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THE ROLE OF LOW DOSE BUSULFAN (BU) (4MG/KG) WITH CYCLOPHOSPHAMIDE (CY) AS A CONDITIONING REGIMEN FOR SEVERE APLASTIC ANAEMIA (SAA)

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Bu is an agent currently used in BMT.Bu have minor toxicity to the lymphoid system and cause little immunosuppression. However, successful allogeneic engraftment after the Tutschka regimen suggests that Bu can enhance the immunosupressive properties of Cy. Graft rejection has been a problem in SAA who were conditioned with Cy alone.Aiming to reduce graft rejection and to improve the immunosuppressive activity of Cy, we used Bu/Cy 4mg/Kg and 200mg/Kg, respectively, as a conditioning in 81 pts with SAA.Pts were 3-53 years of age (mean, 24). The previous transfusions number was 1-276 (mean, 39), and 48% had therapy immunossupressive before transplant. MTX/CSA were used in order to prevent GVHD.12/81 (15%) pts rejected their transplants in a mean time of 317 days (28-1001), 2 primarily (3%) and 10 (12%) as a late rejection. aGVHD grades 2 or 3 occurred in 20/67 (31%) pts and cGVHD in 24/61 (39%). The actuarial survival rate at 2850 days was 56% (CI 46-68%). In the univariate analysis age, previous treatment, number of previous transfusions, time of CSA use and aGVHD were statistically significant for survival. The OS for patients who received less and more than 15 transfusions was 78% and 50%, respectively (P=0, 001); less and more than 50 transfusions was 67% and 28%, respectively (P=0, 002). The cumulative incidence of rejection was 22% (CI 10-33%). In the univariate analysis number of previous transfusions and time of CSA were statistically significant for rejection. The cumulative incidence of rejection for patients who received less and more than 50 transfusions was 15% and 43%, respectively (P=0, 06). The following risk factors were statistically significant for survival in the multivariate analysis: number of previous transfusions, time of CSA, and aGVHD; for rejection: number of previous transfusions, and time of CSA.Low dose Bu/Cy showed to be effective and safe as conditioning regimen for SAA with acceptable rejection and survival rates. Moreover, higher number of transfusions showed to be associated with poor survival and more frequent rejections.

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QUANTITATIVE DETECTION OF CHIMERISM AFTER ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

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For peripheral blood stem cell transplantation (PSCT), several methods for engraftment analysis, including detection of restriction fragment length polymorphisms and amplification of polymorphic genetic loci, have been described previously. We report here a quantitative, non-isotopic method using short tandem repeat (STR) marker to facilitate the monitoring of engraftment. The DNA from the donors and the pre-transplant recipients were amplified with the AmpFlSTR Profiler Plus kit that contained 9 STR markers. The fluorescent polymerase chain reaction products were then fractionated on the polyacrylamide gels in the ABI PRISM 377 DNA Sequencer and were analyzed by the GeneScan 2.1 software. The best markers were selected as the informative alleles to distinguish donor from recipient. For quantitative analysis of engraftment, the chimeric samples were prepared by mixing pretransplant recipient and donor DNAs in different ratio to generate a standard curve. After amplifying the post-transplant recipient DNA, the extent of engraftment was determined by interpolating the percent peak area of the informative alleles according to the standard curve. This method was evaluated by the samples from 30 patients who received allogeneic PSCT during 1999 to 2002. Four of them were informative for some degree of mixed chimerism indicating leukemic relapse. Of these 4 cases, 35%, 6.5%, 15.5%, and 2.7% recipient DNA were first detected in the third, tenth, fourth, and fourth month after PSCT, respectively. These four mixed chimeric patients were soon treated with a rapid taper of immunosuppression. Three of them were then allowed a second PBST. In summary, this method provided an accurate, quantitative, and early assessment of mixed chimerism in post-transplant patients. Such information may be useful to guide implementation of additional treatment to circumvent graft failure or relapse in the future.

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STEM CELL TRANSPLANTATION IN AUSTRALIA AND NEW ZEALAND: AN UPDATE FROM THE AUSTRALASIAN BONE MARROW TRANSPLANT RECIPIENT REGISTRY (ABMTRR)

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The ABMTRR has captured data for an estimated 99.5% of transplants performed in the region from 1992-2001. In 2001, 1042 transplants were performed, fewer than the peak of 1094 in 1998. This change is due to a reduction in staged, autologous transplants (mainly for breast cancer) and allografts for CML, despite an increase in unrelated donor and reduced-intensity allografts. For adult patients, the most common indications for single autografts (total 578) were NHL (42%), myeloma (35%) and Hodgkin lymphoma (9%). There were 17 transplants performed for solid tumours (3%) and 4 for autoimmune disease, while 34 staged autografts were done for myeloma (6), germ cell tumours (6), breast cancer (4) and others (18). Of 232 related donor allografts, AML (31%), CML (16%), ALL (11%) and NHL (10%) were the most common indications. In 1996, 97 patients were transplanted for breast cancer while in 2001 there were only 6. In 1999, there were 81 allografts for CML, 73 in 2000, but only 38 in 2001 coinciding with the initiation of trials with imatinib mesylate. For CML in 1st chronic phase, the numbers dropped from 63 in 1999 to 29 in 2001. Outcome data were available with a minimum of 12 months follow-up. For allografts, the major cause of death in the first and second year was relapse (26%, 68%) with GVHD responsible for 15% and 5% respectively. For autografts disease-related deaths were 78% and 86% in the first and second years. Of 1673 adult recipients of related donor allografts, the estimated probability of survival at 9 years was 42% and was 32% for 406 VUDS. We conclude that the ABMTRR is a valuable resource in identifying trends in transplantation.

	2001 Australia	2001 New Zealand
Total transplants	937	105
Male:Female	582:355	61:44
%male	62.1	58.1
Number of autografts	611	67
Allo related	234	29
Allo unrelated	92	9
1st transplants (allo&auto)	871	104
2nd or 3rd transplants	66	1
staged autografts	57	3
mismatched allografts	24	4
age range (yr)	.3-75	1-64
Aged 0-15 yr	109	22
Aged 16-49 yr	391	45
Aged 50+ yr	437	38
% aged 50+ yr	46.6	36.2
Number of contributing centres	31	6

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EFFECT OF ALLOGENEIC STEM CELL TRANSPLANTATION ON BONE MARROW (BM) ANGIOGENESIS IN CHRONIC MYELOGENOUS LEUKEMIA

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Aim: Angiogenesis or new vessel formation is integral to the biology of many malignancies. Increased BM angiogenesis has

been demonstrated in patients with chronic myelogenous leukemia (CML). Allogeneic stem cell transplantation (ASCT) can be curative for patients (pts) with CML. It is not known if the abnormal BM angiogenesis changes following SCT. We studied the effect of SCT on BM microvessel density (MVD) in CML and examined the prognostic value of pre and post transplant (Tx) MVD. **Patients and Methods**: We evaluated MVD from BM samples obtained just prior to and at 3-5 months after ASCT in 38 CML pts.31 pts each had either pre-Tx or post-Tx biopsies and 24 pts had both pre and post Tx biopsies available for comparison. Microvessels were stained using immunohistochemical staining for vonWillebrand factor, using a labeled streptavidin-biotin peroxidase method. MVD was estimated by determining the average number of vessels in 3 hot spots examined at 400x magnification. The angiogenesis was graded as low (MVD < 10), intermediate (MVD < 20) or high (MVD >= 20). **Results**: The median MVD pre Tx for 31 pts was 14 (4-37); with 13 pts having high-grade angiogenesis, 12 intermediate-grade and 6 low-grade. The median post Tx MVD in 31 pts was 20 (range 5-36) with 16 pts having high-grade angiogenesis, 13 intermediate-grade and 2 low-grade. The median time between biopsies was 4 months (range 1-6 months). There was no significant change in the following Tx (P =0.8, paired t-test). The MVD increased in 12 pts (median increase 8), decreased in 10 (median decrease 9) and remained unchanged in 2. The pre or post Tx MVD did not predict relapse free or overall survival following SCT. The microvessels in the post Tx BM demonstrated striking dilatation compared to the pre-Tx marrow. **Conclusion**: BM angiogenesis seen in CML does not change significantly following allogeneic SCT. These results are consistent with what we have observed in patients with multiple myeloma receiving high dose or conventional therapy and also with other reports. It is possible that persistent vessels may serve as a nidus for tumor relapse, however we were not able to demonstrate such an effect in our small group. This data provide further evidence against using regression of angiogenesis as a marker of therapeutic response, even in the context of anti-angiogenic therapy.

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INFECTIONS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (ALLO-BMT) IN SOUTH BRAZIL: LOW INCIDENCE OF INVASIVE FUNGAL INFECTIONS DESPITE LOW-DOSE FLUCONAZOLE PROPHYLAXIS

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Infections remains a main cause of morbidity and mortality after allo-BMT. Herein we describe our data concerning infections incidence until D+100 after BMT. We retrospectively reviewed 98 patients transplanted from 12/94 to 04/02 with an HLA-identical (n=96), 1-HLA allele mismatch (n=1) or singeneic (n=1) sibling donor. Graft source was bone marrow in 88 patients. Median follow-up was 3 years. Median age was 31 years. Seventy-six patients had malignancies (CML 45, ALL/AML 27, MDS 4). Eight-eight patients were CMV positive. GVHD prophylaxis was cyclosporine + methotrexate in 94 patients. Uni- and multivariate analysis were performed with Kaplan-Meier/log rank and Cox models respectively. Transplant-related mortality (TRM) and overall survival (OS) were 24% (100 days) and 54% (5 years). Acute grade 2-4 GVHD incidence was 32%. Fifty-four patients presented at least one episode of a severe bacterial infection (septicemia, septic shock, and pneumonia), with a cumulative incidence of 56% until D+100. Most frequent bacteria isolated were Staphylococcus sp. (n=53), E. coli (n=7) and Klebsiella sp. (n=6). In univariate analysis, no variable influencing incidence of bacterial infections, however, there was a tendency of less bacterial infections with a higher nucleated cell dose graft (p=0.11). Thirty-five patients presented at least one episode of CMV reactivation with a cumulative incidence of 36%. Six patients presented a CMV disease. In multivariate analysis, presence of acute GVHD grade 2-4 (HR=2.8; CI95: 1.43-5.46; P=0.003) and recipient age more than 18 years (HR=0.09; CI95: 0.01-0.64; P=0.02) were associated with a higher incidence of CMV infection/disease. Only three patients presented a proven or probable invasive fungal infection: 1 candidemia, 1 Aspergillus sp. infection and 1 Fusarium sp. infection until D+100. The incidence of bacterial and CMV infections were comparable to the literature. On the other hand, despite use of a relatively lower dose of fluconazole, incidence of fungal infection was significanty lower than expected. In our experience, use of low-dose fluconazole (200mg/d) is sufficient to provide adequate prophylaxis for fungal infections, decreasing BMT costs. Low incidence of mold infections might be explained by factors other than fluconazole use.

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DNA BASED HIGH RESOLUTION TYPING : OPTIMIZED PRESELECTION FOR CONFIRMATORY TYPING (CT)

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Search speed and monetary resources can be minimized by inserting a DNA based high resolution typing before requesting an unrelated stem cell donor for confirmatory typing. Therefore the DKMS offers the possibility to request high resolution typing class I and II since 1992 (at least on four digits level). These typings are performed with new drawn blood samples and not with "stored samples", because we think that donors should be aware of the importance to become a potential stem cell donor soon. With the introduction of optimized software and changing the workflow in 2000, the turnaround time from donor contact to the transmission of the typing results could be halved from over 20 to about 10-13 days. The results of the high resolution typings give a very precise preselection of donors for an allogenic stem cell transplantation. So the donor selected for confirmatory typing after a request for high resolution typing is more propable to be the suitable donor. We observe a steadily increasing number of high resolution typing requests that over-strides the increasing number of the confirmatory typings. High resolution typings and the returning of the confirmatory typing results by the search centers heighten the quality of our donor data base, reduce the workload for the search centers and accelerate donor searches in the future.

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CASE REPORT OF A PATIENT WITH ADULT T-CELL LEUKEMIA/LYMPHOMA (ATLL) 18 MONTHS AFTER ALLOGENEIC MATCHED RELATED DONOR PERIPHERAL STEM CELL TRANSPLANT

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The long term prognosis for persons affected with Adult t-cell leukemia/lymphoma associated with HTLV -1 disease is dismal. We report a case in which a patient is without evidence of disease at 18 months after peripheral blood stem cell transplant. Her brother was her matched donor. She was treated with Fludarabine and Busulfex conditioning regimen prior to transplant. She is thiry nine years old, African American, diagnosed in November 2000. She presented with palpable adenopathy, hepatomegaly, elevated WBC of forty-four thousand and eight nine percent lymphocytes, hypercalcemia, elevated LDH, pleural effusion, pericardial effusion and multiple areas of adenopathy in her chest. HTLV 1 antibody was reactive. Bone marrow biopsy and thoracentesis were both consistent with ATLL . Complex chromosome abnormalities were present including trisomy 3 and 7. She received 2 cycles of adriamycin, VP-16, dexamethasone with decrease in adenopathy and was brought to transplant after passing the requisite pretransplant organ testing. She received 16 doses of high dose Busulfex per pharmacokinetic dosing every six hours, Fludarabine at 40mg/m2, one dose daily for 5 consecutive days, and antithymocyte globulin 15mg/kg for four doses. Her graft versus host disease prevention included long course methotrexate and cyclosporine. Evaluations at 100 days, 200 days, 1 year and 18 months show no radiographic evidence of disease. FISH studies consistently show 100% male donor chromosomes. Bone marrow aspirate and biopsy at one year shows normal tissue morphology