemically modified to covalently attach monoclonal antibodies or other receptors capable of binding to specific cell surface antigens on subpopulations of blood and bone marrow cells. We have evaluated AIS CELLectors for their ability to deplete CD3+ and CD8+ cell subsets from human peripheral blood mononuclear cells (PBMC) and human bone marrow mononuclear cells (BMMC). A cell load of $4x10^9$ PBMC was processed serially in two 3000 cm^2 AIS CELLector CD3, AIS CELLector CD8, or AIS CELLector Goat anti-Mouse devices. Anti-CD3 and anti-CD8 monoclonal antibodies were used to coat and target the CD3+ or CD8+ cell subsets from PBMC and BMMC for capture by the AIS CELLector Goat anti-Mouse. With PBMC, the AIS CELLector CD3+ gave a 1.4 log reduction of CD3+ cells; the AIS CELLector CD8+ gave a 1.6 log reduction of CD3+ CD8+ cells. Results with the AIS CELLector Goat anti-Mouse device were similar to those obtained by the direct capture method of T depletion. BMMC processed by the devices showed similar T cell depletion as that attained with PBMC, with corresponding enrichment of CFU-GM, CFU-GEMM, and BFU-E proportional to the amount of T cell depletion.

D 121 DIFFERENT SUBSETS OF T CELLS IN THE ADULT MOUSE BONE MARROW (BM) AND SPLEEN INDUCE OR SUPPRESS ACUTE GRAFT VERSUS HOST DISEASE, Varghese Palathumpat, Sussan Dejbakhsh-Jones, Bari Holm, and Samuel Strober, Department of Medicine, Division of Immunology & Rheumatology, Stanford University School of Medicine, Stanford, CA 94305

Fractionation of normal adult mouse spleen or BM cells (C57BL/Ka) was performed by discontinuous percoll density gradients. The fractionated low density C57BL/Ka spleen cells completely suppressed acute lethal GVHD when coinjected with unfractionated C57BL/Ka spleen cells into sublethally irradiated BALB/c mice but the high density spleen fractions induced acute GVHD. All the BM fractions protected the BALB/c recipients but unfractionated BM cells showed only modest protection. The low density fractions of both BM and spleen cells had a marked depletion of typical $\alpha\beta TcR^+CD4^+$ or $CD8^+$ T cells, and a predominant population of $\alpha\beta TcR^+CD4^-CD8^-$ T cells. Purified populations of the latter cells suppressed GVHD. The protected recipients were chimeric.

Unfractionated BM and spleen cell mixtures (1:1), or high density fractions of these cells induced acute GVHD in lethally irradiated BALB/c recipients. In contrast, low density fractions of BM and spleen cell mixtures reconstituted the irradiated hosts. BALB/c mice injected with the BCL_1 B cell leukemia/lymphoma were lethally irradiated and transplanted with unfractionated BALB/c or C57BL/Ka BM and spleen mixtures or low density fractions of C57BL/Ka mixtures. The survival of BALB/c mice given BCL_1 cells, irradiation, and a low density fraction of the C57BL/Ka mixture was markedly prolonged as compared to those recipients given unfractionated allogeneic or syngeneic mixtures. Similar results were obtained with leukemic C57BL/Ka x BALB/c F_1 hybrid mice. Thus, the low density fraction fails to induce acute lethal GVHD, but retains graft versus leukemia activity.

D 122 *CYTOKINES VERSUS CYTOTOXIC* T LYMPHOCYTES (CTL) IN THE PATHOGENESIS OF ACUTE GRAFT-VERSUS-HOST DISEASE (GVHD), Robertson Parkman, Carl Lenarsky, Bernadette Barrantes, Reynaldo Santos and Kenneth I. Weinberg, Division of Research Immunology/Bone Marrow Transplantation, Childrens Hospital Los Angeles, Los Angeles, CA 90027

The classic hypothesis is that acute GVHD is due to CTL with specificity for histocompatibility antigens uniquely expressed on recipient cells. Recent evidence from both animal models and human transplant recipients has implicated a role for cytokines in the pathogenesis of acute GVHD. To determine the role of cytokines in acute GVHD, recipients of histocompatible, T cell depleted haploidentical, and matched unrelated donor (MUD) transplants were assessed for cytokine production. Plasma cytokine levels were determined by ELISA assays, and leukocyte cytokine by reverse polymerase chain reaction (PCR) to detect cytokine RNA. Patients with acute GVHD were compared to patients without GVHD (both autologous and allogeneic transplant recipients) and to the same patients after the resolution of their acute GVHD.

Patients were assessed for *IL-1α, IL-1β, IL-2, IL-4, IL-6,* TNF-α, TNF-β and γ-INF. Cytokines were demonstrated in the serum of all MUD patients with acute GVHD: IL-1α, 6/6; IL-2, 0/6; IL-4, 0/6; IL-6, 2/6; TNF-α, 1/6; and γ-INF, 6/6. Cytokine RNA was detected in the same patients' leukocytes: IL-1α, 5/6; IL-1β, 5/6; IL-2, 0/6; IL-4, 0/6; IL-6, 2/6; TNF-α, 5/6; TNF-β, 0/6; and γ-INF, 2/6. The two assays were confirmatory for the presence of IL-1α, TNF-α, and γ-INF in patients with acute GVHD, while IL-2 and IL-4 were found in no patients. Thus, cytokines derived from either monocytes or T lymphocytes may have an important role in the pathogenesis of acute GVHD.

D 123 ENGRAFTMENT AND GRAFT-VS-HOST DISEASE AFTER SEQUENTIAL AUTOLOGOUS/ALLOGENEIC BONE MARROW TRANSPLANTATION IN PATIENTS WITH LYMPHOMA, D. Przepiorka, S. Giralt, M. Andreeff, Y. O. Huh, M. Luna, C. Reading, M. Thomas, R. E. Champlin, University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030

In several animal models, T-cell-depleted autologous marrow has been used successfully as a source of natural suppressor cells to prevent GVHD following subsequent transplantation of allogeneic marrow. We report 2 patients with relapsed lymphoblastic lymphoma treated using this approach without other posttransplant immunosuppression. The patients received cyclophosphamide 60 mg/kg IV daily X 2 and total body irradiation 2.0 Gy twice daily for 6 fractions (total dose 12.0 Gy) followed on Day 0 by infusion of autologous marrow depleted of cells positive for CD2, CD3, CD4 and CD8 by immunomagnetic separation. On Day 8, unmanipulated marrow from an HLA-matched sibling was infused. The ratio of allogeneic to autologous cells infused was 22:1 for Patient 1 and 6:1 for Patient 2. No additional immunosuppressive drugs were administered initially. Patient 1 developed Gr 2 GVHD with onset on Day 14. On Day 20, the peripheral blood lymphocytes (PBLs) were positive for CD2, CD3, CD8, CD38 and DR by flow cytometry and were 95% donor in origin by RFLP. The absolute lymphocyte count (ALC) decreased when the patient was treated with high-dose steroids for GVHD but remained donor in origin when last tested on Day 46. Patient 1 had an absolute neutrophil count (ANC) of 112 on Day 29, and the marrow was >95% donor in origin by RFLP. GM-CSF was started, and the ANC was >1000 by Day 39. He was never transfusion-independent and required GM- or G-CSF to support engraftment through Day 89 when he died with disseminated Aspergillus. Patient 2 developed Gr 3 GVHD with onset on Day 11. The skin rash resolved with high-dose steroids, but the bilirubin continued to rise. A liver biopsy showed ductopenia. Administration of cyclosporine was followed by exacerbation of the hyperbilirubinemia. On Day 20, the PBLs had the same phenotype as above and were 97% donor in origin by in situ hybridization (ISH) for the Y chromosome. Patient 2 had an ANC of 60 on Day 28, and the marrow was 98% donor in origin by ISH. G-CSF was given, and the ANC was >1000 by Day 36. Off G-CSF, the ANC was 8118 on Day 53, and the marrow was still 98% donor-positive by ISH, but she remained transfusion-dependent until death on Day 58 from a GI bleed. At autopsy, evidence of both GVHD and VOD was present in the liver. This experience indicates that sequential autologous/allogeneic marrow transplantation is feasible in man, but this approach alone is not sufficient to prevent acute GVHD.

D 124 ROLE OF THE CD4+ CELLS IN GVHD: GENERATION OF CD4-DEFICIENT MICE BY GENE TARGETING

A.Rahemtulla, A.Arabian and T.W. Mak. The Ontario Cancer Institute, Toronto, Ont. Canada M4X 1K9.

Preliminary evidence from studies using antibodies to the CD4 molecule suggest that the $CD4^+$ cells are involved in anti-class II Graft versus Host disease. This role of the $CD4^+$ cells would become more clear if we were to mutate the CD4 gene in the germ line and generate a strain of mice that did not have any $CD4^+$ helper T cells. It is possible to mutate the mouse CD4 gene in the pluripotent embryonic stem (ES) cells through a targeted mutation of the CD4 gene by homologous recombination. We have achieved this by electroporating ES cells with a genomic construct of the CD4 gene in which the coding region is disrupted by insertion of the neomycin resistance gene. The G418 resistant ES cell colonies were screened by the polymerase chain reaction (PCR) to detect those that had undergone a homologous recombination event. We have established eight independent ES cell lines in which the CD4 gene has been mutated. We have generated 3 independent strains of mice that do not express CD4. Functional studies have shown that these mice have markedly reduced helper function and further studies to characterize these mice are under way. These mice are being back-crossed to C57Bl/6 and C57Bl/10 backgrounds so that bone marrow transplantation experiments could be carried out in these animals. Mice lacking $CD4^+$ cells would provide important information about the role of these cells in the pathogenesis of Graft versus Host disease.

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D 125 ADDITION OF FOLINIC ACID TO A METHOTREXATE/CYCLOSPORINE REGIMEN FOR PREVENTION OF ACUTE GRAFT-VERSUS-HOST DISEASE, J.A. Russell, A.R. Jones, M.C. Poon, R.C. Woodman, B.A. Ruether, Foothills Hospital, Calgary, Alberta, Canada.

When used to prevent acute graft-versus-host disease (GvHD) after bone marrow transplantation (BMT) methotrexate (MTX) may exacerbate myelosuppression and stomatitis. Its toxicity may be potentiated not only by renal dysfunction but also by Trimethoprim/Sulfamethoxazole (TMP/SMX). Folinic Acid (FA) reverses the effect of both MTX and TMP/SMX in human cells and is often used to allow the safe administration of high doses of MTX. The present study was to ascertain whether FA allows the administration of all doses of MTX without increasing the incidence of GvHD.

Sixty patients (pts) aged 18-54 years (median 37) who engrafted after a first BMT from a sibling or unrelated donor (6 pts) were studied. They received MTX 15 mg/m² on D +1, 10 mg/m² on D +3, +5 and +11. Cyclosporine A (CSA) 2.5 mg/kg i.v. or 6.25 mg/kg p.o. b.i.d. was started on D -1 and adjusted thereafter to therapeutic levels. FA 5 mg q.6 h. p.o. or i.v. was given 24 h. after each MTX dose until 24 h. before the next dose. After D +12 FA was continued until granulocyte count > 0.5×10^9/L or discharge from hospital. All patients receiving sibling BMT received TMP 160 mg and SMX 800 mg p.o. twice daily. The 6 unrelated BMT pts were given twice weekly TMP/SMX. No MTX doses were omitted because of renal dysfunction or severe stomatitis. Grade II to IV acute GvHD occurred in 19 pts (32%) and contributed to death in one. The incidence of GvHD is similar to reported rates using MTX/CSA as prophylaxis. We conclude that FA does not seem to abrogate the effect of MTX in preventing GvHD and that it allows the administration of full doses of MTX. The degree to which it may help prevent stomatitis and accelerate engraftment could only be established in a randomized study.

D 126 DEMONSTRATION OF A BONE MARROW PRECURSOR OF THE T-CELL IN ANTI-TCR Vß MAb TREATED TCRVß8.2 TRANSGENIC MICE. Smith, R.T., Lincoln, G., Sheng, B.D., Odebralski, J. Whisenant Tumor Biology Unit, Dept. of Pathology, U. of Florida HSC., Gainesville FL, 32610.

TGß transgenic mice suppress rearrangement of all other Vß and some Vr genes in constructing functional TCR molecules (Von Boehmer, et. al. 1989). In order to determine whether this process was reversible by cross linking the TG TCR, purified MAb to the transgene product was administered in large amounts to TGVß8.2 mice. When IP injection was started at birth, 100 mgc./week, complete deletion of all T-cells occurred, and partial depletion lasted up to 16 weeks. 7-12 days after last injection at 5 weeks, a population of cells was found first in the BM, and then in PBL having the phenotype TCRVß8.2+, Thy1-, B220-, T200+, TCRr-, Sca-1+, CD3-, CD4-, CD8-, FcR2a-. An average of 8% (range 2-17%) of BM cells were found of this type, over a 5-14 day period after cessation of injection, after which the level rapidly returned to the low but detectable levels found in normal TCRVß transgenic mouse BM. These putative T-stem cells were sorted by FACS and placed in two syngeneic thymic environments to determine whether they would undergo normal T-cell development. In (a) syngeneic C57L mice, introduced by intrathymic injection in vivo, and (b), in culture with a thymic stromal cell line, in vitro, they developed in sequence over a 3-7 day period, with the TGVß8.2+, the thymus phenotypes Thy-1+, HSA+, CD3+, CD4+/8+, and CD8+/4-, CD8-/4+.

Normal TCRαß rearrangement occurs only after entry into the thymus. Therefore, these findings indicate that TCRVß transgene expresses in a T-cell precursor generated in the BM. In this model, the TG appears to provide a marker for a T-stem cell.

D 127 IMMUNOLOGIC EFFECTS OF PROLONGED INFUSION OF IL-2 FOLLOWING ALLOGENEIC AND AUTOLOGOUS BMT. R.J. Soiffer, C. Murray and J. Ritz. Dana-Farber Cancer Institute, Boston, MA 02115

Experimental and clinical studies have shown that immunologic factors play a role in preventing relapse post-BMT. This phenomenon has been termed the graft-versus-leukemia (GVL) effect. IL-2 has been shown to induce GVL in vitro but high dose IL-2 administration is often associated with significant toxicity in vivo. We have conducted a clinical trial to assess the immunologic effects of rIL-2 given by continuous infusion at very low doses for 3 months post-BMT. 22 pts (12M, 10F, ages 22-50y) received rIL-2 at doses of 2-4 x 10^5U/m²/day. 11 pts received autologous marrow, 10 CD6-T cell depleted allogeneic marrow, and 1 syngeneic marrow. IL-2 was infused through an indwelling catheter with a portable pump in an outpatient setting, beginning a median of 73 days post-BMT. Infusions were generally well tolerated and only one pt required hospitalization for rIL-2 related toxicity. Side effects included: fatigue (9 pts), weight gain (7 pts), cough (6 pts), nausea/vomiting (5 pts), fever >39° C (4 pts), rash (3 pts), thyroid dysfunction (3 pts) and hypotension (2 pts). No pts developed azotemia, jaundice, pulmonary capillary leak syndrome or GVHD. Platelet counts fell by >25% within one week in 16/22 pts but then stabilized. All pts developed marked eosinophilia. At least a 2-fold increase in circulating lymphocytes occurred in 20/22 pts and NK cells ($CD56^{bright}$+CD16+CD3-CD8+) became the predominant circulating lymphoid subset in all pts. After 3 months of therapy, the median NK cell count increased 15-fold. Cytolytic activity against NK sensitive (K562) and NK resistant (COLO) tumor cells increased in all pts tested and was markedly enhanced after overnight incubation with additional rIL-2 in vitro. Effects of rIL-2 were similar following either autologous or allogeneic BMT. We conclude that prolonged infusion of low dose rIL-2 can be safely administered post-BMT and can selectively increase NK cell number and activity without inducing GVHD. Further studies to assess the impact of these immunologic effects on disease relapse post-BMT should be undertaken.

D 128 PRETRANSPLANT ASSESSMENT OF HUMAN MINOR HISTOCOMPATIBILITY ANTIGEN-REACTIVE MEMORY AND NATIVE INTERLEUKIN-2-SECRETING T HELPER CELL PRECURSOR WITHIN CLASS I MHC-RESTRICTED $CD8^+$ AND CLASS II MHC-RESTRICTED $CD4^+$ T CELL SUBSETS. Matthias Theobald, Donald Bunjes and Renate Arnold, Department Internal Medicine III, BMT-Unit, Ulm University Hospital, D-7900 Ulm, F.R.G.

Nine HLA-identical MLC-negative sibling donor-host-pairs were investigated for pretransplant expression of anti-donor and anti-host mH-reactive IL-2-secreting T helper cell (Th) function. Anti-donor mH-reactive host-Th-precursor (Th-p) (HvG-direction) occurred in 6/9 sibling pairs (f: 1/906 - 1/31.113), whereas anti-host mH-reactive donor-Th-p (GvH-direction) were detectable in lower frequency (f: 1/18.049 - 1/53.882) among 5/9 pairs. Since 8/9 recipients suffering from acute leukemia have been previously transfused with multiple blood products, this difference may in part result from in vivo priming of the host. In fact, circulating anti-donor mH-reactive host-Th-p were enriched within the $CD45RO^+$ memory T cell subset as compared to naive $CD45RO^-$ T cells. In contrast, unprimed anti-host mH-reactive donor-Th-p as well as allo-MHC-reactive normal control Th-p occurred in almost equal frequencies within $CD45RO^+$ and $CD45RO^-$ T cell subsets. Extensive specificity analyses sustained the specificity (90%) of responding Th-p for mH-antigen recognition. Both, $CD8^+$ and $CD4^+$ T cells contributed in substantial frequency and exclusive specificity to mH-reactive Th-p. Based on panel studies and inhibition experiments with appropriate monoclonal antibodies, the analysed mH-specific $CD8^+$ Th-p appeared to be class I MHC-restricted whereas mH-specific $CD4^+$ Th-p operated in a class II MHC-restricted fashion.

D 129 ACUTE GRAFT-VERSUS-HOST DISEASE INDUCED BY HUMAN CD56(+),CD16(+),CD8(+),CD3(-) CELLS IN SCID MICE. JS Thompson, CQ Xun, SA Brown, CD Jennings, VA Medical Center and Department of Medicine, University of Kentucky, Lexington, KY 40511.

To study the effect of NK cells and T cells on the induction of aGVHD, we isolated NK and T cells from human peripheral blood by complement-mediated lysis with either group A antibodies (T10B9/CD3,TCR + T12A10/CD6) or group B antibodies (Go22/CD16 + Leu19/CD56) respectively. Each isolated cell group was pulsed for 16 hours with ionomycin and phorbol 12,13-dibutyrate followed by continuous culture for 2 weeks with rIL-2. The phenotype and NK activity were assayed before IV injection into 3-4 month old SCID mice previously irradiated with 400 rad. The phenotype of group A treated cells was CD16(+)65%,CD56(+)52%,CD8-dim(+)59%, CD4(+)5.6%,CD3(+)14% and specific lysis to K562 target cells was 84.5%(1:40),95.9%(1:20),96.4%(1:10) and 59.0%(1:4). The phenotype of group B treated cells was CD3(+)97.7%,CD4(+)42.5%,CD8(+)39%,CD16(+)5% and CD56(+)8.6%. Minor to severe skin lesions, diarrhea and weight loss appeared 24 hours to 10 days after injection of $1x10^6$,$1x10^7$ or $5x10^7$ group A cells, but not group B cells nor in control mice (400 rad, no cell injection). The mice were sacrificed by day 12 if they did not die. Histological examination of group A mice revealed cellular infiltration, crypt or single cell necrosis and vili architecture disorder in gastrointestinal tissue; epithelial necrosis and cellular infiltration in the skin; nodular proliferation of small lymphocytes underneath the capsule of spleen. These findings were not present in group B cell injected animals. Thus, in this SCID mice animal model, human CD16(+),CD56(+), CD8(+),CD3(-) cells produced aGVHD-like lesions that are seen in clinical aGVHD. This is additional evidence that NK cells, not T cells are the direct effector cells in aGVHD.

D 130 CYTOTOXIC T CELLS (CTC) ACTIVATED WITH OKT3 AND IL-2 PROVIDE HELP FOR IG SYNTHESIS AND EXPRESS mRNA LEVELS FOR LYMPHOKINES AND PERFORIN (CYTOLYTIC PORE-FORMING PROTEIN): IMPLICATIONS FOR IMMUNE RECOVERY. Mikio Ueda, Lawrence G. Lum, Neng-Ren Jin, Indira D. Joshi, Joseph P. Uberti, Frank Martilotti, Ta-Hsu Chou, and Lyle L. Sensenbrenner. Wayne State University and DMC BMT Program, Detroit, MI 48202

The prevention of relapse remains a major clinical problem in BMT. Similar to murine models, we demonstrated that human CTC activated with OKT3/IL-2 have strong non-MHC restricted cytotoxicity against Daudi and K562 and inhibited the development of allospecific cytotoxic lymphocytes similar to the activity of LAK cells maintained in high dose IL-2 (1500 IU/ml) (Uberti et al, Am Soc Hemat Abst, 1991). This study asks whether CTC generated by activating PBL with OKT3 in the presence of IL-2 (50-300 IU/ml) provides help to normal B cells for Ig synthesis or suppresses Ig synthesis by normal T and B cells stimulated by pokeweed mitogen (PW); and whether the activated CTC produce factors (IL-2, IL-3, IL-6, and perforin) that help B cell differentiation or mediate cytotoxic functions. Activated CTC provided help to autologous or allogeneic B cells after PW stimulation as measured by an ELISA-Plaque assay. Activated CTC did not suppress Ig synthesis when cocultured with normal T and B cells after PW stimulation. The helper activity mediated by activated CTC was radioresistant. mRNA levels for IL-2, IL-3, IL-6, and perforin in activated CTC were increased as detected with a reverse transcriptase-PCR procedure. In summary, activated CTC helped B cells produce Ig and did not suppress Ig synthesis by normal T and B cells. The helper activity observed in activated CTC may be due to increased levels of mRNAs for IL-2, IL-3, and IL-6. Additionally, the increased expression of mRNA for perforin may be responsible for the cytotoxicity mediated by activated CTC. The results of our studies suggest that activated CTC may be clinically useful after BMT to mediate a graft-vs-leukemia effect or to accelerate immune reconstitution.

D 131 TOLERANCE INDUCTION IN THE HUMAN T CELL COMPARTMENT OF SCID-HU MICE. Bart Vandekerckhove, Reiko Nawikawa* and Maria-Grazia Roncarolo, DNAX Research Institute, Palo Alto, CA 94304 and *Systemix, Inc., Palo Alto, CA 94304

Animal studies have shown that tolerance induction by clonal deletion and/or anergy occurs predominantly in the thymus. Macrophage (Mϕ) and dendritic cells (DC) are thought to induce tolerance by clonal deletion, whereas thymic epithelium seems to mediate tolerance by clonal anergy. It is unclear whether these findings also apply in man. To address this question we used SCID-hu mice constructed with fetal liver (FL) and fetal thymus (FT) co-implanted under the kidney capsule and obtained from the same or from 2 different donors. HLA phenotyping of the resulting thymus-like organ showed that all thymocytes are of liver origin. In addition, immunohistological studies demonstrated that all DC and most of the Mϕ are of FL origin, whereas thymic epithelium remains of FT origin. Thymocytes or human T cells in the peripheral blood of SCID-hu mice transplanted with donor A (FL_A/FT_A animals) did not proliferate upon stimulation with the autologous B cell line (B-LCL), but responded vigorously upon stimulation with various allogeneic B-LCL. Furthermore, thymocytes or human T cells from the blood of SCID-hu mice transplanted with fetal liver and fetal thymus from 2 different donors (FL_A/FT_B animals) are nonresponsive to A and B B-LCL, whereas the proliferation against allogeneic B-LCL was normal. No suppressive activity was observed in mixing experiments. However, limiting dilution analysis of $CD4^+$ or $CD8^+$ thymocytes activated with PHA and expanded in IL-2, showed specific reactivity against the HLA antigens expressed on the B-LCL derived from the thymus donor. The frequencies of these cells was 1.5 to 3 times lower than those observed for T cells reactive against allogeneic B-LCL. Collectively, these data indicate that human T cells become tolerant for the HLA antigens expressed in the thymus in which they mature. This tolerance is not or only partially due to clonal deletion, suggesting that clonal anergy may play a role.

D 132 ADHESION MOLECULE-MEDIATED EFFECTOR-EFFECTOR INTERACTIONS REGULATE THE LYTIC ABILITY OF LAK CELLS.

°Andrea Velardi, °Ricciarda Galandrini, °Nicola Albi, *Adelmo Terenzi, °Fausto Grignani, *Massimo F. Martelli, and +Carlo E. Grossi. Depts of °Medicine and *Hematology, Univ. of Perugia and +Anatomy, Univ. of Genova, Italy.

Adhesion molecules are known to play a central role in effector cell to tumor target adhesive interactions. In addition, adhesion molecule-mediated signals between T lymphocytes and APC are known to promote T-cell proliferation. Here, we show that adhesion molecule-mediated homotypic interactions among effector cells can regulate the cytolytic functions of CD3+ and CD3- LAK cells. Pre-treatment of effectors with Ab to LFA1 and LFA3, or to ICAM1, downregulated or, respectively, upregulated the cytolytic ability of CD3- LAK cells or CD3+/CD8+ cytolytic clones against tumor targets. In order to focus on effector-effector homotypic interactions and avoid interference on the effector to target cell adhesion, targets were selected according to their negativity for the complementary ligands, e.g. anti-LFA3-treated effectors were tested against CD2- targets and anti-LFA1-treated effectors were tested against ICAM1-/ICAM2- targets. Therefore, interactions occurring among cytolytic effectors are able to modulate their lytic ability against tumor targets.

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R. Galandrini is a recipient of a fellowship from "Associazione Italiana per la Ricerca sul Cancro".

D 133 RABBIT ANTI MOUSE BRAIN IS NOT A T CELL DEPLETING AGENT Sherry M. Wren, Mary Lynn Hronakes, and Suzanne T. Ildstad. University of Pittsburgh, Department of Surgery. Pittsburgh, PA 15261.

Rabbit Anti Mouse Brain (RAMB), has been utilized as an agent for T cell depletion since it was first described in the 1950's. It is a polyclonal antisera prepared by immunizing rabbits with homogenized mouse brain. RAMB binds to the theta (θ) antigen on T cells and is capable of mediating antibody and complement(C') depletion. It is cross-reactive on a number of other cell types which are Thy 1 positive. RAMB has been frequently utilized in animal models of bone marrow transplantation for T cell depletion of bone marrow. We previously reported that RAMB treatment of mouse bone marrow removes cells which facilitate allogeneic stem cell engraftment in lethally irradiated recipient mice. Classically, RAMB activity has been assessed in vitro by documentation of loss of Con A mitogenic responses after RAMB and C' treatment.

We have now performed flow cytometric analysis of both bone marrow and spleen after treatment with RAMB and guinea pig complement (GPC') to determine which cellular subsets are depleted by RAMB treatment. In both spleen and bone marrow there was no significant reduction in the T cell population (CD4, CD8, and Thy1.2 positive cells) after RAMB+GPC' treatment (Table 1). However, when the same RAMB+GPC' treated spleen cells were tested in Con A assays there was total abrogation of the Con A response. In striking contrast, the allo MLR response was intact. These data suggest that RAMB may be depleting an Antigen Presenting Cell (APC) since there was loss of the Con A response in RAMB+GPC' treated splenocytes but full preservation of the anti-allo response when an alternative source of APC and/or cytokines were provided by the stimulator population. Therefore, these data suggest that the phenotype of the cell type which facilitates allogeneic stem cell engraftment is in fact, not a $CD4^+$ or $CD8^+$ cell, but instead a cell which can function a an APC (ie. dendritic cell). Studies are in progress to further characterize the cell phenotype.

	RELATIVE PERCENTAGE T CELLS		
BONE MARROW TREATMENT	CD4	CD8	THY 1.2
UNTREATED	1.6%	2.1%	6.6%
RAMB + GPC'	1.5%	1.7%	4.7%
THY 1 + RC'	0.0%	0.0%	0.0%

Hematopoiesis

D 200 PERIPHERAL BLOOD STEM CELL MOBILIZATION: RAPID ENRICHMENT OF PROGENITOR CELLS USING A UNIQUE BIOTIN-AVIDIN IMMUNOAFFINITY SEPARATION SYSTEM, Ronald Berenson, Robert Andrews, William Bensinger, Barbra Fogarty, Kirsten McGuire, Sheryl Williams and Shelly Heimfeld, CellPro, Inc., Bothell, WA 98021 and Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Treatment of patients with chemotherapy and/or growth factors leads to significant increases in the level of progenitor cells in the blood, which makes possible the use of blood as a source of precursor cells for transplantation. Analyses have been done on peripheral blood samples from baboons being given Stem Cell Factor, and from human patients given G-CSF. Methylcellulose culture indicates a 10-1000 fold increase in colony-forming cell (CFC) progenitors per ml of blood following treatment with either growth factor. We have developed a CD34 antibody and a biotin-avidin column immunoadsorption system to provide rapid enrichment of these progenitor cells. Up to 10^9 white blood cells have been processed on a small 1.5 ml research device, with a total processing time of 2-3 hours. By FACS analysis the separated cells are between 50-90% CD34+. Colony assays indicate a 50-100 fold enrichment for CFC, with >50% recovery of the total CFC activity. These results indicate that the biotin-avidin immunoaffinity system can be used to rapidly enrich for peripheral blood stem cells, both for basic research and for transplantation.

D 201 RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEPO) ADMINISTRATION DOES NOT AFFECT PERIPHERAL LEUKOCYTE COUNTS IN PATIENTS WITH DELAYED ERYTHROPOIETIN AFTER BONE MARROW TRANSPLANTATION. M.R. Bishop, P.J. Stiff, R.S. McKenzie, J.A. Sosman, and D. Koch Loyola University - Chicago BMT Program, Maywood IL.

Anemia (Hb<8.0 gm/dl) requiring RBC transfusions is a common problem post-BMT. Recent data suggests that EPO levels are inappropriately low post-BMT and may not normalize for up to 6 months. Experience in dialysis-dependent patients with anemia demonstrates a decrease in transfusion requirements with supplemental rHuEPO administration. However, there is a theoretical concern that rHuEPO administration may adversely affect leukocyte and platelet production in BMT patients. We undertook a pilot study to determine the potential benefit of rHuEPO in patients requiring RBC transfusions beyond 21 days post-BMT. Six patients (3-allogeneic, 3-autologous) were treated with 150 U/kg rHuEPO t.i.w. for 3 wks. If there was less than 1.0 gm/dl rise in Hgb at the end of this period, the dose was increased to 300 U/kg t.i.w. for 3 wks. Prior to therapy the median EPO level was 154 U/ml (32-298). One patient died from septic shock during therapy.

	Pt Rx* Start Day	Pre Rx WBC	Pre Rx plt	1wk post D/C of Rx WBC	1 wk post D/C of Rx plt
1	+21	0.4	23	1.7	14
2	+22	0.9	10	1.7	38
3	+65	1.4	56	5.0	33
4	+21	1.6	84	3.7	213
5	+30	1.1	12	1.2	5

* Day 0 = day of BM infusion

These data suggest that rHuEPO administration has little effect on peripheral leukocyte or platelet numbers. There were no adverse affects of SQ rHuEPO administration. Data suggests that RBC transfusion requirements were decreased during the period of rHuEPO administration. Platelet transfusion requirements did not change, and patients who were refractory to platelet transfusions prior to Rx remained refractory. These data suggest that rHuEPO can be administered safely and has minimal effect on peripheral leukocyte or platelet number.

D 202 HUMAN HERPESVIRUS SIX (HHV-6) AND BONE MARROW TRANSPLANTATION: SUPPRESSION OF GROWTH FACTOR INDUCED MACROPHAGE MATURATION IN HUMAN BONE MARROW CULTURES. Eileen M. Burd and Donald R. Carrigan. Department of Pathology, Medical College of Wisconsin; Milwaukee, Wisconsin 53226.

Seroprevalence of HHV-6 in adult marrow transplant patients is at least eighty percent, and reactivation of latent HHV-6 is common after transplantation. These reactivations are associated with a variety of clinical manifestations, including interstitial pneumonitis. However, a more common complication associated with HHV-6 infection in these patients is poor marrow function or marrow failure. Patients with this viral syndrome respond poorly to growth factors such as GM-CSF. To explore suppression of marrow cell responses to such growth factors by HHV-6 infection, marrow mononuclear cell cultures were infected with several different strains of HHV-6 immediately prior to the addition of GM-CSF. Infection by all strains of virus suppressed the outgrowth of nonspecific esterase positive adherent macrophages induced by the factors by more than 90%. Nonadherent cell populations in the infected cultures, which were numerically similar to those in uninfected control cultures, contained only rare cells expressing HHV-6 antigens. Infectious virus could not be isolated from the infected marrow cultures, and infectious center assays with infected marrow cells demonstrated that fewer than one cell in ten million was productively infected. Treatment of infected marrow cell cultures with ganciclovir or foscarnet at concentrations sufficient to completely block HHV-6 replication failed to abrogate suppression of macrophage outgrowth in response to GM-CSF. Therefore, full replication of the virus in the marrow cultures was not necessary for the inhibition of the growth factor responses. Preliminary experiments have indicated that alpha, beta and gamma interferons and tumor necrosis factor are not involved. The interference of HHV-6 infection with the responses of marrow cells to growth factors may play roles in both the poor marrow function seen in the patients and in their weak responses to growth factor therapy.

D 203 MOBILIZATION AND TRANSPLANTATION OF PERIPHERAL STEM CELLS IN MICE FOLLOWING IN VIVO IL3 TREATMENT. David A. Crouse, J. Graham Sharp, John D. Jackson, Jim Rogers, Bruce Gordon. Univ. Nebraska Med. Center, Omaha, NE 68198.

Patients with tumor in the marrow or marrow hypocellularity are not candidates for autologous marrow transplantation. The collection of circulating hematopoietic stem cells (HSC) and progenitor cells may be an alternative in these patients and exogenous hematopoietic cytokines may raise the numbers in blood, allowing more efficient harvest and transplantation. The objective of this project was to determine, in a mouse model, if IL3 was effective in promoting the mobilization of HSC and progenitor cells into the blood and if the post-transplantation recovery was improved when such mobilized peripheral stem cells were used for transplantation. We evaluated mobilization during and after cytokine treatment (rmIL3, BioSource; 0.71 μgm, s.q. twice a day for 7 days) in normal BDF_1 mice. The number of progenitors and stem cells were assayed using in vitro assays (Meg-CFC, M-CFC, Eo-CFC, GM-CFC, HPP-CFC, LTC-IC) as well as spleen colony assays (day 8 & 12 CFUs) to allow the assessment of the relative content of more differentiated versus primitive HSC. We found that there were small increases (1.2 to 1.5 fold) in marrow HSC and progenitor cell frequencies with much larger increases observed in the spleen (8 to 30 fold) and the blood (4 to 6 fold). Although both progenitor cells and HSC were elevated in number, the more primitive classes appeared to be relatively enriched in the post-IL3 animals. Lethally irradiated mice transplanted with IL3 mobilized peripheral stem and progenitor cells exhibited more rapid and complete recovery than animals transplanted with unmobilized cells. Enhanced recovery for some endpoints was evident as early as seven days post-transplantation. These experiments support the use of the mouse model to develop rational and efficient in vivo cytokine applications, including combinations for mobilization which may have use in the clinical setting. (Supported by NIH Grant CA46686 and the Edna Ittner Pediatric Research Fund)

D 204 A NEW SERIES OF CD34 MONOCLONAL ANTIBODIES DESIGNED FOR IMMUNOMAGNETIC ISOLATION OF HUMAN AND RHESUS MONKEY BONE MARROW STEM CELLS.

Torstein Egeland and Gustav Gaudernack, Institute of Transplantation Immunology, Rikshospitalet National Hospital, Oslo, Norway.

Isolation of human bone marrow $CD34^+$ cells by means of paramagnetic beads (Dynal) coupled with a monoclonal antibody (Mab) against CD34 is an established technique, but is limited by the availability of suitable CD34 Mabs. High purity $CD34^+$ cells can be recovered from the bead-rosetting population after detachment with anti-murine Fab antibodies (1). We wanted to generate new anti-CD34 Mabs that could a) further increase purity and recovery of human $CD34^+$ cells after isolation and b) that could also be used for isolation of Rhesus monkey $CD34^+$ cells.

A series of hybridomas were generated after hyperimmunization of BALB/c mice with KG1A and were screeened for optimal reactivity in a bead-rosetting assay. After screeening of KG1A-reactive Mabs with a panel of 23 cells/cell lines including HL-60 and HL-60 transfected with the gene encoding for CD34, 8 candidate CD34 Mabs were used for immunomagnetic isolation of $CD34^+$ cells from human and Rhesus monkey bone marrow. Mabs 553-A10, 563-A10, and 581-B9 yielded cell populations that displayed a bright staining with the reference CD34 Mabs 12.8, My10, and BI3C5 in flow cytometry. Beads coupled with 553-A10 and 563-A10 can also be used to isolate Rhesus monkey bone marrow-derived cells. All cells are positive with 12.8 after isolation. The purity and recovery after isolation are comparable to or higher than for cells isolated with other anti-CD34 Mabs.

1. E. Smeland et al, in prep.

D 205 SYNERGISTIC ACTION OF IL-1, IL-6, AND B-FGF ON ABNORMAL BONE MARROW HISTIOCYTES IN A PATIENT WITH HURLER'S SYNDROME (HS) RECEIVING GM-CSF THERAPY UNDERGOING BONE MARROW TRANSPLANTATION. Vincent S. Gallicchio, Nedda K. Hughes, Eric Lang, Michael L. Cibull, and P. Jean Henslee-Downey, Bone Marrow Transplant Program, Departments of Medicine and Pathology, University of Kentucky Medical Center, Lexington, KY 40536-0084.

We investigated a 2-yr old male with HS who received 8 mcg/kg/day GM-CSF for allogeneic bone marrow engraftment. After 3 wks of therapy pre- and post morterm bone marrow samples showed the almost complete replacement of marrow space with abnormal histiocytes demonstrating metachromatic cytoplasmic granules. *In vitro* culture of bone marrow from a second HS patient revealed a GM-CSF dose-related increase in colony formation that peaked at 250 U/ml. Microscopic examination of these colonies revealed the presence of histiocytes identical to what was observed in the patient's marrow. We report additional data that demonstrates these abnormal histiocytes when cultured in the presence of optimal doses of IL-1, IL-6 and B-FGF, the number of histiocyte containing colonies was increased significantly compared to cell cultures with GM-CSF alone. These results indicate GM-CSF, and factors known to act synergistically with GM-CSF, increase the number of abnormal histiocyte derived colonies in culture and highlight the need for caution in the use of these factors in patients with HS and perhaps other metabolic disorders associated with the potential proliferation of abnormal storage cells.

Group	#Histiocyte Colonies
Neg. control	7.7 + 0.6
GM-CSF, 250 U/ml	24 + 3.2
IL-1, 30 ng/ml	39 + 5.8
IL-6, 30 ng/ml	59 + 11.8
B-FGF, 10 ng/ml	45 + 2.1

D 206 LONG TERM RECONSTITUTION OF ALL HEMATOPOIETIC LINEAGES IN HAPLOTYPE MISMATCHED MICE USING PURIFIED BONE MARROW STEM CELLS. Jane S. Lebkowski, Maureen A. McNally, Karla Knobel, Diane Rood, Lydia Kilinski, and Thomas B. Okarma. Applied Immune Sciences, Inc. Menlo Park, CA 94025.

Based on the clinical need to extend allogeneic bone marrow transplantation to recipients with mismatched donors, we examined the capacity of purified murine stem cell populations to reconstitute lethally irradiated haplotype mismatched mice. Murine stem cell populations were purified by cell sorting nonadherent nucleated total bone marrow (TBM) cells for the presence of the antigen Thy1.2 and the absence of lineage-specific antigens B220, Lyt-2, Mac-1, J11D.2, and L3T4 ($Thy1^+Lin^-$). Three types of transplants were performed: $B6D2F_1 \rightarrow B6C3F_1$; $B6C3F_1 \rightarrow B6D2F_1$; and $CByB6F_1 \rightarrow C3D2F_1$. The data show that both TBM and $Thy1^+Lin^-$ enriched stem cell populations are capable of rescuing all three lethally irradiated haplotype mismatched strains of mice for greater than six months. $Thy1^+Lin^-$ sorted stem cells were effective both at hematopoietic lineage reconstitution and rescue from lethal irradiation at doses ten fold lower than TBM cells. 50% survival was achieved with $2.5x10^5$ TBM cells, but required only $2.5x10^4$ $Thy1^+Lin^-$ sorted stem cells in allogeneic recipients. 50% survival was achieved with $5.0x10^4$ TBM cells, but required only $5.0x10^3$ $Thy1^+Lin^-$ sorted stem cells in syngeneic recipients. Both TBM and $Thy1^+Lin^-$ sorted stem cells facilitated the long term engraftment of all hematopoietic lineages. In > 95% of the allogeneic reconstituted chimeras, the majority of the hematopoietic cells was of donor origin. There was no significant change in the percent donor hematopoietic engraftment in mice transplanted with mismatched bone marrow between two and six months post transplant. This data indicates that purified stem cells can be successfully used in murine bone marrow transplants to rescue one full haplotype mismatched recipients.

D 207 NORMAL BLOOD AS A CONVENIENT SOURCE FOR HIGHLY PURIFIED HEMATOPOIETIC PROGENITOR CELLS, Dale Peterson, Dale Kalamasz, Barbra Fogarty, Shelly Heimfeld, Ron Berninger, Ron Berenson, Pat Maloney, Hod Namdaran, and Tony Goffe, CellPro Inc., Bothell, WA 98021

Research into human hematopoiesis, growth factors, and many other topics has been inhibited by the limited supply of bone marrow for research use. Using an affinity separation method, we have successfully obtained large numbers of purified progenitor and stem cells from blood components obtained from regional blood banks. Ten 1-2 day old units of buffy coat cells were ficolled, yielding roughly 10 billion nucleated cells. The cells were incubated with biotinylated anti-CD34 monoclonal antibody at $2x10^8$ cells/ml and separated using 5-7 CEPRATE™ LC avidin columns (1.5-2 billion cells/column). The captured cells, 20-45% CD34+, were released from the columns, pooled, re-incubated with biotinylated anti-CD34 antibody, and then separated again on a single CEPRATE™ LC column. The captured cells were analyzed by FACS and short term colony-forming assays. The complete procedure requires only 5 hours and yields approximately one million cells which are up to 96% CD34+. This represents over a 20,000-fold enrichment of CD34 cells. The short term colony forming assays show a similarly dramatic enrichment of colony-forming cells. Such cells can be a valuable research tool for those who do not have access to bone marrow samples or who are interested in the differences between the progenitor pools in marrow and peripheral blood.

D 208 RAPID ISOLATION OF HUMAN HEMATOPOIETIC CD34+ CELLS: PURGING OF HUMAN TUMOR CELLS. Lisa Schain, Jane S. Lebkowski, Mark Harvey, David Okrongly, Roland Levinsky* and Thomas B. Okarma. Applied Immune Sciences, Inc. Menlo Park, CA and *Institute for Child Health, London UK.

Human $CD34^+$ cells were isolated in over 65 experiments from bone marrow samples using a process which employs sterile polystyrene devices containing covalently immobilized soybean agglutinin (SBA) or the CD34 monoclonal antibody, ICH3. In this process, bone marrow mononuclear cells (BMMC) were first depleted of many differentiated cells such as B, myeloid, certain T, fibroblasts, and stromal cells, by capture on SBA devices. The nonadherent SBA^- population, which was thereby 1.5-5.0 fold enriched in $CD34^+$ cells, was then loaded into similar devices containing covalently attached ICH3. After $CD34^+$ cell capture, the nonadherent $CD34^-$ cells were removed, and the $CD34^+$ cells released by physical agitation. The purified $CD34^+$ cells, representing 0.3-2.2% of the input BMMC, were greater than 90% viable, had low to high forward and low 90 degree light scatter properties, were 10-50 fold enriched in CFU-C and long term bone marrow culture initiating (LTBMICs), and lacked detectable CD34 mAB on their surface. $CD34^+$ cells purified from bone marrow samples deliberately seeded with 5 different tumor cell lines were 99.0->99.9% depleted of these contaminants. This sterile closed system offers a convenient means to separate $CD34^+$ cells from even up to $1.5x10^{10}$ BMMC allowing the recovery of all fractionated cells and yielding functional $CD34^+$ cells of high purity.

D 209 GRANULOCYTE MACROPHAGE COLONY STIMULATING FACTOR IN PERIPHERAL STEM CELL COLLECTION FOR TRANSPLANTATION: HIGH INCIDENCE OF APHERESIS CATHETER THROMBOSIS. L.Stephens, W.D. Haire, G. Kotulak, K. Schmit-Pokorny, A. Kessinger. Department of Internal Medicine, University of Nebraska Medical Center, Omaha NE. 68198

Most patients requiring peripheral stem cell (PSC) transplant have inadequate peripheral venous access for repeated apheresis procedures. Apheresis catheters placed in the inferior vena cava (IVC) via the percutaneous, translumbar route are highly effective for PSC collection. Aspirin (ASA) (325 mg daily), reduces the frequency of IVC catheter thrombosis from 12/60 without ASA to 2/40 with ASA ($p=.007$). Since introducing Granulocyte Macrophage Colony Stimulating Factor (GMCSF) for mobilization of committed hematopoietic precursors, our thrombotic catheter occlusion rate has increased. Patients on GMCSF continue to take ASA as before, but when compared to historical controls receiving ASA but no GMCSF, the thrombosis rate rose from 2/40 (5%) to 11/21 (52%) ($p=.0005$). Kaplan-Meyer curves of thrombus-free apheresis procedures indicate the ASA only users had significantly more thrombus free apheresis procedures than the ASA plus GMCSF users ($p=.0001$). The GMCSF user's occluding thrombi appear physically unusual as 9/11 (81%) cleared with mechanical aspiration and did not require lytic agents. Histological examination of one thrombus revealed accumulations of fibrin and peripheral blood leukocytes. Conclusions: 1) GMCSF is associated with an increased rate of thrombotic catheter occlusions despite ASA use. 2) Occluding thrombi can often be mechanically removed without lytic agents. Further study into thrombosis prophylaxis in this population is indicated.

D 210 MYELOTOXICITY IN A PHASE I TRIAL OF RADIOLABELED MONOCLONAL ANTIBODY B72.3. M. Tempero, S. Joshi, G. Dalrymple, S. Augustine, K. Harrison, K. Holdeman, D. Jacobson, J. Jackson, and D. Colcher. Depts of Internal Medicine, Cell Biology and Anatomy, Pathology and Microbiology, Radiology and Radiation Safety, University of Nebraska Medical Center, Omaha, Nebraska, 68198-3330.
Myelosuppression is a dose limiting feature in therapeutic trials with radioimmunoconjugates. Hoping to optimize dose to tumor, we have incorporated autologous bone marrow transplant (ABMT) into a phase I trial of monoclonal antibody B72.3 (recognizing TAG-72 antigen) labeled with ^{131}Iodine (^{131}I MAb B72.3) given intravenously to patients with metastatic gastrointestinal cancer (>30% tissue TAG-72 positive). Prior treatment was limited to 5-FU (+/- leucovorin). Blood counts were followed weekly and bone marrow cultures (expressed as % surviving fraction) were performed before treatment and at week +2. Two of six planned dose levels are completed:

PT#	Dose	Prior Rx	CFU-GM	BFU-E	Toxicity Grade	BM Tx
1	50 mCi/M^2	yes	5%	22%	IV AGC, IV plts	no
2	50 mCi/M^2	yes	19%	14%	0 AGC, 0 plts	no
3	50 mCi/M^2	no	8%	14%	IV AGC, III plts	no
4	100 mCi/M^2	no	0.1%	17%	IV AGC, III plts	no
5	100 mCi/M^2	no	0.7%	15%	IV AGC, IV plts	yes
6	100 mCi/M^2	yes	0.5%	0.8%	IV AGC, IV plts	no

When bone marrow colony cultures were obtained, peripheral counts were normal. In all cases, myelotoxicity became evident after week +3 and reached a nadir between 4 and 6 weeks. CFU-GM cultures appear to be a sensitive indicator of myelotoxicity and the effect on the % surviving fraction is dose dependent. These results suggest that the maximum tolerated dose based on myelotoxicity with ^{131}I MAb B72.3 was exceeded at 50 mCi/M^2. It is anticipated that autologous bone marrow transplant will be necessary for all remaining patients in this trial.

D 211 **ISOLATION AND ENRICHMENT OF PRIMITIVE CD34+/HLA-DR- HUMAN HEMATOPOIETIC STEM CELLS BY COUNTERFLOW ELUTRIATION FOLLOWED BY A SOYBEAN AGGLUTININ DEPLETION AND CD34+ POSITIVE SELECTION.** **John Wagner, Jane Lebkowski, Shawn Fahey and Tom Okarma. The University of Minnesota Medical School, Minneapolis, MN and Applied Immune Sciences, Menlo Park, CA.**
Murine pluripotential hematopoietic stem cells have been separated from CFU-S and CFU-GM by counterflow elutriation (CE) (Nature 1990, 347:188-9) and then enriched on the basis of surface antigen expression. Using the same approach, we attempted to isolate human hematopoietic stem cells. Low-density bone marrow (BM) mononuclear cells (2.8-7.7 x 10^8) were injected into a Beckman JE5.0 elutriator at 15 mL/min, rotor speed 3000 rpm. Cells were collected at 29 mL/min (FR29), 37 mL/min (FR37) and by turning the rotor off (R/O). Cells from each fraction (FR) were first incubated in AIS CELLector devices coated with soybean agglutinin (SBA) with the SBA- cells subsequently incubated in AIS CELLector devices coated with murine anti-CD34 (ICH3). In 7 separate experiments, adherent (CD34+) cells were collected and evaluated for coexpression of CD19, CD33, CD38 and HLA-DR as well as clonogenicity in short-term culture (per 10^5 cells).

BM FR	%RECOV	%CD19+	%CD33+	%CD38+	%DR+	CFU-GM	BFU-E
UNS	100	7±3	7±5	21±2	9±1	185±23	113±10
FR29	12±1	3±1	0	4±1	2±1	0	0
34+	.06±.01	0	0	9±2	18±4	6±1	14±1
FR37	28±2	8±1	1±0	17±3	5±2	4±2	16±6
34+	.40±.20	13±7	3±2	33±10	39±10	248±27	142±46
R/O	60±5	4±1	21±4	39±14	15±3	170±13	410±98
34+	.80±.18	4±1	62±11	83±2	80±3	3220±420	5300±512

As with murine BM, most committed progenitors are found in the R/O CD34+ FR with a 20-59 fold enrichment of mature CFCs. These cells represent most of the CD34+ cells in the BM, have an intermediate nuclear:cytoplasmic ratio, have rare nucleoli and frequently coexpress HLA-DR. In contrast, FR29 CD34+ cells represent only a minor fraction of the CD34+ BM cells, have scant cytoplasm, contain 1-2 prominent nucleoli, rarely co-express HLA-DR (ie, 76-90% DR-) and produce few colonies. Thus, CE followed by SBA depletion and CD34 selection is a useful method for separating primitive stem cells from committed hematopoietic progenitors. The self-renewal of FR29 34+ cells in long-term BM culture is currently under study.

Leukemia

D 300 **IMPROVED SURVIVAL IN AML AFTER AN INTENSIVE INDUCTION AND CONSOLIDATION REGIMEN FOLLOWED BY ALLOGENEIC OR AUTOLOGOUS BONE MARROW TRANSPLANTATION.** **Joseph H. Antin, Anna J. Mitus, David P. Schenkein, Richard T. Grapski, Kenneth B. Miller. Division of Hematology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; New England Medical Center, Tufts School of Medicine, Boston, MA; University of Massachusetts School of Medicine, Worcester, MA**
The role of marrow grafting in the therapy of AML in first remission remains unsettled. Both allogeneic and autologous bone marrow transplantation (BMT) can provide effective consolidation, but each is associated with unique advantages and disadvantages. In 1987, we initiated a prospective, multi-institution study to evaluate a novel induction regimen and to compare allogeneic to autologous BMT. Induction chemotherapy consisted of: daunorubicin 45 mg/m^2 (d1-3), ara-c 100 mg/m^2 (d1-7) followed by high-dose ara-c 2 gm/m^2 x 6 doses (d7-10). Patients entering complete remission received 2-3 cycles of: daunorubicin 60 mg/m^2 (d1-2) and ara-c 200 mg/m^2 (d1-5) alternating with ara-c 2 gm/m^2 x 6 doses (d1-3) and etoposide 100 mg/m^2 (d4-5). All patients under age 55 with an HLA compatible donor were offered allogeneic BMT, while those patients without a histocompatible donor or between 55-65 yrs of age were offered autologous BMT. To date, 64 patients have been enrolled. The median age is 43 yrs (range 19-63). 53/60 (88%) evaluable patients achieved CR. Survival data were analyzed by intention to treat. 27 patients (median age 43) were assigned to autologous BMT and 22 (median age 31.5) to allogeneic BMT. Actuarial event-free survival (EFS) for the autologous arm is 85 ± 8% at the median follow-up of 514 days (range 41-1522) and 57 ± 13% at 2 yrs. Actuarial EFS for the allogeneic group is 65 ± 11% at the median follow-up of 421 days (range 129-1378) and 53 ± 12% at 2 yrs. 11 patients have relapsed: 7 in the autologous arm and 4 in the allogeneic arm. Only 2 patients actually receiving autografts relapsed at days 193 and 202, respectively, and no recipient of an allogeneic BMT has relapsed. If only transplanted patients are considered, actuarial EFS at 2 yrs is 71 ± 18% for the autologous arm and 66 ± 12% for the allogeneic arm. Thus, intensive induction and consolidation therapy followed by allogeneic or autologous BMT can result in substantial improvements in EFS compared to historical data. Further accrual and follow-up will help to establish the relative roles of autologous and allogeneic BMT.

D 301 DOES LEUKAEMIC RELAPSE INDUCE THE EXPRESSION OF ANTI LEUKAEMIA CYTOTOXIC EFFECTOR MECHANISMS?
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A 21 years old male with AML (FAB M2) was induced with high dose Ara-C after failure of first line induction chemotherapy. After 1. remission was reached, he immediately underwent combined T depleted allogeneic bone marrow transplantation after conditioning with TBI and Cyclophosphamide. 4 months later leukaemic relapse occured. Relapse was regarded as refractory to chemotherapy and IL-2 therapy was started (3 cycles of IL-2 9 x 10^6/m^2 d 1-5). Under IL-2 therapy the patient cleared the leukaemic blasts from peripheral blood and bone marrow evaluation 6 weeks later showed no increase of leukaemic burden now infiltrated by lymphatic cells. Phenotyping of the peripheral blood showed increasing numbers of NKH 1 (CD 56) positive cells. We tested in vivo expressed LAK activity by Cr^{51} release assay with NK sensitive and resistant targets as well as the patient's leukaemic blast target cells from diagnosis. Following IL-2 therapy leukaemic blasts and NK resistant targets were lysed efficiently. Surprisingly, the same lysis pattern was endogenously expressed in vivo at diagnosis of relapse prior to IL-2 therapy. However, before relapse no LAK activity was found at all. In vivo expressed cytotoxic anti-leukaemia reactivity is probably induced by the expansion of the leukaemic clone.

D 302 T-DEPLETED BMT FOR ACUTE LEUKEMIA. EVALUATION OF A NEW CONDITIONING REGIMEN THAT INCLUDES THIOTEPA. Franco Aversa, Adelmo Terenzi, Alessandra Carotti, Rita Felicini, Paolo Latini°, Cynthia Aristei, Yair Reisner * and Massimo F. Martelli. Istituto di Ematologia, University of Perugia. ° Radioterapia Oncologica, Policlinico Monteluce 06100 Perugia, Italy. * Dept. of Biophysics Weizman Institute, Rehovot, 76100 Israel.

33 patients (25M, 8F), median age 27 years (range 14-50), with acute leukemia (18 ANLL, 15ALL), received a HLA-identical BMT depleted of T-lymphocytes by the soybean agglutinin and E-rosetting technique between July 1985 and July 1991.
25 (75%) were high risk because of disease status (10 relapses, 11 CRII and 4 secondary leukemias). The first 13 patients (Group I) were conditioned with ATG, 36 mg/kg procarbazine, 14.4 Gy hyperfractionated TBI (HFTBI) and 120 mg/kg Cyclophosphamide (Cy); the last 20 (Group II) received 14.4 Gy HFTBI, 10 mg/kg Thiotepa, ATG and 100 mg/kg Cy. All patients achieved a full-donor chimerism engraftment at DNA polymorphism analysis. Neither acute nor chronic GVHD were observed. 8 (61.5%) Group I patients survive leukemia-free at a median follow-up of 68 months (range 55-72); 5 (38.5%) relapsed. 12 Group II patients (60%) survive leukemia-free at a median follow-up of 12 months (range 2-30). 2 ALL relapsed; 6 non-relapse deaths occurred (1 VOD, 1 CMV-IP, 1 Lymphoma, 3 infections).
In view of the fact that the majority of our patients were high-risk, the mortality and the relapse may be considered low. Furthermore, we believe that the introduction of the strong myeloablative drug thiotepa in the conditioning regimen contributed to eradicating the neoplastic cells and so, to reducing the incidence of relapse frequency, despite the total absence of GVHD.

D 303 AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA USING MONOCLONAL ANTIBODY-PURGED BONE MARROW. Edward D. Ball, Witold Rybka, Letha Mills, David Hurd, Robert McMillan, Roger Gingrich, Bradley Slease, and Eric Martin. Departments of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, Dartmouth-Hitchcock Medical Center, Hanover, NH 03756, Bowman-Gray School of Medicine, Winston-Salem, NC 27103, and Scripps Clinic and Research Foundation, La Jolla, CA 92037, U. of Iowa Medical Center, Iowa City, IO 52242, and Medical Center of Delaware, Newark, DE 19713.

Fifty-four patients with acute myeloid leukemia (AML) were treated from August, 1984 until June, 1991 using high-dose chemotherapy and autologous bone marrow transplantation (ABMT) with monoclonal antibody (mAb) (PM-81 and AML-2-23, anti-CD15 and 14, respectively) and complement treated bone marrow. At the time of transplant seven patients were in first complete remission (CR), 36 in second CR, seven in third CR, and five in first relapse. The median age of all patients was 33 years (range 11-57 years). Preparative regimens included busulfan (64 mg/kg) and cyclophosphamide (120 mg/kg) (18 patients), cyclophosphamide (120 mg/kg) and total body irradiation (1200 cGy) (36 patients) and VP-16 (60 mg/kg) and busulfan (64 mg/kg) (1 patient). Median overall and relapse-free survival of first CR patients (n=7) was 24 months post-ABMT and the two and three-year actuarial overall and relapse-free survival was 68% and 51%. Median survival for 43 patients in second or third CR was 7.9 months post-ABMT and 10.9 months since CR. Fifteen patients survive disease-free from 9 to 86 months post-ABMT (median followup=30 mo). In the second and third CR group, 11 patients showed "inversions", where their post ABMT remission lasted longer than the previous one. Actuarial two and three year disease-free and overall survival of patients in second and third CR was 34% (± 8%) and 30% (± 9%), and 34% (± 8%) and 30% (± 8%), respectively. Of 14 patients age 30 years or under, 9 are disease-free at times ranging from 9 to 86 months (median followup=24 mo) with a 64% (± 13%) actuarial disease-free survival. Of four patients treated with mAb-purged marrow at early first relapse after induction/ablation with busulfan (64 mg/kg) and cyclophosphamide (120 mg/kg), three survive disease-free in CR2 at 4, 20 and 29 months post-ABMT. One patient died in CR two months post-ABMT of CNS hemorrhage. ABMT avoids the problems of graft-versus-host disease, of finding suitable donors for allogeneic marrow transplantation, and yields outcomes, especially in younger age groups, that are comparable to allogeneic BMT.

D 304 STRATEGY TO IMPROVE THE UTILITY OF BONE MARROW TRANSPLANTATION (BMT) FOR PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA (CML) IN BRITISH COLUMBIA (BC). Michael Barnett, Stephen Nantel, Allen Eaves, Connie Eaves, Donna Reece, Hans Klingemann, John Shepherd, Daphne Brockington, and Gordon Phillips. The Leukemia/Bone Marrow Transplant Program of British Columbia, Division of Hematology, Vancouver General Hospital, British Columbia Cancer Agency, Terry Fox Laboratory, and the University of British Columbia, Vancouver, BC V5Z 4E3

The Leukemia/BMT Program of BC is the sole BMT facility for the province (population ~ 3 million). The incidence of CML in the ≤ 60 age group is ~ 25/year (BC Cancer Agency Annual Report, 1990-91). Our strategy for CML (Can Med Assoc J 143:187-193, 1990) involves early (i.e., ≤ 1 year of diagnosis) BMT for chronic phase (CP) disease with either: 1) related donor (RD) marrow (preferably) or 2) unrelated donor (UD) marrow for those aged ≤ 50 years or 3) cultured autologous (Au) marrow for selected patients aged ≤ 50 years who lack a donor and those aged 51-60 years [Blood 76 (Suppl 1):526a, 1990]. Seventy-three BC residents (57 aged ≤ 50 years, 16 aged 51-60 years), diagnosed between 01/87 and 04/91 as having CML in CP-1, were referred. Fifty-five of 57 pts aged ≤ 50 years were HLA typed: 27 had a suitable RD -- 22 underwent RD-BMT and 5 are about to; 26 of the other 28 had an UD search and an UD was found for 15 -- 9 underwent UD-BMT (1 whose CML transformed in the interval is not included in the results) and 3 are about to, 2 declined (1 chose Au-BMT), and 1 had a medical contraindication to BMT. Twelve pts aged ≤ 50 years lacking a donor and 16 aged 51-60 years were screened for Au-BMT: 8 of the former were eligible -- all underwent Au-BMT (1 screened in CP-2 is not included in the results); 10 of the latter were eligible -- 6 underwent Au-BMT, 3 declined, and 1 died of transformed CML.

	RELAPSE HEM.	RELAPSE CYTO.	2-YEAR SURVIVAL	2-YEAR EFS
1) RD-BMT (n=22)	1	1	68%	57%
2) UD-BMT (n=8)	0	0	43%	43%
3) Au-BMT (n=13)	4*	4	85%	48%
4) No BMT (n=20)	-	-	66%	-

* 2 after infusion of unmanipulated cells for graft failure

This strategy has allowed the majority of pts with CML aged ≤ 60 years to undergo early BMT; its impact on the disease remains to be determined.

D 305 **LOW DOSE METHOTREXATE WITH CYCLOSPORINE AND METHYLPREDNISOLONE AS GRAFT VERSUS HOST DISEASE PROPHYLAXIS FOR PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA RECEIVING ALLOGENEIC BONE MARROW TRANSPLANTS.** **Stephen Bulova, David Topolsky, Pamela Crilley, Michael Styler, and Isadore Brodsky, Department of Neoplastic Diseases, Hahnemann University, Philadelphia, PA, 19102.**

Thirty-five patients with chronic myelogenous leukemia (CML) received unmanipulated allogeneic bone marrow transplants from HLA identical siblings using a conditioning regimen of Busulfan - 16 mg/kg and Cytoxan - 120 mg/kg. The first 20 patients received graft versus host disease (GVHD) prophylaxis with Cyclosporine (CSA) and methylprednisolone (MP). Nine patients (45%) developed ≥ grade II acute GVHD. GVHD was the major cause of death in 8 of these 9 patients (89%) which occurred a median of 90 days (56-118) post-transplant. One patient expired with an early relapse. Of the 11 other patients, 4 developed extensive and 4 limited chronic GVH (73%) causing death in 1. The incidence and severity of GVH was significantly higher than in patients transplanted for AML using identical conditioning and GVH prophylaxis regimens. Low-dose methotrexate (MTX) 7.5 mg/M² IV day 1, and 5 mg/M² IV day 3 and 6 was added to the CSA, MP regimen for the next 15 patients. There was no difference in the rate of engraftment or incidence of infection, but there was an increase in mucositis. One of 15 patients (7%) developed ≥ grade II acute GVHD which led to his death with all patients being followed for >120 days. One patient expired with relapsed disease. Of the 13 other patients, 3 developed extensive and 4 limited chronic GVH (54%) leading to death in 1 patient. Low dose methotrexate appears to enhance the effectiveness of CSA and MP and reduces the incidence and severity of acute and chronic GVHD in patients receiving allogeneic bone marrow transplants for CML.

D 306 GRAFT-VERSUS-LEUKEMIA EFFECT IN VITRO: ANALYSIS OF EFFECTOR CELLS BY LIMITING-DILUTION ANALYSIS

Donald Bunjes, Dieter Schaarschmidt, Mathias Theobald, Gerhard Heil, Renate Arnold and Hermann Heimpel. Department of Internal Medicine III Ulm University, D 7900 Ulm, FRG.

The therapeutic efficacy of allogeneic BMT has been partially attributed to the graft-versus-leukemia effect extended by graft lymphocytes, especially T-lymphocytes. We have analysed the effector cells in a limiting-dilution culture system using HLA-mismatched donor-recipient pairs (n=7). In all cases the leukaemic blasts were capable of stimulating proliferation and IL-2 secretion but were not susceptible to lysis by cytotoxic T-cells. Segregation analysis and blocking experiments demonstrated that the T-cells induced by the leukaemic blasts were responding to alloantigens and not leukaemia specific antigens. Since the leukaemic blasts were not intrinsically resistant to lysis by cytotoxic T-cells we investigated whether this resistance could be due to a low expression of adhesion molecules. Using a large panel of MoAb we were able to demonstrate no or a low level expression of adhesion molecules and no upregulation by gamma-interferon and/or TNF-alpha could be demonstrated. Since cytotoxic T-cells seem to be poor effector cells in this model, we are investigating the effects of allo-antigen-specific T-helper cells on clonogenic leukaemic cells in coculture studies.

D 307 VERAPAMIL MODULATION FOR PURGING MULTIPLE DRUG RESISTANT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) AND PREPARATIVE THERAPY FOR AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT): A PILOT STUDY, Mitchell S. Cairo, J. Michael Plunkett, Eva Knoppel, Carmella van de Ven, and Leonard Sender, Children's Hospital of Orange County, Orange, CA 92668.

ABMT for ALL is associated with a high relapse rate (70-75%) and is in part limited by ineffective methods of bone marrow purging. Recently, Rothenberg, et al (*Blood* 74, 1989) and Cairo, et al (*AACR* 32,1991) have demonstrated an increased expression of the multiple drug resistant gene (MDR) at relapse in patients with ALL. Verapamil (VPL) (2-10 μM) has been shown *in vitro* to reverse multiple drug resistance associated with chemotherapeutic agents, but is in part limited *in vivo* (>3μM) by cardiac toxicity (Cairo, et al, *Cancer Research* 49, 1989). L_{100}, a T-lineage ALL line, was developed after repeated passages of L_0 to sub-lethal concentrations of Vincristine (V). L_{100} was determined to be an MDR line by expression of MDR1 mRNA by Northern blot analysis (pHDR5A kindly provided by Michael Gottesman, NCI) and p-glycoprotein by Western blot analysis (C219 monoclonal antibody). VPL (10 μM) significantly reduced the V-IC_{50} of L_{100} from 251 ± 93 μM to 79 ± 10 μM ($p<.001$) and VP-16 IC_{50} from 18.5 ± 3.3 μM to 7.5 ± 2.4 μM ($p<.05$). Bone marrow CFU-GM IC_{90} for V was 7.3 μM and for VP-16 80 μg/ml. VPL (10 μM) only increased V and VP-16 BM CFU-GM toxicity by 11.1 and 9.4% respectively (6-fold selective cytotoxicity) ($p<.001$). Dose limiting BM CFU-GM IC_{90} cytotoxicity with VPL (10 μM) was 1.5 μM of V and 50 μg/ml of VP-16. 5 patients with ALL (9.8 ± 2.3)(5 to 19 yrs) (four 2nd CR and one 4th CR) have undergone ABMT with purged bone marrow utilizing the above doses of V, VP-16, and VPL. Preparative treatment consisted of 1200 TBI, VPL (bolus 0.15 mg/kg + 0.005 mg/kg/min x 4 hr infusion) + VP-16 (1800 mg/m^2) (4 hr infusion) and Cytoxan (50 mg/kg x 2). 1 patient relapsed 4 months from ABMT (4th CR), 1 patient died on day 60 secondary to aspergillosis pneumonia, and 3 patients are engrafted and NED 2-6 months post-ABMT. Recovery of ANC ≥500/mm^3 x 2 days was 28.8 ± 6.0 days and platelets ≥ 20,000/mm^3 was 32 ± 9.7 days. I.V. VPL during VP-16 infusion was tolerated well and resulted in 15' level of 65.6 ± 16.3 ng/ml, 1 hr level of 449 ± 130 ng/ml, and 5 hr (1 hr post) was 180 ± 100 ng/ml. The addition of VPL may provide a unique method to *ex vivo* purge MDR-positive leukemia and modulate preparative therapy during ABMT for ALL.

D 308 A COMPARISON OF ALLOGENEIC BONE MARROW TRANSPLANTATION, AUTOLOGOUS TRANSPLANTATION, OR MAINTENANCE CHEMOTHERAPY FOR THE TREATMENT OF CHILDHOOD ACUTE LYMPHOCYTIC LEUKEMIA IN SECOND REMISSION, William F. Cassano, William Treloar, and the U.F. Pediatric Marrow Transplant Team, Department of Pediatric Hematology-Oncology University of Florida College of Medicine, Gainesville, Florida 32610

We compared three treatment options in 31 patients (pts) to determine optimal therapy for relapsed, childhood acute lymphocytic leukemia (ALL). Patients who had a bone marrow relapse on or less than 6 months off therapy (18 pts) or more than 6 months after completing therapy (13 pts) were enrolled in this study at the time of achieving second remission of ALL. 13 pts were treated with maintenance chemotherapy (chemo), 12 pts with allogeneic transplantation (allo), and 8 pts with autologous transplantation (auto). A statistical comparison of means in all three treatment groups failed to identify any significant differences between the groups' mean age at diagnosis, mean leukocyte count at diagnosis, or mean duration of first remission. Analysis of relapse free survival data by log rank analysis did show a significant survival advantage for allo over chemo and auto over chemo, but no significant difference between auto versus allo treatment arms. Proportional hazards regression analysis revealed that treatment modality (allo, auto, or chemo) was the most significant prognostic variable ($P<0.001$) followed by age at diagnosis ($P=0.013$), duration of first remission ($P=0.017$) and leukocyte count at diagnosis ($P=0.04$). Analysis of financial data indicates that patient charges are not significantly different for allo and auto treatment arms, but both are about twice as costly as the maintenance chemotherapy arm. We conclude that both allogeneic and autologous bone marrow transplantation are superior to maintenance chemotherapy for the treatment of childhood ALL in second bone marrow remission.

D 309 CONVENTIONAL VERSUS T-CELL DEPLETED ALLOGENEIC BONE MARROW TRANSPLANTATION FOR EARLY REMISSION ACUTE LEUKEMIA. B. Childs, H. Castro-Malaspina, N. Kernan, N. Collins, J. Brochstein, N. Flomenberg, J. Young, E. Papadopoulos, M. Carabasi, D. Emanuel, A. Gillio, T. Small, I. Cunningham, P. Black, R.J. O'Reilly. Bone Marrow Transplantation Service, Departments of Medicine and Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, NY.

From 1986-90, 43 pts., aged 2-43, with early remission acute leukemia and an HLA-identical sibling were prospectively randomized to receive either a T-cell depleted (via soybean lectin agglutination and E-rosette formation) or a conventional (unfractionated) allogeneic marrow graft. The 21 pts. randomized to receive a conventional transplant consisted of: 12 with ANLL/1st CR, 4 with ANLL/2nd CR and 5 with ALL/2nd CR. The 22 pts. receiving T-cell depleted grafts included 10 with ANLL/1st CR, 5 with ANLL/2nd CR and 7 with ALL/2nd CR. Age and sex distributions of the two groups were comparable. All pts. were conditioned prior to transplant with hyperfractionated TBI 1500 cGy followed by cyclophosphamide 120 mg/kg. Nine of 17 recipients of T-cell depleted grafts from male donors received graft rejection prophylaxis. Recipients of unmodified grafts received GvHD prophylaxis with methotrexate and cyclosporin-A. Of the 22 recipients of T-cell depleted marrow, 3 pts. developed acute GvHD (one Grade I and two grade II), while among the 18 evaluable conventional graft recipients, 11 pts. developed acute GvHD (Grade I, 3; Grade II, 4; Grade III, 4). Extensive chronic GvHD developed in three of 13 evaluable conventional transplant recipients. No cases of chronic GvHD were observed among recipients of T-cell depleted grafts surviving greater than 100 days post-BMT. In the group receiving T-cell depleted marrow, 2 pts. died of bacterial sepsis with 1 death attributable to interstitial pneumonitis. In the group receiving conventional grafts, there were 6 septic deaths and 2 deaths due to interstitial pneumonia. No significant difference in survival, disease-free survival, or relapse rate exists *between the conventional and T-cell depletion arms. There are 10 surviving pts.* in the conventional arm (median F/U 31 months), while 11 recipients of T-cell depleted marrow have survived, disease-free, a median of 31 months following BMT. One pt. (ALL/2nd remission) in the conventional arm relapsed 12 months following BMT, while 4 pts. (2 with ANLL/2nd CR, 1 with ALL/2nd CR and 1 with ANLL/1st CR) in the T-cell depletion arm relapsed, all within the first six months of transplantation. Disease free survival is 47% for the conventional transplant recipients and 46% for the T-cell depletion pts. In this trial, advantages achieved through the abrogation of GvHD were offset by increased rejection of *T-cell depleted grafts. However, the antileukemic efficacy of conventional and* T-cell depleted marrow grafts was equivalent. Recently we have identified treatment approaches preventing graft rejection. The impact of these approaches on the efficacy of T-cell depleted grafts is now under study.

D 310 BONE MARROW TRANSPLANTATION FOR CHILDHOOD HODGKIN'S DISEASE AND NON-HODGKIN'S LYMPHOMA, Kenneth B. De Santes, Finn Bo Petersen, Frederick R. Appelbaum, C. Dean Buckner and Jean E. Sanders, Fred Hutchinson Cancer Research Center, Seattle, WA 98104. Sixty-five children with malignant lymphoma were transplanted at the Fred Hutchinson Cancer Research Center between February 1973 and December 1989. Fifty-four patients (83%) had non-Hodgkin's lymphoma and 11 had Hodgkin's disease. Twelve patients are currently surviving free of disease with a median follow-up of 36 months (range 9 - 114 mo). The 5 year actuarial probabilities of disease-free and overall survival were 16.4% and 19.3%, respectively. Patients who were transplanted while in complete remission had a greater probability of disease-free survival than patients who had residual tumor at the time conditioning was initiated (33.9% vs 5.3%, p=.0007). Improved survival was also noted among children whose initial relapse occurred $\geq$ 6 months after diagnosis (19.3% vs 10.3% p=.02). Recurrent lymphoma was the most frequent cause of treatment failure with the 5 year actuarial probability of relapse being 71.4%. Children transplanted in complete remission had a 56.5% probability of relapse, compared to 88.9% for all other patients (p=.03). The type of marrow graft (i.e. autologous vs allogeneic) and the occurrence of acute graft vs host disease did not significantly affect the risk of relapse. Twenty-one patients died of transplant-related toxicity. CMV pneumonia and idiopathic interstitial pneumonitis accounted for the majority of transplant-associated deaths. The risk of developing a fatal pulmonary complication was greater among children previously exposed to $\geq$ 15 Gy of chest radiotherapy (61.3% vs 20.8%, p=.05). Transplant-related mortality was also higher among patients whose initial relapse occurred < 6 mo after diagnosis (79.1% vs 35.5%, p=.03) and for those who had persistent disease at the start of conditioning (61.5% vs 30.5%, p=.005). Bone marrow transplantation can be successfully employed to salvage children with recurrent lymphoma, especially if implemented early in the disease course. Methods to reduce the incidence of post-transplant relapse are required.

D 311 IS BUSULFAN-CYCLOSPHAMIDE REGIMEN FOR TRANSPLANT IN CHRONIC MYELOID LEUKEMIA BETTER THAN CYCLOPHOSPHAMIDE TOTAL BODY IRRADIATION ? RESULTS OF A RANDOMIZED FRENCH MULTICENTRIC TRIAL, A. Devergie, J.P Jouet, D. Guyotat, D. Blaise, M. Attal and the members of GEGMO. Centre Hayem, Hospital SAINT-LOUIS, 1 avenue Cl. Vellefaux 75475 PARIS CEDEX 10, FRANCE.

There is a general agreement that cure of chronic myeloid leukemia (CML) in chronic phase can be achieved in a relatively high proportion of patients receiving an allogeneic bone marrow transplant (BMT) after conditioning regimen with cyclophosphamide and total body irradiation (CY TBI). There are now data regarding efficacy of Busulfan and cyclophosphamide (BUCY) in BMT for acute or chronic myeloid leukemias. BU CY association has been shown to have a good antileukemic activity with a good tolerance. We initiated a prospective randomized multicentric study comparing effects of BU 16 mg/kg and CY 120 mg/kg versus CY 120 mg/kg and TBI 10 Gy in CML in 1st chronic phase. 127 patients were randomized and 105 were evaluable (BU CY 56 - CY TBI 49). No significant difference was observed between the 2 groups in terms of age, sex, interval between diagnosis and BMT. All patients were grafted with an HLA identical sibling donor and received cyclosporin A and "short" Methotrexate for prophylaxis of GVH. Overall actuarial survival is 65,5 %, respectively 69 % (CY TBI) and 62 % (BU CY) with a median follow up of 18 months. No difference was observed in terms of early toxicity, infection, acute or chronic GVHD. No rejection was observed after CY TBI. On the contrary, 4 patients, in the BU CY arm presented either no take (1 patient) or late rejection (3 patients). Longer follow up will be necessary to evaluate relapse rate. The ocurrence of marrow failure after "little" BU CY could indicate that this conditioning regimen is insufficient ; a subsequent trial could compare CY-TBI to "big" BU CY (BU 16 mg/kg and CY 200 mg/kg).

D 312 **ALLOGENEIC BMT FOR ACUTE NON LYMPHOBLASTIC LEUKEMIA (ANL) IN CHILDREN: ITALIAN EXPERIENCE**, G. Dini, E. Lanino, O. Abla, C. Uderzo, P. Polchi, F. Locatelli, P. Di Bartolomeo, W. Arcese, F. Rossetti, G. Rosti, M. Andolina, L. Boni, P. Paolucci, M.T. Van Lint, A. Bacigalupo for the AIEOP BMT group and the GITMO.

Between '80 and '89, 70 children (43 males, 27 females), 12-180 mos old with ANL received matched allo BMT at one of the Italian Centres participating in this study. At diagnosis (dx) WBC count was higher than $100x10^9/l$ in 13 children, lower in 57. According to the FAB classification, 12 children had ANL M1, 24 M2, 9 M3, 8 M4, 14 M5, 1 M6, 2 M7. Forty-nine children were grafted in first CR, 21 in "advanced" disease (3 in 2nd CR, 18 in relapse). Conditioning regimens prior to BMT included TBI in 56 children; 14 children received a non- containing TBI regimen. Prophylaxis for GVHD included CSA alone in 58 children, other regimens in 12 children. Twenty-six children (37%) developed grade II-III acute GVHD, while one child developed grade IV.

Twenty children out of the 57 surviving more than 100 days (35%) developed chronic GVHD. The overall survival (OS) from the date of BMT was 55% at 121 mos. The progression-free survival was 47% at 121 mos, with a median of 66 mos. In detail it was 57% for the 49 children grafted in 1st CR, while it was 28% for the other 21 children ($p = 0.006$).

We analyzed 8 prognostic factors, including sex, FAB classification, WBC count at dx, conditioning regimen, sex matching, age, status at BMT and the dx-BMT interval. In univariate analysis the FAB classification (FAB M 1,2,3) the number of WBC ($< 100x10^9/l$), the dx-BMT interval ($\leq$4 mos) and the status at BMT (1st CR) favourably influenced the OS. In multivariate analysis only FAB classification and status at BMT favourably influenced the OS.

D 313 AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) FOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN 1ST OR 2ND REMISSION (REM): FACTORS AFFECTING SURVIVAL AND RELAPSE. Kristine Doney, Jean Sanders, Finn Bo Petersen, Frederick Appelbaum and C. Dean Buckner for the Seattle Bone Marrow Transplant Team, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

We have analyzed the results of ABMT at our institution for 55 patients (pts) with ALL in 1st (n=22) or 2nd (n=33) marrow rem who received a preparative regimen which included 12-15.75 Gy fractionated total body irradiation (TBI). Median age was 18.5 yrs (2-45 yrs) and median time from diagnosis to BMT was 27 mos (4-122 mos). 31 of the 33 pts in 2nd rem had their marrow harvested while in 2nd rem. Prior to cryopreservation, 34 pts had their marrows treated with anti-T (n=6), anti-B (n=13), anti-CALLA (n=13), or anti-T and anti-B (n=2) cell monoclonal antibodies. Median times to achieving 200 and 500 granulocytes/mm^3 were 18 and 22 days post-ABMT, respectively. Median time to achieving a platelet count $\geq$20,000/mm^3 was 39 days. Currently, 18 patients are alive and 37 have died. The major cause of death was relapse (27 pts). Event-free survival at 2 years is 26% (1st rem pts = 36%, 2nd rem pts = 18%). The actuarial probability of relapse at 2 years is 68% (1st rem pts = 50%, 2nd rem pts = 79%). Pre- and post-ABMT variables were evaluated for their effect on survival and relapse. Variables significantly associated ($p<0.05$) with improved survival in univariate analyses include preparative regimens using 14.4 or 15.75 Gy TBI, no extramedullary disease at the time of ABMT, and a shorter time to achieving 200 granulocytes/mm^3. In a proportional hazards regression model, only the absence of extramedullary disease at the time of ABMT was significantly associated with improved survival. None of these variables was significantly associated with relapse post-ABMT. These analyses were also performed after redefining each patient's rem/relapse status by counting extramedullary relapses as independent relapses. Using this definition of disease phase, only 8 pts were transplanted in 1st overall rem and 27 in 2nd rem. Improved survival was significantly associated with no active extramedullary disease at the time of ABMT and with a shorter time to achieving 200 granulocytes/mm^3, whereas relapse post-ABMT did not correlate with any of the variables analyzed.

D 314 BUSULFAN (BU) - CYCLOPHOSPHAMIDE (CY) CONDITIONING AND ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) FOR CHRONIC MYELOID LEUKEMIA (CML), Mohamed B. Elmongy, John D. Shepherd, Michael J. Barnett, Donna E. Reece, Stephen H. Nantel, Hans-G. Klingemann, and Gordon L. Phillips. The Leukemia/Bone Marrow Transplant Program of British Columbia, Division of Hematology, Vancouver General Hospital, British Columbia Cancer Agency, and the University of British Columbia, Vancouver, BC V5Z 4E3

Between 3/87-5/91, 35 patients (pts) with CML underwent BMT following a BU (16 mg/kg) and CY (120 mg/kg) conditioning regimen. Marrow source was HLA-identical related (33) or one-antigen mismatched related donors (2). Graft-vs-host disease (GVHD) prophylaxis was with cyclosporine (CSP) and methotrexate (MTX) in all pts. Ten pts received folinic acid rescue following MTX (Blood 76 Suppl 1:569A, 1990). Two other pts who received marrow from parous female donors received XomaZyme (o.1 mg/kg x 14) in addition to CSA and MTX as a part of a pilot study for pts at high risk for acute GVHD. There were 15 females and 20 males; median age 40 (range 13-50). Thirty-one pts were in first stable phase (SP), and two were in accelerated phase. One pt was in second SP, and one was in myeloid blast phase. Of the 31 pts in SP1, one pt received prior BU, and 27 were transplanted within one year from diagnosis. All pts engrafted with median day (range) to PMN > 0.5 x 10^9/L and platelets > 20 x 10^9/L was 17 (12-32) and 12 (0-31) respectively. Sixteen pts (45%) developed grade ≥ II acute GVHD; 48% of the pts developed grade II mucositis, and 31% of the pts developed ≥ grade II hepatic toxicity. Only 4 pts developed grade III or IV regimen-related organ toxicity (2 hepatic, 1 bladder, and 1 pulmonary). Three pts died of early complications; 1 of acute GVHD, 1 of hepatic toxicity, and 1 of lung toxicity. Four pts died of chronic GVHD complications at +6, +9, +12, and +21 months. Two pts relapsed; probability of relapse is 7% ± 10%. Twenty-six pts (74%) are alive and disease free (median follow-up of 17 months, range 3-39). The two year actuarial event-free survival is 69% ± 17%. We conclude that: 1) BUCY is a well-tolerated regimen and its toxicity is not excessive when used with CSA and MTX for GVHD prophylaxis, and 2) BUCY has an excellent antileukemic effect against CML and should be considered as an alternative preparative regimen for this disease.

D 315 IMPROVED RESULTS IN ALLOGENEIC BMT FOR LEUKEMIA BY MODULATING TBI DOSE AND GVHD PREVENTION F Frassoni, A Bacigalupo, A Marmont. Centro Trapianti Midollo Osseo, Divisione Ematologia 2, Ospedale San Martino, Genova Italy

The outcome of allogeniec BMT depends upon many factors: among them the TBI dose and the allograft(including GVHD , immunodeficiency , immunosuppressive therapy ,etc.)affect substantially either the relapse and the transplant related complications. We were concerned that increased doses of TBI were associated with high transplant related mortality and threfore we deliberately have choosen to use mild TBI doses as conditioning regimen for allogeneic BMT. The dose we have used is probably a threshold one and small variations produce major effects in term of relapse and survival . The results are the following. Using fractionated TBI(fTBI) consisting of 333 cGyx3(effective dose 1000-1100 cGy) the incidence of interstitial pneumonitis was 5%. We observed an actuarial eight years relapse incidence(RI) of 0% and 10% in CML chronic phase(CP) and in AML first complete remission(1CR) respectively but with doses of TBI lower than 990 cGy the RI was around 50% in both diseases. Being the above schedule a threshold dose, different GVHD prevention schemes produce great variations in the results. In fact, providing a TBI dose of 1000-1100 cGy ,in AML 1CR 1mg/kg vs 5mg/kg of Cyclosporin A(CS)as GVHD prophilaxis produced 68% and 0% versus 30% and 50% DFS and RI respectively. Conversely in CML 1CP 5mg/kg of CS produced <10% relapse rate only. From these data it is suggested that the GvHD protocol have a different impact on different diseases probably because remission and cure of AML and CML in chronic phase is achieved and maintained with BMT by non-superimposible mechanisms. Concerning AML 1CR we have initiated a trial comparing two different schemes of GVHD prevention: CS 1mg/kg vs CS 1mg/kg+Methotrexate to evaluate whether we can improve the transplant related mortality without increasing RI. Twenty patients have been enrolled and the DFS is until now >90% in both arms with a median follow-up of 15 months. Until new methods of eradicating leukaemia, free from the toxicity associated with currently used conditiong regimens, will become availbale, a modulation of the present therapeutic tools provide significant improvement of results in allogeneic BMT.

D 316 REPRODUCIBILITY OF PCR FOR DETECTION OF MINIMAL RESIDUAL DISEASE IN PATIENTS TRANSPLANTED FOR CML, G Gehly, J Gabert, C Haskovek and E D Thomas, Exp Path, Fred Hutchinson Cancer Research Center, Seattle, WA and Dept. of Biology, Institut Paoli-Calmettes, Marseille, FR.

Reproducibility of PCR results will be increasingly important as the use of the technique becomes more widespread. We evaluated PCR reproducibility by assaying for minimal residual disease in two independent laboratories. Thirty-seven marrow and 30 blood specimens from twenty posttransplant, remission CML patients were evaluated in a blinded fashion by both laboratories. The time after transplant ranged from 3 yr to 9 yr. Both laboratories employed a single-step PCR method followed by Southern transfer and oligonucleotide probing. Assay sensitivity was between 10^{-4} and 10^{-5} as determined by diluting chronic phase CML cells into BCR-abl negative HL60 cells.

Comparative results for marrow specimens are summarized below.

Site	Positive	Negative	Equivocal	Inadequate
USA	7	11	0	1
FR	5	12	1	0
Total	12	23	1	1

The results for blood specimens were:

Site	Positive	Negative	Equivocal	Inadequate
USA	4	8	3	0
FR	3	8	2	2
Total	7	16	5	2

Equivocal specimens had signals too faint to be clearly positive or gave conflicting results on two repeat experiments. Inadequate specimens were those failing to amplify a control gene such as beta 2 microglobulin or c-abl. The results in Seattle and Marseille were identical in 14/20 patients. In three cases, the results were identical except one laboratory found a single specimen to be inadequate. These results indicate that for a majority of patients, PCR results can be obtained reproducibly by two laboratories using similar methods.

D 317 COMPLETE REMISSION AFTER TREATMENT WITH TRETINOIN (ALL-TRANS-RETINOIC ACID) IN A AML(M3) PATIENT RELAPSED POST ABMT IN II CR. PRELIMINARY REPORT. Teodosio Izzi, Giuseppe Rossi, Bruno Roncoli, Pierino Ferremi, Maria Adele Capucci, Mariarosa Mariano, Cecilia Carbone, Vittorio Ferrari, Alberto Zaniboni. Sezione Autonoma di Ematologia e Trapianto di Midollo Osseo, III Divisione di Medicina Generale, Spedali Civili di Brescia, Italia.

A 32 year old man affected by AML(M3), achieved a complete remission after DNB+ARAC and relapsed after one year during the maintenance treatment (DNB+6THIOG+ARAC+MITOX+VP16).

The patient achieved a II CR and underwent an autologous bone marrow transplant (ABMT) with the frozen bone marrow harvested in I CR. The transplant was performed after Busulfan 14 mg/kg and CY 200mg/kg and the patient left the isolation at +20 days from BMT.

15 months after ABMT the patient relapsed in bone marrow and treatment with tretinoin was started at a dose of 45mg/m2 per day. For 20 days the drug was given once in the morning and for the following 30 days divided into two equal doses of 45mg/m2 administered six hours apart.

After 50 days of treatment a bone marrow biopsy showed a complete remission with normal trilinear cellular proliferation.

The peripheral blood count shows a complete recovery in WBC with 3x10^9/l neutrophils and a low platelet count. No adverse side effects were observed during the treatment.

Tretinoin is an effective agent for inducing complete remission in patients with acute promyelocitic leukemia, our report shows the possibility of achieving a CR also in patients relapsed after an ABMT.

D 318 Ig- AND TCR-REARRANGEMENTS IN BONE MARROW HARVESTED FOR AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) - A PROGNOSTIC PARAMETER.

Hanne Jørgensen, Peter Hokland, Arne Willy Jensen and Marianne Hokland. Department of Immunology, University of Aarhus and Department of Hematology and Medicine, Aarhus Amtssygehus, Denmark.

Given the fact that about 40% of patients relapse after ABMT it was interesting to know, whether detection of minimal residual disease (MRD) in the bone marrow (BM) harvested was useful as a prognostic parameter. We therefore analysed 50 consecutively harvested BMs from patients with various malignant diseases for rearrangements of the genes encoding the Ig- and/or T-cell receptor. This kind of rearranged genes demonstrate the presence of clonally derived cells, and thus indicates MRD. Surprisingly, we demonstrated rearrangements in 16 (32%) of these 50 BMs even though they were judged by immmunophenotyping not to include malignant cells. Rearrangements of the Ig-genes were rarely seen, while TCR-β rearrangements were seen in 50% of AML patients and in 27% of ALL patients. As expected, the presence of rearrangements clearly indicated a higher probability of relapse. However, despite the presence of rearrangements, 6 patients (37%) still remained in complete remission 2 years after BM harvest. From our results it appeared, that very few of the patients receiving bone marrows with the presence of TCR-β rearrangements (when present as the only rearrangement) developed overt disease. Thus, our results clearly demonstrate, that a) the presence of clonally derived cells in the BM does not necessarily imply early relapse, and b) that the type of rearrangement is of great importance.

D 319 HIGH DOSE BUSULFAN AND ETOPOSID AS A CONDITIONING FOR THE BONE MARROW TRANSPLANTATION.

K.Kogure, H.Nakamura, N.Aotsuka, T.Asai, H.Oh. 2nd Department of Internal Medicine, Chiba University School of Medicine, Chiba, Japan.

The relapse of the primary disease is one of the major complications in autologous and syngeneic bone marrow transplantation (BMT). The second BMT against relapsed disease after allogeneic BMT also faces high frequency of relapse. We studied the efficacy and safety of high dose busulfan and etoposid as a conditioning regimen for autologous, syngeneic and the second allogeneic BMT. Six patients with hematological malignancies were treated with this regimen. The median age of patients was 29(range 18-38). One patient was transplanted with autologous marrow graft and two patients were grafted from monozygous twin siblings. Three patients with relaps underwent the 2nd BMT from the same donor as the first allogeneic BMT conditioned with endoxan and total body irradiation. Busulfan (16mg/kg) was given oraly over 4 days, followed by continuous infusion of etoposide (60mg/kg) for two days. In the second allogeneic BMT, cyclosporin A and methylpredonisolone were administered as a prophylaxis against graft versus host disease. All the patients were tolerable to this regimen and major side effects were transient fever and mucositis. Immediate hematological recovery was observed. Four patients had been disease free(2-21 mo.: median 13mo.) and two patients relapsed; one in early phase after syngeneic BMT and the other 210 days after autologous BMT. This conditioning regimen seems to be effective and safe especially in the setting where we may not need to consider graft rejection.

D 320 G-CSF-combined conditioning regimen for acute myelogenous leukemia

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We conducted high doses of granulocyte colony-stimulating factor (G-CSF)-combined conditioning for autologous blood stem cell transplantation (ABSCT) in an attempt to increase the chemosensitivity of myelogenous leukemia cells to cell cycle-specific cytotoxic drug, cytosine arabinoside (Ara-C). Consecutive 9 AML patients received the continous infusion of Ara-C (100mg/sqm/24h x 6d) following by the high doses of Ara-C (3g/sqm/1h x 2/d x 2d) in addition to busulfun (4mg/kg/d x 4d) and enocitabine (10mg/kg/d x 2d) (BU-AraC-VP16). G-CSF was escalated up to 20 ug/kg during Ara-C administration. Five patients received ABSCT with this conditioning during 1st CR, and the other 3 cases during 2nd or 3rd CR. G-CSF could support the colony formation of leukemic blast cells from all of 5 patients examined. One case (Case 2) was conducted G-CSF-combined BU-AraC-VP16 as a 2nd conditioning because he had relapsed 8 months after ABSCT with BU-AraC-VP16 without G-CSF. Two cases has relapsed and the remaining 7 cases are now continuing complete remission (median follow up: 5 months). Notably, Case 2 has already retained 2nd CR for 9 months. No additional adverse effects were seen as compared to BU-AraC-VP16 without G-CSF. Despite a limited number of patients, G-CSF-combined BU-AraC-VP16 is worth trying as a conditioning for not only ABSCT but allogeneic bone marrow transplantation in the treatment of AML.

D 321 MICROSATELLITE DETECTION OF DONOR LEUKEMIA FOLLOWING ALLOGENEIC BMT.

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Donor Leukemia is a rare event following allogeneic Bone Marrow Transplantation(BMT). We report on two cases where PCR analysis of dinucleotide repeats (microsatellites) combined with cytogenetics confirmed the donor origin of cells post BMT.
In the first case a patient was transplanted for severe aplastic anaemia from his HLA identical female cousin. Successful engraftment was achieved at day 28 with donor haematopoiesis. Nine months post transplant, blast cells were documented and cytogenetic analysis indicated a 9;11 translocation characteristic of acute monocytic leukemia. However the abnormality was in donor (female) cells only. PCR of microsatellites confirmed the donor origin of the cells.In the second case archival slide material from a patient who had been transplanted in 1984 for Chronic Myeloid Leukemia was examined retrospectively using PCR. At the time of transplant,in situ hybridisation suggested a leukemic relapse in donor cells. Our retrospective analysis seven years later using microsatellites confirmed that the relapse was indeed of donor origin.In both cases the donor remains hematologically normal. PCR of microsatellites is a novel highly reliable method of assessing chimerism following BMT and has allowed us to confirm the donor origin of cells in two cases of leukemic relapse.

D 322 BUSULFAN/ETOPOSIDE PREPARATIVE REGIMEN FOR AUTOLOGOUS PURGED BONE MARROW TRANSPLANTATION FOR TREATMENT OF ANLL. C. A. Linker, L. E. Damon, C. A. Ries, H. S. Rugo*
University of California, San Francisco, CA

We evaluated the efficacy and toxicity of a new preparative regimen in the treatment of patients in remission of ANLL with high dose chemotherapy and autologous bone marrow rescue. Chemotherapy consisted of busulfan 1 mg/kg q 6h for 4 days (total dose 16 mg/kg) on days -7 to -4 followed by an IV infusion over 6 - 10h of etoposide 60 mg/kg on day -3. Autologous bone marrow, treated *in vitro* with 100 μg/ml of 4-hydroperoxycyclophosphamide, was infused on day 0. We have treated 46 patients, 27 in first CR and 19 in second or subsequent CR. Twenty three of the 27 first remission patients acheived CR with high dose Ara-C combined with either daunorubicin or mitoxantrone and 4 received standard dose regimens. One patient required two courses of therapy to achieve initial remission, and two patients received one course of consolidation therapy, which in neither case included high dose Ara-C, prior to BMT. Median age was 39 (17 - 59) and median time from CR to BMT was 3 months (1.5 - 7). Of the first CR patients, one died of diffuse gut injury and 4 of 26 survivors have relapsed (at 8, 12, 14, 23 months). One patient died of unrelated causes and 21 remain in CCR. With median follow-up of 20 months (2^+ - 57^+) and ten patients in CCR over 2 years, actuarial DFS is projected to be 72 ± 11%. Included in this group are 14 patients with favorable FAB types M3 and M4EO who all remain in CCR with median follow-up of 20^+ months. Median time to 100 and 500 neutrophils was 24 and 38 days respectively, time to hospital discharge was 41 days, and time to platelet independence was 55 days. Of the 19 second or subsequent remission patients, 7 died during treatment and 3 of 13 survivors have relapsed (at 2, 3, 9 months). Ten patients remain in CCR. With median follow-up of 34 months (3^+ - 46^+) actuarial DFS is projected to be 45 ± 12%. Busulfan/etoposide is an effective and acceptably toxic regimen whose use with autologous purged BMT may improve the long term DFS of patients with ANLL.

D 323 ALLOGENEIC BMT FOR HUMAN T-LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1) POSITIVE ADULT T-CELL LEUKEMIA (ATL); A CASE REPORT. Per Ljungman, Johan Aschan, Karin Karlsson, Berit Lönnqvist, Claes Malm, Olle Ringdén, Olle Vikrot, Birgitta Åsjö, Gösta Gahrton. Deps. of Medicine, Clinical Immunology, and Transplantation Surgery, Huddinge Hospital, Huddinge; Dep of Medicine, University Hospital, Linköping; Dep of Virology, National Bacteriological Laboratory, Stockholm, Sweden.

The patient was a 48 year old female of japanese origin who in January 1990 developed leukocytosis, fever and hypercalcemia. She was HTLV-1 positive. Morphology, cytochemistry and immunology supported the diagnosis of ATL. BMT was performed in first complete remission July 13,1990 with an HLA-identical, HLTV-1 negative sister as donor. The conditioning regimen was TLI 3 x 2 Gy, cyclophosphamide 60 mg / kg x 2, and TBI 7.5 Gy. T-cell depletion was performed with monoclonal antibodies. The patient received foscarnet from day -10 to day +49 after BMT, in an attempt to reduce the risk for infection of the marrow graft with HTLV-I. She had an uncomplicated course with engraftment on day +16 and developed acute GVHD grade I and later limited chronic GVHD, which both were treated with corticosteroids and resolved. Blood group antigens showed only donor erythropoesis. She remained healthy until the end of May, 1991 when she developed intermittent fever up to 39°C. No explanation could be found for the fever. She remained intermittently febrile until June, 30 when she was readmitted due to confusion and signs of encephalitis. An extensive search for the cause of the encephalitis was negative. Neuroradiologic examinations were normal. CSF did not contain any leukemic cells and the bone marrow was normal. The patient rapidly deteriorated despite antiviral, antibacterial and antifungal treatment and died on July, 20. Autopsy showed slight atrophy of the cerebral cortex, gliosis, and perivascular lymhocyte infiltration. No infectious agent was found. The patient was after BMT followed repeatedly with serology for HTLV-1 (ELISA). All serum samples tested showed presence of HTLV-1 antibody. However, there was no HTLV-1 antibody detected in the cerebrospinal fluid. Bone marrow and PBL specimens were examined by PCR and co-cultivation experiments with normal PBL. All bone marrow samples and PBL samples was positive for HTLV-1. This case indicates that HTLV-1 can not be erradicated by allogeneic BMT despite antiviral prophylaxis with foscarnet. Whether another anti-retroviral agent would give better results is unknown.

D 324 CYTOGENETIC AND MOLECULAR REMISSION OF CML AFTER T-DEPLETED BMT. THE ROLE OF A NEW MYELOABLATIVE REGIMEN THAT INCLUDES THIOTEPA. Massimo F. Martelli, Franco Aversa, Piergiuseppe Pelicci #, Adelmo Terenzi, Alessandra Carotti, Nicola Albi, Amedea Mencarelli #, Emilio Donti #, Paolo Latini °, Franco Checcaglini °, Yair Reisner *. Istituto di Ematologia e #Clinica Medica I, University of Perugia. ° Radioterapia Oncologica, Policlinico Monteluce 06100 Perugia, Italy. * Department of Biophysics, Weizman Institute, Rehovot, 76100 Israel.

The majority of CML patients treated with standard conditioning regimens and donor marrow depleted ex vivo of T lymphocytes relapse after BMT. We report the chimerism pattern, Ph-chromosome status and molecular genetics of 22 CML patients (16 M, 6 F; median age 36 years, range 16-49) who received a lectin-separated BMT after a conditioning regimen that included 14.4 Gy hyper-fractionated TBI, 10 mg/kg Thiotepa, ATG and 100 mg/kg Cyclophosphamide. One patient was mismatched at 1 locus. All but one, who was in second CP, were in 1st CP. All patients achieved a full-donor chimerism engraftment at DNA polymorphism analysis. Neither acute nor chronic GVHD were observed. 16 (72%) survive event free at a mean follow-up of 12 months (range 2-35). Molecular and cytogenetic relapse was documented in one patient 7 months after transplant. 5 non-relapse deaths occurred (1 aspergillus IP, 1 CMV-IP, 1 idiopatic IP, 1 sepsis and 1 Lyell syndrome). Three monthly check-ups have failed to reveal Ph-chromosome or PCR positivity in either peripheral blood or bone marrow of the 16 survivors.

It seems reasonable to hope that, despite the total absence of GVHD, our conditioning schedule that included the myeloablative agent thiotepa will be accompained by a relapse-rate as low as that encountered with unmanipulated bone marrow.

D 325 COMPARISON OF CYTOGENETICS, RFLPs AND PCR FOR ASSESSING CHIMERISM FOLLOWING BMT FOR CML. **Shaun R. McCann, Mark Lawler, Peter Humphries, Philip McGlave and Bruce Blazar. Depts of Haematology/Genetics St. James's Hospital and Trinity College Dublin Ireland, Depts of Pediatrics and Hematology, University of Minnesota, Minneapolis.**

We conducted a blind study between 2 institutions to assess the relative sensitivities of cytogenetics,RFLPs and PCR in the assessment of chimerism post BMT in a cohort of 17 patients who had received allogeneic BMT for CML. In all cases recipients were male and donors were female.All patients were conditioned with fractionated TBI and cyclophosphamide.Follow up time ranged from 27-744 days. Cytogenetic markers of engraftment were the Y and/or Philadelphia chromosomes.For RFLP analysis a panel of DNA markers which were informative for donor / recipient were used to assess chimerism post BMT. PCR was performed on archival slide material following rapid lysis after scraping of slide material into an tube using a sterile scalpel blade.PCR was executed with a panel of 3 Ychromosome specific oligonucleotide primer sets and autosomal material was coamplified to allow levels of recipient cells to be estimated. The results of cytogenetics and RFLP analysis were unknown at the time of PCR study.Results indicated that MC was detectable in one patient using cytogenetics, in a second patient using RFLPs and in a further three patients using PCR In no case did we fail to detect MC by PCR. The earliest detection of MC was by PCR at day 27.This study indicates that PCR is more sensitive than RFLP analysis which in turn is more sensitive than cytogenetics in the detection of MC in sex mismatched transplants.

D 326 ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) FOR HEMATOLOGIC MALIGNANCIES IN PATIENTS (PTS) OVER AGE 45--T.W. Ratliff, G.S. Harman, R.D. Halvorson, M.J. Snyder, J.H. Essell, and J.M. Thompson, Dept. Hematology SGHMMH, Wilford Hall USAF Medical Center, San Antonio, Texas 78236. Advanced age has been used as a factor in denying allogeneic bone marrow transplantation. Contraindications to the procedure in older patients were thought to include excessive toxicity of the preparative regimen and excessive risk of severe graft-versus-host disease (GVHD). From July 1987 thru Aug 1990, we performed allogeneic BMT on 17 patients, ages 45-61, all with good performance status, no severe organ dysfunction, and lack of excessive prior cytotoxic chemotherapy. Underlying diagnoses included chronic myelogenous leukemia (CML) in chronic phase (7 pts), CML in advanced phase (4 pts), acute myelogenous leukemia (AML) in complete remission (2 pts), AML in relapse (2 pts), and agnogenic myeloid metaplasia (1 pt). 7/17 (41%) patients are alive currently, 348-1508 days post-BMT. Causes of death included bacterial infection (1 pt), fungal infection (1 pt), cytomegalovirus pneumonitis (3 pts), mixed infection (1 pt), veno-occlusive disease of the liver (VOD) (1 pt), cerebrovascular accident (1 pt), and relapsed disease (1 pt with CML in blast crisis and 1 pt with AML in relapse). 3/7 living patients and 3/10 dead patients had $\geq$ grade 2 GVHD. 3/17 patients (all dead) had $\geq$ grade 2 VOD. Our results indicate that allogeneic BMT can be offered to selected patients over age 45. These results will be further compared to our results in younger patients and reported results in older patients.

D 327 AUTOLOGOUS BMT FOLLOWING AN "ad hoc" CONDITIONING REGIMEN FOR EXTRAMEDULLARY RELAPSES OF CHILDHOOD ALL: A PRELIMINARY REPORT,

Rossetti F., Colleselli P., Messina C., Cesaro S., Sotti G. and Zanesco L., Department of Pediatrics, University of Padova, Padova Italy

INTRODUCTION. Early extramedullary ALL relapses treated with second-line chemotherapy have resulted in 20-40% long-term survival. We planned to treat CNS and testis relapses with ABMT following high-dose cytosine arabinoside plus hyperfractionated TBI to test efficacy and toxicity of such conditioning regimen. PATIENTS & METHODS. From July 1987 to July 1991, ten children who relapsed in CNS (8 patients) or testis (2 boys) during front-line chemotherapy or within the 6th month from the cessation of chemotherapy, underwent ABMT following one dose intrathecal methotrexate, HD Ara-C (3 gr/m2 twice daily for 4 consecutive days) and TBI (1,2 Gy three times daily for 4 further consecutive days). No further CNS prophylaxis was administered following ABMT. Their ages at time of diagnosis ranged from 0,7 to 14,8 yeras and, at time of ABMT, from 3,1 to 16,7 years. The 1st remission-to-relapse intervals ranged from 13 to 30 months and th relpase to ABMT intervals from 5 to 10,5 months. RESULTS. One patient died due to streptococcus plus Candida Albicans sepsis on day +5 and another one died due to bone marrow relpase occurred 4 months after ABMT. The remaining 8 patients are alive and well 2 to 48 (median 18) months following ABMT. In particular, no CNS relapse has been so far observed even though we did not perform any CNS prophylaxis following ABMT. The toxicity of this conditioning regimen was acceptable. A slight to moderate conjunctivitis, nausea and diarrhea were the most frequent complications. No patient had evidence of CNS toxicity. CONCLUSION. This preliminary report would suggest a possible efficacy of HD Ara-C plus hyperfr-TBI as a conditioning regimen to children undergoing ABMT for an earlyextramedullary ALL relapse. A longer follow-up and more cases could be able to confirm this hypothesis.

D 328 SECOND BONE MARROW TRANSPLANTS (BMT) FOR LEUKAEMIA. Marcus Vowels, Mathew Shing, Reg Lam Po Tang, Hedy Mameghan, David Ford and Annette Trickett. Pediatric Bone Marrow Transplant Unit, Prince of Wales Children's Hospital, Sydney, 2031, Australia.

Nine patients with ALL (n=2), AML (6) and CML (1) received a second BMT and one of these a third BMT, following failure of sustained engraftment (4) or relapse (6). The initial transplant utilised matched (7), one antigen mismatched (2) and autologous (AUTO) (1) marrow. Four patients who failed to have sustained engraftment received an AUTO BMT. These patients had had an AUTO marrow collected as a backup prior to their first BMT because of increased risk of rejection. No conditioning therapy was given. Two patients died before engraftment, one relapsed at 17 mths and one remains relapse free for 62+ mths. Six patients who relapsed 13 to 47 mths (median 18 mths) from first BMT received a second allogeneic (ALLO) BMT. Source of donor marrow was from the same donor (5) as the first BMT or an ALLO instead of an AUTO (1). Conditioning consisted of chemotherapy alone (3) if total body irradiation (TBI) was used for the first BMT, or TBI and melphalan $\pm$ antilymphocyte globulin (3). Successful engraftment occurred in all six patients. Acute graft versus host disease (GVHD) was more frequent as compared with the first BMT. Three patients given chemotherapy conditioning relapsed at 7, 8 and 16 mths whereas of three patients given TBI, two are relapse free for 20+ and 25+ mths (both longer than there first renmission) and one died from pneumonitis after BMT. One patient has severe chronic GVHD and pneumonitis. Thus, collection of an AUTO backup marrow rescues patients who are at increased risk of failure of sustained engraftment and second ALLO BMT leads to relapse free survival in a significant number of patients.

D 329 INTERLEUKIN-2 AS ADJUNCT IMMUNOTHERAPY FOLLOWING AUTOLOGOUS MARROW TRANSPLANTATION FOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Despite intensive chemoradiotherapy conditioning and ex vivo marrow purging, leukemia relapse remains the most frequent cause of failure following autologous BMT for acute lymphoblastic leukemia (ALL). In order to induce in vivo antileukemic immunologic activity which could prevent relapse, we have performed a phase I study infusing recombinant human Interleukin-2 (IL-2) in the immediate post-transplant period. This early time period was chosen because the disease burden is lowest. After conditioning with hyperfractionated total body irradiation and cyclophosphamide and reinfusion of cryopreserved, purged autologous marrow, we initiated continuous infusion IL-2 therapy (96 hrs/week x 3 weeks) on day +1. Fourteen patients with high-risk, relapsed ALL were treated at 3 dose levels of IL-2; 0.5, 1.0 and 2.0 x 10^6 U/m^2/day (supplied by Hoffman LaRoche). Of the patients evaluable, those treated with IL-2 had more rapid neutrophil recovery, earlier platelet and RBC transfusion independence, and earlier hospital discharge compared to control ABMT recipients not receiving IL-2. However, toxicity associated with the combination of IL-2 and the inherent toxicity of the BMT conditioning regimen was substantial. Grade III/IV liver, renal and pulmonary toxicity was observed in approximately 20% of patients. IL-2/BMT toxicity was excessive at the higher doses resulting in 2 deaths at 2 x 10^6 U/m^2 and may have contributed to 2 others (one at 1.0 and one at 2.0 x 10^6 U/m^2). Assessment of immune activation induced by in vivo IL-2 demonstrated no accelerated recovery of natural killer function. In contrast, proliferation of CD8+, HLA-DR+ T cells with enhanced in vitro cytotoxicity against leukemia targets was seen in the majority of IL-2 patients studied. These data suggest that IL-2 given immediately post-transplant is associated with variable, but significant clinical toxicity at lower doses than in the non-BMT setting. This toxicity may possibly reflect T cell rather than NK cell activation in conjunction with the toxicity of pre-BMT conditioning. IL-2 therapy after BMT, though associated with rapid engraftment, must be undertaken cautiously and its use requires further study of appropriate doses, schedules and measures to reduce toxicity.

Lymphoma and Solid Tumors

D 400 MARROW TRANSPLANTATION FOR HODGKIN'S DISEASE RESULTS OF SEQUENTIAL TRIALS.

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108 consecutive patients with advanced Hodgkin's Disease unresponsive to or relapsing after initial therapy were stratified into high risk (resistant relapse or primary refractory HD, HR) and good risk (sensitive relapse, SR) groups. 91 pts were treated with BCNU 400-600mg/m^2, Etoposide 1.8-2g/m^2 and Cytoxan 5g/m^2 (BEC). The initial 24 pts, including 17 HR and 7 SR, were treated with BEC alone. 17 pts relapsed and 1/24 pts is in CCR for 60 months. The next cohort of 67 patients was followed for a minimum of 13 months. 21/67 pts had SR and were treated in subsequent CR/PR. 10/21 relapsed and 9/21 are alive in CCR. 46/67 pts had HR HD and were eligible for a BMT2 using either ThioTEPA 900/m^2, Velban 0.4-0.6mg/kg_2 and Ara-C 3-6 gm/m^2, (TAVe) or ThioTEPA 750mg/m^2, Mitoxantrone 40/m^2 and Carboplatinum (JM8) 1000/m^2 (TMJ). 30/46 pts underwent BMT2. 14/46 pts (including 10/30 BMT2 pts) are alive in CCR up to 54 months. 6/30 pts undergoing sequential BMT relapsed post BMT2. The final cohort of 17 pts was treated with TMJ initially and HR patients were offered BMT2 after Cytoxan 7.5g/m^2 and VP-16 2400 mg/m^2. 3/17 pts have relapsed. Sequential BMT is associated with a lower risk of relapse in pts with HR HD compared with pts receiving a single BMT for SR HD. TMJ is associated with less toxicity when given as the initial conditioning regimen rather than prior to BMT2. Given the lack of pulmonary toxicity with TMJ, it should be evaluated further in patients with HD.

D 401 PENTOXIFYLLINE (PTX), CYCLOPHOSPHAMIDE (CY), CARMUSTINE (BCNU), AND ETOPOSIDE (VP-16) "CBV REGIMEN" IS AN EFFECTIVE BONE MARROW TRANSPLANT (BMT) REGIMEN FOR RELAPSED LYMPHOMA J. A. Bianco, J. Nemunaitis, D. F. Andrews, M. Lilly, A.Shields, F.B.Petersen, C. D. Buckner and J. W. Singer. The Marrow Transplant Program, V.A. Med Ctr, Fred Hutchinson Cancer Ctr and University of Washington School of Medicine, Seattle, WA.

A recent analysis of Seattle patients with advanced lymphoid malignancy treated with CBV showed a 32% day 100 non-relapse mortality (NRM) in autologous (A) BMT patients and a 40% NRM in allograft recipients. The development of regimen related toxicity (RRT) has been associated with elevations in assayable levels of tumor necrosis factor (TNF). The methylxanthine derivative PTX reduces the production of TNF in response to a variety of stimuli in vitro and in vivo. We therefore administered prophylactic PTX (2000mg/day) to 10 patients with advanced lymphoid malignancy receiving the CBV regimen (CY 7200mg/m^2, BCNU 300-600mg/m^2, VP-16 2400mg/m^2). The median age was 37 years, range 25-56; 4 received ABMT, 5 had allografts and 1 had a syngeneic donor. With a median follow up of 270 days (range 60-502) 8 patients survive, 7 are disease free. The day 100 NRM was 10% with 1 patient dying of aspergillus on day 52. Overall toxicity of this regimen was low with no patient experiencing ≥ grade II RRT (see table) while historical data suggests an expected grade III-IV RRT of > 50% . These data suggest that PTX can reduce NRM among high risk patients receiving CBV and that the maximum tolerated doses of these agents may be higher than the standard regimen when PTX is added.

Toxicity	*PTX group [n=10]*
Bilirubin‡	1.5 ± 0.5 (1.6)
Creatinine ‡	1.2 ± 0.3 (1.1)
MSO4@	0.4 ± 0.4 (0.0)
Fever (days)^	1.6 ± 3.7 (0.0)
Day discharged•	19.6 ± 6.7 (18.0)

Mean ± S.D. (median) ‡ Maximum value days 0-28. @Days morphine
^ Temperature ≥ 38.3°C days 0-28 . • 1 patient died prior to discharge

D 402 MOLECULAR DETECTION OF TUMOR SPECIFIC IMMUNOGLOBULIN GENE SEQUENCES FOLLOWING AUTOLOGOUS TRANSPLANTATION OF MULTIPLE MYELOMA,

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Autologous transplantation is increasingly being investigated in the treatment of multiple myeloma (MM). To identify tumor specific molecular markers for sensitive detection of residual malignant cells, we performed nucleotide sequence analysis of the expressed immunoglobulin (Ig) variable region (V) heavy chain in two cases of Ig A MM. The first MM sequence, Alpha 6.3, demonstrated 94% homology to a germline VH-1 gene, HG3 which was originally identified in a human fetal liver DNA library. The second MM sequence, 914.18, demonstrated its greatest homology (88.8%) with the VH-3 gene V-H-1.9 III which is a fetally expressed V-region gene known to encode for anti-DNA reactivity. The V-region gene sequence of both MM cases (alpha 6.3, 914.18) appeared highly diversified from germline with a non-random distribution of amino acid substitutions which were clustered in the CDR regions. To verify that these MM VH genes were in fact somatically mutated, specific oligonucleotide (oligo) primer pairs corresponding to mutated regions of the CDR and the likely germline precursor sequence were made. With granulocyte DNA from each patient as a template, PCR analysis demonstrated appropriate amplification products using the "germline" primers, but no amplification using the "mutated" primers. These preliminary data suggest that the B cells of MM may be heavily mutated from germline in a pattern consistent with an antigen driven response. The patient with MM sequence 914.18 was treated on an autologous transplantation protocol previously described by the Dana-Farber Cancer Institute. Following this treatment, DNA extracted from a peripheral blood buffy coat was used as a template in a PCR reaction with tumor specific primer pairs derived from the Ig gene sequence. In one reaction the "mutated" CDR2 oligo (sense primer) and a joining region consensus oligo (anti-sense primer) were used. In a 2nd reaction a consensus framework 1 oligo (sense primer) and a tumor specific oligo corresponding to the junctional sequence of the diversity (D) and joining (J) regions of this neoplastic clone were used (anti-sense primer). In both reactions an amplification product of the appropriate size was obtained indicating the presence of residual disease. Sequencing of these amplification products is in progress to further confirm their tumor origin. These data suggest that oligonucleotide primers made from either somatically mutated CDR regions or D-J junctional sequences can be used in PCR reactions to identify minimal residual disease in multiple myeloma.

D 403 AN ANALYSIS OF COMPLICATIONS OF OUTPATIENT BONE MARROW HARVESTING, Brian Bolwell, Alan Lichtin, Karen Sands, Amy Murar, Renee Burwell, Cleveland Clinic Foundation, Cleveland, OH 44195

Bone marrow transplantation requires an adequate marrow harvest, yet there are little data concerning the side effects of the procedure itself. We routinely send patients a quality assurance questionnaire about potential side effects of the bone marrow harvest, in an effort to quantitate the complications of the procedure. From January of 1990 through July of 1991 64% of 211 patients undergoing bone marrow harvest(135 total patients) responded. The median age of the patients was 35 years old. There were 72 males and 63 females. All patients underwent outpatient bone marrow harvesting using a posterior iliac crest approach. 95% of patients underwent general anesthesia and 5% epidural anesthesia. All patients received autologous red cell transfusions obtained at the time of harvest using a cell separator; only one patient required homologous red blood cell transfusion. The median harvest yield was 3.27 X 10^8 nucleated cells per kilogram(range 0.67-5.87). The most common side effect was pain at the site of donation(90% of respondents). Other symptoms noted were low back pain-60%, nausea-43%, vomiting-31%, sore throat-43%. Less frequent complications included fever-18% and bleeding at the donation site-6%. After an average time of two weeks post-harvest, 39% of patients stated that they were still unable to perform all normal physical activities. We conclude that 1) outpatient bone marrow harvesting is feasible; 2) homologous red blood cell transfusions can be reduced by utilizing the red blood cells collected at the time of harvest; 3) while bone marrow harvesting has few major complications, a variety of minor problems persist for weeks and merit continued study.

D 404 RENAL DYSFUNCTION (RD) DURING HIGH DOSE CISPLATIN THERAPY (HDCP) AND AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT): EFFECT OF AMINOGLYCOSIDES (AG), Brenda W. Cooper, Hillard M. Lazarus, Wida Soegiarso, Richard J. Creger, Ireland Cancer Center, University Hospitals of Cleveland, Case Western Reserve University, Cleveland, OH 44106.

Aminoglycoside antibiotics often are avoided in pts who have recently received cisplatin-based chemotherapy because of possible synergistic nephrotoxicity. We reviewed the incidence of RD in 102 consecutive pts who received HDCP-containing regimens followed by ABMT at our institution between 1985 and 1991. RD was defined as an increase in serum creatinine $\geq$ 0.5 mg/dl. All pts received HDCP administered as 40 mg/m^2 for 5 consecutive days in 3% saline with saline hydration and mannitol diuresis. Other high-dose chemotherapy administered included BCNU and VP-16 in 56 pts with lymphoma, VP-16 in 28 pts with lung cancer, and BCNU in 18 pts with other non-hematologic malignancies. 41% of the pts received AG for fever and neutropenia after HDCP. Characteristics of pts who did or did not receive AG were similar with respect to age, sex, duration of neutropenia, cancer type, and previous cisplatin therapy. Mean increase in serum creatinine was 1.11 mg/dl in pts who received AG and 0.62 mg/dl in pts who did not receive AG. A logistic regression analysis was performed to determine independent risk factors for RD:

Variable	Multivariate Effect (p value)
Aminoglycosides	0.537
Amphotericin B	0.004
Vancomycin	0.534
Cephalosporin	0.232
Cancer Type	0.829
Age	0.006
Days Neutropenic	0.037

Age, duration of neutropenia, and amphotericin B were independent predictors of developing RD. AG antibiotics did not significantly increase the risk of developing RD. Our data suggest that AG can be safely administered to febrile, neutropenic ABMT pts who have recently received HDCP administered in this fashion.

D 405 OUTCOME OF AUTOLOGOUS (AUTO) VS ALLOGENEIC (ALLO) BONE MARROW TRANSPLANTATION (BMT) IN 25 CHILDREN WITH NEUROBLASTOMA (NB) AND UNFAVORABLE FEATURES (UPF). P Dinndorf, L Johnson, P Gaynon, J Nachman, E Morgan, R Quinones, Children's National Medical Center, Wash,DC, 20010 and University of Chicago Med Ctr, Chicago, Il 60637. 25 children with NB and UPF (defined by age, stage, Shimada classification, amplified n-myc) were treated with an intensive chemotherapy regimen (23 received 3-8 courses of 6-in-1 CCG-321 P4; 2 received CCG-321 P2). Patients (pts) were restaged and surgical removal of residual tumor was attempted. 13 pts were treated with radiotherapy (XRT) to the tumor bed and areas of suspected residual disease. BMT conditioning consisted of Cy (50/kg x 4 days) and fractionated TBI (200 cGy BID x 3 days). 8 pts with matched sibs were rescued with allo marrow; 4 received auto purged (au p) marrow; 13 received auto nonpurged (au np) marrow. Follow-up ranges from 2-59 mos. Estimated DFS at 24 mos by Kaplan-Meier analysis of the allo group is 67%; of the auto group is 41%; and of the entire cohort is 51%. There were two early deaths due to transplant related toxicity (sepsis, CMV pneumonitis). Relapse occurred in 7 patients from 6-18 mos post BMT (1/8 allo pts; 2/4 au p pts; and 4/13 au np pts). We conclude: [1] This multimodality approach to the treatment of NB pts with UPF, using aggressive initial chemotherapy, surgical excision, +/- XRT and BMT with cytoxan and TBI improved the DFS of children at 2 years compared to historical controls. [2] 4 of 5 relapses which occurred in sites of old bulk disease were in pts who did not receive boost XRT to these sites. This suggests the need for XRT to potential sites of residual disease prior to BMT. [3] None of 6 pts in CR at time of BMT relapsed. 4/13 in VGPR and 3/4 in PR have relapsed. This suggests pts with a greater amount of residual tumor at the time of BMT were more likely to relapse.

D 406 CYCLOPHOSPHAMIDE(Cy), CARBOPLATINUM(Cb), AND MELPHALAN(L-PAM) +/- FRACTIONATED TOTAL BODY IRRADIATION(fTBI), A NEW CYTOREDUCTION REGIMEN FOR CENTRAL NERVOUS SYSTEM TUMORS(CNS) OR NEUROBLASTOMA(Nb)
RE Duerst, A Eskenazi, H Weinstein, P Savina, and A Guaspari. Univ. of Rochester School of Medicine, Rochester, NY, Univ. of Maryland School of Medicine, Baltimore, MD, Dana Farber Cancer Inst. Boston, MA, and Burroughs-Wellcome Cancer Therapy Dept, Research Triangle Park, NC.

A regimen employing Cy (1800 mg/m^2) Days -4 and -3, Cb (400 mg/m^2 initial dose level) Days -4 and -3, and L-PAM (140 mg/m^2) Day -2 was developed for treatment of patients (<21 yo) with recurrent or progressive CNS tumors. The Cb will be escalated in 100 mg/m^2 intervals every 4 patients to a dosage of 800 mg/m^2 dy. This regimen was based on a regimen consisting of Cy, continuous infusion Cisplatinum, VM26, and L-PAM followed by fTBI for children with metastatic Nb (DFCI 84-011). Five patients (2-19 yo) with recurrent CNS tumors have been treated with this regimen. (DX=Medulloblastoma -2, Ependymoma, glioblastoma multiforme and choroid plexus carcinoma). Four were treated at the initial dose level, one at Cb, 500 mg/m^2. There has been one toxic death following development of renal insufficiency, pancreatitis and pneumonitis. Three have developed progressive disease after stable disease periods of 3 months duration.

Four children (2-8 yo) with Nb have been treated with the identical chemotherapeutic regimen (Days -6, -5 and -4) followed by fTBI (6x200 cGy = 1200 cGy total). As of 9/91, these patients are in remission 3,4,6 and 8 months from BMT. Pharmacokinetic studies of L-PAM are being performed. Serum concentrations obtained within one hour after the end of a one-hour infusion, range from 1-6 mcg/ml. CSF samples (at 60 min) have been obtained from 4 patients; 3 have had levels from 60-120 ng/ml. This regimen appears to be well tolerated at the initial dose level of Cb. Longer follow-up will be required to document its efficacy.

D 407 "PRIMING" FOR PERIPHERAL BLOOD STEM CELL COLLECTION: A COMPARISON OF METHODS.
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Autologous bone marrow "transplant" (ABMT) following high dose cytotoxic therapy is proving to be a useful therapeutic modality in the treatment of a variety of metastatic diseases. The usefulness of ABMT is limited by the quality and quantity of bone marrow which may be harvested from the candidate patient. For patients whose marrow is non-collectable (hypocellular or fibrotic) or non-usable (metastatic disease in marrow), we have instituted a program in which stem cells are collected from the peripheral circulation and used as a substitute for autologous bone marrow. From July 1990 to July 1991, 19 patients have had peripheral blood stem cells (PBSC) harvested. To elicit stem cells into the peripheral circulation, we have "primed" patients with cytoxan (50mg/Kg/day x 2 days), cytoxan followed by administration of leucovorin, or cytoxan followed by administration of G-CSF. The number of nucleated cells, granulocyte-macrophage colony forming cells (GM-CFC) and CD34+ cells collected was followed. Similarly, the course of hematologic recovery following high dose cytotoxic therapy and peripheral blood stem cell "transplant" was followed. We cannot demonstrate any significant difference in CFU-GM or CD34+ cell production following priming with cytoxan alone or with cytoxan combined with either leucovorin or G-CSF. Moreover, we cannot demonstrate any difference in rates of hematologic recovery with these different priming modalities. We have found that using any of these priming techniques, that with a reasonable number of pheresis procedures (6-8) we can collect sufficient PBSC for hematopoietic reconstitution in 80% of patients who can not be treated by ABMT.

D 408 RELATIONSHIP BETWEEN GRAFT PROGENITOR CONTENT AND HEMOPOIETIC ENGRAFTMENT FOLLOWING AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR LYMPHOMA. Helen U. Enright, Elizabeth H. Perry, Susan K. Fautsch Larry C. Lasky, Daniel J. Weisdorf. Bone Marrow Transplant Program, University of Minnesota, Minneapolis, MN 55455.

The influence of graft progenitor content on hemopoietic engraftment following autologous bone marrow transplantation (BMT) was analysed for 99 patients with lymphoma (30 with Hodgkin's disease and 69 with non-Hodgkin's lymphoma (NHL)). Grafts were assayed for progenitor content before (fresh) and after cryopreservation (thawed). Correlations were sought (Pearson's correlation coefficient) between the logarithim of the graft progenitor content of CFU-GM, CFU-GEMM, BFU-E and CFU-E and the following engraftment end-points: days to achieving a WBC > 1 x 10^9/L for 3 consecutive days (time to WBC); days to an absolute neutrophil count > 0.5 x 10^9/L (time to ANC); and time to red blood cell and platelet transfusion independence. All but 5 patients who died before engraftment were evaluable and achieved an ANC of 0.5 x 10^9/l following BMT (at a median of 21 days; range 9-50), although 4 patients with NHL required second infusions of bone marrow (at 35,44,45 and 62 days) before successful complete engraftment occurred. The median time to WBC was 23 days (range 11-86). There was no statistically significant correlation between bone marrow dose (nucleated cells/kg infused) and any engraftment end-point. The CFU-GM progenitor content of the fresh samples did not correlate with the time to ANC or to WBC. However, there was a statistically significant correlation between the CFU-GM and CFU-GEMM contents of the thawed samples and time to ANC (r=-.307, p=.025 and r=-.418, p=.008 respectively). The CFU-GEMM content of the thawed samples was a better predictor of time to ANC and WBC than the CFU-GM content of the same samples. There was no statistically significant correlation between the BFU-E, CFU-E or CFU-GEMM progenitor content of either fresh or thawed samples and time to red blood cell transfusion independence. Similarly, CFU-GEMM contents did not correlate with platelet transfusion independence. These data suggest that CFU-GEMM cultures, especially cultures of bone marrow samples after cryopreservation may be the most useful predictors of engraftment following autologous BMT for lymphoma.

D 409 CYTOTOXIC AND CYTOTOXIC/G-CSF MOBILIZATION OF AUTOLOGOUS BLOOD STEM CELL AND THEIR AUTOGRAFTING Tetsuya Etoh, Takanori Teshima, Mine Harada, Yasushi Takamatsu, Seiji Kondo, Shoichi Inaba, Yoshiyuki Niho First Department of Internal Medicine and Blood Transfusion Service, Faculty of Medicine, Kyushu University Fukuoka 812, Japan.

We analyzed a total of 90 courses of leukapheresis consisting of 305 collections of peripheral blood stem cells (PBSC) mobilized by chemotherapy or chemotherapy/G-CSF from 60 patients with hematologic tumors or solid malignancies. The mean yields of CFU-GM collected with cytotoxic mobilization was $8.43x10^4$/kg in 49 courses of leukapheresis. The cytotoxic/G-CSF mobilization significantly expanded CFU-GM yields up to $16.4x10^4$/kg in 41 courses. When CFU-GM yields were compared between cytotoxic and cytotoxic/G-CSF mobilization in a single patient, in whom 2 successive courses of leukapheresis were performed: the first course with cytotoxic mobilization and the second one with cytotoxic/G-CSF mobilization, CFU-GM yields with cytotoxic/G-CSF mobilization were greater than those with cytotoxic mobilization in 10 out of 11 patients. These results indicate the effectiveness of G-CSF for the expansion of a PBSC pool. Next, we analyzed factors affecting the efficacy of PBSC collections. Synchronous recovery of both leukocytes and platelets during a course of leukapheresis was significant for achieving a high CFU-GM yield in both cytotoxic and cytotoxic/G-CSF mobilizaion. The MNC yield was not significantly different among the hemopoietic recovery patterns. In such successful collections from patients showing rapid recovery of both leukocytes and platelets, increasing ages of the patients decreased CFU-GM yields. The optimum timing of collection was different between cytotoxic and cytotoxic/G-CSF mobilization; WBC levels on the day of maximum CFU-GM collection were higher in cytotoxic/G-CSF mobilization than in cytotoxic mobilization. Following PBSC transplantation, hemopoietic reconstitution was observed in all of the cases and the rate of trilineage reconstitution was significantly correlated with the dose of CFU-GM insfused.

D 410 IFOSFAMIDE, CARBOPLATIN AND ETOPOSIDE (ICE) WITH AUTOLOGOUS STEM CELL RESCUE (ASCR): PHASE I RESULTS. Karen K Fields, Gerald J Elfenbein, Paul E Zorsky, John W Hiemenz, Ramy A Saleh, Brenda W Cooper, Janelle B Perkins, Lori E Kronish, Mary C Machak. Div of Bone Marrow Transplantation, Univ of S Florida, Tampa, Fla 33612

We have treated 58 patients(pts) in a Phase I-II study with escalating doses of ICE with ASCR at the following doses: Ifosfamide 6-17.1g/M^2, Carboplatin 1.2-2.1g/M^2, and Etoposide 1.8-2.1g/M^2. Mesna was dosed equivalently with Ifosfamide. Autologous stem cells were reinfused 48 hours after completion of chemotherapy. 4 pts received peripheral blood stem cells(PBSC); 52 received autologous bone marrow. At least 4 pts per dose level were evaluated for toxicity(tox). Pt characteristics follow: median(med) age=41 (range 15-56); females=48, males=8; breast cancer(ca)=37, non-Hodgkin's disease=9, ovarian ca=5, acute leukemia=3, Hodgkin's lymphoma=2, germ cell ca ovary=1, osteosarcoma=1, and sarcoma=1. For the 30 pts not receiving growth factors(CSF) or PBSC, the med time to granulocyte (AGC) recovery was 23 days(d) (range 15-47) for AGC >500 and 24d (range 17-58) for AGC >1000. The med time to platelet recovery >20,000 and >50,000 was 20d (range 13-46) and 24d (range 15-108), respectively. The med time to discharge(d/c) from the hospital following transplant was 27d (range 19-95). With the addition of CSF, med d/c day decreased from 27d to 25d despite increase in other tox. 4 pts(7%) died during therapy, 3 from sepsis and 1 from acute renal failure(ARF). Other grade 3/4 tox included: 21 pts with reversible(rev) enteritis, 5 with rev hepatic tox, and 7 with fluid overload associated with rev cardiopulmonary tox. Severe metabolic acidosis and electrolyte wasting was prominent at upper dose levels. Carboplatin doses of 2.1g/M^2 were associated with grade 3/4 ototox and symptomatic peripheral neuropathy in 4/6 pts; therefore the maximum tolerated dose(MTD) of Carboplatin in ICE was determined to be 1.8g/M^2. The present dose of Ifosfamide in ICE is 17.1g/M^2 due to the development of irrev coma and ARF with death in 1 pt and rev gr 4 CNS tox and grade 2 ARF in another pt at this dose level; both pts had been heavily pretreated with cisplatin. None of the other 6 pts at this dose level exhibited this tox. The present dose of Etoposide is 2.1g/M^2. Further dose escalations are ongoing to determine the MTD of Ifosfamide and Etoposide in ICE.

D 411 PROTEIN C DEFICIENCY IN CHILDREN UNDERGOING AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT), B.G. Gordon, W.D. Haire and R. Bagin, Departments of Pediatrics, Internal Medicine, and Societal and Preventive Medicine, University of Nebraska Medical Center, Omaha, NE 68198.

Protein C (PC) is an important *in vivo* regulator of coagulation. Decreases in PC anticoagulant activity (PCClot) and antigen (PCAg) occur in adults undergoing ABMT, which may contribute to the morbidity of ABMT. We studied 7 children (4 yrs-16 yrs) undergoing ABMT, on the day of ABMT and weekly thereafter. PCClot and PCAg were decreased below the normal range by day 0 in 8 patients, and continued to drop by day 7 (PCAg 66% ± 6 d0 to 39% ± 7 d7, p=0.01; PCClot 64% ± 6 d0 to 40% ± 7 d7, p=0.02). The fall in PCClot from day 0 to 7 correlated very strongly with decrease in PCAg (p=0.0002). Since the PCClot assay can yield spuriously low values in the face of high factor V (FV) and VIII (FVIII) levels, we also assayed these factors. Neither FV nor FVIII rose from day 0 to day 7, suggesting that the fall in PC was not an artifact of the assay. The low PC levels may reflect decreased hepatic synthesis or synthesis of a poorly carboxylated PC molecule with impaired function. Factor VII (FVII), which is also carboxylated during hepatic synthesis, decreased from day 0 to day 7 (86% ± 8 d0 to 41% ± 5 d7, p=0.01), and this fall correlated with both PCClot and PCAg (p=0.007 and p=0.013 respectively). Prealbumin, an index of hepatic synthetic function, also decreased significantly (19.2 ± 2.7 mg/dl d0 to 11.9 ± 2.4 mg/dl d7, p=0.04), but the decrease in prealbumin did not correlate with PCClot or PCAg (p=0.14 and p=0.18 respectively) with this small number of patients, suggesting that decreased hepatic synthesis was not the sole cause of decreased PC. In summary, both PCClot and PCAg decreased after ABMT in children, and the decrease was not an artifact of the functional anticoagulant assay. Lack of correlation with prealbumin suggest that decreased synthesis is not the sole cause of PC deficiency. The correlation with FVII suggests defects of a similar nature in synthesis of both proteins. Since both require gamma carboxylation, defects in this system may partially explain the PC deficiency. However, a component of increased peripheral consumption or altered extravascular distribution of the protein cannot be ruled out.

D 412 MINIMAL DISEASE DETECTION BY PCR PREDICTS FOR RELAPSE AFTER ABMT IN B-CELL NHL. John G Gribben, Arnold S Freedman, Sunhee Woo, Kelly Blake and Lee M Nadler. Division of Tumor Immunology, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

PCR amplification of bcl-2 translocations were performed to assess if detection of minimal residual disease in the bone marrow predicted for relapse after high dose therapy and purged autologous bone marrow transplantation. Criteria for inclusion in the present study were that patients had a PCR amplifiable breakpoint at bcl-2 in diagnostic tissue and that samples were available for analysis at least six months after ABMT. 113 pts have been included in the study to date. Samples were repeatedly negative by PCR from 47 pts, (42%) all of whom remain disease free at a median of 27 months (range 7-103 months) after ABMT. PCR detected bone marrow involvement at six months after ABMT in an additional 9 pts, but subsequent samples had no evidence of residual disease although these patients received no further therapy. All 9 of these pts remain disease free. Samples from 12 pts were initially negative when assessed by PCR, but later samples developed evidence of disease infiltration. 5 of these patients (42%) subsequently relapsed. Samples from 45 pts have consistently shown evidence of infiltration with cells bearing the bcl-2 translocation. 24 of these pts (53%) relapsed at a median of 13 months after ABMT. 21 pts have had consistent evidence of bone marrow infiltration without evidence of relapse from 6 to 104 months after ABMT. All patients to date who have relapsed after ABMT had PCR detectable evidence of bone marrow infiltration prior to relapse. The detection of cells with the translocation was associated with the re-infusion of autologous marrow which contained residual lymphoma cells after immunologic purging. These data suggest that PCR detection of minimal disease in the bone marrow is a prognostic indicator of disease free survival following ABMT. The finding of a number of residual cells with the bcl-2 translocation at prolonged periods after ABMT in pts who remain disease free lends evidence to the hypothesis that the bcl-2 translocation is necessary, though insufficient for the induction of tumor cell growth.

D 413 IMMUNOTHERAPY WITH ANTI-B4-BLOCKED RICIN AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) FOR B-CELL NON-HODGKIN'S LYMPHOMA: PHASE I TRIAL OF 7 DAY CONTINUOUS INFUSION, Michael L. Grossbard, John G. Gribben, Jeanne M. Kinsella, Walter A. Blattler and Lee M. Nadler, Division of Tumor Immunology, Dana-Farber Cancer Institute, Boston, MA 02115, and ImmunoGen, Inc., Cambridge, MA, 02139

Although ABMT yields high rates of complete remission in B-cell NHL, only 25-40% of these patients have 2 yr disease free survival. Anti-B4-blocked Ricin (Anti-B4-bR) is an immunotoxin (IT) comprised of the anti-B4 (CD19) monoclonal antibody and blocked ricin, a modified toxin derived from native ricin. The CD19 antigen is expressed on nearly all normal and neoplastic B-cells. In blocked ricin, the non-specific binding capability and toxicity of whole ricin is blocked. In a Phase I dose escalation trial we are administering Anti-B4-bR by 7d continuous infusion to pts who are in clinical CR 60 to 210 days post-ABMT. Because Anti-B4-bR exerts its cytotoxicity by inhibiting protein synthesis, we hypothesize that it may kill residual NHL cells resistant to high dose therapy. These pts also present an optimal setting for the delivery of IT to sites of minimal residual disease (MRD). In order to detect MRD and assess its eradication, we have employed polymerase chain reaction (PCR) analysis using oligonucleotide primers and probes for bcl-2 to detect t(14;18) seen in 85% of follicular and 30% of diffuse NHL. To date, 9 pts have been treated. Three pts received Anti-B4-bR at 20 ug/kg/d x7d, 5 at 40 ug/kg/d X 7d, and 2 at 50 ug/kg/d X 7d and 5d respectively. Serum levels were 0.25-0.37nM in the pts treated at 20 ug/kg, 1.4-1.8nM in the pts treated at 40 ug/kg, and 1.8-2.7nM in the pts treated at 50 ug/kg. Based on in vitro cytotoxicity studies, these levels are therapeutic. Serum levels were remarkably uniform in pts treated at a given dose level. Toxicity has included transient Grade 2-4 elevations of SGOT/SGPT, hypoalbuminemia, peripheral edema, fatigue, nausea, and fever. Despite initial plt counts as low as 32K, only 3 of 9 pts required plt transfusions for counts < 20K lasting 5-10d following therapy. At a dose of 50 ug/kg/d, we have exceeded the MTD as defined by the development of Grade 4 thrombocytopenia. We continue to treat patients at 40 ug/kg/d to clarify the toxicities of therapy post-ABMT. Moreover, PCR analysis of bone marrow samples obtained from patients indicates that 6 of 7 evaluable patients had a detectable breakpoint at the major breakpoint region of bcl-2 prior to ABMT. Following Anti-B4-bR therapy, all 6 pts no longer had a PCR detectable breakpoint. Of note, all 9 pts remain in CR at 5 to 14 months post-ABMT.

D 414 PROTEIN C DEFICIENCY AND PROTHROMBIN ACTIVATION IN ADULT HEMATOPOIETIC STEM CELL TRANSPLANTATION: A STUDY OF 45 CONSECUTIVE PATIENTS. W.D. Haire, B.G. Gordon, R. Bagin, L. Stephens, G. Kotulak, P.C. Comp, Departments of Internal Medicine, Pediatrics and Societal/Preventive Medicine, University of Nebraska Medical Center, Omaha, NE 68198 and University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

To determine if deficiency of the naturally-occurring anticoagulant protein C (PC) may contribute to thrombotic complications of stem cell transplantation, we assayed PC (antigenic assay, PCAg; and anticoagulant activity, PCClot) and prothrombin fragment 1.2 (PF1.2, the prothrombin activation peptide) prior to and weekly after starting treatment in 37 adult autologous and 8 adult allogeneic transplants patients. Enzymatic activity of PC was determined with a chromogenic substrate (PCChrom) assay on day 0 and 14 with the first 20 patients.

	PCAg	PCClot	PCChrom	PF1.2
day 0	104±5%	100±4%	105±4%	1.02±.09 ng/ml
day 7	115±6%	98±5%	---	1.23±.09
day 14	69±4%*	57±4%*	70±3%*	1.00±.08
day 21	56±3%*	46±3%*	---	1.26±.14#

*p<0.0001, #p=0.06 compared to day 0

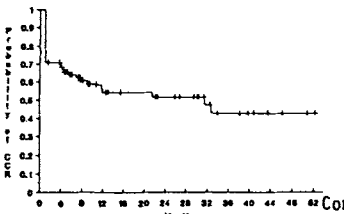
The ratio of PCChrom to PCClot rose from 1.01 to 1.40 by day 14; the ratio of PCAg to PCClot rose from 1.05 to 1.31 (p<0.001). This suggests production of a PC molecule that is more active in the chromogenic than the clot-based assay. This pattern of activity is characteristic of incompletely carboxylated PC. In summary, significant PC deficiency, due both to a drop in PCAg and production of a dysfunctional molecule, occurs during transplantation. The rise in PF1.2 indicates that prothrombin activation, possibly related to the drop in PC levels, also occurs during transplantation. These data support the hypothesis that acquired PC deficiency may contribute to thrombotic complications of stem cell transplantation.

D 415 AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION AS A POSTREMISSION THERAPY IN THE TREATMENT OF LYMPHOID MALIGNANCY, Mine Harada, Takanori Teshima, Yasushi Takamatsu, Koichi Akashi, Shuichi Taniguchi, Masahiro Murakawa, Seiji Kondo, Shoichi Inaba, Takashi Okamura, Tsunefumi Shibuya and Yoshiyuki Niho, First Department of Internal Medecine and Blood Transfusion Service, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

Thirteen patients(10 ALL and 3 malignant lymphoma) were treated with marrow-ablative chemotherapy followed by autologous blood stem cell transplantation(ABSCT). Peripheral blood stem cells were collected during a recovery phase after consolodation chemotherapy of 1st or 2nd complete remission(CR). Pretransplant conditioning regimen consisted of 4 mg/kg of busulfan on days -8 to -5, 10-20mg/kg of etoposide on days -4 and -3 and 3 g/m^2 of Ara-C every 12 hour on days -3 and -2. All patients were maintained in a protective environment with partial gut decontamination. On day 0, patients were infused with ABSC which had been cryopreserved at -80°C. 4 of 5 patients, who received ABSCT during 1st CR, are now surviving in continuing CR for 3-24 mo. 4 patients received ABSCT during 2nd CR; 2 of them developed relapse at 8 and 10 mo while 2 other patients are continuing CR for 12 and 3 mo. The remaining 4 patients received ABSCT when their diseases were refractory. 2 died of regimen-related toxicity (congestive heart failure and veno-occlusive disease of the liver) shortly after ABSCT; 2 other patients with malignant histocytosis and Burkitt's lymphoma are now surviving in CR for 16 and 3 mo respectively. From these observations, it is indicated that our conditioning regimen(BEA) is active for lymphoid malignancies and that ABSCT can be safely performed except for heavily-treated patients. Despite a limited number of patients, our data suggest that marrow-ablative chemotherapy and ABSCT are useful as a postremission therapy in the treatment of lymphoid malignancy.

D 416 MULTICENTER AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) TRIAL FOR RELAPSED LYMPHOMA USING VP-16, CISPLATIN, AND BCNU. H. M. Lazarus, P. Crilley, N. Ciobanu, R. Creger, R. Fox, D. Shina, S. Bulova, R. Gucalp, D. Topolsky, W. Soegiarso, B. Cooper, I. Brodsky. Ireland Cancer Center, Case Western Reserve University, Cleveland, OH 44106; Hahnemann University, Philadelphia, PA 19102; Albert Einstein Cancer Center, New York, NY 10467.

79 pts age 15-64 yr with relapsed or refractory non-Hodgkin's lymphoma (NHL) (N=36) & Hodgkin's Disease (HD) (N=43) were treated. 59 pts received 1500-2000 cGy involved-field radiotherapy to active or previously bulky (>5 cm diameter) disease immediately before high-dose chemotherapy. All pts received IV BCNU 600-1050 mg/m^2, VP-16 2400-3000 mg/m^2, cisplatin 200 mg/m^2, and ABMT. Toxicities included fever, nausea/vomiting, hearing loss, stomatitis, esophagitis, diarrhea, pneumonitis, hepatic veno-occlusive disease, bacteremia (N=17) & fungemia (N=7). One pt died within 30 d of marrow infusion. 56 pts (71%) achieved complete response (CR) [N=25 HD; N=31 NHL]. 40 pts (51%) [N=16 HD; N=24 NHL) remain in continuous CR a median of 13 (4-53) mo after ABMT (see Kaplan-Meier plot below). This regimen is effective for relapsed lymphoma, even for primary refractory disease (pts never in CR).



Status at ABMT	No.	CR	Continuous CR	Duration Median (range)
Primary Refractory	22	15	9	13 (5-42) mo
Untreated Relapse	16	12	10	8 (4-44) mo
Resistant Relapse	18	9	6	30 (7-53) mo
Sensitive Relapse	23	20	15	13 (5-47) mo

D 417 **Y-90 LABELED B1 IMMUNOCONJUGATES IN LYMPHOMA AUTOTRANSPLANTATION:**
Roger M. Macklis, S. Glenn, B. Beresford, J. Humm and L. Nadler, Harvard Medical School and Dana Farber Cancer Institute, Boston MA and Coulter Immunology Co, Hialeah, FL

We are evaluating the radiochemistry, radiobiology and pharmacokinetics of murine IgG2a MoAbs specific for the CD20 (B1) antigen. These MoAbs have been labeled with the high energy beta emitting radiometal Y-90 (particle range approximately 1 cm in tissue) through a SCN-Benz-Alk-DTPA chelate system. The resulting radioiommunoconjugates (RICs) recognize more than 90% of human B cell NHL. TLC and HPLC studies show that this radiochemical linkage is extremely stable under physiologic conditions, and radiobiological studies show that CD20+ B cell NHL lines are extremely sensitive to this form of targeted radiation. This high radiosensitivity may be due to 2 unusual features of this form of protracted low dose-rate radiation exposure: (1) shifts in cell cycle kinetics into the radiosensitive G2M phase of the cell cycle, and (2) the induction of endonuclease-mediated DNA fragmentation and programmed cell death (apoptosis) in susceptible cell types. Animal models of malignant lymphoma show that the RICs are distributed very heterogeneously in target tissue, though the long-range beta particles from Y-90 partially correct for this inhomogeneity. Based on these preclinical data, we are now initiating a phase I dose-escalation trial in which these RICs will be used to deliver biologically targeted TBI as preparation for autotransplants in relapsed CD20+ NHL patients.

D 418 RADIOLABELED ANTIBODY THERAPY FOLLOWED BY AUTOLOGOUS MARROW TRANSPLANTATION FOR RELAPSED B CELL LYMPHOMAS, Press O.W., Janet F. Eary, Christopher C. Badger, Paul J. Martin, Frederick R. Appelbaum, Ron Levy, Richard Miller, Wil B. Nelp, Darrell R. Fisher, Greg Wiseman, Dana Matthews, and Irwin D. Bernstein. University of Washington, Seattle, WA 98195, The Fred Hutchinson Cancer Research Center, Seattle, WA 98104, Stanford University, Stanford, CA 94305, Battelle Pacific Northwest Laboratories, Richland WA 99352, and IDEC Pharmaceuticals, Mountain View, CA 94043

Despite dramatic advances in curative combination chemotherapy for newly diagnosed patients with aggressive non-Hodgkin's lymphomas (NHL), few patients with low-grade lymphomas or relapsed NHL can be cured with conventional therapy. We have evaluated the therapeutic potential of radiolabeled anti-idiotypic, anti-CD20 & anti-CD37 monoclonal antibodies (MoAbs) followed by autologous marrow reinfusion in 33 patients with advanced NHL who failed conventional chemotherapy. On successive weeks, patients were infused with escalating amounts of antibody (0.5, 2.5, 10 mg/kg) trace-labeled with 5-10 mCi I-131. Absorbed radiation doses to tumor sites and normal organs were estimated by standard MIRD techniques based on data obtained by serial whole body gamma camera imaging, serial tumor biopsies, and computed tomography. In 16 of the 33 patients, every assessable tumor site received more radiation than any critical normal organ, and these patients were considered candidates for therapeutic infusions of I-131-MoAbs. The 17 patients who did not achieve favorable MoAb biodistributions generally had large tumor burdens (>0.5 kg, 11 pt) or massive splenomegaly (13 pt). To date, 14 patients have received high dose radioimmunotherapy with 211-1168 mg of MoAbs labeled with 232-628 mCi of I-131. Acute toxicity was limited to transient mild nausea, pruritis, and low grade fever. All treated patients experienced significant myelosuppression 2-4 weeks following treatment and 10 had elective reinfusion of autologous, purged marrow. Eleven patients achieved clinical complete remissions, one a partial response, one a minor response and one is too early to evaluate. Response durations have varied from 4-37+ months.

We conclude that the tolerable toxicity and encouraging efficacy warrant further dose escalation in this phase I trial. In addition, future studies will attempt to improve the proportion of patients who achieve favorable MoAb biodistributions by performing splenectomies in patients with splenomegaly and administering cytoreductive chemotherapy to patients with tumor burdens >0.5 kg.

D 419 Autotransplant for lymphoma: The University of Rochester experience, Aaron P. Rapoport, M.D., Jacob M. Rowe, M.D., Peter A. Kouides, M.D., and John F. DiPersio, M.D., Ph.D, Bone Marrow Transplant Unit, University of Rochester Medical Center, Rochester, NY 14642.

72 patients with relapsed or refractory Hodgkin's disease (HD) or non-Hodgkin's lymphoma (NHL) underwent autologous marrow (62) or blood stem cell (10) transplants between November 1988 and September 1991. 43 patients entered with "minimal" disease or in complete remission, following 1 or more courses of salvage therapy and 29 patients entered with "bulky" disease (at least one disease area >2 cm). 67 patients (93%) received the BEAC preparative regimen consisting of BCNU (300 mg/m^2) on day -7, etoposide (200 mg/m^2) days -6 to -3, cytarabine (200 mg/m^2) days -6 to -3, and cyclophosphamide (35 mg/kg) days -6 to-3. Five patients (7%) received TBI. Of 35 patients with HD, 20 (57%) are surviving event-free at a median follow-up of 331 days (29-945). 7 patients (20%) died from transplant-related complications; all had bulky disease on admission. 8 patients (23%) have relapsed at a median of 157 days post-transplant (71-250), all in sites of prior disease. Of the 19 patients with "minimal" disease, 15 (79%) are surviving event-free as are 5 of 16 (30%) with "bulky" disease. Of 37 patients with NHL, 18 (49%) are surviving event-free at a median of 194 days post-transplant (21-1022). 3 patients (9%) died during the transplant procedure, and 4 patients died after discharge from non-relapse causes. 12 patients (33%) have relapsed, 9 in sites of previous disease. Of 24 patients with "minimal" disease, 15 (63%) are surviving event-free as are 3 of 13 patients (23%) with "bulky" disease. While additional follow-up is needed, this database supports previous reports suggesting that patients with "bulky" residual disease are more likely to die from complications of therapy or suffer early relapse. In addition, the BEAC regimen was well-tolerated by the 43 patients with "minimal" disease as only 2 (5%) succumbed to complications of transplant. A significant proportion of these patients may be long-term survivors.

D 420 THE EFFECTS OF CONTINUOUS INFUSION GM-CSF IN ADULT PATIENTS RECEIVING HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS BONE MARROW TRANSPLANT, Elizabeth Reed, James Armitage, William Vaughan, Julie Vose, Philip Bierman, Anne Kessinger and Karel Dicke, Section of Oncology/Hematology, University of Nebraska Medical Center, Omaha, NE 68198

Seventeen patients treated with high dose chemotherapy and autologous bone marrow transplant for Hodgkin disease (6 patients), non-Hodgkin lymphoma (2 patients), breast cancer (6 patients) and other solid tumors (3 patients), received GM-CSF by continuous infusion at the dose of 125 ug/M^2/day. GM-CSF was started within 2 hours after marrow infusion and was continued a median of 14 days (range 2-30 days). Absolute neutrophil counts reached 100 x 10^8 cells/liter and 500 x 10^8 cells/liter a median of 11 days (range 8-20 days), and 13 days (range 10-30 days) after transplant, respectively. All patients survived the transplant hospitalization and were discharged a median of 22 days (range 14-40 days) after marrow infusion. Proven infections occurred in 7 patients. Weight gain of $\geq$ 5 kg. occurred in 8 patients. New pleural effusions occurred in 7 patients a median of 10 days (range 3-19 days) after transplant. Three patients had symptomatic effusions and GM-CSF was discontinued after 2, 4, and 13 days of continuous infusion. Two of the patients had malignant effusions and the other patients were thought to have effusions secondary to GM-CSF. These patients had recovery of neutrophils that was comparable to previous reports in transplant patients using GM-CSF as a bolus at twice the dose. However, there may be an increased incidence of weight gain and pleural effusions when GM-CSF is given by continuous infusion.

D 421 HIGH-DOSE CARBOPLATIN, ETOPOSIDE AND CYCLOPHOSPHAMIDE WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) FOR REFRACTORY MALIGNANCIES: EXPERIENCE WITH 63 PATIENTS. Saez R, Selby G, Strnad C, Slease R and Epstein R, University of Oklahoma, Presbyterian Hospital, Veterans Administration Medical Center, Oklahoma City, OK, Tulsa Medical Center, Tulsa, OK.

Last year we completed a Phase I study with high-dose continuous infusion carboplatin (1.2-2 g/M^2) d-7 to d-4, etoposide (1.2-2.4 g/M^2) d-7 to d-5 and cyclophosphamide (120 mg/kg) d-9, d-8 in 32 patients with refractory malignancies.

Mucositis/enterocolitis was dose limiting. Hepatic and pulmonary dysfunctions were minimal. MTD was etoposide 2.1 g/M^2 and carboplatin 2.0 g/M^2 in combination with cyclophosphamide (120 mg/kg). We have extended our experience to 63 patients: breast (24), Hodgkin's disease (12), ovarian (9), NHL (7), testicular (5) and others (6).

Responses were seen in 26 of 31 patients with measurable disease, including 7/8 with testicular/ovarian cancer, 7/8 with breast cancer and 6/8 with Hodgkin's disease. Twenty-seven patients remain free from progression 1-22+ months post ABMT.

Detailed data on toxicity and responses per disease type will be presented.

Conclusion: high-dose carboplatin when combined with etoposide and cyclophosphamide has significant anti-tumor activity in testicular, ovarian and breast cancer and in Hodgkin's disease.

D 422 INTERSTITIAL PNEUMONITIS (IP) FOLLOWING HIGH-DOSE CHEMOTHERAPY (CT) WITH CYCLOPHOSPHAMIDE, BCNU, ETOPOSIDE $\pm$ CISPLATIN (CBV $\pm$ P) AND AUTOLOGOUS BONE MARROW TRANSPLANTATION (AuBMT) FOR ADVANCED HODGKIN'S DISEASE (HD): INCIDENCE, RISK FACTORS AND OUTCOME, Antoine Sayegh, Donna Reece, Michael Barnett, Joseph Connors, John Shepherd, Randall Fairey, Susan O'Reilly, Stephen Nantel, Hans-G. Klingemann, John Spinelli, Nicholas Voss, Gordon Phillips. The Leukemia/Bone Marrow Transplant Program of British Columbia, Division of Hematology, British Columbia Cancer Agency, Vancouver General Hospital, and the University of British Columbia, 910 West 10th Avenue, Vancouver, BC V5E 4E3.

Between 03/85-08/91, 90 consecutive patients (pts) with advanced HD were treated with high-dose CT and AuBMT. All patients had evidence of disease progression following multiagent primary therapy. CT consisted of CBV (C:1.8 g/m^2 x 4; B:600 mg/m^2; V:2.4 g/m^2) in 26 pts and CBVP (C and V in same doses as above with V given as a 34-hour continuous infusion; B:500 mg/m^2; P:50 mg/m^2 x 3) in 59 pts. Five pts received other CT. 6 pts had previous B exposure, and 54 pts had received chest radiotherapy (RT) -- including 19 pts who received chest RT immediately before high-dose CT. Median age was 28 years (yrs) (range: 13-35). IP occurred in 16 pts and was fatal in 6. The actuarial cumulative incidence of IP was 19% (95% confidence interval: 11-28%) with a median follow-up of 22 months (range: 0.5-75 mos). Median time to onset of IP was 53 days post AuBMT (range: 8-160). Fifteen pts received corticosteroid therapy early in the development of IP, and 10 of these recovered. In a preliminary statistical analysis, the only predicting factor for IP was prior B exposure (p=.0002; chi-square). The incidence of IP was not influenced by prior chest RT (p=.33) or by dose of B used with high-dose CT (p=.58). IP is a serious complication in pts receiving high-dose CVB $\pm$ P and AuBMT, particularly in pts with a history of B exposure, and therapeutic alternatives should be considered in such pts. Rapid recognition of IP and early treatment with corticosteroids are essential in preventing irreversible pulmonary failure.

D 423 EFFECTS OF INTERLEUKIN-3 ON LONG-TERM CULTURES OF HUMAN BONE MARROW AND APHERESIS HARVESTS FROM LYMPHOMA PATIENTS. JG Sharp, SL Mann, DA Crouse, DD Weisenburger, A Kessinger, L Garrison. University of Nebraska Medical Center, Omaha, NE and Immunex Corp, Seattle, WA.

The risk of stimulating tumor growth is an important concern in the use of cytokines to ameliorate hematopoietic aplasia and to mobilize stem cells into the circulation for more efficient collection of peripheral stem cells. We have employed a sensitive culture technique which detects occult lymphoma cells to monitor the bone marrow and apheresis harvests of lymphoma patients receiving 125, 250 or 500 $\mu g/m^2$ of recombinant human interleukin-3 (IL-3) to facilitate peripheral stem cell collections. Samples of both marrow and apheresis harvests obtained prior to IL-3 administration (PRE) and similar harvests following administration (POST) were placed into long-term culture with or without IL-3 (200 U/ml). In 6 fully evaluable Hodgkin's disease patients studied to date, suspected tumor cells were found in the aphereses harvests of 2 (33%) which is the predicted frequency in such harvests. However, these cells were present in the PRE as well as POST apheresis harvests and did not show differential growth in the presence of IL-3. Consequently, we concluded that in this small sample, IL-3 did not stimulate lymphoma growth. Administration of IL-3 to the patients changed the yield of harvested cells by increasing the POST apheresis and decreasing the POST marrow harvests. Addition of IL-3 to the cultures leads to a greater cumulative production of cells from all harvests. Lymphocytes showed a relative increase in the POST apheresis harvests at the expense of granulocytes. In contrast, culture of both PRE and POST apheresis and marrow cultures with IL-3 was associated with a relatively greater production of granulocytes. This increase occurred at the expense of both lymphocyte and monocyte/histiocyte production. Therefore, IL-3 is able to modulate hematopoiesis in lymphoma patients with no evidence of an increased risk of lymphoma growth. (Supported by the Immunex Corporation.)

D 424 PHASE I TRIAL OF HIGH-DOSE CYCLOPHOSPHAMIDE(CPA), VP-16, CARBOPLATIN(CBDCA) AND TOTAL BODY IRRADIATION (TBI), WITH AUTOLOGOUS STEM CELL SUPPORT, IN PATIENTS WITH ADVANCED MALIGNANCIES. AM Storniolo, JR Mason, BA Newton, MD Mullen, SL Seagren, and TC Shea. University of California, San Diego, CA 92103.

Four previously treated patients(pts), 3 with relapsed non-Hodgkin's lymphoma and 1 with relapsed metastatic cervical cancer, received CPA(6g/m^2), VP-16(1.8g/m^2), CBDCA(1g/m^2), and escalating doses of TBI(8 Gy - 3 pts; 10 Gy - 1 pt). Because pts had documented bone marrow involvement (2), skeletal involvement (1), or had received extensive pelvic radiation (1), the conditioning regimen was followed by infusion of peripheral blood stem cells (PBSC) which were collected during G-CSF administration (3 pts) or during steady state (1 pt). PBSC reinfusion was followed by daily administration of either G-CSF (3) or GM-CSF(1). Median time to >500 neutrophils = 11.5 days (d); to >20,000 platelets (plt) = 52.5d; and number of hospital days = 27. Pt #1 remains plt-transfusion dependent >180 days. Pt #2 demonstrated prolonged thrombocytopenia which resolved following splenectomy. Maximum tolerated dose has not been reached. Reversible grade 3 toxicity was limited to mucositis in 2 pts. No significant hepatic, renal or neurologic toxicity was observed. Two pts had transient drops in left ventricular ejection fraction which subsequently normalized. Three of 4 pts were evaluable for response; all 3 achieved CR and are alive and progression-free 4-6 months post treatment. Day 14 colony counts and CD34+ cells reinfused per Kg are summarized below:

Pt	CFU-GM/Kg	CFU-GEMM/Kg	CD34+ cells/Kg	Mononuc. cells/Kg	ANC>500 (d)	Plts>20K (d)
1	$0.75x10^4$	$0.49x10^4$	$0.70x10^7$	$10.2x10^8$	19	>180
2	$10.7x10^4$	$2.12x10^4$	$1.46x10^7$	$4.51x10^8$	14	92
3	$1.53x10^4$	$1.26x10^4$	$1.23x10^7$	$3.64x10^8$	9	13
4	$205x10^4$	$11.6x10^4$	$11.2x10^7$	$13.9x10^8$	8	8

This regimen appears effective and well tolerated, especially in heavily pretreated hematologic malignancies.

D 425 ROLE OF INTERLEUKIN-6 IN VIVO DURING HEMATOPOIETIC RECOVERY AFTER CHEMOTHERAPY AND AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION. Yasushi Takamatsu, Koichi Akashi, Mine Harada, Takanori Teshima, Kazuya Shimoda, Shoichi Inaba, Tetsuya Eto, Koji Nagafuji, Yoshiyuki Niho. The First Department of Internal Medicine, Blood Transfusion Service, Faculty of Medicine, Kyushu University, Fukuoka, Japan.

We studied interleukin(IL)-6 production by peripheral blood monocytes during hematopoietic recovery after chemotherapy-induced marrow aplasia. Thirteen adult patients with hematological malignancies including 8 AML, 3 ALL and 2 NHL were studied in complete remission. During a recovery phase following intensive chemotherapy, peripheral blood mononuclear cells(PBMC) were collected by leukapheresis. Monocytes were separated as adherent cells and incubated in culture medium with 10% FCS for 24 hours. High concentrations of IL-6 could be detected in these monocyte culture supernatants. Furthermore, plasma IL-6 levels were also elevated. These data indicate that monocytes were activated to produce IL-6 in vivo during the hematopoietic recovery.

PBMC collected during the hematopoietic recovery include a large number of hematopoietic stem cells and are used for autologous blood stem cell transplantation(ABSCT). Morphological examination of the PBMC revealed that a half of them consisted of monocytes. Since previous data showed that these monocytes produced IL-6 in vivo, we investigated plasma IL-6 levels after ABSCT in 5 patients. Plasma IL-6 levels began to increase from day 3 after transplantation and reached peak levels on day 7-9(madian 186pg/ml). Hematopoietic recovery was usually rapid after ABSCT compared with those after allo- and auto-BMT. Therefore, it is suggested that these monocytes infused with stem cells may accelerate hematopoietic reconstitution through producing IL-6.

D 426 AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION IN ADVANCED NEUROBLASTOMA. Takanori Teshima, Eiichi Ishii, Mine Harada, Ikuko Minamishima, Akinobu Matsuzaki, Yasushi Takamatsu, Shoichi Inaba, Kohji Ueda, Yoshiyuki, Niho. First Department of Internal Medicine, Department of Pediatrics and Blood Transfusion Service, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan.

Recent clinical trials indicate that survival of patients with advanced neuroblasroma can be improved with the use of marrow-ablative chemotherapy plus autologous bone marrow transplantation (ABMT). Accordingly, autologous blood stem cell transplantation (ABSCT) will privide a chance of long-term survival or cure for bad-prognosis patients with neuroblastoma. Seven patients with stage 4 neuroblastoma were treated with marrow-ablative chemotherapy followed by ABSCT. Peripheral blood stem cells (PBSC) were collected by apheresis during a recovery phase after chemotherapy, when tumor cells disappeared morphologically in the blood and the marrow. Collected PBSC were cryopreserved without purging by a simplified method at -80^{o}C. Pretransplant conditioning regimen consisted of 400 mg/m^2 of CBDCA on day -7 to -5, 200mg/m^2 of VP-16 on day -7 to -4 and 180 mg/m^2 of l-PAM on day -2. Rapid reconstitution of trilineage hemopoiesis was observed in all of the patients. Four of the 7 patients, who received ABSCT at complete remission (CR), are now surviving in continuing CR for 6 to 17 mo. The remainding 3 patients, who received ABSCT at relapse, died of progression of the disease. All patients tolerated well the conditioning regimen without severe regimen related-toxicity. These results suggest that marrow-ablative chemotherapy with ABSCT can be performed safely and this treatment modality may be effcetive in the treatment of advanced neuroblastoma.

D 427 PILOT TRIAL OF URSODIOL TO PREVENT VENO-OCCLUSIVE DISEASE OF THE LIVER (VOD) IN PATIENTS (PTS) RECEIVING BUSULFAN/CYCLOPHOSPHAMIDE. J.M. Thompson, J. H. Essell, G.S. Harman, R. D. Halvorson, and M. J. Snyder. Dept. Hematology SGHMMH, Wilford Hall USAF Medical Center, San Antonio, Texas 78236.

Ursodiol is a hydrophilic, nonhepatotoxic bile salt indicated for the treatment of cholesterol gallstones. We are performing a pilot study to determine if the prophylactic use of ursodiol is effective in decreasing the incidence and severity of VOD following allogeneic bone marrow transplantation (BMT). Fifteen consecutive pts undergoing BMT for hematologic malignancies were prepared with busulfan 4mg/kg/day x 4 days and cyclophosphamide 60mg/kg x 2 days between 2/91 and 7/91. All received cyclosporine and methotrexate (MTX) (10mg/M^2) D+1,3,6 for GVHD prophylaxis. Ursodiol 300mg po bid was begun at least one day prior to beginning the preparative regimen. Clinical parameters for this group were compared to 28 consecutive pts transplanted 6/89-1/91 with an identical regimen without ursodiol. There were no significant pre-transplant differences in disease or clinical status between the groups. However, pre-transplant AST levels were significantly different, 31.5mg/dl in the ursodiol group, vs 18.1mg/dl in the historical control group ($p = 0.0001$). MTX doses were held in either group for hyperbilirubinemia ($\geq$2mg/dl). The mean total dose of MTX given was 28.7mg/M^2 in the ursodiol group and 27.5mg/M^2 in controls. The median maximum bilirubin was 1.9mg/dl (range 0.9-45) in the ursodiol group and 5.05mg/dl (range 0.7-29.4) in controls. The incidence of VOD was 2/15 (13.3%) in the ursodiol group and 18/28 (64.3%) in controls ($p = 0.004$). Death due to VOD occurred in 1/15 (6.7%) in the ursodiol group and 6/28 (21.4%) in controls ($p=0.41$). Overall survival was 13/15 (86.7%) in the ursodiol group and 15/28 (53.6%) in controls ($p=0.067$). Our data suggest that ursodiol may decrease the incidence of VOD in pts receiving this regimen. When this pilot study is concluded, we plan a double-blind randomized trial to further investigate this possibility.

D 428 PHARMACOKINETICS OF HIGH DOSE PO AND IV HYDROXYUREA (HU) IN BMT PREPARATIVE REGIMENS, William P. Vaughan, Edward S. Kris, Peter R. Gwilt, U of NE Med Center, Omaha, NE 69198

Short term po or iv HU single agent produces only marrow toxicity. This toxicity pattern, its broad spectrum of clinical activity, and its potential for synergy with other drugs makes HU worthy of investigation in BMT preparative regimens. Pharmacokinetic studies were done in a phase I/IV trial of iv HU (6-12 g/m^2 over 72 h) with BCNU (B), VP-16 (E), and Cytoxan (C) followed ABMT for large cell lymphoma, and a phase I clinical trial of iv single agent HU (loading doses of 600 or 900 mg/m^2 followed by 200 or 300 mg/m^2/h, respectively for 48, 60 or 72 h). At the same time, we studied the pharmacokinetics of fixed dose po HU (18 g/m^2 over 72 h) combined with C and thiotepa (T) followed by ABMT for breast cancer. In the phase I/IV trial combining iv HU with BEC, mucositis was dose limiting and was also seen in the trial using po HU combined with CT. Average steady state serum levels and AUC in pts receiving iv infusion HU increased in a non-linear fashion. Average steady state concentrations achieved with 72 h iv infusions of 6, 8, 10.2, 12.6, 17.5, 18.9, and 22.5 g/m^2 were 0.11, 0.31, 0.52, 0.53, 0.60, 0.58, 0.79, and 0.8/ mM, respectively. Mean serum levels and AUC/dose in pts receiving po HU were not statistically different from pts receiving continuous infusion. However, peak levels achieved with po HU ranged widely from 0.55 to 1.65 mM with a mean of 0.96 ± 0.33 mM. The pharmacokinetics appeared to be zero-order uptake and first-order elimination. The half-life was 2.4 ± 0.7 h. However, there were peaks observed following the primary peak that might indicate atypical absorption or disposition. The recommended dose of iv HU for use in other high dose chemotherapy/ABMT combinations is 10 g/m^2 total dose over 72 h. Our data are consistent with a completely available po dosage form, but difficulty of po administration during nausea and vomiting from other high dose chemotherapy and more erratic pharmacokinetics favors the iv formulation in this setting.

D 429 CONTINUOUS INFUSION VP-16 AND CYTOXAN FOR AUTOLOGOUS BONE MARROW TRANSPLANTATION OF HIGH RISK PATIENTS WITH LYMPHOMA, RS Weiner, RB Mowat, W Cassano, DJ Oblon, AM Miller, WD Noyes, NS Conley, L Myers, P Phillips, N Mendenhall, University of Florida College of Medicine, Gainesville, FL 32610

Prior use of multi-modality therapy to treat high risk pts with Hodgkin's Disease and non-Hodgkin's lymphoma presents challenges when contemplating ablative therapy for relapse. Too often total body irradiation (TBI) is unsafe and options limited for involved field radiotherapy as well. As a result of extensive institutional experience with continuous infusion (CI) VP-16 and intermittent cytoxan in a broad based phase I-II study, we developed a preparative regimen consisting of CI VP-16 150-200 mg/m^2 over 11 hrs q 12 hrs x 12 doses and intermittent cytoxan 350-500 mg/m^2 q 12 hrs x 12 doses for use in pts for whom involved field radiation therapy followed by cytoxan and TBI was inappropriate. Sixteen pts with lymphoma (4 pts with intermediate to high grade non-Hodgkin's lymphoma and 12 pts with Hodgkin's Disease) were treated with this regimen between 1987 and 1991. All pts presented in first or subsequent relapse. All pts had responding disease as judged by objective tumor regression after combination chemotherapy. Two pts (both with Hodgkin's disease) died in the peri-transplant period. Six of 12 pts with Hodgkin's disease remain in continuing complete remission from 8 - 25 months with a median of 11 months. Relapses occurred 3, 5, and 12 months in relapsed Hodgkin's disease pts. Two of 4 pts treated for non-Hodgkin's lymphoma are in continuing complete response at 8 and 32 months. One pt relapsed at 11 months and the second pt relapsed after 2 months and died after a second transplant. CI VP-16 and cytoxan is a safe and effective transplant regimen for previously irradiated pts with Hodgkin's disease who relapse. Further studies are warranted in non-Hodgkin's lymphoma as well.

D 430 DOSE VERSUS TREATMENT OUTCOME WITH HIGH DOSE CYCLOPHOSPHAMIDE (C), CARMUSTINE (B), ETOPOSIDE (V) IN HODGKIN'S DISEASE (HD) AND NON-HODGKIN'S LYMPHOMA (NHL): OBSERVATIONS IN A CONSECUTIVE SERIES OF 160 PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION. C. Wheeler, J.P. Eder, L. Ayash, K. Ault, K. Antman, A. Elias, R. Mazanet, G. Schwartz, P. Mauch, L. Gaynes, I. Tepler,W.H. Churchill, C. Brugnara, L.Schnipper and J.H.Antin. Beth Israel and Brigham and Women's Hospitals, Harvard Medical School, Boston Ma. and Maine Medical Center, Portland, Maine.

Since 1986 we have treated 160 patients (pts) with HD (83) and NHL (77) with CBV.at seven dose levels..Of the entire group, histology was NHL high grade13, intermediate grade 63, low grade 1; HD, NS 74, HD other 7. 4 pts with NHL were transplanted in first complete response . All other had relapsed or refractory disease. All but 2 HD pts had failed at least 7 concommitant or sequential agents. 29 pts with HD and 30 with NHL were primarily refractory to therapy. C(6 gm/m2) B(450 mg/m2) V(1600 mg/m2) (dose level III) appeared to define a breakpoint in the tradeoff of dose vs response. There were 5/90 early deaths at dose level III or below (3/50 NHL, 2/40 HD) and 12/70 at dose levels greater than III (3/27 NHL, 9/43 HD) (p=.022). Kaplan-Meier curves of time to failure are not statistically different for the two groups. Patient groups at were not statistically different with respect to the major prognostic variable of chemotherapy sensitivity These data suggest that, within the high dose range, moderate doses are as effective as very high doses with more manageable toxicity.

Alternative Donors

D 500 HAPLOIDENTICAL BMT IN CHILDREN: AN ITALIAN EXPERIENCE. Andolina M., Agosti E. de Manzini A., Locatelli F., Zecca M., Bonetti F., Rossi R., Porta F. Istituto per l'Infanzia di Trieste, Clinica Pediatrica di Pavia, Clinica Pediatrica di Brescia. Italy.

If a patient has no other choice but a BMT, any effort should be done in order to find a donor. A minority of children has a compatible sibling and few acute leukemias can wait months searching a MUD. Within the pediatric italian group for BMT an experience of mismatched BMT using haploidentical parents and siblings as donors is being developed. Up to now 37 children underwent such BMT, mismatched for 2-3 loci. 24 marrows were treated in vitro with vincristine and methylprednisolone, 5 were T depleted with Campath 1, 2 with soy bean lectins; 6 were not treated ex vivo. Cyclosporin was given to all patients. 22 received also I.V.I.g. (100 mg/kg/day). Acute and chronic GVHD were a minor problem in most patients and were direct or indirect cause of death in 6. A marrow rejection was a problem in a first series of genetic disease and chronic leukemias; recently an increased immune-suppression (TAI) before the transplant seemed to facilitate the take.

Since most patients had advanced disease the overall survival is poor due to early deaths and relapses. 7 patients are alive 3-56 months without GVHD. 3 patients survived more than 1 year (and relapsed) with no or irrelevant problems of chronic GVHD.

In conclusion we can state that an haploidentical BMT is a concrete chance for children in whom an autologous or an HLA matched BMT is not feasible.

D 501 THE ROLE OF HLA DISPARITY IN TRANSPLANT OUTCOME: IMPORTANCE OF MOLECULAR ANALYSIS OF HLA POLYMORPHISM, Lee Ann Baxter-Lowe, James Casper, Jack Gorski, David Eckels, and Robert Ash, The Blood Center of Southeastern Wisconsin and The Medical College of Wisconsin, Milwaukee WI 53233

HLA disparity between bone marrow recipients and donors has been associated with graft rejection, graft-versus-host disease (GVHD), and inadequate immune reconstitution. This study examines the effect of differences among HLA molecules, including single amino acid differences, on transplant outcome. These subtle differences among HLA molecules were detected using the polymerase chain reaction to amplify HLA genes and sequence-specific oligonucleotide probe hybridization to detect polymorphic sequences in the amplified products. This method was utilized to detect oligotypes that correspond to all known alleles for HLA-DRB and -DQB. HLA-DR polymorphism was correlated with response in mixed lymphocyte cultures (MLC) and with the incidence and severity of acute GVHD following bone marrow transplantation. There was a highly significant relationship between HLA-DR mismatching and reactive MLC ($p<0.00001$). Further, the magnitude of relative responses was influenced by the nature of the HLA-DR polymorphism. In preliminary studies of the HLA-DR matched samples, reactivity in MLC was also associated with HLA-DQ and/or -DP disparity. HLA-DR and -DQ matching was evaluated in 66 donor/recipient pairs and the results were compared with the incidence and severity of acute GVHD. Univariate analysis revealed that HLA-DR disparity was significantly associated with severe aGVHD (grades III and IV, $p=0.004$). In contrast, HLA-A, -B, or -DQ disparity were not significantly associated with the most severe forms of acute GVHD. Empirical studies based upon molecular analysis of HLA polymorphism along with theoretical considerations of HLA structure and function may permit the development of systems for predicting the impact of HLA disparity on the outcome of marrow transplants.

D 502 AN IMPROVED MLR TEST FOR DETECTION OF NON-MHC ANTIGEN DIFFERENCES IN BONE MARROW TRANSPLANTATION (BMT). A. Bishara, B. Leshem, C. Brautbar, S. Slavin, I. Cohen, E. Rosenkovitch, and E. Kedar. The Tissue Typing Unit and the Dept. of Bone Marrow Transplantation, Hadassah Hospital, and The Lautenberg Center of Immunology, Hebrew University, Jerusalem, Israel.

Selection of donors for BMT is based on serological determination of MHC class I and class II antigens and on the mixed leukocyte reaction (MLR). The standard MLR assay, which is used as the final indicator of donor-patient compatibility, is both time-consuming (6 days) and relatively insensitive since it does not detect non-MHC antigen differences. Such differences may be *the major cause for graft* versus host disease (GVHD) and graft rejection in BMT performed between HLA genotypically identical siblings or in phenotypically identical unrelated combinations. Therefore, the aims of our study have been: a) to shorten the MLR test, and b) to increase its sensitivity for better selection of compatible donors, and for predicting BMT outcome more accurately. Three modifications were employed in the MLR test: a) measuring IL-2 secretion in the MLR supernatants as an early T-cell activation signal; b) addition of lymphokines (IL-1, IL-2, IL-4) to the cultures, to amplify weak proliferative responses; and c) using stimulator cells pretreated for 24 hr with IFN γ, TNFα or both, to enhance MHC and non-MHC antigen expression. With these modifications, results could be obtained after 3 days; weak responses in histoincompatible combinations were markedly enhanced, and positive responses were observed in several MHC *matched recipient-donor* pairs that were not reactive in the standard MLR test. The positive reactions in the modified test correlated (80%) with post BMT complications in recipients of grafts from HLA "matched" siblings.

D 503 UNRELATED DONORS FOR BONE MARROW TRANSPLANTATION IN CHILDREN. J. Casper, L.A. Baxter-Lowe, R. Truitt, K. Murray, C. Lawton, N. Bunin, J. Hunter, B. Camitta, R. Ash. Medical College of Wisconsin, Children's Hospital and The Blood Center of SE Wisconsin, Milwaukee, WI 53226

Two-thirds of children in need of a bone marrow transplant (BMT) lack an HLA-matched sibling. Since 1986 we have performed 60 BMTs utilizing matched and mismatched unrelated donors. There were 41 male and 19 females who ranged in age from 6 months - 18 years (median-7). Malignant diseases included: ALL-23, AML-3, CML-9, JCML-3 and myelodysplasia-9. Non-malignant diseases were: SAA-7, SCID-2, WAS-2, congenital neutropenia-1 and metachromatic leukodystrophy-1. Histocompatibility was determined using serologic analysis, oligotyping and mixed lymphocyte cultures (MLC) . An HLA-A,B,DR,DQ match was located for 17 patients, an A mismatch for 16, a B mismatch for 12 and a D-region mismatch for 7. The median percent relative response index in the donor to host direction was 15% (0-56) and in the host to donor direction 8% (0-98). The conditioning regimen included: cytosine arabinoside 3 gm/m^2 q12hr x 6; cyclophosphamide (CY) 45mg/kg/ day x2; methylprednisolone 1gm/m^2 q12hr x 4 and total body irradiation 14 GY/9 fractions/3 days with lung, liver and kidney attenuation. Patients with higher risk leukemia also received busulfan 2-4mg/kg/day x2. Marrows were T-cell depleted (≈1.5 log) using a monoclonal CD3 antibody and normal rabbit serum. IV cyclosporine (3 mg/kg/day) was begun on day-1. The median number of mononuclear cells infused was 1.6 x 10^8/kg recipient weight. Fifty-nine of the 60 patients engrafted. Median times to PMN >500 mm^3 and platelets >25,000/mm^3 were 17 and 17 days respectively. Acute GVHD occurred in the majority of the patients but it was ≥ grade II in only 24%. Similarly, chronic GVHD was seen in 67% of the evaluable patients but was extensive in only 4. The 23 deaths were attributed to: infection-5, infection/GVHD-7, GVHD-1, lymphoma-2, recurrent ALL-2, pancreatitis-1, VOD-3, interstitial pneumonia-1 and non-engraftment-1. Disease free survival for lower risk leukemia (ALL or AML in 1st or 2nd CR, CML in chronic phase), higher risk leukemia (ALL or AML > 2nd CR, CML in accelerated or blast phase, JCML and myelodysplasia) and non-malignant disease are 70%, 35% and 73% (overall 55% at 20 months). These results compare favorably with matched sibling transplants.

D 504 RETROSPECTIVE ANALYSIS OF BONE MARROW TRANSPLANTS (BMT) USING EITHER MATCHED UNRELATED DONORS (MUD) OR PARTIALLY MISMATCHED RELATED DONORS (PMRD) IN PATIENTS (PTS) WITH HEMATOLOGIC MALIGNANCIES. Jan Gyarfas, P.Jean Henslee-Downey, Edward Romond, Edward Harder, Michael Bishop, Gil Ciocci, Ewa Marciniak, John Thompson and the Bone Marrow Transplant Program, University of Kentucky, Lexington, KY 40356.

Alternative donors have been sought since a histocompatible HLA matched sibling donor is available for only 25% of pts who could benefit from allogeneic BMT. The ability to identify a MUD has improved but the development of such a donor in a timely fashion appears to be limited. Therefore, a more readily available alternative source might be a PMRD. Over the last five years, 90 pts with hematologic malignancy have undergone BMT using either a MUD (N=20) or a PMRD (N=70). Serological typing demonstrated similar antigens (ag) at all 6 major HLA loci for all except 2 (5/6) of the MUD group. Genotypic identity was present for one haplotype in all of the PMRD group with 1 (N=21), 2 (N=34), or 3 (N=15) ag mismatched on the unshared haplotype. Both groups were treated with the same conditioning regimen including 14 Gy hyperfractionated total body irradiation followed by high dose chemotherapy with VP-16, AraC, Cytoxan and Methylprednisolone (MPD). All grafts were depleted using a complement mediated, IgM, anti CD-3 monoclonal antibody (MoAb), T10B9, achieving between 1.5-2.0 log depletion of mature T-lymphocytes. Post grafting immunosuppression was given with either Orthozyme CD-5+, Cyclosporine or both in combination with MPD. The MUD group had a mean age of 32 yrs (14-46) consisting of 6 pts with chronic myelogenous leukemia(CML), 6 with acute myelogenous leukemia(AML), 6 with acute lymphoblastic leukemia(ALL) and 3 with myelodysplastic syndrome in leukemic transformation(MDSLT). The PMR group had a mean age of 19 yrs (1-49) consisting of 22 pts with CML, 11 with AML, 32 with ALL and 5 with MDSLT. All evaluable pts in the MUD group and 90% in the PMR group engrafted. No obvious difference in the risk for aGVHD was seen in the two groups: grade I-II occurred in 53% and grade III-IV in 6% of pts in the MUD group and 44% and 17% in the PMR group, respectively. Likewise no obvious difference in chronic GVHD was seen: 57% vs 46%. Six pts (30%) in the MUD and 34 (49%) in PMR group are alive at a median observation time of+453 days (89-929) and +335 days (7-1,293) respectively. Since no difference in outcome could be correlated with the degree of mismatch in the PMR group, this analysis suggests that results with either alternative donor are comparable and therefore the donor most readily available might be the best choice.

D 505 SUCCESSFUL BONE MARROW TRANSPLANT (BMT) UTILIZING DONORS RECOGNIZING A FULL HAPLOTYPE HLA MISMATCH IN PATIENTS WITH FAR ADVANCED HEMATOLOGIC MALIGNANCY. P. Jean Henslee-Downey, Edward Romond, Edward Harder, Michael Bishop, Jan Gyarfas, Ewa Marciniak, and John Thompson, Department of Medicine and Pediatrics, University of Kentucky, Lexington, KY 40536.

Patients (pts) with a hematologic malignancy who have relapsed have little hope for cure with conventional dose chemotherapy. Allogeneic BMT may provide a hope for disease free survival but a HLA histocompatible sibling donor or a HLA phenotypically similar unrelated donor is only available for up to half of the pts who could benefit. If a related haploidentical donor with complete lack of sharing for one chromosome could be used, then the majority of patients would have a donor readily available as this would be the relationship between parents and children as well as many first degree relatives. In June 1990, we began a study to evaluate the use of such donors. Eleven pts between 2 and 42 years of age (mean 22 yrs) have been transplanted following conditioning therapy with 14 Gy total body irradiation followed by high dose chemotherapy including VP-16, Cytosine Arabinoside, Cyclophosphamide, and Methylprednisolone (MPD). Five pts had acute lymphoblastic leukemia, 3 in refractory relapse (RR) & 2 in 3rd remission; 5 had chronic myelogenous leukemia, 3 in blast crises and 2 in accelerated phase; and 1 had acute myelogenous leukemia in RR. As prophylaxis against graft versus host disease(GVHD) a combination of immunomodulating techniques were employed. All marrow grafts were incubated with a complement mediated, IgM, anti-CD3, monoclonal antibody (MoAb), T10B9, to achieve an approximate 2 log depletion of mature T lymphocytes. Post grafting systemic immunosuppression commenced with constant infusion Cyclosporin on day -2 followed by *in vivo* administration of an anti-CD5 MoAb conjugated to ricin A chain, Orthozyme CD-5+, given in combination with moderate dose MPD beginning on day +5 for a twelve day course. Ten pts engrafted with a mean day to a peripheral count > 500 occurring on day +12.6. Failure to engraft occurred in a pt with persistent leukemia. Four pts demonstrated signs of GVHD consisting of skin only in three pts (Grade I) and involving skin and gut (Grade II) in one pt. Seven pts (64%) are surviving between 37 and 445 days (median 235 days). Deaths appear to be correlated with the far advanced stage of malignancy: 2 with aspergillus and 2 with relapse. These surprising results show promise that 3 antigen mismatched related donors can be successfully used for marrow transplantation. Results may be further improved if the transplant is performed before the pt reaches an end-stage of the underlying malignancy.

D 506 EFFICACY OF COMBINED CYCLOSPORINE (CSP), METHOTREXATE (MTX) AND XOMAZYME-H65 PROPHYLAXIS FOR PATIENTS (PTS) AT HIGH RISK OF ACUTE GRAFT-VERSUS-HOST DISEASE (GVHD) AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT), Thomas Nevill, Michael Barnett, Ka-wah Chan, Hans-G. Klingemann, Stephen Nantel, Donna Reece, John Shepherd, Hans Messner, Jacinta Meharchand, and Gordon Phillips. The Leukemia/BMT Program of British Columbia: Division of Hematology, Vancouver General Hospital, British Columbia Cancer Agency, the University of British Columbia, British Columbia Children's Hospital, Vancouver, British Columbia and The BMT Program of the Ontario Cancer Institute, Toronto, Ontario, Canada.

Between 10/90 and 8/91, 20 pts at high risk for acute GVHD after allogeneic BMT received as GVHD prophylaxis: continuous infusion CSP 5 mg/kg/d x 7 days and then 3 mg/kg/d; MTX 10 mg/m^2 x 3; and XomaZyme-H65 0.1 mg/kg/d x 14 doses. As well, 16 pts received Pentoxifylline 2 g/d until day +100 as tolerated. There were 11 males (M) and 9 females (F), median age (range) 33 years (1-48). High-risk features were: unrelated donor (18 pts; 15 seromatched, 3 one-antigen mismatched) or M recipient with related parous F donor (2 pts). Nine pts had chronic myeloid leukemia (CML), 7 acute leukemia and 4 other. Conditioning was cyclophosphamide (Cy)/total body irradiation (TBI) (17 pts), Busulfan/Cy (2 pts), or other (1 pt). There was 1 early death (candidal sepsis, day +19) but all others engrafted. Median days to WBC > 1.0 x 10^9/L, ANC > 0.5 x 10^9/L, and platelets > 20 x 10^9/L were 19, 18 and 17, respectively. XomaZyme was moderately well tolerated. Seven pts (35%) experienced significant fluid retention (≥ 10% of baseline weight), and 2 pts had XomaZyme discontinued due to side effects {anaphylaxis (1 pt) and renal failure (1 pt)}. Five pts required CSP dose modification due to nephrotoxicity with 1 pt remaining dialysis-dependent. Six pts also had MTX doses reduced because of renal impairment (4 pts) or mucositis (2 pt). Although 14 pts (70%) developed ≥ grade II acute GVHD, only 3 (15%) had grade III-IV disease. Seven of 12 evaluable pts (58%) experienced chronic GVHD (5 limited, 2 extensive). There have been 5 post-engraftment deaths (2 acute GVHD, 1 chronic GVHD, 2 pneumonitis), and one pt has relapsed CML. Fourteen other pts remain alive with a median follow-up (range) of 236 days (19-327). Although toxicity is significant, the addition of XomaZyme-H65 to standard CSP/MTX GVHD prophylaxis appears to decrease the severity of acute GVHD in high-risk pts.

D 507 CORRELATION OF OLIGONUCLEOTIDE HLA TYPING WITH MLC IN UNRELATED BONE MARROW TRANSPLANTATION, Afzal Nikaein, Robert Collins, Joseph Fay, Transplantation Immunology, Baylor University Medical Center, Dallas, TX 75246

Oligonucleotide typing (OG) was correlated with MLC in 116 unrelated bone marrow donor-recipient combinations. 19/63 (30%) HLA-DR and 12/56 (21%) HLA-DQ matched pairs by serology were mismatched (MM) by OG. The mismatching was due to dif-ferences in gene subtypes among serologic HLA-matched antigens. MLC compatibility in various degrees of matches for DR, DQ, and DP occurred as follows:

#	OG DR		OG DQ		OG DP	
MATCH	#	Comp.MLC	#	Comp.MLC	#	Comp.MLC
2	75	55(72%)	85	57(67%)	15	10(67%)
1	37	15(41%)	23	8(35%)	38	28(74%)
0	4	0(0%)	0	-	6	6(100%)

As shown above, the degree of matching for DR and DQ was predictive of MLC compatibility. By contrast, the degree of HLA-DP matching was not predictive of MLC results. Similarly, HLA-DP compatibility had no effect on the degree or incidence of acute GVHD in 32 patients who received UBMT. T cell clones (111 total) generated from skin biopsies of two patients with acute GVHD showed no restriction toward donor's nor recipient's Class I or Class II (DR, DQ, DP) antigens. One of these two patients received HLA-DP mismatched bone marrow. 50% of T cell clones of this patient expressed donor's and 40% expressed recipient's HLA-DP phenotype with 10% of the clones undetermined. The above studies suggest that: 1) OG is a reliable technique for accurate HLA typing and can successfully be applied to select fully matched donors for UBMT 2) HLA-DP matching does not predict MLC compatibility or incidence and severity of GVHD and 3) the nature of the antigen responsible for acute GVHD remains undetermined.

D 508 HIGH DOSE METHYLPREDNISOLONE (MP) THERAPY FOR ACUTE GRAFT VERSUS HOST DISEASE (aGVHD) ASSOCIATED WITH MATCHED UNRELATED (MU) BONE MARROW TRANSPLANTATION (BMT). David J. Oblon, David Felker, Kathy Coyle, Lesley Myers, Division of Medical Oncology, University of Florida School of Medicine, Gainesville, Fl. 32610

There is a high incidence of aGVHD associated with matched, unrelated (MU) BMT. We have treated aGVHD in 7 patients (pts) undergoing MU BMT with high dose MP, an extremely efficacious regimen for aGVHD in matched, sibling BMT. Our regimen is: MP [5mg/kg/day (D) x 4D]; → response: R_x x 8 D, then taper 20% Q 8 D; If no response by D 4, then escalate to 10 mg/kg/d x 4 D. Response R_x as detailed above. If no response by D 4, pts go off study. Nine pts underwent MU BMT. The median age was 29 years (range 21-36) Male: Female, 6:3; Diagnosis was acute leukemia 1 pt; Chronic myeloid leukemia 8 pts. The conditioning regimen was cytoxan/total body irradiation in 9/9 pts and aGVHD prophylaxis was cyclosporin and methotrexate for 9/9 pts. One pt died of sepsis on day 5. Eight of 8 pts at risk developed severe aGVHD (grade ≥II). One died of sepsis before therapy for aGVHD was instituted. The median time of onset of aGVHD was 13 days (range 11-53) All (100%) pts responded to MP; 5/7 pts at 5mg/kg, and 2/2 pts at 10 mg/kg. Three of 7 pts experienced a flare of aGVHD during the MP taper. None responded to re escalation of MP. All 3 pts died with aGVHD and /or aGVHD associated complications, such as sepsis. Four pts underwent taper of MP without a flare of aGVHD. All 4 pts are alive at 3+, 17+, 19+ and 19+ months after BMT. Two pts underwent taper at 10% /week and did not flare. Although the number of pts is small, we could not detect any relationship between the following factors and the development of a flare of aGVHD: Sex or age of donor; MLC reaction (+ or -); previous therapy; or duration of leukemia. We conclude that 1) MP can control aGVHD associated with MU BMT; 2) Flare of aGVHD during MP taper is frequent and contributes to a fatal outcome; 3) A slower (10% /week) MP taper may yield fewer flare reactions.

D 509 INFLUENCE OF GENDER AND MINOR HISTOCOMPATIBILITY FACTORS ON GVHD IN ALTERNATIVE DONOR TRANSPLANTATION. James P. OKunewick, Deborah L. Kociban, Laurie L. Machen and Mary J. Buffo. Allegheny-Singer Research Institute & Medical College of PA, Pittsburgh, PA 15212.

Using a murine model for matched unrelated-donor transplantation we have compared the effect of differences between the sex of the donors and of the recipients on the incidence and severity of GvHD in several different MHC-matched, but minor histocompatibility mismatched, mouse strain combinations. For each strain combination examined paired sets of pooled marrow and spleen cells were prepared according to the respective sexes of the donors. These were then injected into paired male and female recipients, such that GvHD occurence could be directly compared for each of the four possible combinations: male to male, male to female, female to female, and female to male. The results indicated that while in some cases it was possible to detect a significant difference in the incidence of GvHD based on donor/recipient gender disparities (in particular with male versus female AKR recipients of cells from female B10.BR donors), the occurence of such differences was by no means predictable solely on the basis of gender factors alone. To the contrary, with the other strain combinations tested either no significant gender related differences could be found or the effects were reversed with respect to relative sexes of the donors and recipients. Furthermore, in all cases the effects of these gender related differences were much smaller than those that could be attributed to other non-MHC antigenic disparities. These results suggest that other minor histocompatiblity factors, rather than the sex-chromosome related factors, may exert a much greater influence on the incidence and intensity of GvHD, and may in fact affect the expression of any such sex-chromosome factors in GvHD development across minor histocompatibility barriers. (Supported by NIH/NIAID Grant #1R01 AI28425.)

D 510 T-CELL ADD-BACK AFTER T CELL DEPLETION BY ELUTRIATION USING HLA DISPARATE ALLOGENEIC BMT: PILOT STUDIES. R. Quinones, R. Gutierrez, C. Carter, S. Karandish, N. Luban, R. Gress, P. Dinndorf, G. Reaman, Children's National Medical Center, George Washington University, Washington, D.C. and National Institutes of Health, Bethesda, MD.

T cell depletion (TCD) can abrogate the severe graft-versus-host disease (GVHD) that limits HLA disparate BMT, but with high risk of rejection and relapse. We show durable engraftment following add-back of donor T cells and in vivo immunosuppression. We treated 10 children with refractory leukemia with TBI, Ara-C, cyclophosphamide (CY), ATG, and steroids, followed by hematopoietic rescue with TCD, HLA disparate, related BMT. TCD by double elutriation reproducibly separated ≥ 3 logs of T cells from marrow, to which 0.8 to 6.5 x 10^5 donor T cells/kg were added back. All patients (pt) engrafted (ANC > 500 by Day (D) 31 ± 16 and platelet > 2 x 10^5 by D 77 ± 59). No pt rejected, 1 pt required a boost. Three pt developed grade II AGVHD; 1, grade III. Two pt have CGVHD (quiescent on therapy). Only 1 pt relapsed (juvenile CML, 2nd blast crisis). Disease-free survival is 58% (6/10 pt, D 100 to 810). A second group of pt (N=5) was treated with Busulfan, Ara-C, CY, ATG, and steroids. Hematopoietic rescue was with elutriated HLA disparate related BMT with 0.1 to 2.5 x 10^5 donor T cells/kg. Two pt engrafted: a SCID pt as a mixed chimera and an infant with ALL, who relapsed on D 32. A pt with congenital aplasia failed to engraft. A SCID variant pt and a Chediak-Higashi pt had autologous reconstitution. We conclude that TCD by double elutriation with T cell add-back is an effective means of using HLA disparate related marrow to allow BMT in patients with refractory leukemia. TBI appeared superior to Busulfan for achieving sustained engraftment.

D 511 ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) USING UNRELATED DONORS (UDS): THE VANCOUVER EXPERIENCE. Donna E. Reece, John D. Shepherd, Hans-G. Klingemann, Michael J. Barnett, Ka-Wah Chan, Stephen H. Nantel, Gordon L. Phillips. Leukemia/Bone Marrow Transplantation Program of British Columbia, Division of Hematology, British Columbia Cancer Agency, Vancouver General Hospital, B.C. Children's Hospital and University of British Columbia, Vancouver, B.C. V5Z 4E3 Canada.

Between 07/85 and 08/91, searches for UDs were carried out in 195 patients (pts), of whom 51 (26%) have undergone allogeneic BMT. The median pt age was 33 (range 1-51) years. In general, selection criteria and management techniques were similar to those used for pts with matched related donors. Diagnoses included: chronic myelogenous leukemia (21 pts), acute myelogenous leukemia (11 pts), acute lymphoblastic leukemia (10 pts), myelodysplasia (4 pts), severe aplastic anemia (2 pts), other (3 pts). Median interval from initation of search to BMT was 5.5 (range 1.2-69.3) months (mos). HLA matching included serologic 6/6 antigen identity (HLA-A,-B,-DR) in 41 pts; mixed leukocyte reaction was nonreactive (≤15% relative response index) in 31. Eighteen pts received conditioning with VP-16 (1.2-1.8g/m^2) + CY (180 mg/kg) + total body irradiation (TBI) (1000-1200 cGy) while 26 pts received CY(180 mg/kg) + TBI (1200 cGy), 3 pts CY (200 mg/kg) + TBI (300 cGy), 3 pts busulfan (BU) + CY, and 1 pt BU + CY + total nodal irradiation (750 cGy). Prophylaxis for acute graft-versus-host disease (AGVHD) included cyclosporine and methotrexate in all pts; 17 pts also received pentoxifylline and/or XomaZyme. Neutrophil recovery (≥ 0.5 x 10^9/L) occurred on a median of day 20 (range 15-37) post-BMT while the last platelet transfusion was given on a median of day 26 (range 12-128). At a median follow-up of 12 (range 0-36) mos, the actuarial event-free survival is 40% (95% confidence interval [CI] 26 to 55%). The cumulative incidence of grades II-IV AGVHD was 75% (95% CI 62 to 88%) while the cumulative incidence of relapse was 17% (95% CI 2 to 32%). Chronic GVHD has occurred in 13 of 33 evaluable pts. Twenty-three pts are alive; 16 of 17 pts ≥ 6 mos post-BMT have a normal performance status. Two pts died < day 28 without evidence of engraftment while 3 died > day 28 with inadequate graft function. Other causes of death included the following: AGVHD (9 pts), regimen-related toxicity (4 pts), infection (1 pt), chronic GVHD (3 pts), relapse (4 pts), other (1 pt). We conclude that UD-BMT is an acceptable technique in pts lacking related donors. Strategies to improve the therapeutic index will be helpful.

D 512 HLA-MISMATCHED CORD BLOOD TRANSPLANTATION IN A PATIENT WITH ADVANCED LEUKEMIA.
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A 3 year old boy presented an ALL with a worst prognosis : the first CR lasted 2 months and the second CR was difficult to achieve. No HLA matched donor was available. A cord blood (CB) transplantation was considered in this patient whose mother was pregnant after autologous BM had been cryopreserved. The procedure of CB collection was similar to that described by BROXMEYER. Prior to this actual CB collection maternal cell contamination was sought by PCR amplification of HLA DQB1 exon 2. Results on two separate previous samplings indicate that in both cases, contamination was lower thant 10^{-6}. The recipient and his sibling (the CB) have inherited different maternal HLA haplotype. The conditioning regimen consisted of TBI, ARA-C, MELPHALAN. The patient received $0,6.10^{4}$CFU-GM per kg. The complete engraftment was slow and showed an intensive erythropoiesis with an increased level of fetal haemoglobin. Different chimerism studies demonstrated only the presence of donor lymphocytes and hematopoietic cells. Despite HLA disparity, the patient presented only grade I GVHD and transiently some features of limited chronic GVHD. The immune reconstitution was delayed . Lymphocyte proliferative response to antigens was achieved at 36 week post-graft. This patient remains well and disease free with a Karnofsky index at 90% 15 months post graft. Concerning the possible GVL effect of CB transplant, other similar transplantations will provide clues to this question.

Issues

D 600 PROPHYLACTIC VANCOMYCIN PREVENTS BACTEREMIA BY GRAM POSITIVE ORGANISMS IN NEUTROPENIC BONE MARROW TRANSPLANT PATIENTS,
Luke Akard, Fran Newton, John Black, and Jan Jansen, Methodist Hospital of Indiana and Indiana University School of Medicine, Indianapolis, IN.
Since gram positive (GP) infections are the major bacterial causes of morbidity and mortality in bone marrow transplant patients (pts), nontoxic therapies to prevent GP infections are needed. Based on results of our prior study that showed ciprofloxacin 500 mg po bid did not prevent GP infections, we treated consecutive BMT patients with the addition of vancomycin 15 mg/kg/d intravenously beginning the day after marrow infusion and continuing 14 days. Only 3 of 53 patients (13 allogeneic, 40 autologous) developed GP bacteremia (6%) during initial neutropenia. In these 3 patients, neutropenia and bacteremia occurred prior to the planned administration of vancomycin. Organisms identified were: patient 1-Staph cohnii, Strep sanguis, and Staph haemolyticus; patient 2-Staph epidermidis; patient 3-Strep viridans. No gram negative infections occurred and there were no infectious deaths. Despite intravenous vancomycin, all 53 patients demonstrated persistent GP organisms in posterior pharyngeal and nasal surveillance cultures. In our previous study of ciprofloxacin prophylaxis, 24 out of 63 patients (35%) developed GP bacteremia (1 death due to viridans streptococcus) and no patient developed GN bacteremia. These data strongly support that prophylactic vancomycin decreases GP infection in BMT patients and thereby reduces the morbidity and mortality of this procedure.

D 601 FOSCARNET FOR TREATMENT OF CYTOMEGALOVIRUS INFECTION IN BONE MARROW TRANSPLANT RECIPIENTS.
J. Aschan, O. Ringdén, P. Ljungman, B. Lönnqvist and J. Tollemar. Dept. of Clinical Immunology, Transplantation Surgery and Medicine, Karolinska Institute, Huddinge Hospital, S-141 86 Huddinge, Sweden.

Forty-two episodes of verified or clinically suspected cytomegalovirus (CMV) infection in 40 bone marrow transplant (BMT) recipients were treated with foscarnet (trisodium phosphonophormate hexahydrate). CMV infection was verified in 31 out of 42 treatment episodes by immunofluoroscence, serology and/or isolation from blood, urine, bronchoalveolar lavage fluid or autopsy material. Symptoms treated were pneumonia (n=17), pancytopenia with or without fever (n=12), enteritis (n=5), fever (n=4), encephalitis (n=2), retinitis (n=1) and hepatitis (n=1). Foscarnet was given as a continous intravenous infusion. Daily dose ranged from 69-300 mg/kg (median 162), treatment duration from 1-49 days (median 14), total dose from 4.4-510 g (median 92.5) and the average steady state level was 55-240 µg/ml (median 95).
Side-effects observed were increase in serum creatinine (38%), decrease in serum calcium (19%), increase in serum bilirubin (12%), decrease in hemoglobin concentration (7%), increase in serum calcium (5%), increase in serum transaminase (5%), hypophosphataemia (2%) and tremor (2%).
CMV was eradicated from blood and/or urine in 11/25 (44%) of assessable treatment episodes with infection verified by isolation. Overall clinical improvements including eradication of CMV, afebrility and/or improvements in laboratory abnormalities were seen in 14/31 (45%) episodes of verified infection.
All 15 patients with CMV interstitial pneumonia (CMV IP) died.
We conclude that foscarnet is nephrotoxic but otherwise well tolerated with moderate clinical and virostatic effects on CMV infection. The effect on CMV IP is discouraging.

D 602 UPDATE ON OUTCOME FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANT (ABMT) FOR PATIENT'S WITH HURLERS SYNDROME (HS) USING ALTERNATIVE MARROW DONORS. Gil H. Ciocci, P. Jean Henslee-Downey, Anjana L. Pettigrew, Chris Miara, Jennifer Morrow, and the Bone Marrow Transplant Program, University of Kentucky, Lexington, KY 40536.

Between 1987 and 1991, fourteen patients (pts) with absence of serum alpha-L-iduronidase resulting in accumulation of glycosaminoglycans (GAG) producing characteristic manifestations of HS who did not have HLA histocompatible siblings underwent BMT from alternative donors: 10 from partially mismatched related donors (PMRD) and 4 from phenotypically similar unrelated donors (PSUD). Amongst recipients of PMRD, 4 presented 1; 5, 2; and 1,3 major HLA antigen mismatches in the graft versus host disease (GVHD) direction and all recipients of PSUD grafts presented no serologic difference. At the time of BMT, the pts ranged from 12 to 44 months of age (mean 23.6). All pts received conditioning therapy with 14 Gy fractionated total body irradiation followed by high dose chemotherapy including Ara-C, Cytoxan and Methyl-prednisolone (MPD). All marrow grafts were incubated with an anti-CD3 monoclonal antibody, T10B9, to produce between 1.5 to 2 log reduction in mature T lymphocytes. Additional GVHD prophylaxis included systemic post grafting therapy with Orthozyme CD-5+ in 10, cyclosporine in 3, and both in 1 pt. Thirteen pts (93%) demonstrated evidence of engraftment with a peripheral white count >500 at mean of +13.8 days. However, 3 of these pts experienced graft failure, 1 within 3, & 2 by 12 mos post BMT. Most pts were protected from GVHD with minimal manifestations of skin only (Grade I) in 4, skin and gut (Grade II-III) in 2, and severe (Grade IV) multi-organ involvement in 1 pt. Eleven pts (79%) are surviving but 3 have autologus recovery. Of 8 pts surviving with sustained engraftment, all have demonstrated biochemical correction of urinary GAG and white cell enzyme production comparable to donor levels. In addition, morphologic signs of substrate removal has been observed including disappearance of organomegaly, gum hypertrophy and upper airway obstruction, increased mobility, cornea clearing, relief of hydrocephalus, correction of glaucoma, and softening of course facial features. Several pts do have persistent orthopedic abnormalities. Serial developmental testing in some of the pts has demonstrated progress at a rate similar to normal development and in the pt with longest follow up (4 yrs) the intellectual quotient has advanced from a moderate-severe to low normal level. At this time, favorable outcomes appear to be appreciated in about half of the pts. These results might be improved with earlier ABMT but also provide support for the future promise of less toxic autologous transplantation with genetic gene transfer.

D 603 HUMAN HERPESVIRUS-6 (HHV-6): A NEW PATHOGEN IN ALLOGENEIC BONE MARROW TRANSPLANT (BMT) RECIPIENTS, William R. Drobyski, Eileen Burd, W. Michael Dunne, Konstance K. Knox, and Donald R. Carrigan, Departments of Medicine and Pathology and the Bone Marrow Transplant Program, Medical College of Wisconsin, Milwaukee, WI 53226.

Human herpesvirus-6 (HHV-6) is a recently described member of the herpesvirus family, which bears close homology to cytomegalovirus (CMV). Early studies revealed HHV-6 to be the etiologic agent in exanthem subitum, a self-limited childhood illness. Whether HHV-6 is able to infect and cause clinically significant disease in bone marrow transplant recipients, however, is largely unknown, although recently we have shown that HHV-6 appears to play an etiologic role in selected cases of interstitial pneumonitis after BMT. In this study 20 adult patients who underwent allogeneic bone marrow transplantation were prospectively followed for the first 100 days posttransplant to define the incidence and severity of infection due to HHV-6. HHV-6, as documented by IgM seroconversion or viral isolation, occurred in 80% of patients. HHV-6 was directly isolated from the blood of five patients, one of whom also had virus cultured from the bone marrow. Concurrent HHV-6 and CMV infection were documented in four of 16 patients, suggesting that these viruses might be capable of coreactivation. HHV-6 infection was temporally associated with the development of specific clinical syndromes in these patients. The most serious of these was marrow suppression or marrow graft failure which was not due to graft rejection or attributable to any other known etiology. Interstitial pneumonitis and meningoencephalitis were also observed in several patients. This study identifies HHV-6 as a new pathogen in allogeneic marrow transplant recipients and implicates it as the etiologic agent in potentially life-threatening infections.

D 604 APPLICATION OF PCR TO EARLY DIAGNOSE CMV INFECTION AND TO RAPIDLY DETECT TREATMENT FAILURES OF ANTIVIRAL THERAPY, Hermann Einsele, Helmuth Schmidt, Michael Steidle, Michael Müller, Gerhard Ehninger, Hans Dierck Waller, Claudia A.Müller, Medizinische klinik und Poliklinik, Abteilung II, und Sektion Transplantationsimmunologie und Immunhämatologie, University of Tübingen, D-7400 Tübingen, Germany

63 patients undergoing allogeneic bone marrow transplantation were followed up for the development of CMV infection and CMV disease. In 46 out of these 63 patients CMV could be detected in the blood and urine samples a median of 29 days after BMT. In 36 of these patients the virus could be additionally cultivated from blood and/or urine samples a median of 45 days after BMT. Antiviral therapy of 18 episodes of CMV disease were followed-up clinically and by virus culture and PCR technique. Clinical improvement, negative culture and PCR assays were assessed for their ability to predict the efficacy of ganciclovir therapy in the individual patient. In 11 successfully treated episodes of CMV disease clinical improvement was associated with an effective suppression of virus replication as demonstrated by negative culture and PCR technique. One patient who did not improve clinically when receiving antiviral therapy remained positive for CMV as demonstrated by culture and PCR technique. Six patients with either early relapse of or fatal CMV disease after an initial improvement were found to be PCR-positive after termination of therapy. In contrast, these patients were culture-negative for CMV after cessation of antiviral therapy.

Demonstration of CMV in blood and urine by PCR technique after cessation of antiviral therapy - in spite of negative findings in the culture technique- indicates incomplete suppression of virus replication. Thus PCR technique not only allows earlier detection of CMV after BMT, but was also found to better predict the efficacy of antiviral therapy than culture assays or clinical assessment.

D 605 COMPARISON OF CIRCULATING CMV NEUTRALIZING ACTIVITY (CMVNA) IN A RANDOMIZED DOUBLE-BLINDED STUDY OF FOUR IVIgG PRODUCTS IN BONE MARROW TRANSPLANT (BMT) RECIPIENTS

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Forty-two CMV seronegative BMT recipients were randomized to receive 1 of 4 commercially available IVIgG products (Gammimmune N , ARC-Hyland , Gammagard , or Sandoglobulin ,) at a dose of 500 mg/kg every other week starting the week prior to BMT. The 4 groups were comparable in distribution of patient ages, weights, autologous vs. allogeneic donors and underlying diseases. Every other week dosing provided total IgG levels within the physiologic range for age and stable CMV titres by latex agglutination (average geometric mean titre of 4.2 after the second IVIgG dose), with no statistically significant differences among the 4 product groups for these parameters. CMVNA and ELISA titres were determined on a subset of sera from 27 study patients representing the 4 product groups. Serum samples obtained prior to IVIgG infusions (presumed negative controls) and samples obtained 2 weeks after IVIgG dose #2 (ie. 3 weeks post BMT) were assayed in a blinded manner and the identity of products remains unknown until all analyses have been completed. CMVNA was assessed by inhibition of plaque formation in a standardized human fibroblast culture inoculated with HCMV, Towne strain. Mean titres of CMVNA varied among product groups. The highest mean 50% CMVNA was 1:43 (with predicted range at 95% confidence level of 1:26 - 1:61) whereas the lowest mean 50% CMVNA was 1:14 (1:6 -1:22); two of the IVIgG product groups showed intermediate 50% mean titres of 1:27 (1:14 - 1:39) and 1:26 (1:14 - 1:38) for an overall p=0.02. Comparison of 50% CMVNA with ELISA values yielded a linear correlation, R=0.566. However, this degree of correlation does not appear to be adequate to recommend substitution of the simpler ELISA determination of circulating CMV titres for the actual measurement of CMVNA. We conclude that commercially available IVIgG products provide passive CMVNA, and that the level of circulating CMVNA may be affected by the IVIgG product used.

D 606 ALLOGENEIC MARROW TRANSPLANTATION IN CATS. PW Gasper, MA Thrall, R Fulton, BJ Rose, TN Thomas, and the CSU Marrow Transplant Team. Marrow Transplant Lab., Pathology Dept., CVMBS, Colorado State University, Fort Collins, CO 80523.

One hundred and eight allogeneic marrow transplants were performed in cats with two objectives:
1) as therapy for inherited metabolic diseases—cats affected with Chediak Higashi Syndrome, GM1 Gangliosidosis, Mucopolysaccharidosis types I and VI, α-mannosidosis, and Niemann-Pick type C—and,
2) as therapy for retrovirus infections—cats infected with feline leukemia virus or with feline immunodeficiency virus). The overall rate of stable donor-origin lymphohematopoietic engraftment was 79%. The longest living BMT-recipients are over seven years post-transplantation. The incidence of graft-versus-host disease was 19%. Our present preferred regime is pre-transplant enrofloxacin for gastrointestinal decontamination(from day -6 through day +14 or onset of fever), total body irradiation (TBI) (10.00 Gy divided into six 1.67 Gy fractions delivered twice, six hours apart on days -2, -1, and 0 via a 6 Mev. linear accelerator), and cyclosporin (15 mg/kg once daily on days -1, 0, and +1 and then from the day of engraftment until day +100). Marrow is harvested from the donor and administered to the recipient cat on day 0 (following TBI) and again on day +5. We house the cats conventionally, provide food and water ad lib., evaluate them at least twice daily, provide nutritional support, fluids, and antibiotics as needed.
Cats appear uniquely suited for marrow transplantation. Although one can identify the usual components of the histocompatibility complex in cats, our results suggest that cats have a lower incidence of graft-versus-host disease and they are more likely to accept nonsibling marrow grafts than other outbred mammals.

D 607 REDUCTION OF GRAFT VERSUS HOST DISEASE (GVHD) INCIDENCE AND SEVERITY LOWERS COST OF BONE MARROW TRANSPLANTATION. K. Gorman, P.J. Henslee-Downey, G. Carey, K. Longson, B.J. Ransil. Putnam Associates, Burlington, MA, University of Kentucky BMT Program, Lexington, KY, Ortho Biotech, Raritan, NJ and Harvard Medical School, Beth Israel Hospital, Boston, MA.
In order to measure the effect of acute GvHD and the effect of prophylactic use of Orthozyme-CD5+ (CD5+) on the charges for allogeneic bone marrow transplantation (BMT), we studied the costs of services provided to 57 BMT patients (pts) during their initial inpatient period at the University of Kentucky. Pts underwent BMT between January 1986 and January 1990, and prices of services were adjusted to 1989 dollars. All pts received T cell depleted, partially matched related donor grafts. No additional GvHD prophylaxis was given to the first 17 pts who constitute the control group. All subsequent pts included in the cost analysis (n=36) received CD5+, an immunotoxin composed of an anti-CD 5 MoAb conjugated to ricin A chain. The CD5+ group had a significant reduction in the occurrence and severity of acute GvHD when compared to the control group. We found the occurrence of acute GvHD in pts prolonged inpatient length of stay and increased the costs associated with the following: pharmaceutical products, lab fees, blood products, medical products and physician fees. Increased severity of acute GvHD resulted in higher initial inpatient costs. For the total patient sample, each incremental level of acute GvHD severity, from no GvHD to mild GvHD to severe GvHD, increased inpatient costs by approximately $56,400. The costs per level of acute GvHD increased at a rate 3 times greater for the control group than for the CD5+ group, as shown below.

GvHD	Pts with GvHD (%)				Charges (Mn ± SD, in 000's)		
Level	Control		CD5+		Control	CD5+	p Value
None	0	0%	12	35%	---	$121 ± $29	
Mild	2	12%	17	46%	$124 ± $20	$170 ± $57	
Severe	15	88%	7	19%	$247 ± $75	$197 ± $61	
All Patients	17	100%	36	100%	$233 ± $82	$159 ± $57	<0.001

Because the CD5+ group had a lower occurrence and severity of acute GvHD, the CD5+ group had a lower average initial inpatient BMT cost by approximately $73,900 and a shorter average length of inpatient stay by 20 days (62.4 ± 20.6 days vs. 82.4 ± 25.4 days; p<0.01). The reduction in average costs for the CD5+ group was associated with significant reductions in room costs, pharmaceutical products, lab tests, blood products, medical supplies and physician fees. We conclude that CD5+ was a cost-effective GvHD prophylactic agent and that it produced a significant cost benefit when utilized in this manner.

D 608 PROPHYLACTIC INTRAVENOUS IMMUNOGLOBULIN (IVIG) AFTER ALLOGENEIC MARROW TRANSPLANT (BMT): PRELIMINARY ANALYSIS OF A DOSE-RANDOMIZING STUDY. J Graham-Pole, M Amylon, G Elfenbein, J Gallo, S Gross, J Jansen, M Klemperer, H Lazarus, T Pick, W Spruce, R Weiner, Bone Marrow Unit, University of Florida, Gainesville, FL 32610. We randomized patients (pt) transplanted at 9 centers to receive either 250 mg/kg (gp 1) or 500 mg/kg (gp 2) IVIG weekly for 17 weeks (wk). Pt were stratified by age, cytomegalovirus (CMV) serology and HLA match. We have compared the first 122 pt for frequency, duration and severity of bacterial, viral, fungal and protozoal infections (inf) and frequency of acute graft-vs host disease (GVHD). There were 57 gp 1 and 65 gp 2 pt; 34 had ALL, 34 ANL, 31 CML, and 23 other diseases. 67 were aged 0-18 years, 70 were male, 78 CMV+ and 109 HLA-matched. At 24 wk post-BMT 81 (66%) had developed 1-5 inf (27 fatal); 20 (16%) had died of other cause or had primary disease recurrence; 75 (61%) remained well. 49 developed bacterial (4 fatal), 31 viral (7 fatal), 37 fungal or protozoal (7 fatal) inf; 9 mixed inf were fatal. 70% gp 1 and 63% gp 2 developed inf (p=NS). 55 pt (45%) developed GVHD (53% gp 1, 38% gp 2, p=NS). More CMV+ than CMV- pt developed inf (p.037); more gp 1 than gp 2 (p.053) and more black than white pt (p.017) developed viral inf; more pt aged 18+ than pt aged 0-18 (p.012) and more mismatched than matched pt (p.020) developed fungal or protozoal inf. More pt aged 0-18 (73%) and more HLA-matched pt (68%) survived to 24 wk post-BMT than pt aged 18+ (47%) or HLA mismatched pt (8%)(p.001). There was no intergroup difference in duration of inf or GVHD. This preliminary analysis suggests pt variables are more significant predictors than IVIG dose for patterns of inf after BMT. We are conducting a multivariate analysis of a larger pt cohort to identify factors independently predicting for frequency, duration and severity of inf and of GVHD in this population.
Supported by a grant from Baxter-Travenol.

D 609 THE ASSOCIATION BETWEEN HUMAN HERPES VIRUS 6 (HHV-6) AND PNEUMONIA IN MARROW TRANSPLANT PATIENTS. RC Hackman[1,2], RW Cone[3], D Myerson[1,2], M-L Huang[3], R Bowden[1,4], L Corey[3]; (1) Fred Hutchinson Cancer Research Center, Seattle, Washington 98104; (2), (3), (4) Departments of Pathology, Laboratory Medicine, and Pediatrics, University of Washington, Seattle, Washington 98105

Pneumonia remains a significant complication of bone marrow transplantation and is frequently unassociated with an identified infectious agent (idiopathic pneumonia). We evaluated the hypothesis that HHV-6 plays a role in post transplant pneumonia by retrospective polymerase chain reaction analysis of lung tissue from 15 patients who had undergone open lung biopsy for diffuse pneumonia in comparison to analysis of lung tissue from 15 non-transplant normal individuals. HHV-6 DNA was present in 15/15 patients and 14/15 controls. The mean HHV-6 DNA level in 7 of the 15 transplant recipients was 400,000 HHV-6 genomes per million cells in comparison with a mean of 500 genomes in the controls. In situ hybridization for HHV-6 demonstrated scattered infected cells in the lung tissue most positive by PCR analysis. Six of the 7 transplant recipients which were highly positive for HHV-6 by PCR had pneumonia previously classified as idiopathic, suggesting a causal role for HHV-6 in this disorder.

D 610 BONE MARROW TRANSPLANTATION (BMT) FOR METABOLIC DISEASES: RESULTS OF THE EBMT.
Peter M.Hoogerbrugge[1], Oebo F.Brouwer[2], Alain Fischer[3], Dept. of Pediatrics[1] and Pediatric Neurology[2], Univ. Hospital, 2300 RC, Leiden, The Netherlands; Hopital Necker Enfants Malades[3], Paris, France.
Successful BMT for metabolic diseases results in replacement of enzymatically deficient blood cells and tissue macrophages of the recipient by those of an enzymatically competent donor. Since 1981, patients with metabolic dieseases have been transplanted. In the present survey, updated results of the EBMT will be reported. Special attention will be focussed on patients with metabolic diseases primarily affecting the CNS. So far, 12 patients transplanted for metabolic diseases with severe neurological involvement were reported. At the time of submission of the abstract, detailed questionaires are send, the results of which will be presented. At least 4 patients (age 3 to 9 years) were transplanted for **Adrenoleukodystrophy (ALD)**. Two patients, hardly showing neurological symptoms at BMT, remained asymptomatic during a follow-up period of 6 and 27 months. One patient deteriorated rapidly following BMT, the fourth patient improved. Five patients were transplanted for **Metachromatic Leukodystrophy (MLD)**. In three patients, the effect of BMT could not be evaluated due to graft rejection or short follow-up. In one patient, neurological deterioration was present 2 years after BMT, in the other patient, the disease process stabilized following BMT. Of two patients with **Sanfilippo-A** disease, the disease process continued in one and stabilized in the other patient. In a patient with **Sanfilippo-B** disease, the neurological deterioration continued following BMT. Additional follow-up of these patients will be shown; the relevance of transplantation early in the disease process, which was also observed in animal model studies, will be discussed as well.

D 611 THE ADVERSE EFFECT OF AZOTEMIA UPON GRANULOCYTOPENIC SURVIVAL IN BONE MARROW TRANSPLANTATION.
P.J.Manno, W.D.Haire, J.R.Campbell, L.Stephens, A.Kessinger, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68198
Serum from uremic patients can suppress CFU-GM and megakaryocytic proliferation in vitro (Kidney Int 29:641 1984). Azotemia is often encountered during the bone marrow transplantation process. To investigate the possible relationship of azotemia to engraftment time and survival, we reviewed the records of 419 consecutive marrow and perpheral stem cell transplant patients at our institution. We employed the proportional odds model (Bone Marrow Transplantation 1990, 6, 185-191) in our analysis. An absolute granulocyte count of 0.5 x 10^9/L for two consecutive days was used to define bone marrow recovery. Deaths occurring in the first seven days of transplant were censored. The 71 patients who survived the first week but died prior to marrow engraftment were assigned an endpoint of 500 days for recovery. Patients were stratified according to the peak serum creatinine during the week following stem cell infusion. A serum creatinine of $\geq$1.5 mg/dl in 74 patients during this time was associated with a decreased granulocytopenic survival compared with 345 patients with a peak of <1.5 mg/dl (p=.05 log rank). We conclude that early onset of azotemia had an adverse effect upon granulocytopenic survival, but the precise contribution cannot be ascertained from this data due to other factors that also influence outcome. Further study is needed to determine whether early dialysis for transplant patients with mild azotemia would improve survival.

D 612 **LATE PSYCHOSOCIAL EFFECTS OF ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT),**
Michael L. Nieder, Howard R. Hall and Jeffrey Margolis, Department of Pediatrics, Case Western Reserve University Medical School, Cleveland, OH 44106
Serious medical complications often occur during BMT and have immediate ramifications for the patients and their families. Occasionally, these problems lead to lifelong physical disabilities. Just as important but more difficult to assess are the emotional and psychosocial problems which may result from these medical problems. In 1991, 46 surviving patients (median age 13.5 years, range 5-30) who had BMT from 1980-90 were examined and queried about their present emotional and psychological condition. In addition to a specially created questionnaire, the Child Behavior Checklist (Achenbach, 1988) was used for patients presently aged 5-11 years and the Symptom Checklist-90-R (Derogatis, 1975) was used for patients aged 12 and older. Results were compared to standard norms and standard cancer patient norms. The results suggest that those patients who suffered more complications during the transplant now display more global personality distress than other BMT or cancer patients. Patients who had chronic graft versus host disease (CGVH) also exhibit more chronic emotional distress. Patients who had no serious complications during BMT and no CGVH do not seem to manifest signs or symptoms of significant psychological or emotional distress as compared to standard norms. In this group of all BMT patients, 95% were either in school or had completed at least high school. This data reinforces the need for early psycho- logical support for children undergoing BMT. For patients with CGVH or who had serious complications during the BMT, ongoing counseling or psychotherapy should be considered.

D 613 SCID PATIENTS RECONSTITUTED BY FETAL LIVER STEM CELLS: POSSIBLE ROLE OF IL-10 IN TRANSPLANTATION TOLERANCE,
Maria-Grazia Roncarolo, Rosa Bacchetta, Jean-Louis Touraine*, Mike Bigler and Bart Vandekerckhove, DNAX Research Institue, Palo Alto, CA 94304 and *INSERM U80, Lyon, France 69374
Severe combined immunodeficiencies can be treated by fetal liver and thymus transplant when HLA identical donors are not available. We studied the mechanisms of tolerance in two patients in whom stable engraftment of T cells of donor origin was obtained after transplantation, whereas B cells, monocytes and NK cells were of recipient origin. Despite the HLA mismatch between donor and recipient cells, no acute or chronic graft versus host disease was observed and the patients acquired normal T and B cell responses against different antigens. The phenotype of PBMC showed normal percentages of B cells, monocytes, NK cells and T cells, although the CD4/CD8 ratio was consistently inverted. *In vitro* experiments with PBMC stimulated by the recipient EBV-LCL showed specific unresponsiveness for the HLA antigens expressed by the recipient cells, whereas the proliferative and cytotoxic responses against allogeneic EBV-LCL were normal. However, polyclonally activated PBMC responded normally to recipient EBV-LCL, indicating that donor T cells reacting against the recipient HLA antigens were present in the peripheral blood of both patients. An extensive clonal analysis showed that $CD4^+$ and $CD8^+$ hostreactive T cell clones recognized class II and class I HLA molecules of the recipient, respectively. Limiting dilution experiments indicated that the frequency of $CD8^+$ hostreactive cells was in the same range as that observed for $CD8^+$ alloreactive T cell clones. In contrast no selfreactive (anti-donor HLA) $CD8^+$ T cells could be isolated. Hostreactive $CD4^+$ clones were normal in their capacity to produce IL-2, IFN-γ, GM-CSF and IL-5, but they failed completely to synthesize IL-4. In addition these cells secreted very high levels of IL-10. Interestingly, exogenous IL-10 was able to inhibit the proliferative responses of the $CD4^+$ hostreactive T cell clones. Our data demonstrate that hostreactive cells are not deleted from the donor T cell repertoire following allogenic fetal liver tranplantation. Therefore, *in vivo* tolerance between the host and the recipient is maintained by clonal anergy or suppression in which IL-10 may play a role.

D 614 ENZYME REPLACEMENT IN HURLERS SYNDROME USING BONE MARROW TRANSPLANTATION. E. F. Saunders, M.A. Kirby, H.S. Solh, J.T.R. Clarke. Division of Hematology-Oncology and Clinical Genetics, The Hospital for Sick Children, Toronto.

Two female infants aged 20 mo (Pt 1) and 23 mo (Pt 2) with Hurlers Syndrome (α iduronidase deficiency) received bone marrow from HLA-MLC matched siblings heterozygous for α iduronidase deficiency. Pts were conditioned with Busulfan 16 mg/Kg and Cyclophosphamide 200 mg/Kg. GVHD prophylaxis consisted of Cyclosporin A and Methotrexate. Hematopoietic recovery was prompt with no GVHD. Both pts showed mixed hematopoietic chimerism. Pt 1 had 75% donor cells 2 mo post BMT based on RFLP analysis of peripheral leukocytes decreasing to 25% by 19 mo. Pt 2 had a progressive increase in donor leukocytes from 45% at 2 mo post BMT to 80% at 8 mo, based on sex chromosome analysis of peripheral lymphocytes (male donor). Follow-up is 24 mo for Pt 1 and 17 mo for Pt 2. Both had marked improvement in general health. Upper airway obstruction and sleep apnea disappeared. Liver and spleen size decreased to normal. Alder-Reilly bodies disappeared from marrow cells and liver biopsies became normal. The α iduronidase level of peripheral leukocytes was 4.10 nmoles/mg/hr 24 mo post BMT in Pt. 1 and 13.9 9 mo post BMT in Pt. 2 (normal control 26-63). MRI of the brain showed normal progression of myelinization and improvement in white matter lesions. Both pts had no change in corneal clouding, rate of head and linear growth or spinal deformity. Neuropsychological development has continued below normal. Although these pts had a systemic improvement in their disease any effect on CNS function is not yet evident.

D 615 POST-BMT HUMORAL IMMUNODEFICIENCY - PATHOGENETIC INSIGHTS, Jan Storek & Andrew Saxon, Dep. of Medicine, UCLA, L.A., CA 90024.

B-cell immunity is impaired for 1-2 years in healthy BMT survivors and even longer in patients with chronic GVHD. Can this long-lasting reconstitution be simply explained by the repetition of B-cell ontogeny?

1. Phenotype of circulating B-cells from BMT recipients has many similarities to that of neonates and infants. By flow cytometry, both the posttransplant and normal neonatal/infant cells were large, underexpressed CD21 (C3d/EBV receptor), Leu8 (addressin for high endothelial venules) and overexpressed CD38 (marker of immaturity in this setting). Activation markers like CD23 (FcεRII) or CD25 (IL-2R) were not overexpressed in either neonates/infants or BMT recipients. Interesting, yet unexplained differences were also found: CD71 (transferrin receptor) was overexpressed on posttransplant but not on neonatal/infant B-cells. The percentage of $CD5^+$ B-cells was high in neonates/infants and after autografting but not after allografting.

2. The earlierst step of the B-cell program (activation → proliferation → Ig production) is defective. This was shown by decreased Ca^{++} flux into the B-cell cytosol upon membrane Ig crosslinking in comparison to normal adults. Nevertheless the number of membrane Ig molecules per cell was not decreased. By inference, immaturity of the signaling pathway may account for the activation defect.

3. Early posttransplant, stem cells differentiate primarily into the myeloid rather than B-cell lineage. This was documented by flow cytometric analysis of bone marrow at the time of neutrophilic engraftment (usually 3-5 weeks posttransplant) - no $CD19^+$ cells were found in the marrow at that time. Circulating $CD19^+$ cells reached normal numbers only 2-4 months after autologous BMT and at >4 months after allogeneic BMT. Contrary to BMT recipients, the number of bone marrow and circulating B-cells in neonates/infants was reported not to be decreased compared to normal adults (Caldwell CW et al: Am J Clin Pathol, 1991, 95:816).

In Summary, the humoral immunodeficiency in BMT recipients results from both relative immaturity (i.e. ontogenic recapituation) and superimposed defects in B-cell maturation.

D 616 IMMUNE RESPONSE TO *CANDIDA ALBICANS* BEFORE, DURING AND UP TO 5 YEARS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

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The aim of the present study was to follow the immunologic recovery against *Candida* after bone marrow transplantation (BMT), since invasive *Candida* infections contribute to the mortality and morbidity among allogeneic BMT recipients.

Material. We followed 133 consecutive BMT recipients with *in vitro* lymphocyte stimulations, before BMT, after engraftment, at 1, 2, 3, 6 and 12 months and at annual controls after BMT. During the first year, 213 stimulations were performed in 96 patients and between the 2nd to 5th year, 64 patients were followed with 99 stimulations. The normal range of stimulation was calculated as a geometric mean ±2 SE from 45 healthy bone marrow donors and 8 healthy volunteers. Peripheral blood lymphocytes were stimulated by *Candida* protein antigen (CP) and *Candida* mannan mitogen (CM) prepared from an isolate of *Candida albicans* group A. As a control mitogen *Staphylococcus aureus* protein A (SPA) was used. DNA synthesis was measured by ^{3}H-thymidine incorporation at day 7 in culture.

Results. In all patients lymphocyte responses to CP and SPA normalized within 6-12 months, while CM responses were within the normal range already one month after BMT. Patients colonized with *Candida* regained cellular stimulation capacity to CP and CM within 1-3 months after BMT while non-colonized patients required > 6 months. There was a tendency for low CP stimulation among patients receiving T cell depleted bone marrow as compared to patients receiving methotrexate + cyclosporin as graft-versus-host disease (GVHD) prophylaxis. Patients who later developed grade II-IV of acute GVHD had significantly higher responses to CP and CM before BMT ($p=0.02$ for both) compared to patients with grade 0-I of acute GVHD. No impact on stimulation was recorded by chronic GVHD or by cytomegalovirus seropositivity.

Conclusion. Cellular immune response to *Candida* is restored early after BMT and was mainly influenced by superfiscial fungal colonization. High cellular response to *Candida* before BMT was associated with a higher incdience of ≥ grade II acute GVHD.

D 617 TREATMENT OF EAE WITH BONE MARROW TRANSPLANTATION, D.W. van Bekkum, M. van Gelder, E.P.M. Kinwel-Bohre, TNO Institute for Applied Radiobiology and Immunology, Lange Kleiweg 151, P.O. Box 5815, 2288 HV Rijswijk, The Netherlands.

We have shown previously that complete and lasting regression of the autoimmune disease adjuvant arthritis (AA) can be obtained in rats by treatment with high dose total body irradiation (TBI) and transplantation of syngeneic or autologous bone marrow. Analogous experiments have been performed in Buffalo rats suffering from severe paralysis following induction of Experimental Allergic Encephalomyelitis (EAE). On day 20 after immunisation with spinal cord and complete Freund adjuvant - when paralysis was progressive - rats were subjected to 9 or 10 Gy of TBI and syngeneic bone marrow. Shortly after treatment the paralysis aggravated causing the death of a variable proportion of the animals. The survivors showed complete regression of all symptoms within 10 days, while spontaneous regression in the controls took 30 days. Rechallenge with spinal cord and adjuvant at 20 days after bone marrow transplantation resulted in relapses in both the treated and non treated groups, but the incidence of the relapses as well as the associated paralytic score and mortality were considerably less in the treated animals. These results are encouraging in that they indicate a comparable response of EAE to bone marrow transplantation as was seen in AA. Modification of TBI conditioning and numbers of bone marrow cells grafted are presently under investigation.

D 618 TESTICULAR FUNCTION AFTER BUSULFAN (BU) PLUS CYCLOPHOSPHAMIDE (CY), John R. Wingard, Dawn F. Miller, George W. Santos, Emory University School of Medicine, Atlanta, GA 30322 and Johns Hopkins University School of Medicine, Baltimore, MD 21205

Testicular function was assessed in 45 adult males after bone marrow transplantation (BMT) using BU 16 mg/kg plus CY 200 mg/kg. Assays were performed singly or on multiple occasions (42%) 0.5 to 6.5 years after BMT. Serum testosterone levels were 586 ± 226 ng/dl with subnormal levels in only 1 of 42 patients (2.4%). Serum luteinizing hormone levels (LSH) were elevated in 12 of 34 patients tested (35.3%). Serum follicle stimulating hormone levels (FSH) were elevated in 32 of 38 patients (84%) and normal in the remainder. These findings are comparable to data from 68 men treated by CY plus total body irradiation. There were no significant correlations between LH, FSH, testosterone, time out from BMT and age except for an association between LH and FSH ($r = 0.91$, $p < 0.00001$). Sperm counts were obtained in 21 patients. No detectable sperm were found in all but two. One had one visible sperm on smear 3 years after BMT; the second had no sperm at 2 years, but counts of 10 and 12 million/ml at 3 and 4 years respectively. Four of the 6 men with semen analysis 2+ years demonstrated no sperm. Two men reported fathering normal children after full-term pregnancy (at 2.5 and 3 years respectively after BMT). The former had a sperm count of 0 at 6 months (no assays subsequently) and the latter ten million/ml at 3 years after BMT. None of the progeny were HLA typed. We conclude that although luteal function is relatively well preserved with normal serum LH and testosterone levels, follicular function is markedly impaired, resulting in aspermia in most men treated by BU plus CY. However, some recovery of spermatogenesis appears possible with the passage of time (2+ years after BMT), apparently enabling some individuals to father children.

D 619 THE MONOCLONAL ANTIBODY HA-1A AS ADJUNCTIVE THERAPY FOR IMMUNOCOMPROMISED PATIENTS WITH SEPTIC SHOCK, J. Wood, D. Przepiorka, M. Kessler, C. Ippoliti, R.O. Wallerstein, A.B. Deisseroth, S. Giralt, B.S. Andersson, R.E. Champlin. University of Texas, M. D. Anderson Cancer Center, Houston, TX 77030

HA-1A, a human monoclonal IgM with specificity for bacterial endotoxin, has been shown to improve outcome for nontransplant patients with gram-negative bacteremia and septic shock when used in conjunction with appropriate antibiotics. We have used HA-1A as adjunctive therapy for treatment of 19 episodes of septic shock in 16 immunocompromised adults. The study group included 7 autologous marrow recipients, 6 allogeneic marrow recipients, and 3 patients receiving growth factor support following high-dose chemotherapy without marrow rescue. The median time from transplantation (or treatment) was 22 days (range 2-1114). All allogeneic recipients had received a course of steroids. The absolute neutrophil count was <1000 in 12/19 episodes (<200 in 8/19), creatinine ≥2.0 in 7/19, and total bilirubin ≥2.0 in 10/18. Pressors were required during 15/19 episodes and mechanical ventilation during 12/19. Within 24 hours prior to receiving HA-1A, the mean APACHE score was 30 ± 8 (± SD). Gram-negative bacteremia was documented during 7 episodes, gram-positive bacteremia during 6, and no bacteremia during 6. All patients were treated with broad spectrum antibiotics. Each patient received 100 mg HA-1A IV over 30 minutes at a median of 18 hours (range 1-101) from the diagnosis of sepsis. There were no complications noted during the infusion of HA-1A. Survival exceeded 6 hours after infusion of HA-1A for 17/19 episodes. At 6 hours, 9/13 had a decrease in pressor requirements, and 10/17 had an increase in blood pressure and/or systemic vascular resistance. Survival exceeded 1 week for 12/19, and sepsis was the cause of death for 6 patients. For those patients with documented gram-negative bacteremia, at 6 hours 4/6 had a decrease in pressor requirements, and 5/7 had an increase in blood pressure and/or systemic vascular resistance. Survival exceeded 1 week for 5/7, but 3/7 had persistant or recurrent gram-negative bacteremia. There was no excess early mortality amongst those with gram-positive bacteremia. Overall, survival was best for those with an APACHE score less than 30 (9/9 vs 3/10, $p<0.01$). HA-1A was tolerated well, but the single-dose schedule may not be sufficient for neutropenic patients when given late after the onset of sepsis.

Late Abstracts

GRAFT-VERSUS-LEUKEMIA EFFECT IN ALLOGENEIC BONE MARROW TRANSPLANTATION OF RADIATION-INDUCED LEUKEMIA-BEARING MICE. Shiro Aizawa and Toshihiko Sado, Division of Physiology and Pathology, National Institute of Radiological Sciences, Chiba 260, Japan

Manifestation of graft-versus-leukemia (GVL) effect in MHC-compatible and -incompatible, allogeneic bone marrow transplantation (BMT) and the roles of T cell subsets contaminated in the donor bone marrow were studied using radiation-induced leukemia-bearing C3H mice maintained under specific-pathogen-free (SPF) condition. The results indicated that BMT from MHC-incompatible allogeneic (B10) donor significantly improved the survival of the treated mice as compared to that from syngeneic (C3H) donor. When donor (B10) bone marrow cells were treated with either anti-Thy 1.2 or anti-Lyt 2.2 antibody plus complement prior to BMT, a beneficial GVL effect was completely abolished. On the other hand, BMT from MHC-compatible allogeneic donors (B10.BR, CBA, AKR) failed to show an improvement in survival. However, intentional enhancement of GVH reaction by pre-immunization of B10.BR donor mice with relatively small number of C3H spleen cells or by an addition of B10.BR lymph node cells to the donor bone marrow resulted in a significant improvement in survival. The depletion of all T cells completely abrogated the GVL effect, while the depletion of either Lyt 2^+ or L3T4$^+$ T cells from donor (B10.BR) bone marrow resulted in only partial, if any, abrogation of GVL effect. The results indicate that GVL effect observed in leukemic mice treated with allogeneic BMT from MHC-compatible and -incompatible donors was totally dependent on T cells and suggest that the roles of T cell subsets in the induction of GVL effect were different between MHC-compatible and -incompatible, allogeneic BMT.

In further experiments, GVL effect was studied with other radiation-induced leukemia isolated from C3H mice in our laboratory. The results indicate that GVL effect can be observed in BMT from MHC-compatible allogeneic donor but the degree of GVL effect observed in MHC-compatible allogeneic BMT is variable among types of leukemia and strains of bone marrow donors.

ICAM-1: A CENTRAL MEDIATOR OF GvHD

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Microangiopathic changes are fundamental components of early complications after BMT. The underlying endothelial cell damage is suggested to be a result of HLA dependent donor T-cell stimulation and early activation of host macrophages. Cytokines such as IFNg and TNFa mediate both the specific and unspecific immune response. They induce the expression of histocompatibility antigens and adhesion molecules on a variety of cells thereby enhancing leucocyte cooperation and interaction with target tissue.

We demonstrate here, that in GvHD cytokine producer cells and cellular targets are localized in close neighbourhood. Skin biopsies taken from patients with acute dermal GvHD lesions showed significantly increased TNFa-mRNA and -protein as well as ICAM-1 expression compared to pretransplant specimens. The extent to which TNFa as well as ICAM-1 levels were increased varied markedly between biopsies of different patients although clinically they presented with similar disease. When chronic disease developed these increased levels declined to pretransplant levels.

Using a BMT-mouse-model we have shown that ICAM-1-mRNA upregulation may preceed overt GvHD. Detection of ICAM-1-mRNA might therefore serve as a predictive parameter of GvHD development. Time kinetics of ICAM-1-mRNA expression revealed a significant increase in organs of allogeneic compared to syngeneic mice as early as day two after BMT. ICAM-1-mRNA levels in allogeneic transplanted mice increased with time after BMT and correlated with development of symptoms. Immunohistological staining showed enhanced ICAM-1 expression in allogeneic mice only when symptoms of GvHD were apparent.

In summary our findings support the central role for adhesion molecules in the GvHD pathogenesis and suggest ICAM-1-mRNA as a predictive parameter.

Experiments are under way to test the protective value of anti-ICAM-1-antibodies in GvHD therapy and prevention.

DNA CROSSMATCHING FOR RAPID SELECTION OF HLA-DR IDENTICAL UNRELATED MARROW DONORS AND ITS APPLICATION IN DR-Dw TYPING. B A Bradley, T Clay, N Wood, and J Bidwell. UKTSSA, Bristol, UK.

PCR amplification products of HLA-DRB gene second exons show HLA-DR/Dw phenotype-specific patterns (PCR fingerprints) when electrophoresed on non-denaturing polyacrylamide gels (1,2). We recently applied this technique to selecting HLA-DR/Dw-matched marrow donors (3). PCR fingerprinting and its supplementary technique DNA crossmatching are more accurate, faster, and cheaper than DNA-RFLP typing (2,3). Fingerprinting, requires no expensive equipment, restriction enzymes, probes, radio-isotopes, or Southern blotting and results can be obtained in less than eight hours. In assessment of the technique (3) in 53 unrelated HLA-A and HLA-B matched patient-donor pairs, 42 pairs gave the same results with PCR fingerprinting as with DNA-RFLP analysis. In the eleven other pairs DR-Dw mismatches were detected with PCR fingerprinting but not by the standard DNA-RFLP method; PCR-SSO typing with selected sequence-specific oligonucleotides (SSO) confirmed that mismatches that passed unrecognised in DNA-RFLP were due to different subtypes of HLA-DR4. These methods have potential as tissue typing techniques. We demonstrate that crossmatching a test DNA with DNA from a range of homozygous typing cells (HTC) can reveal the HLA-DR type of the individual. HLA-DR phenotypes can be deduced both from the unique pattern of the PCR fingerprint and from DNA crossmatches with a range of HTCs. Incompatible HTC will give additional bands as a consequence of interaction with non-self DRB gene products whereas compatible HTC will give no bands over and above those seen in the test DNA PCR fingerprint. Thus the HLA-DR type may be deduced. These methods are being developed for typing unrelated donor-recipient pairs for marrow transplantation.

1. Bidwell JL and Hui K, 1990. 2. Wood N et al, 1991.
3. Clay T et al, 1991

HIGH DOSE BUSULFAN-CYCLOPHOSPHAMIDE WITH AUTOLOGOUS BONE MARROW OR BLOOD STEM CELL RESCUE FOR CHEMOSENSITIVE STAGE IV BREAST CANCER.

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Alkylating agents are among the most active agents in the treatment of breast cancer. Although the high dose alkylating agent combination Busulfan and Cyclophosphamide (BuCy) is routinely used to prepare patients with myeloid malignancies for bone marrow transplantation (BMT), the activity and toxicity of BuCy in the treatment of stage IV breast cancer has not been reported. In a Phase 2 pilot study, we administered Bu (16 mg/kg total dose, p.o.) and Cy (6 gm/M^2 total dose, i.v.) over eight days to 13 patients with measurable and chemosensitive stage IV breast cancer. Patients were rescued with either autologous unpurged bone marrow (n=10) or peripheral blood stem cells (n=3). Five patients received yeast-derived GM-CSF post transplant to accelerate marrow recovery. All patients showed evidence of engraftment by serial marrow biopsies. Absolute neutrophil count $\geq 500/mm^3$ recovered at 16.5±6 days (mean ± 1 S.D.) (range, 11-28 days). Platelets recovered to greater than $20,000/mm^3$ unsupported at 25.5±13 days (range, 9-47 days). There were two toxic deaths, one at day +57 (hepatic venocclusive disease) and another at day +19 (diffuse alveolar hemorrhage). Eleven other patients experienced only grade I or II toxicity and were discharged after an average hospital stay of 40 days (range 28-59 days). Ten patients were evaluable for response. Nine patients achieved a 50% or greater reduction of visceral metastases and/or stable bone disease defined as loss of bone pain and no progression on bone scans. Five patients relapsed between days 164 to 244 post transplant (mean 191 days). Four other patients remain in progression-free survival at 347, 304, 70 and 63 days post transplant. We conclude that BuCy has acceptable toxicity (15% toxic deaths) and activity (90% complete and partial remission rates). However, further follow-up is required to assess durability of response.

GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR and ERYTHROPOIETIN VS PLAACEBO IN AUTOLOGOUS BONE MARROW TRANSPLANTATION

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12 patients with lymphoma (7 Hodgkin's and 5 non-Hodgkin's) were enrolled and randomized to GM-CSF plus EPO (9 patients) or to GM-CSF and placebo (3 patients). rhGM-CSF was administered at 10mg/kg/d as a 4-6 hour IV infusion for up to 30 days. EPO was given intravenously at 150 units/kg/d for up to 30 days. The mean time for patients on the GM-CSF/EPO arm to reach an absolute neutrophil count of 500 cells/ul was 15.3 days (range 10-29) and 14 days (range 10-18) for patients on the GM-CSF/placebo arm. Patients receiving GM-CSF/EPO had a mean of 11 red blood cell transfusions (range 4-31 excluding a patient who developed hemorrhagic cystitis) while patients on the GM-CSF/placebo arm had a mean transfusion requirement of 8 units (range 6-12). All patients experienced fevers and were treated with broad spectrum antibiotics including Amphotericin B. One patient discontinued GM-CSF because of possible adverse effects. With a median follow-up of 8 months, two poor-risk patients (on GM-CSF/EPO arm) with Hodgkin's disease have relapsed. These preliminary data suggest that the combination of GM-CSF and EPO is well tolerated in patients undergoing autologous bone marrow transplantation. The number of patients is too small to conclude that the combination of the two growth factors leads to clinical benefit. Large scale clinical trials are now warranted.

HIGH-DOSE WEEKLY INTRAVENOUS IMMUNOGLOBULINS (IVIG) FOR THE PREVENTION OF INFECTION IN PATIENTS (PTS) UNDERGOING AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) AND INTENSE THERAPY FOR ACUTE LEUKEMIA: A STUDY OF THE NORTH AMERICAN BONE MARROW TRANSPLANT GROUP. Steven N. Wolff, Joseph W. Fay, John P. Greer, Randy A. Brown, Geoffrey P. Herzig and Roger H. Herzig. Vanderbilt University, Nashville, TN 37232, Baylor University Medical Center, Dallas, TX 75246, Washington University, St. Louis, MO 63110 and the University of Louisville, Louisville, KY 40292.

IVIG can prevent bacterial and fungal infections in pts with hypogammaglobulinemia and possibly in pts undergoing allogeneic bone marrow transplantation. Based on these observations, we designed a prospectively randomized study to evaluate whether weekly IVIG could prevent infections in pts with severe myelosuppression. Eligible pts could not have an active infection and were to undergo ABMT or intense therapy for acute leukemia. Pts were randomized to receive IVIG or serve as control. IVIG was begun prior to beginning cytotoxic therapy and administered once weekly at a dose of 500 mg/kg until recovery of neutrophils to >500/ul. The study was designed to detect a 20% reduction in infection with a power of 80%. The study end-points are proven infections, bacteremia and fungemia. Since 1/90, 165 patients have entered the trial. The IVIG group and the control group are comparable for diagnosis, treatment, age, base-line IgG level, use of prophylactic antibiotics and duration of neutropenia. The median (range) of serum IgG prior to treatment was 921 mg/dl (336-1848) for the IVIG group and 900 mg/dl (402-1995) for the control group. 75% of all patients entered underwent ABMT. All patients entered will be evaluable. Both the IVIG group and the control group had a similar ($p > .05$) incidence of proven infection (45% vs. 46%) and bacteremia (35% vs. 35%). Gram-negative and gram-positive bacteremia occurred in 12% and 23% of each group. Fungemia occurred in 7% of the IVIG group and 9% of the control group ($p > .05$). Death from infection occurred in 5% of the IVIG group and 2% of the control group ($p > .05$). This completed study demonstrates no benefit for the prevention of severe infection with the use of high-dose weekly IVIG in pts with severe myelosuppression.