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TWO-DIGIT RESOLUTION TYPING OF CLASS-I HUMAN LEUKOCYTE ANTIGEN (HLA) DOES NOT COMPROMISE OUTCOMES IN ADULTS UNDERGOING MATCHED UNRELATED ALLOGENEIC BONE MARROW TRANSPLANTATION

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HLA allele mismatching may be associated with inferior outcomes in unrelated stem cell transplantation. Since July 2005, our transplant unit performed 4-digit (allele level) HLA Class-I typing for all unrelated donors from National Marrow Donor Program and 2-digit Class I typing was on all others. All donors had four-digit Class-II typing. We reviewed all patients who received matched unrelated transplants (HLA-A, B, C, DR, and DQ) between July 2005 and February 2009. We compared overall survival and cumulative incidences of relapse, acute and chronic graft versus host disease (aGvHD, cGvHD) between the 4- and the 2-digit typing cohorts. The mean age is 44.9 yrs for the 86 consecutive pts (17 and 69 respectively). Most pts (61/86) have acute myeloid/lymphoid Leukemia or myelodysplastic syndrome. Conditioning regimens included: BUCY (Busulfan 12.8 mg/kg, Cyclophosphamide 120 mg/kg, 5 pts), CY-TBI (Cy 120 mg/kg, Total Body Irradiation 12 Grays, 21 pts), Flu-Bu2 (Fludarabine 120 mg/m^2, Bu 6.4 mg/kg, TBI 200 cGy, 38 pts), Flu-Bu4 (Flu 200 mg/m^2, Bu 12.8 mg/kg, TBI 400 cGy, 19 pts) and Flu-Cy (Flu 120 mg/m^2, Cy 40 mg/kg, 3 pts). Most grafts are peripherally collected (68/86 pt) and most (63/86 pts) received Alemtuzumab/CyA (Alemtuzumab 30 mg, Cyclosporine 5 mg/kg/d) for GvHD prophylaxis. There are no statistically significant differences between the baseline characteristics of the two groups (age, sex, graft source, diagnosis, preparative regimen, GvHD prophylaxis and CMV seropositivity). The median follow up is 25.3 months. Forty-two deaths occurred in both groups. The overall survival between the two groups is not statistically different (log rank test, p = 0.58). There were 13 relapses with no statistically significant difference in the cumulative rate of relapse between the two groups, log-rank test p value = 0.91. Grade 2-4 aGvHD is seen in 52 pts and the cumulative rates are not different between the groups, log-rank P value = 0.85. Thirty-three pts experienced limited or extensive cGvHD with no difference in the cumulative rates, log-rank P value = 0.81. In a Cox model of overall survival using age, diagnosis, CMV seropositivity, GvHD prophylaxis, Conditioning regimen and the level of matching; only diagnosis and CMV seropositivity are statistically significant prognostic factors. In this single institution retrospective study, 2-digit typing of HLA class-I is not associated with inferior outcomes in matched unrelated transplantation.

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IS HLA MATCHING BY SBT A NECESSITY FOR BMT?

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Hematopoietic stem cell transplantation (HSCT) outcome has improved dramatically since the introduction of allele level HLA typing. Currently, HLA laboratories are using three different technologies for allele level matching, PCR amplification with sequence-specific primers (PCR-SSP), polymerase chain reaction-sequence-specific oligonucleotide probes (SSOP), and sequence-based typing (SBT). However, it is important to emphasize that SBT is the only HLA typing method so far that allows scrutiny of every nucleotide in the HLA gene sequence, therefore, it allows us to recognize new alleles and potential gene mutations that would ensure allele level typing to be as good as claimed.

HLA high resolution typing was performed by SBT using Protrans sequencing kits. Patient DNA samples were first PCR amplified by group-specific primer mixes to separate two HLA alleles. The PCR products were then cycle-sequenced and processed on an ABI 3100 genetic analyzer. Sequencing data were further analyzed with the Assign 3.5 + HLA analysis software.

Our SBT data showed that the occurrence of new sequences in patients and donors is not negligible. We found it to be 3% in our local ethnically diverse population, where a few patients even had two novel sequences. Family analysis of sequencing data revealed that some sequence changes are actually HLA gene mutations that occur only in the patient and are not found in the parents or siblings sharing the same haplotype. By comparing patient peripheral blood samples with buccal swabs, we confirmed that HLA gene mutations also occurred in patient somatic cells. Had we used PCR-SSP or PCR-SSOP for HLA typing these new alleles or mutations would have been missed.

We argue that spontaneous HLA gene mutations found in leukemia or immunodeficiency patients that are not inherited from parents should not be reported as new alleles, unless the gene is known to be passed on to the next generation that is healthy, or carried by at least two normal individuals. If every mutation were reported as a new allele, the already long lists of alleles would continue to increase, and unnecessarily slow down the HLA matching process.

We speculate that HLA gene mutations might affect immune surveillance and impact disease severity, because our patients with these mutations had poor prognosis. Further study is required to clarify the significance of HLA gene mutations.

IMMUNE RECONSTITUTION

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FLT-3 LIGAND AND ANDROGEN WITHDRAWAL ENHANCE IMMUNE RECONSTITUTION AFTER HSCT

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Impaired thymopoiesis contributes to immune deficiency following HSCT. Clinical data suggest that thymic insufficiency may contribute to graft-versus-host disease and infectious clearance. We have previously shown that androgen withdrawal enhances thymus renewal by thymic epithelial cell (TEC) proliferation, increased entry of thymic progenitors and accelerated thymocyte development. We now demonstrate that FMS-like tyrosine kinase 3 (Flt-3) enhances thymopoiesis in conjunction with androgen blockade. Flt-3 increases stem cell number (LSK, Lineage- Sca-1+ cKit+), B cell precursors (Lineage- Sca-1 + IL-7ra+), and T cell directed stem cells (Lineage- Sca-1+ cKit+ CCR9+) in the marrow of older mice without altering thymic function. Immature granulocyte and dendritic cell precursors were also enriched in the marrow. In contrast, in young mice, Flt-3 increased the emigration of stem cells without altering the distribution of marrow cell populations. To test the effect of the Flt-3-treated marrow on thymic reconstitution, aged mice were pre-treated with lupron or saline and conditioned with 900 cGy radiation. One million congenic T-cell depleted marrow cells were adoptively transferred from Flt-3 treated or saline treated donors. Flt-3-treated marrow in combination with lupron led to the most rapid thymic reconstitution, nearly doubling total congenic thymocytes by 5 weeks post-transplant. Thymic subsets were increased including: CD4 single positive (2.67x), CD8 single positive (2.69 x), double positive (1.92x), and double negative subsets (1.59x) compared to saline-treated marrow and recipient controls. Thymocyte subsets and total numbers were significantly greater in lupron-treated hosts receiving Flt-3-treated marrow than in lupron-treated hosts receiving saline-treated marrow. In contrast, Flt-3-treated marrow transferred in the absence of lupron did not increase thymopoiesis. These data suggest that: T cell directed precursor number and precursor entry may be critical points of thymic regulation post-HSCT. By enhancing TEC proliferation, T precursor emigration, and thymocyte maturation via androgen withdrawal with lupron, it appears that precursor number becomes limiting. Flt-3 increases T directed precursors in the marrow thereby further enhancing thymic recovery. These data may present a clinical opportunity to improve thymic renewal following stem cell transplantation by Flt-3 treatment of donors and androgen withdrawal in hosts.

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IMMUNE RECONSTITUTION FOLLOWING REDUCED INTENSITY CONDITIONING WITH CAMPATH-1H VERSUS TOTAL LYMPHOID IRRADIATION/ANTI-THYMOCYTE GLOBULIN PRIOR TO ALLOGENEIC STEM CELL TRANSPLANTATION

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