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Recent evidence has suggested that allogeneic engraftment failure of purified hematopoietic stem cells (HSC) can be decreased by including a BM-derived $CD8^+/\alpha\beta TCR^-$ facilitating cell (FC) or $CD8^+/\alpha\beta TCR^+$ T cell populations. Therefore, the present study investigated the clinically important question of GVHD effector activity of these donor $CD8^+$ populations in the setting of HSC transplantation. METHODS: A purified cell component-based model of lethal GVHD was established using a $P \rightarrow F_1$ ($B6 \rightarrow B6D_2F_1$) murine model. All recipients were lethally irradiated and reconstituted with as few as 2000 purified HSC (Sca^+ $ckit^+$ Lin^-) of donor alone or together with one of the following lymphoid subsets: 1) $\alpha\beta TCR^+$ splenic T cells; 2) $CD8^+/\alpha\beta TCR^+$ BM-derived T cells (T_{BM}) and 3) the $CD8^+/\alpha\beta TCR^-$ FC population. RESULTS: As expected, all recipients of purified HSC exhibited little evidence of GVHD with clinical scores of 1.6 ± 0.3, on a five parameter clinical scale ranging from 0 to 10. Addition of purified $\alpha\beta TCR^+$ splenocytes in the donor inoculum, as a positive effector control, resulted in lethal GVHD with a >50% mortality and a clinical score of 4.3 ± 0.9. Similarly, recipients of HSC plus BM-derived $CD8^+$ T cells rapidly exhibited lethal GVHD with decreased survival and significant morbidity (4.3 ± 0.7). In marked contrast, no clinical evidence of GVHD was present in recipients of HSC+FC (1.55 ± 0.1). Histological analysis of the gut demonstrated minimal to no evidence of GVHD in 85% of these recipients whereas 57% of HSC+T_{BM} demonstrated moderate to severe GVHD. CONCLUSION: These findings suggest that in contrast to $CD8^+$ $T_{BM,}$ the FC, which expresses a unique FCp33-TCRβ heterodimer in place of αβTCR, does not result in significant GVHD even in an aggressive $P \rightarrow F_1$ model. Therefore, supplementation of the donor inoculum with the FC has the potential to offer a safer, more efficient approach to enhance alloengraftment in the absence of GVHD.

HISTOCOMPATIBILITY/ALTERNATIVE STEM CELL SOURCES

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RAPID CONVERSION TO FULL CHIMERISM AFTER REDUCED INTENSITY CONDITIONING (RIC) AND TRANSPLANTATION OF T-CELL DEPLETED LARGE NUMBERS OF CD34+ STEM AND CD56+ NATURAL KILLER (NK) CELLS OBTAINED FROM MOBILIZED HAPLOIDENTICAL DONORS

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Since alloreactive NK cells have recently been described to exert an anti-leukemic effect, to reduce GvHD and to facilitate engraftment across HLA-barriers, we have designed a protocol to promote rapid engraftment of haploidentical stem cells after Reduced Intensity Conditioning (RIC) in patients with refractory hematological malignancies. Haploidentical donors (if available, NK-alloreactive donors were preferentially chosen and 5 out of 8 donors had a constellation of NK-alloreactivity) were mobilized using G-CSF and/or G+GM-CSF. In order to cotransplant large numbers of CD56+ NK cells together with stem cells, PBSCs were negatively depleted of T-lymphocytes using an anti-CD3 monoclonal antibody conjugated to magnetic microbeads and high gradient magnetic cell sorting utilizing the automated CliniMACS device (Miltenyi Biotec).The RIC regimen included Fludarabine (200 mg/m^2), Thiotepa (10 mg/m^2) and Melphalan (60 to 140 mg/m^2). Rituximab (375 mg/m^2) was given prior to the stem cell infusion. Immunosuppressive regimen included Muromonab-CD3 (OKT3) and Cyclosporine or Mycophenolate mofetil. We transplanted 8 patients (5 AML-relapse, 1 therapy-related secondary MDS, 1 Biphenotypic leukemia-CR2, 1 CML-blastic crisis). 5 patients have received a previous allogeneic HSCT. The mean total MNC, CD34+ and CD3+ cell counts per kg infused were 8.16×10^8 ($2.6 - 15 \times 10^8$), 15.13×10^6 ($2.14 - 37.54 \times 10^6$) and 0.243×10^6 ($0.01 - 0.476 \times 10^6$) respectively. The number of infused CD56+ NK cells ranged from 44 to 200 x 10^6/kg. All patients achieve early 100% peripheral blood donor chimerisms with a median of 14.5 (8-20) days. 7 patients achieve primary engraftment (ANC > 500) at a mean of 11.14 (7-14) days. One patient had only early lymphoid engraftment. He was successfully engrafted by day 45 after repeated plasmapheresis and a stem cell boost. No early transplant-related mortality was seen. 3 patients had transient grade 1-2 skin GVHD that was controlled with immunosuppression and 1 patient developed liver GvHD. 5 of the 8 patients are alive with short follow-up. Overall, patients tolerated the RIC well and achieved very rapid chimeric engraftment. Haploidentical transplantation of T-cell depleted PBSC after RIC might be a therapeutic option for patients with advanced malignant diseases and a platform for further exploiting the role of alloreactive NK cells in the reduction of GvHD and the facilitation of engraftment in patients with nonmalignant diseases.

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ALLOGENEIC STEM-CELL TRANSPLANTATION WITH MATCHED UNRELATED DONORS IN ELDERLY PATIENTS (55-67 YEARS); AGE BY ITSELF IS NO LONGER A CONTRAINDICATION

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Allogeneic stem-cell transplantation (SCT) is a potentially curative approach for patients (pts) with hematologic malignancies. The introduction of low-intensity conditioning (LIC) regimens reduced SCT-related toxicity and allowed treatment of elderly pts. However, there is only limited data on the feasibility of this approach in SCT using unrelated donors, in elderly pts over age 55 years. Thirty-five pts were included in this study. The median age was 58 years (range, 55-67). Eleven pts were age 60 and above at the time of SCT. Diagnoses included myeloid malignancies (n = 22); AML (n = 13, 8 secondary), MDS (n = 4), CML (n = 3), myelofibrosis (n = 2), and lymphoid malignancies (n = 13); multiple myeloma (n = 9) and non-Hodgkin's lymphoma (n = 4). Conditioning regimens included fludarabine (F) and busulfan (FB, n = 16), F and melphalan (FM, n = 12), F and treosulfan (FT, n = 5), and F and low-dose TBI (n = 2). All pts were also given ATG during conditioning. With a median follow-up of one year, 18 pts are alive and 16 are disease-free. The 1-year OS and DFS are $46 \pm 10\%$, and $33 \pm 11\%$, respectively. Five pts died of disease progression, and 12 of treatment-related causes (TRM), including acute GVHD (n = 2), infection (n = 2), liver toxicity (n = 3), pulmonary toxicity (n = 2), TTP (n = 2), and heart failure (n = 1). The estimated risk for TRM was $37 \pm 9\%$. A limited multivariant analysis identified conditioning with FM (rather than FB or FT), and diagnosis of lymphoid malignancy as risk factors for TRM with hazard ratios of 21.6 (p = 0.02) and 11.2 (p = 0.05), respectively. Age category was not predictive of TRM; only 2 of the 11 pts over age 60 had TRM. Pt or donor gender and disease status at SCT were also not significant risk factors. Pt number and follow-up duration are too small to assess risk factors for OS and DFS. When only the 20 pts with myeloid malignancies conditioned with FB or FT were analyzed, the estimated TRM, OS and DFS rates were $28 \pm 11\%$, $60 \pm 12\%$ and $60 \pm 12\%$, respectively. In conclusion, SCT from unrelated donors is feasible in selected groups of elderly patients when using LIC regimens. Toxicity is acceptable and not significantly different from that observed in younger patients. Favorable outcome was observed in myeloid malignancies. Age by itself should not rule out SCT from unrelated donors.

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A COMBINATIONAL METHOD TO IDENTIFY HLA-DRB1*0401 INDIVIDUALS FOR IMMUNOTHERAPEUTIC STUDIES

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HLA-DR4 is present at high frequencies in many populations and presents many well-characterized peptide antigens to T cells in diseases including infection, autoimmune disorders, transplant rejection and cancer. We have established a combinational, simple and eco-