PR1 is also processed and presented by NE, which may result in enhanced immunogenicity of the peptide compared to peptides derived from a single protein. Redundancy of proteins may also lessen the impact of tumor-loss variants after PR1-based immunotherapy.

93

TREATMENT OF DONORS WITH INTERLEUKIN-18 REDUCES ACUTE GRAFT-VERSUS-HOST DISEASE VIA STAT6 AND PRESERVES CD8+ MEDIATED GRAFT-VERSUS-LEUKEMIA EFFECTS

Reddy, P.[1]; Teshima, T.[1]; Hildebrandt, G.[1]; Williams, D.[1]; Liu, C.[2]; Cooke, K.[1]; Ferrara, J.L.[1] 1. Internal Medicine, University of Michigan Medical Center, Ann Arbor, MI; 2. University of Florida, Gainesville, FL.

Interleukin 18 modulates both Th1/Th2 responses and stimulates hematopoietic growth factor secretion. We investigated the effect of pretreating bone marrow transplant (BMT) donors with IL-18 on the severity of acute GVHD using a well-characterized irradiated experimental BMT model (BALB/c(H-2^d→ B6 (H-2^b). Pretreatment of allogeneic BMT donors with IL-18 (1μg/day of rmIL-18 for 10 days, day -11 to -1) significantly improved survival, and caused significant reduction in serum levels of TNF-α(212 ±18pg/ml vs 524 ±69pg/ml, P<0.05)and LPS (5.3 ±1.1U/ml vs 12.4 ±1.3U/ml, P<0.05) and GVHD related intestinal histopathology (P<0.05). All surviving mice showed complete donor engraftment, thus ruling out graft rejection and mixed chimerism as causes for decreased GVHD. IL-18 pretreatment was also associated with reduced IFN-γ (1987 ±387pg/ml vs 4686 ±964pg/ml, P<0.03) and greater IL-4 secretion (54.8 ±9.3pg/ml vs 14.5 ±3.4pg/ml, P<0.04) by donor T cells after BMT. Surprisingly, when IFN-γdeficient mice were pretreated with rmIL-18 and used as donors acute GVHD mortality was reduced thus ruling out the role of donor IFN-γin this effect. Acute GVHD mortality was also reduced when IL-18 was administered to donors deficient in STAT4. Treatment of STAT6 deficient donors with IL-18 did not alter IFN-γ secretion (14.3 ±2.1 ng/ml vs 10.2 ±3.8ng/ml, P=NS) or enhance IL-4 production. Furthermore pretreatment of STAT6-/- donors with IL-18 did not reduce acute GVHD mortality thus confirming the role of STAT6 signaling in IL-18's protective effect. We next investigated whether IL-18 can maintain a graft-versus-leukemia (GVL) effect using the same model and by injecting recipients with host-type (H-2^b) EL-4 MHC II-/-lymphoma cells at the time of BMT. IL-18 treatment did not alter donor $CD8^+$ cytotoxic T lymphocyte (CTL) activity and preserved graft versus leukemia (GVL) effects after allogeneic BMT (70% v 10%, P < 0.01). Our results demonstrate that pretreatment of donors with IL-18 preserves the $CD8^+$ mediated GVL effects but reduces acute GVHD in a donor derived IFN-γ independent but in a STAT6 dependent mechanism.

94

TACROLIMUS (FK-506) AND MYCOPHENOLATE MOFETIL (MMF) GVHD PROPHYLAXIS IN ALLOGENEIC SCT (ALLOSCT) RECIPIENTS: ALTERED MMF PHARMACOKINETICS (PK) ASSOCIATED WITH AGVHD

Bessmertny, O.; Osunkwo, I.; Harrison, L.; Wolownik, K.; Wischhover, C.; Garvin, J.; George, D.; Del Toro, G.; Bradley, M.; Cairo, M.S. Children's Hospital of New York-Presbyterian, Columbia University, New York, NY.

FK-506/MMF is an effective salvage therapy for steroid-refractory chronic (C) GVHD (Mookerjee et al, BMT 1999; 24:517-20) and has been used for prophylaxis of acute (A) and C GVHD following nonmyeloablative conditioning in adults (Rini BI et al, JCO 2002; 20:2017-20). We investigated safety and efficacy of FK-506/MMF regimen for GVHD prophylaxis to spare the use of methotrexate (MTX) and steroids in 29 pts (mean age 8.5 [range 0.5-22 years]; 21 M, 8 F) undergoing 32 AlloSCT for hematologic malignancies (n=20), non-malignant disorders (n=7), and neuroblastoma (n=2). GVHD prophylaxis included FK-506 0.03 mg/kg/day continuous IV on Day -1 or 1st day of conditioning plus MMF 15 mg/kg/dose PO/IV BID starting Day +1. Doses of FK-506 and MMF were adjusted to maintain concentrations within reference ranges of 5-20 ng/mL for FK-506 and 1-3.5 mcg/mL for mycophenoleic acid (MPA). HLA typing was serologic for A and B and high resolution DRB1. Stem cell sources were 6/6 UCB (n=4), 5/6 UCB class I mismatch (n=4), 4/6 UCB (mismatch: class I [n=8], class II [n=1], double class I/II [n=3]), 6/6 related (R) BM (n=3), 6/6 RPBSCT (n=8), 5/6 RPBSCT class I mismatch (n=1). Among 28 evaluable pts, mean days to ANC ≥500/mm^3 x 2 days was 14 (11-17) and 27 (8-79) following related RPBSCT/BM and UCB, respectively. Fifteen pts (53.6%) developed ≥grade II AGVHD: 7 RBM/PBSC and 8 UCB. The incidence of grade III/IV AGVHD was 32% (5 RBM/PBSC and 4 UCB) and 6% for CGVHD (n=2). FK-506/MMF was well tolerated, with 2 episodes of grade III-IV neurotoxicity (disorientation and leukoencephalopathy). MPA trough concentrations were monitored in the second half of the study (n=12). Eight pts developed AGVHD and 7 had MPA below target range (<1 mcg/ml) at GVHD onset. MMF doses ≥900 mg/m^2 IV/PO q6h (4 times standard dose) were required to achieve target MPA trough concentrations in the early AlloSCT period (< Day +30). Five of 12 pts monitored achieved target MPA levels before Day +30 and the incidence of ≥Grade III AGVHD was 20%. The estimated 1-year OS is 52.1%. These results suggest that FK506/MMF is a safe and effective MTX and steroid-sparing GVHD prophylaxis regimen following R and UCB AlloSCT. Further pharmacokinetic and pharmacodynamic studies are ongoing in AlloSCT recipients to define the optimal dosing regimen of MMF.

95

CD83 ANTIGEN: A POTENTIAL NEW TARGET FOR GRAFT VERSUS HOST DISEASE PREVENTION

Munster, D.J.; Kato, M.; MacDonald, K.P.; Rice, A.M.; Hart, D.N. Cancer Biotherapy, Mater Medical Research Institute, South Brisbane, QLD, Australia.

Novel strategies are required to overcome the limitations imposed on hematopoietic stem cell transplantation by graft versus host disease (GVHD). Current GVHD therapies focus on elimination or attenuation of the effector T cell. There is emerging evidence in the literature to suggest a primary role for dendritic cells (DC) in the initiation of GVHD. CD83 is a marker of DC differentiation/activation. Polyclonal anti-CD83 antibody (RA83) inhibits the T lymphocyte proliferative response in the mixed leukocyte reaction (MLR) stimulated by allogeneic monocyte derived DC (MoDC). We investigated the mechanism of this RA83 mediated immunosuppression and found that it was not due to functional blockade of CD83. It required the presence of NK-cells, Fab fragments of RA83 did not inhibit the MLR, and treatment with an Fcγ receptor III (CD16) blocking monoclonal antibody (3G8) prevented the blockade, indicating that it was due to antibody dependent cellular cytotoxicity (ADCC) of $CD83^+$ cells. In additional experiments we showed that activated NK-cells lyse ^{51}-Cr labelled $CD83^+$ MoDC in the presence of RA83, but not in the presence of non-immune polyclonal antibody. Immature $CD83^-$ MoDC were not lysed in the presence of RA83. However, lysis of MoDC did not account for all the effect because stimulation with allogeneic MoDC fixed with paraformaldehyde also exhibited inhibition of T lymphocyte proliferation in the presence of RA83. This implied that $CD83^+$ targets of ADCC must exist or arise in the responder cell preparation. We then established that activated blasting T-cells ($CD25^+$, $Ki67^+$) stimulated by allogeneic MoDC also express CD83 and that they become targets of NK-cell mediated ADCC. Thus, CD83 is expressed by both stimulator and responder cells in the MLR. Simultaneous targeting of both activated cell populations by a single reagent such as anti-CD83 may be a potent means of achieving therapeutic immunosuppression for allogeneic hematopoietic stem cell transplantation.

96

IMPROVED OUTCOME IN ACUTE LETHAL MURINE GRAFT VERSUS HOST DISEASE (GVHD) FOLLOWING ADMINISTRATION OF THE PROTEASOME INHIBITOR PS-341

Welniak, L.A.[1]; Sun, K.[2]; Sayers, T.J.[2]; Murphy, W.J.[1] 1. Microbiology, University of Nevada, Reno, Reno, NV; 2. SAIC-Frederick, Frederick, MD.

Proteasome inhibitors have recently shown much promise by selectively acting on various neoplastic cells, particularly myelomas. We have recently found that the use of proteasome inhibition with PS-341 sensitizes neoplastic cells to TRAIL mediated

killing and can be used to purge leukemic cells from hematopoietic stem cell (HSC) products in an experimental murine transplantation model. We now wish to extend our studies to examine the use of PS-341 administration post-HSC transplant. We hypothesized that PS-341 would reduce GVHD associated mortality by preventing degradation of IkB leading to decreased NF-kB DNA binding activity and NF-kB dependent cytokine production. We tested the efficacy of PS-341 administration in a full MHC mismatched murine model of acute lethal GVHD. C57BL/6 bone marrow and splenocytes were transplanted into lethally irradiated BALB/c hosts. PS-341 (1mg/kg) was administered on the day of transplant and also at later time points in some groups. Under experimental conditions where animals that receive vehicle control succumb to acute GVHD within 12 days of transplant, mice that received PS-341 on the day of transplant are protected from lethal disease (log rank test p< 0.0001). However, administration of PS-341 during the first week post-transplant resulted in markedly accelerated mortality due to GVHD. These studies demonstrate that the proteasome inhibitor PS-341 can be given concurrent with allogeneic HSC transplantation and can reduce early lethal acute GVHD although timing of the administration is critical.

97

TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA (CML) RELAPSING AFTER MYELOABLATIVE STEM CELL TRANSPLANTATION WITH STI-571 (IMATINIB MESYLATE) WITH OR WITHOUT DONOR LYMPHOCYTE INFUSION (DLI)

Barrett, J.A.; Battiwala, M.; Solomon, S.; Childs, R.; Hensel, N.; Wehrlen, L.; Kurlander, R. Stem Cell Allogeneic Transplantation Section, Hematology Branch, NHLBI, National Institutes of Health, DHHS, Bethesda, MD.

CML relapsing after allogeneic stem cell transplantation (SCT) can be successfully retreated with DLI. However DLI can cause GVHD and is less effective for hematological relapse. We therefore used STI-571 to treat patients with CML relapsing after SCT following myelobalative preparative regimens. Ten patients (9 who had not received STI-571 pretransplant) relapsed 4-36 months post transplant. Five were molecular relapses, five were more advanced (karyotypic =1, chronic phase (CP) = 2, blast crisis (BC) =2). STI-571 300-600 mg/day was given to all patients who had a minimum of two positive RT-PCR assays *2 months apart, or who developed hematological relapse. Prior to treatment, immunosuppression was stopped on all patients (n=9) without active GVHD. STI as a single agent was given to 5 molecular relapses. Two had failed prior DLI. Four patients promptly became PCR negative within 6 weeks. However three had a molecular relapse after STI was stopped and are currently retreated. STI-571 combined with 1-5 x107/kg CD3+ DLI was given to 5 patients relapsing with more advanced CML. Three (CP or karyotypic relapse) achieved complete molecular remissions, sustained in two. One myeloid BC patient developed extensive chronic GVHD after DLI given day 100 to treat persisting molecular disease, STI was stopped because of thrombocytopenia, he progressed with chloromas by 5 months post-transplant but has achieved a stable molecular remission persisting >6 months after restarting STI 600mg/day. One patient relapsing in ALL BC achieved a hematological remission but relapsed again 3 months later and died of disease progression.Thus, of 10 STI-treated patients, 7 achieved molecular remission which was sustained in 4. These results suggest that the cytoreductive action of STI can synergize with the graft-versus-leukemia effect. However, the tendency for further relapse when STI is withdrawn suggests that cure of relapsed CML may also require a GVL effect.

98

INFUSION OF HIGH NUMBERS OF G-CSF MOBILIZED BLOOD DENDRITIC CELLS TYPE 2 (DC-2) IS ASSOCIATED WITH AN INCREASED RATE OF CHRONIC GVHD IN ALLOGENEIC PBSC TRANSPLANTATION

Rondelli, D.[2]; Arpinati, M.[1]; Chirumbolo, G.[1]; Urbini, B.[1]; Bonifazi, F.[1]; Stanzani, M.[1]; Falcioni, S.[1]; Bandini, G.[1]; Baccarani, M.[1] 1. University of Illinois at Chicago, Stem Cell Transplant Program, Chicago, IL; 2. University of Bologna, Bologna, Italy.

It was previously shown that dendritic cells type-2 (DC-2) are significantly increased in G-CSF-mobilized leukapheresis products as compared to unstimulated bone marrow. In this study, we analysed whether the numbers of DC-1 and DC-2, as well as of other cell components in the graft, was associated with acute and/or chronic GVHD in 31 adult patients (11 MM, 7 CML-CP, 6 AML, 5 NHL, and 2 MDS) receiving an allogeneic PBSC transplant from HLA-matched siblings. Average cell doses (x106/kg) in the grafts were the following: 283±137 CD3+ T cells, 160±88 CD4+ T lymphocytes, 116±55 CD8+ T lymphocytes, 64±39 CD19+ B lymphocytes, 51±29 CD56+ NK cells, 253±103 CD14+ monocytes, 6.6±4.1 CD34+ cells, 2.1±0.9 HLA-DR+lin-CD11c+ DC-1 and 2.9±1.3 HLA-DR+lin-CD123+ DC-2. Median follow up was 255 days (range: 50-685). Patients were initially divided in three groups according to whether they had shown no signs of acute GVHD (grade 0, n=10), acute GVHD grade I (n=12), or grade II - IV (n=9). Median numbers of CD34+ cells, lymphocyte subsets, and DC-1 and DC-2 received by patients in these three groups did not differ significantly. Of 21 patients with adequate follow up (median 485 days, range: 131-695) 12 developed chronic GVHD (10 extensive, 2 limited). Analysis of cell components of the grafts demonstrated that patients developing chronic GVHD had received a significantly higher dose of DC-2 than patients without chronic GVHD (3.3±1.5 vs 2.2±0.9, p=0.05), while the dose of DC-1 (p=0.7), monocytes (p=0.28), T lymphocytes (p=0.643), B lymphocytes (p=0.939), NK cells (p=0.487) and CD34+ cells (p=0.757) was not different. Also, chronic GVHD did not correlate with recipient or donor age or gender, interval between diagnosis and transplant, presence of ATG or TBI in the conditioning regimen, or type of GVHD prophylaxis. Our results suggest that the presence of large DC-2 numbers in the graft may be associated with a higher risk of chronic GVHD after allogeneic PBSC transplantation. These data might prompt further studies addressing whether depletion of graft DC-2 might be beneficial in this setting.

99

SOLID TUMOR VACCINES ELICIT DISTINCT IMMUNE RESPONSES FROM HOST VERSUS DONOR T CELLS IN MIXED CHIMERAS CREATED BY NON-MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION (NST)

Durakovic, N.[1]; Slansky, J.E.[2]; Pardoll, D.M.[1]; Fuchs, E.J.[1]; Luznik, L.[1] 1. The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD; 2. Dept. of Immunology, Univ. of Colorado Health Sciences Center, Denver, CO.

A growing body of evidence suggests that functional unresponsiveness of the immune system to growing solid tumors may be due to tolerance in tumor-specific $CD4^+$ T cells, whereas tumor-specific $CD8^+$ T cells exist in a state of partial activation with limited effector function. We therefore hypothesized that an allogeneic graft-versus-host reaction may provide a helper effect that augments the anti-tumor immune response of host $CD8^+$ T cells in mixed hematopoietic chimeras. To test this hypothesis, we analyzed host versus donor T cell responses to AH1, an H-$2L^d$-restricted tumor antigen expressed by CT-26, a colon cancer of BALB/c mice. BALB/c mice with pre-established subcutaneous tumor underwent NST from MHC-compatible B10.D2 donors. Two weeks later, groups of chimeras received nothing, B10.D2 splenocytes IV, CT-26 tumor vaccine (irradiated CT-26 cells mixed with a GM-CSF secreting bystander cell line), or both. By staining with H-$2L^d$ tetramers loaded with the AH1 423-431 a.a. peptide, AH1-specific $CD8^+$ T cells were found to comprise 2-3% of total spleen $CD8^+$ T cells three weeks after vaccination. In contrast, AH1-specific T cells could not be detected in the non-transplanted, tumor bearing mice. To further characterize AH1-specific T cells we characterized T cell avidity for antigen, as measured by binding of AH1 peptide-loaded, L^d IgG dimmers. AH1 specific T cells were easily expanded in vitro using AH-1 peptide pulsed irradiated BALB/c splenocytes. After four weeks of stimulation the highest percentages of cultures containing AH1-specific T cells were derived from tumor-bearing chimeras that received B10.D2 splenocytes and a CT-26 vaccine. Expanded cultures contained AH-1 specific $CD8^+$ T cells derived from the host and the donor, based on the differential expression of Ly9.1 marker on the