oligonucleotide arrays. Applying both an unsupervised hierarchical clustering analysis and a supervised Bayesian ANOVA analysis, 300 genes from these analyses were classified as being either membrane bound or soluble gene products based on the database of membrane-associated/soluble gene products generated by Diehn et al. (PLoS Genetics 2006). Thirty-two of these were annotated to be secreted gene products. Several novel growth factors not typically associated with hematopoiesis were identified. We have found that one of these factors, adrenomedullin, a vasodilator not known to have hematopoietic function, induces the proliferation of human BM CD34+CD38-lin-HSCs in single cell cultures and promotes the quantitative expansion of human BM CD34+ progenitor cells in vitro. This molecular signature of HUBECS provides a unique resource for the discovery and characterization of novel soluble factors that regulate human HSC fate.

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FLT3 EXPRESSION DISCRIMINATES HSC SUBPOPULATIONS WITH DIFFERENT ENGRAFTMENT-POTENTIAL

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HSC, sorted as KSL (c-Kit+ Sca-1+ Lin-) cells, are comprised of primitive long-term repopulating cells and short-term and repopulating committed progenitors. Growth factors such as GM-CSF, G-CSF or Flt3-ligand (FL) are utilized to expand and mobilize HSC. We evaluated here the phenotype and function of FL-mobilized HSC. FL significantly expands c-Kithi and c-Kitlo KSL cells in peripheral blood (PB). Only FL-expanded PB c-Kit+ KSL cells exhibited high spleen colony-forming unit frequency, generated high numbers of both lymphoid and myeloid colonies in vitro, and rescued ablated recipients. FL expanded two subsets of c-Kit+ KSL cells: CD34+Flt3− reflective of short-term HSC (ST-HSC) and CD34+Flt3+ long-term HSC (LT-HSC), while the proportion of c-Kit+ CD34+ Flt3+ KSL cells multipotent progenitors (MPP) was significantly decreased in the PB. When 500 CD34+ Flt3−, CD34+ Flt3+ or CD34+ Flt3− c-Kit+ KSL cells were transplanted into ablated syngeneic recipients, all recipients of CD34+ Flt3− c-Kit+ KSL expired within 16 days (n = 5). One of the 5 recipients of c-Kit+ CD34+ Flt3+ KSL (ST-HSC) survived over 120 days. The combination of 50 c-Kit+ CD34+ Flt3− KSL cells (LT-HSC) with 500 c-Kit+ CD34+ Flt3+ or CD34+ Flt3+ KSL cells enhanced recipient survival 4-fold compared to both ST-HSC plus LT-HSC surviving over 120 days and some recipients of MPP + LT-HSC survived up to 68 days. These data suggest that Flt3 expression may be a useful phenotypic marker for selecting critical stem cell populations to ensure rapid and durable engraftment and confirms that both short and long-term repopulating cells are needed for optimal successful transplantation.

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ABILITY OF ANTI C-KIT TARGETING MONOCLONAL ANTIBODY ACK-2 TO TARGET HEMATOPOIETIC STEM CELLS, AND FACILITATE ENGRAFTMENT OF HUMAN CD34+ ENGRAFTMENT AND HEMATOLYMPHOMID DEVELOPMENT IN IMMUNODEFICIENT MICE: A NOVEL ANTIBODY BASED CONDITIONING STRATEGY

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INTRODUCTION: The stem cell receptor factor receptor, c-Kit, is expressed on hematopoietic stem (HSC) and progenitor populations. We also sought to test the hypothesis that treatment with an HSC targeting mAb may serve as an novel antibody based conditioning strategy as well as a means for enhancing human HSC engraftment in immunodeficient mice by selectively inhibiting murine c-Kit.

METHODS: Adult C57BL/6 mice were injected with anti-1-500ug of ACK2 mAb & peripheral blood counts, as well as marrow HSC and progenitor frequency were followed. Subsequently, we examined the ability of ACK2 to enhance human engraftment in sublethally irradiated (4Gy) immunodeficient RAG2/Null Common Gamma-Chain double knock-out (RAG2−/DKO) mice injected intraperipherally with 1×10^5 CD34+ cells from human mobilized peripheral blood (hCD34+).

RESULTS: Administration of ACK2 resulted in rapid development of anemia, neutropenia & thrombocytopenia. In treated mice, the mean number & bone marrow fraction of Long-Term HSC decreased markedly from 0.07% of total marrow cells in controls to 0.03% in ACK2 treated mice as did the marrow number/frequency of CMP, GMP, and CMP. Human CD34+ transplanted RAG2−/DKO mice treated with ACK2 engrafted at a higher rate (46% vs 27% of controls achieved >10% human chimerism) with higher human CD45 + chimerism in bone, bone marrow, spleen, liver, & lymph nodes. Robust human hematopoiesis was observed with a mean of 63% of cells within the thymus human derived. We found differentiation of mature human T-cells occurred via a CD3+4+8+ intermediate pathway identical to that which we found in human stroma in SCID-hu-T-cell development. The ability for host ACK2 conditioning alone to enable donor murine HSC engraftment will be presented.

CONCLUSIONS: Anti c-kit mAb treatment rapidly induces panmyelopenia with correlations in the numbers of marrow HSC and other progenitor populations and may serve as a strategy for host HSC targeted mAb based conditioning. ACK-2 enhances the frequency and degree of human CD34+ engraftment and human hematopoiesis and may serve as a means to enhance this new model of human hematopoiesis.

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MUTATED HOXB4 RESULTS IN INCREASED INTRACELLULAR STABILITY AFFECTING BOTH LONG AND SHORT TERM HEMATOPOIETIC RECONSTITUTION

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The capacity of recombinant Hoxb4 protein to induce ex vivo expansion of hematopoietic stem cells (HSCs) identifies this protein as a potential hematopoiesis regulating factor. However, its short extra- and intra-cellular half-life (3-4 hours and 40-60 minutes, respectively) hampers clinical applications of Hoxb4. The analyses of Hoxb4 molecular structure lead us to generate amino acid substitutions: (A) Leu7→Ala, (B) Tyr23→Ala and (C) Tyr28→Ala in the Hoxb4 protein in order to promote its stability. Indeed, these modifications increased the intracellular stability of Hoxb4 protein 3-fold compared to wild type Hoxb4 (Hoxb4(WT)). The ability of mutated Hoxb4 protein to favor expansion of hematopoietic progenitors was first examined in cultures initiated with 10% Hoxb4(WT)-GFP, 10% mutated Hoxb4-YFP expressing cells and 80% non-transduced cells. After an 18-day culture, the proportion of A and B Hoxb4 mutants cells increased to 50-60% in comparison to 30% for Hoxb4(WT) (P < 0.05), and no differences between the proliferation of C Hoxb4 mutant and Hoxb4(WT) cells could be identified. Western blot analyses showed that the A and B Hoxb4 mutants expressed ~4 fold higher and C Hoxb4 mutant cells ~8-fold lower levels of Hoxb4 protein than Hoxb4(WT) cells. The long-term reconstituting ability of these constructs was then evaluated in vivo using competitive repopulation assays. At 8 and 16 weeks after transplantation, A and B Hoxb4 mutants contributed less peripheral blood and BM leukocytes (PBL) than Hoxb4(WT). Flow cytometry analysis of bone marrow, spleen and thymus revealed that mutated Hoxb4, like Hoxb4(WT) was expressed by all hematopoietic lineages, and that repopulation differences observed between mutated and WT Hoxb4 expressing cells were almost entirely attributable to myeloid lineage cells. However, non-competitive repopulation experiments showed that
the A and B mutated Hoxb4 expressing progenitors had a significant increase in recovery to the PBL recovery in comparison to Hoxb4(WT) (p < 0.05). Together, these studies suggest that different intracellular levels of Hoxb4 protein are affecting different types of hematopoietic progenitors. Early ex vivo expansion of clonogenic progenitors was achieved with mutated Hoxb4 proteins without impairing HSC long-term reconstituting ability. Thus, mutated Hoxb4 could represent a useful tool to accelerate engraftment after HSC transplantation.

**SUPPORTIVE CARE**

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**THE USE OF RECOMBINANT HUMAN ERYTHROPOIETIN (RHUEROPE) AFTER REDUCED INTENSITY CONDITIONING (RIC) ALLOGENEIC HEMATOPOIETIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (ASCT) REDUCES RED BLOOD CELL (RBC) TRANSFUSION REQUIREMENTS**

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We previously reported that hemoglobin (Hb) recovery was hastened after RIC ASCT as compared with ASCT after myeloablative conditioning (Transfusion, 44:501-8, 2004). In this setting pretransplant Hb level becomes the major predictive factor for early Hb recovery posttransplant and RBC transfusion (RBCT) requirements. We subsequently reported the efficacy of early rhHuEpo administration after RIC ASCT to hasten Hb reconstitution (BMT, 36:901-6, 2005). Here we further confirm that early post-transplant rhHuEpo after RIC reduces also RBC requirements.

40 pts surviving at least 60 days were analyzed. Pts characteristics were as follow: age: 50 (27-64); M/F: 28/12; with myeloid (4), lymphoid (29) or solid (7) malignancies. They received a RIC (Fludarabin (150 mg/m²), Busulfan (8mg/kg) and thymoglobulin (2.5 to 5 mg/kg)) followed with an ASCT (all PBSC) from a HLA identical sibling. Aranesp(Amgen, France) was started on day 1.

The 20 first pts received an infusion of 150 mcg/week while the 20 last pts were subsequently treated with 500 mcg/3 weeks. Aranesp was administered intravenously when inpatient and subcutaneously when outpatient. Aranespadministration was sustained until day 60 or when pts reached a Hb level of 140 g/L, whichever occurred first. Overall pts were treated for a median of 7 weeks post transplant. No serious adverse effect or thrombosis episode was reported. This cohort of 40 pts experienced a quicker Hb recovery and lower RBCT requirements than a historical and comparable control group of 27 pts (Day +30 Hb: 114 (94-141) vs. 100 (80-129), p<.0001; pts with 0 or 1 RBCT: 83% vs. 55% (p=0.02)). Thirteen of the 40 pts (33%) presented with an Hb level of 120 g/L or more prior to conditioning. Over the first 60 days, these pts received 0 (0-2) RBCT as compared with 1 (0-2) RBCT for pts with a pre-RIC Hb level < 120 g/L (p=0.05). On this basis, we hypothesized the interest of increasing Hb level prior to RIC by adequate rhHuEpo stimulation. With this perspective, we have treated 13 pts with Aranesp500 mcg, SC 3 weeks prior RIC. Nine of these 13 pts (69%) reached an Hb level of 120 g/L or more on day 7±3 as compared to 35% in patients not receiving Aranesp prior to RIC (p=0.04). This indicates that Aranesp post RIC ASCT is efficient to hasten Hb recovery and decrease RBCTs. In addition, a comprehensive strategy to minimize RBCT in this setting might include pre-transplant stimulation. We will prospectively assess this hypothesis.

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**COST ANALYSIS OF ALLOGENIC PERIPHERAL BLOOD TRANSPLANTATION: IMPACT OF DEGREE OF MUCOSITIS AND USAGE OF PALIFERMIN**

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**Background:** Oral mucositis is associated with increased clinical events and healthcare resource utilization in patients receiving hematologic stem cell transplantation (HSCT) following myeloablative therapy. Palifermin is a recombinant human keratinocyte growth factor approved to prevent severe oral mucositis. The impact of palifermin in the allogeneic peripheral blood (PBSCT) and the costs associated has not been quantified.

**Aim:** To assess the clinical and economic impact of palifermin use in allogeneic HSCT patients.

**Method:** This was a retrospective review of 21 patients undergoing allogeneic HSCT following myeloablative chemotherapy at The Alfred from June 2004–October 2005; versus allogeneic HSCT patients receiving palifermin from October 2005–July 2006. We calculated descriptive statistics on duration and grade of oral mucositis; hospital length of stay (LOS), antibiotic use; antifungal use; and total parentreral nutrition (TPN). Costs were determined through data extracts from the hospital’s clinical costing system and through retrospective medical record review.