16 DONOR PRETREATMENT WITH PROGENIPOIETIN-I PREVENTS ACUTE GRAFT-VERSUS-HOST DISEASE IN AN IL-10 DEPENDENT FASHION AND AUGMENTS THE GRAFT-VERSUS-LEUKAEMIA EFFECT

We have studied the ability of stem cell grafts mobilised with the chimeric G-CSF and FLT-3 receptor agonist Progenipoietin-1 (ProGP-1) to separate graft-versus-host disease (GVHD) and graft-versus-leukaemia (GVL) in the well described B6 → B6D2F1 stem cell transplant (SCT) model. ProGP-1, G-CSF or control diluent was administered to donor B6 mice. ProGP-1 expanded all cell lineages in the spleen and blood with dramatic expansion of dendritic cells (DC) and granulocyte-monocyte lineages (2-log increase). Transplant survival was 0%, 50% and 90% in recipients of control, G-CSF and ProGP-1 treated allogeneic donor splenocytes respectively (P<0.0001). Donor pretreatment with ProGP-1 allowed a 2- to 4-fold escalation in T cell dose over that possible with G-CSF. Donor CD4 T cells from allogeneic SCT recipients of ProGP-1 splenocytes demonstrated an anergic response to host antigen (proliferation and cytokine) while CD8 T cell cytotoxicity to host antigens remained intact. Neither CD11c(+) DC nor "plasmacytoid" CD11c(+)B220(+) DC from ProGP-1 treated donor animals conferred protection from GVHD. Conversely, when equal numbers of purified T cells from control, G-CSF or ProGP-1 treated allogeneic donors were added to allogeneic T-cell depleted control spleen, survival at day 60 was 0%, 15% and 90% respectively (P<0.0001). The improved survival in recipients of ProGP-1 T cells was associated with reductions in systemic TNFα generation (Control v ProGP-1: 227±22 vs 35±14 pg/ml, P<0.01) and GVHD of the GI tract (semi-quantitative GVHD score: 19.5±2.1 v 9.5±0.4, P<0.01). Spleenocytes from ProGP-1, but not control G-CSF treated animals produced large amounts of IL-10 to CpG. Furthermore, ProGP-1 expanded IL-10(-) spleenocytes failed to prevent GVHD relative to wild type (survival: 0% vs 67%, P<0.001). In GVL experiments shown below, host type B6 was added to grafts on day 0 and survival was monitored for 70 days after SCT. Recipients of ProGP-1 spleenocytes containing either low or high T cell doses relative to G-CSF had improved overall survival due to reduced GVHD mortality and leukaemia relapse (P<0.05 v G-CSF). These data confirm that donor pretreatment with ProGP-1 prevents GVHD in an IL-10 and T cell dependent fashion and is superior to G-CSF in separating GVHD and GVL after allogeneic SCT.

17 APPLICATION OF IMMUNE-THERAPEUTIC INTERVENTIONS AFTER ALLOGENIC STEM-CELL TRANSPLANTATION BASED ON MRD DETECTION WITH COMBINED MORPHOLOGICAL AND CYTOGENETIC ANALYSIS

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Recurrent disease remains a major obstacle to cure after allo- genetic transplantation. Detection of MRD allows timely application of immune-therapeutic interventions such as early withdrawal of immunosuppression or DLI to those destined to relapse. We used a novel system (Duet-TM, BioView), which provides combined morphological and cytogenetic analysis on the same cells (Leukemia 2000; 16:1413). Large numbers of cells are automatically scanned and their coordinates saved. MGG staining is removed and FISH is applied to the same slice. Small recipient-derived populations are targeted and their images relocated to identify their morphology. Patients (pts) are informative for Duet analysis if they have cytogenetic abnormalities or sex-mismatched donors. Thirty-five serial tests were performed in 31 leukemia pts. Results were retrospectively correlated with outcomes. Four pts were counted twice due to different results at different time-points. Duet detected minute recipient type populations (<5%) in 28 tests. In ten tests recipient cells were mostly blasts and in 18 they were mature hematopoietic cells (MHC). Seven of the ten pts with blasts relapsed, 3 had TRM before clinical relapse could be documented. Among the 18 pts with MHC morphology, one had early TRM and none of the others relapsed (P=0.002). Duet had increased sensitivity in detection of host population when compared with standard FISH. Duet specificity in predicting relapse was also superior to FISH. Seventy percent of those with recipient type blasts relapsed compared to 25% of those with any recipient population as detected by FISH with no specified morphology (P=0.05). We started applying this system prospectively for clinical decision-making. In two pts minute recipient blast-population (<1%) was detected 1-3 months post-transplant. Immune-suppression was rapidly tapered off. One pt developed GVHD 2-weeks later, when she had 15% blasts, and spontaneously re-entered sustained remission. The other pt progressed despite further interventions. Identification of residual recipient-type cells as blasts predicts imminent relapse and these patients need additional therapy.

HISTOCOMPATIBILITY/ALTERNATIVE STEM CELL SOURCES

18 INTERLEUKIN-10 AND TUMOUR NECROSIS FACTOR ALPHA HAPLO-TYPES PREDICT TRANSPLANT RELATED MORTALITY AFTER UNRELATED DONOR STEM CELL TRANSPLANTS

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There is evidence that serum levels of tumour necrosis factor alpha (TNF-α) and IL-10 influence the probability of transplant related mortality (TRM) and acute graft versus host disease after HLA identical sibling stem cell transplantation (SCT). Certain genetic polymorphisms of these cytokines may also correlate with outcome of HLA matched unrelated donor SCT. We have stud-