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Frequency of acute graft-versus-host disease and the factors for development of acute graft-versus-host disease: single-centre experience

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Introduction: Graft Versus Host Disease (GvHD) is a major cause of morbidity and mortality after allogeneic haematopoietic stem cell transplantation (AHSCT).
Patient and methods: A total of 100 patients who underwent AHCST in University of Erciyes, Faculty of Medicine, Haematopoietic Stem Cell Transplantation Unit were investigated, retrospectively. All transplantation procedures were performed from fullmatch donor and Seattle regimen was used for GvHD prevention. The frequency of acute GVHD (aGVHD) and factors related with development of aGvHD including recipient age, donor age, recipient gender, donor gender, recipient-donor gender match, blood group compatibility, amount of CD34+ given and conditioning regimen were investigated.
Results: The diagnosis of the patients were as follows: acute leukemia in 67 patients (%69,1), aplastic anemia in 7(%7,2), non-Hodgkin lymphoma in 6(%6,2), other diseases in 17(%17,5) (Hodgkin lyphoma, myelofibrosis, myelodysplastic syndrome, chronic myelogenous leukemia, chronic lymphocytic leukemia, paroxysmal nocturnal hemoglobinuria). 36 of patients were female (%36) and 64 patients were male (%64). 57 (%58,8) of patients were given non containing total body irradiation (TBI) conditioning regimen, 40(%41,2) of patients were given total body irradiation (TBI). The median age of 100 patients was found 33,3 years (±11,04). The frequency of aGvHD was %23,7 (23 patient). No statistically significant difference was determined among development of aGvHD and patient age, diagnosis, recipient age, donor age, recipient gender, donor gender, recipient-donor gender match, blood group compatibility, amount of CD34+ given and conditioning regimen. The aGvHD percentage were %18,8 while female donor-female recipient, % 41,2 while male donor-female recipient, % 15 while female donor-male recipient, % 25 male donor- male recipient. It was seemed that the proportions were higher when donors were male. However the differences between these proportions were not found statistically significant (p=0,284).
Conclusion: Transplantation performed between male donor-male recipient and male donor- female recipient may increase risk of aGvHD development.

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Alemtuzumab in conditioning for acute leukaemia: how much is good enough?

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We have recently shown in a single centre prospective study that that in vivo low-dose alemtuzumab (≤ 20 mg total dose) during conditioning combined with only CsA post-transplant may efficiently prevent severe acute and chronic GVHD after sibling and matched unrelated PBSCT for acute leukemia (BMT 2011; 46: 1363–1368). In contrast, a multicenter de-escalation prospective British study (Blood 2010; 116: 3080-3088) in patients with various diagnoses suggested that the minimum dose of alemtuzumab in sibling HCT for efficient GvHD prophylaxis should be 30mg. Probably this conclusion does not apply to patients with acute leukemia, as all patients in the British study who experienced II-IV acute GvHD in the 20mg alemtuzumab dose had a CD52-expressing tumor at the time of transplantation. Here, we update our experience of low dose alemtuzumab as GvHD prophylaxis in 60 consecutive patients (pts). Thirty-two pts were transplanted for AML, 20 for ALL, 2 with biphenotypic leukemia, 1 with untreated MDS RAEB-2, 1 with blast crisis CML (T315I+ mutation), 2 with AA, 1 with PNH and 1 with NHL. 73% of the pts had high risk diseases. Twenty-six patients were transplanted from sibling donors (43%), 33 patients from VUD (55%) and 1 patient from a matched related donor (2%). All pts but 2 received peripheral blood stem cells. The first 10 pts received a total dose of 20 mg alemtuzumab pre-transplant, the next 6 pts a total dose of 15 mg and thereafter 39 pts received a total dose of 10 mg. Overall, 18 (32%) out of 57 evaluable pts developed acute GvHD I-IV. Severe acute GvHD (grade III-IV) was observed in 5 pts (9%) after a median of 32 days (range, 13–95). All pts, but two responded to corticosteroid therapy (88%). There was no significant difference in the aGvHD incidence between sibling and VUD pts. Chronic GvHD was observed in 14 (26%) pts at median on day 159 (120-318). cGVHD was limited in 12/14 pts cGvHD, extensive in 2/14pts, mild in 3/14, moderate in 9/14 and severe in 2/14 pts. Only 2 pts are still receiving immunosuppresion for cGvHD. After a median follow up of 529 days (range 4-1826), 38 out of 60 pts are alive (63%), and 22 (37%) died (TRM n=12, Relapse n=10). The estimated 1- and 2-year OS probability for all pts is 79% ±5.3% and 62% ± 6.9%, respectively. In conclusion, in vivo low dose Campath (≤ 20 mg total dose) confers an efficient GvHD prophylaxis in patients with acute leukemia.

Graft processing and manipulation

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Rapid generation of quadrivirus-specific CD8+ and CD4+ cytotoxic T-lymphocytes (CTLs) for adoptive transfer after stem cell transplantation (SCT)

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Though adoptive therapy with viral specific T-cells has proven safe and effective for both prevention and treatment of post-transplant viral reactivation, improved strategies are still needed to increase the general applicability of this approach. We tested a simplified rapid strategy of the generation of a single product of CD4+ and CD8+ cytotoxic T-lymphocytes (CTL) lines recognizing cytomegalovirus (CMV), Epstein-Barr- (EBV), Adeno- (Ad) and BK- virus. Dendritic cells derived from elutriated monocytes were pulsed with overlapping 15mer pepmixes spanning CMV-pp65 and IE-1; EBV-LMP2, BZLF1 and EBNA1; Adv-Penton, Hexon; BKV-LT, ST and VP1 alone or in combination (Mix) and subsequently were co-cultured with autologous lymphocytes obtained from the initial elutriation in presence of IL-7, IL-15, and low dose IL-2. For detection of antigen specific CTLs, cells were stimulated with test pepmix or control superantigen in the presence of Brefeldin A, anti-CD28 and CD49d, washed, stained for T-cell subset markers and then permeabilized and intracellular-stained for IFNγ, TNFα, IL-2, CD107a. Quadrivirus-specific IFN-γ-producing CD4+ and CD8+ CTLs were successfully induced in all 12 donors (CD4+ 5.8±6.8%, CD8+ 7.3±7.9%). Induction of reactivity in cultures containing individual pepmixes was comparable with results from the cultures containing combination of all studied peptide libraries, supporting the feasibility of developing of a quadrivirus-specific product in a single mixed culture. Two rounds of expansion resulted in significant increase in frequency of CD8+ antigen-specific cells, while there was no change in reactivity of CD4+ cells. The predominant specificity was against CMV in CMV-seropositive donors (n=6; CD3+:8.7±8%, CD4+:3.6±5.9%, CD8+:5.1±3.6%). Significant CMV-reactivity of CTLs was also induced in CMV-seronegative donors (n=6; CD3+:1.6±1%, CD4+:1.2±0.7%, CD8+:0.4±0.3%). The expanded T cells retained predominantly naïve or central memory phenotype and only a small subset demonstrated undesirable markers of terminal differentiation or senescence. Furthermore, the anti-viral cells were polyfunctional, as evidenced by simultaneous production with IFNg of TNF-α and (to a lesser degree) IL-2. In conclusion, we demonstrate a simple, rapid (9 days) GMP-compatible methodology to generate a single preparation of polyclonal CTLs specific for four viruses that are frequent causes of post-transplant mortality or morbidity.