mortality, accounting for about 25%-33% of deaths in long-term survivors of transplants, and that it is the main cause of delay in recovering an adequate quality of life after the transplant.[6] The most important complication associated with chronic GVHD is immunodeficiency, leading to susceptibility to opportunistic infections and second neoplasms.[7] On the other hand, chronic GVHD has been associated with a potent antileukemic effect. Thus, patients developing this complication have a lower incidence of relapse after transplantation.[8] The final clinical result, taking into account these detrimental and beneficial effects, of chronic GVHD has been analysed in patients submitted to allo-BMT. It seems that for those patients who receive a transplant in an advanced phase of disease, chronic GVHD might be associated with a better survival rate.[9] In contrast, for patients at low risk of relapse the potential benefit of the antileukemic effect is counterbalanced by its mortality, with the final result of an adverse impact on survival in this group of patients.[10] Unfortunately, the clinical effect of chronic GVHD depending on its severity (e.g. limited vs extensive) is not specified in these articles. Has chronic GVHD the same clinical consequences after allo-PBT as after allo-BMT? Extensive chronic GVHD after allo-PBT has been associated with high transplant-related mortality.[5,11] However, it is of note that the association of chronic GVHD with reduced relapse rate reported in the allo-BMT setting has not been found in some of the most important series of allo-PBT.[2-4] Two recent reports have shown that extensive chronic GVHD after allo-PBT adversely affected the outcome.[5,11] Although the clinical impact on the outcome of chronic GVHD after allo-PBT requires continued long-term evaluation and a better definition of the effects of limited and extensive chronic GVHD in patients with early or advanced phases of disease, it seems prudent to incorporate methods to decrease the incidence of this complication after allo-PBT. T-cell depletion of the graft is the only known method of GVHD prophylaxis consistently associated with a reduction in chronic GVHD after allogeneic transplants. However, this approach is associated with a higher relapse rate, which limits its use to patients with a low probability of relapse. Another alternative is to employ an *in vivo* modulation of T-cells post-transplant, by prolonging CsA administration. In this issue Mengarelli *et al.*[1] show for the first time in the setting of allo-PBT that this approach reduces the incidence of extensive chronic GVHD. Although overall survival was not modified, we may assume that patients benefited from an improved quality of life.

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Advances in unrelated donor hematopoietic cell transplantation

For patients without an HLA-identical sibling, transplantation of hematopoietic stem cells from HLA-compatible unrelated volunteer donors has become feasible thanks to the expansion of registries of HLA-typed volunteers that now include more than seven million individuals worldwide. The probability of matching patients with at least one donor for HLA-A, B and DR has increased as a function of the logarithm of the donor pool. In the National Marrow Donor Program of the United States, such a probability was 50% with a pool of 100,000 donors and has expanded to 85% with a pool greater than two million donors typed for HLA-A, B and DR. Utilization of unrelated donors as a source of hematopoietic stem cells has also increased because of improved safety of trans-

plantation. The primary factor leading to improved patient outcome in the last decade has been the use of more precise and sensitive HLA typing using DNA-based techniques.

The role of HLA typing and matching on the outcome of unrelated donor transplantation

Results of a study of 1,874 unrelated donor marrow transplants facilitated by the National Marrow Donor Program of the United States was recently reported in abstract form.[1] Treatment regimens were selected by the transplant center. DNA samples from patient and donor were typed at the sequence level for HLA-A, B, C, DRB1, DQB1, DQA, DPB1 and DPA genes. The study revealed three major findings: 1) mismatch for HLA-A, B, C and DRB1 is associated with a worse survival, while mismatch for DQB1, DQA, DPB1 and DPA is not. Based on this finding, it is advisable that future donors will be screened for their matching, not only at HLA-A, B and DRB1, but also at HLA-C. Matching for DQ and DP genes remains of unproven effect on survival; 2) mismatching for one HLA-A, B or DRB1 DNA sequence disparity (allele mismatch) that is not recognized by anti-HLA antibodies is associated with decreased survival, and mismatching for one HLA-A, B and DR locus disparity that is recognized by antibodies (antigen mismatch) is associated with even worse survival. Thus, high resolution DNA typing at the *sequence level* is useful in selecting for more closely matched and safer donors. However, if a fully matched donor is not available, mismatch for an allele (i.e.: A*0101 vs. A*0102) is preferable to mismatch for an antigen (i.e.: A*01 vs. A*02); 3) mismatch for multiple alleles at HLA-A, B, C and DRB1 compounds the risk of mortality. This last finding confirms data in a prior report[2] and its implications are obvious.

The risk of graft failure is increased with donor disparity for HLA-A, B, or C and with patient homozygosity at the mismatched locus.[3] When donor and recipients differ for a single HLA locus, the risk of graft failure varies according to whether the incompatibility is for an HLA antigen or an HLA allele. In a study by Petersdorf *et al.* of patients transplanted from an unrelated donor, there were no episodes of rejection with a mismatch for a single allele (n=47), whereas rejection occurred in 14% of cases with a mismatch for a single antigen (n=51) and in 22% of cases when there was mismatch for multiple alleles (n=9). These data on graft failure from a single center are consistent with the data on survival from the National Marrow Donor Program, and demonstrate that mismatch for an antigen has worse clinical consequences than mismatch for an allele, and that the effect of mismatching for multiple alleles is cumulative.[1-3]

Modern HLA typing using DNA technology can distinguish subtle polymorphisms previously undistinguishable by classical serological typing techniques. It is possible however, that demanding donor matching at the DNA sequence all for HLA-A, B, C and DRB1 loci will constitute an unnecessary stringency, and in some cases will prevent access to transplantation. The allowable limits of genetic disparity will likely differ according to the patient's underlying disease and stage. While patients with low risk disease and fair life expectancy in absence of transplant would want to avoid even the minimal risk associated with a mismatched donor, patients with high risk disease in advanced stage will likely have to tolerate the risk associated with the use of a donor mismatched for a single antigen or multiple alleles, rather than face the greater risks of the disease without transplantation. Therefore, the definition of an acceptable mismatch will require analyses of large number of patients with homogeneous disease risk.

Survival improvement trend over time

In Seattle, better donor matching and prophylaxis of cytomegalovirus disease and candida septicemia have resulted in improved survival in patients transplanted from an unrelated donor.[4] Patients transplanted for chronic myeloid leukemia in chronic phase between 1988 and 1991 (n=61) had a Kaplan Meier estimate of survival at five years of 49%, compared to 65% for patients transplanted between 1992 and 1998 (n=194, p=0.01). Best survival was observed in patients 18 to 40 years old (n=112) with a Kaplan-Meier estimate of 79% at 5 years compared to 54% for patients 41-50 years old (n = 70, p = 0.002), and 20% for patients older than 50 (n=10, p=0.007). Patients above the age of 40 appear to tolerate high-dose whole body irradiation poorly. There is a report in abstract form of decreased morbidity and mortality, despite the use of an unrelated donor, in older patients receiving a regimen of fludarabine 90 mg/m^2 and low dose whole body irradiation 200 cGy.[5]

Role of stem cell dose

Patients with acute myeloid or lymphoid leukemia transplanted with unrelated donor bone marrow enjoyed a significantly improved survival when transplanted with a marrow cell dose greater than 3.7×10^8 nucleated cells per kg of body weight as opposed to a lower cell dose.[6,7] A subsequent single center study was conducted with the hypothesis that the reason for the improved outcome of recipients receiving a high marrow cell dose was related to the dose of CD34 cells.[8] The transplant center requested from the unrelated donor a marrow dose containing 4×10^8 nucleated

cells per recipient body weight. In a cohort of 111 patients older than 20 years of age transplanted with T-replete marrow, the one-year survival was 66% if the CD34 cell dose was greater than 2.5×10^6 per kg of body weight, as opposed to 44% with a lower dose (p=0.003). The dose of CD4, CD8, or CD3 T-cells, B-cells, or monocytes did not affect the probability of a one-year survival in that study. By multivariable analysis, a higher CD34 cell dose was associated with an improved probability of sustained engraftment defined by neutrophils above 500/μL throughout the first 100 days, a lower risk of non-relapse mortality (hazard ratio 0.70, 95% confidence interval, 0.55-0.90, *p*=0.004) and less overall mortality (hazard ratio 0.79, 95% confidence interval, 0.66-0.94, *p*=0.008). These data suggest that human bone marrow is a limited source of hematopoietic progenitor cells for transplantation.

Since mobilization with granulocyte-colony stimulating factor (G-CSF) followed by blood cell apheresis can produce two to three fold higher number of CD34 cells, there is a rationale to testing the use of peripheral blood progenitor cells for transplantation. A single center study in Seattle has tested the use of peripheral blood stem cells (PBSC) from unrelated donors in patients with acute myeloid or lymphoid leukemia. A preliminary survival analysis in patients up to the age of 40 years transplanted in first or second remission (n = 29) is currently showing a Kaplan-Meier estimate of 73% at two years. The National Marrow Donor Program of the United States has launched a multicenter open-label phase II study to evaluate the use of G-CSF-mobilized peripheral blood stem cells in unrelated donor transplantation. A multivariate analysis has retrospectively compared the outcome of patients transplanted with PBSC or marrow over the same period of time at the same centers. The use of PBSC was associated with faster engraftment of neutrophils and platelets, with a suggestion for an increased incidence of acute graft-versus-host disease. The overall survival and disease-free survival were similar.[9] The relative benefits of unmodified bone marrow or peripheral blood components from unrelated donors will be tested in a randomized trial. Pilot studies are needed to test the use of modified components depleted of alloreactive T-cells with the goal of preventing GVHD.

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Editorial note

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