highly DC2 selective. Selective clones were DNA sequenced revealing the random peptide insert. A 1-way MLR was performed with varying dilution of each amplified DC2 specific phage to demonstrate the cellular affects on allogeneic T-cell proliferation. **Results:** Plaque assays from the monocyte adsorbed or non-adsorbed linear random peptide library after the three rounds of panning revealed two consensus sequences in 76 of the 78 (97%) isolated clones that were DC2 selective and one sequence found twice (3%) that was non-DC2 selective. The employed circular random peptide library revealed no DC2 selective sequences from 15 isolated in clones from the monocyte adsorbed and non-adsorbed plaque assays. Preliminary MLR data shows a 35 % reduction in allogeneic T-cell proliferation with the DC2 specific phage compared to control phage. **Conclusions:** Data shows that phage display technology can result in isolating highly specific DC2 peptides from a library of 10,000 different phage clones. Preliminary data suggests that binding DC2 specific peptides may inhibit the function of these immunoregulatory cells, leading to enhanced anti-tumor affect of the transplanted donor graft product by shifting it towards an activated Th1 immune response.

GVH/GVL

28

TREATMENT WITH GRANULOCYTE COLONY-STIMULATING FACTOR AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION FOR ACUTE LEUKEMIA INCREASES THE RISK OF GRAFT-VERSUS-HOST DISEASE AND DEATH

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Purpose: Granulocyte colony-stimulating factor (G-CSF) is given after bone marrow transplantation (BMT) to shorten the neutropenic phase. Its effects have not been evaluated in a large patient population. **Patients and Methods:** We studied 1789 patients with acute leukaemia receiving BMT and 434 patients receiving peripheral blood stem cells (PBSC) from HLA-identical siblings from 1992 to 2002 and reported the findings to the European Group for Blood and Marrow Transplantation (EBMT). Among the BMT and PBSC patients, 501 (28%) and 175 (40%), respectively, were treated with G-CSF during the first 14 days after the transplant. The outcome variables were entered in a Cox proportional hazard model. **Results:** BMT and PBSC patients treated with G-CSF had a faster engraftment of absolute neutrophils $>0.5 \times 10^9$/l ($p < 0.01$), but platelet engraftment ($>50 \times 10^9$/l) was slower ($p < 0.001$). In the BMT patients, acute graft-versus-host disease (GVHD) grades II-IV was 50 ± 5% (±95% confidence interval) in the G-CSF group vs. 39 ± 3% in the controls (relative risk (RR) 1.33, p = 0.007, in the multivariate analysis). The incidence of chronic GVHD was also increased (RR 0.29, p = 0.03).

G-CSF was associated with an increase in transplant-related mortality (TRM) (RR 1.73, p = 0.00016), had no effect on relapse, but reduced the survival (RR 1.7, $p < 0.0001$) and leukaemia-free survival rates (LFS) (RR 1.55, p = 0.0003). No such effects of G-CSF were seen in patients receiving PBSC. **Conclusion:** After BMT, platelet engraftment was delayed, and GVHD and TRM were increased. Survival and LFS were reduced. This suggests that G-CSF should not be given shortly after BMT.

29

SUBEROYLANILIDE HYDROXAMIC ACID REDUCES ACUTE GRAFT-VERSUS-HOST DISEASE AND PRESERVES GRAFT-VERSUS-LEUKEMIA EFFECT BY INHIBITING HISTONE DEACETYLATION

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Pro-inflammatory cytokines and the loss of gastrointestinal tract integrity contribute to acute graft-versus-host disease (GVHD) whereas the donor cytotoxic responses are critical for graft-versus-leukemia (GVL) preservation. Suberoylanilide hydroxamic acid (SAHA) is an anti-tumor agent that inhibits the activity of histone deacetylases (HDAC) and reduces the production of proinflammatory cytokines. Using a well characterized allogeneic murine BMT model B6 (H-2^b) → B6D2F1 (H-$2^{b/d}$) we studied the effects of HDAC inhibition by SAHA on acute GVHD. Recipients were transplanted with $2x10^6$ donor T and $5x10^6$ BM cells after13 GY TBI. Intra-peritoneal injections of 35 mg/kg/day of SAHA from days +3 to day +7 increased histone H3 acetylation in splenocytes harvested 7 days after BMT, confirming the inhibition of HDAC enzymes. SAHA treatment significantly reduced the serum levels of pro-inflammatory cytokines such as TNF-a, IL-1β and IFN-γ ($P < 0.04$) in the allogeneic recipients on day +7 after BMT. Intracytoplasmic staining by flow cytometry and RPA analysis of the host splenocytes on day +7 confirmed the decrease in the cytokine protein and mRNA. SAHA significantly improved the survival ($P < 0.002$) and reduced intestinal damage from GVHD of the allogeneic recipients . However SAHA did not suppress the donor T cell expansion in vivo and the proliferative and cytotoxic responses to host antigens in vitro measured 7 and 14 days after BMT. To test the effect of SAHA on GVL effects, recipients were injected with lethal doses of P815 (H-2^d) tumor cells at the time of BMT. SAHA treatment resulted in significantly improved leukemia-free survival after allogeneic BMT ($P < 0.05$) whereas all the syngeneic BMT recipients of SAHA died of tumor ruling out direct anti-tumor effects of SAHA. Furthermore SAHA increased H3 acetylation in the splenocytes from both the syngeneic and allogeneic leukemic recipients on day +7, confirming that inhibition of HDAC enzymes alone is not sufficient for leukemia free survival in this system. We also tested the effect of SAHA in a second allogeneic BMT model (BALB/c → B6), where it also significantly improved survival ($P < 0.001$) and preserved GVL effects when recipient mice were injected with lethal doses of EL-4 (H-2^b) tumor ($P < 0.04$). We conclude that HDAC inhibition regulates acute GVHD in these models and suggest that this class of pharmacologic agents may provide a novel strategy to reduce GVHD while maintaining the beneficial GVL effects.

30

ABROGATION OF THE INTERACTIONS BETWEEN CXCR3 AND ITS LIGANDS MIG AND IP-10 REDUCES THE SEVERITY OF IDIOPATHIC PNEUMONIA SYNDROME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Chemokines are important mediators in the development of Idiopathic Pneumonia Syndrome (IPS), a major cause of mortality after allogeneic (allo) stem cell transplantation (SCT). We hypothesized, that recruitment of donor T cells to the lung is dependent, at least in part, upon interactions between the chemokines MIG and IP-10 and their receptor CXCR3. CXCR3 is expressed on activated T cells; MIG and IP-10 can be induced in various cell types by IFNγ alone or in combination with TNFa or IL-1ß. We tested this hypothesis using an established murine SCT model wherein lethally irradiated bm1 mice receive SCT from either syngeneic (bm1) or allogeneic (B6Ly5.2) donors. MIG and IP-10 BAL levels were significantly elevated in allo recipients compared to syn controls at weeks 1 (MIG: 162.8 ± 37.6 vs 0; IP-10: 41.1 ± 4.2 vs 0 pg/ml) and 4 (MIG: 153.5 ± 41.7 vs 21.6 ± 9.0; IP-10: 202.0 ± 61.1 vs 3.8 ± 0.9 pg/ml) and correlated with the infiltra-