

Haploidentical Stem Cell Transplantation for Acute Leukemia

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Abstract

Premise: Since March 1993, 133 patients with high-risk acute leukemia (66 AML, 67 ALL) have received a megadose of T-cell depleted hematopoietic stem cells. The 1993-95 conditioning protocol included TBI, thiotepa, ATG and CY for 36 patients who received an inoculum made up of lectin-separated bone marrow and PBPCs. After 1995, to minimise the extra-hematological toxicity of the conditioning and eliminate GvHD, we substituted fludarabine for CY in the conditioning and PBPCs were depleted of T-cells by a positive selection of the CD34⁺ cells using CellPro (n=44 patients) or, since January 1999, CliniMacs (n=53 patients). A later modification to the protocol in January 1999 was the suspension of post transplant G-CSF. **Work in Progress:** We report here the results in the last 53 acute leukemia patients all of whom were transplanted under our modified protocol. Ages ranged from 9 to 62 years with a median of 38 years for the 33 patients with AML and 23 for the 20 with ALL. All were at high risk because 25 were actually in relapse at transplant, 16 were in second or later CR and even the 12 patients in CR1 were at high risk because of the unfavourable prognostic features. Overall 52/53 patients (98%) engrafted. The TBI-Fludarabine-based conditioning was well tolerated even in the 14 patients between 45 and 62 years of age. There was no veno-occlusive disease of the liver and the incidence of severe mucositis was low. Even though no post-transplant immunosuppressive therapy was given, acute GvHD grade \geq II occurred in only 4 cases and only one progressed to chronic GvHD. Overall, 16 patients (30%) have died of non-leukemic causes. Relapses occurred mainly in patients who were already in relapse at transplant (12/25). Only 3 of the 28 who were in any CR at transplant have so far relapsed. As our group has already shown, donor-vs-recipient NK cell alloreactivity exerts a specific graft-vs-AML effect in the absence of GvHD. In fact, leukemia relapse was largely controlled in AML recipients whose donor was NK alloreactive, with only 2 out of 16 relapsing. To date, 13 of 18 AML (72%) and 5 of 10 ALL (50%) who were in any CR at transplant, survive disease-free while 4 of the 15 patients (16%) in relapse at transplant survive. The probability of event-free survival for patients transplanted in CR is 60% in the 18 AML patients and 38% in the 10 ALL. The probability of EFS was significantly better in the 16 AML patients whose transplant included donor vs recipient NK cell alloreactivity than in those whose transplant did not (70% vs 7%). In conclusion, given our current results, the most suitable candidate for the full haplotype mismatched transplant should be in early stage disease and selection of an NK alloreactive donor is recommended.

Although hematopoietic stem cell transplantation (HSCT) from HLA-matched siblings has become treatment of choice for many hematologic diseases, its broader application has been limited by lack of available matched related donors. Attention has been focused on alternative donors, i.e. phenotypically matched unrelated

donors and partially mismatched related donors. Even though the world-wide donor registries includes over 7 million HLA-typed volunteers, the chance of finding a matched unrelated donor depends on the HLA diversity and varies with race, ranging from 60-70% in Caucasians to under 10% for ethnic minorities [1,2]. Other

limitations are the lapse in time from registering to identifying a donor which may lead to disease progression in patients who urgently need a transplant e.g. those with acute leukemia. Age is a further drawback as morbidity and mortality rise with age and paradoxically, so is closer matching, based on DNA techniques, since it reduces the odds of finding a suitable matched donor.

On the other hand, virtually all patients in need for a transplant have a readily available mismatched family donor. Unfortunately, until the 1990's, HSCT from such donors was largely unsuccessful in leukemia patients although results were good in children with severe combined immunodeficiency [3]. Reasons for failure were the high incidence of severe graft-versus-host disease (GvHD) in unmanipulated transplants [4] and graft rejection in extensively T-cell depleted transplants [5].

The turning point in the history of T cell depleted mismatched transplants was the clinical application of a megadose of stem cells. This principle was successfully tested in a series of mouse models which showed escalating doses of T-cell depleted mismatched bone marrow cells were associated with full donor type engraftment [6,7].

In 1993 we applied the cell-dose escalation concept for the first time clinically in 36 adults with advanced leukemia (18 CR \geq II, 18 resistant relapse) [8]. To increase the number of hematopoietic stem cells in the inoculum, bone marrow was supplemented with granulocyte-colony stimulating factor (G-CSF)-mobilized peripheral blood progenitor cells (PBPCs). Both sources of stem cells were depleted of T cells by soybean agglutination and E-rosetting. The final inocula contained a median of 10.8×10^6 CD34⁺ cells/kg recipient body weight which was ten times the number of CD34⁺ cells in T-cell depleted bone marrow cells and a median of 2×10^5 CD3⁺ cells/kg recipient body weight. As for the conditioning regimen, we enhanced the recipient's immunosuppression by adding rabbit anti-thymocyte globulin (rATG) and cyclophosphamide (CY) to a single fraction TBI, and strengthened myeloablation by using thiotepa. Twenty-nine patients (80%) achieved primary sustained engraftment; four of the other seven successfully engrafted after second transplants from different haplo-identical family donors. Even though no post-transplant immunosuppressive therapy was given, only 18% of evaluable patients developed grade II to IV acute GvHD. Six of these otherwise incurable patients (5/12 AML, 1/24 ALL) survive disease-free at 9 years.

Over the years our approach has been modified in several ways. In a search for a substitute for CY, a potential factor in extra-hematological toxicity, we considered fludarabine, which has powerful immunosuppressive effects and low extramedullary toxicity in patients with lymphoproliferative diseases. In a murine model we showed that TBI+fludarabine provide the same immunosuppressive effect as TBI+cyclophosphamide [9]. Accordingly, in October 1995, our standard conditioning protocol in mismatched transplants was modified to include fludarabine instead of CY. At the same time, to reduce the number of T lymphocytes in the inoculum to below

3×10^4 /kg, which has been identified as the threshold for GvHD, we started to positively select the CD34⁺ cells. From October 1995 to August 1997, PBPCs were depleted of T-cells by E-rosetting and positive immunoselection of CD34⁺ cells with the Ceprate-SC (Cell Pro, Bothell, Washington, USA) system (n=44) [10]. In January 1999 we began using the CliniMACS (Miltenyi Biotec, Bergisch Gladbach, Germany) device for graft processing (n=53). This instrument provides a highly purified CD34⁺ cell population without the need for E-rosetting or any other manipulation of leukapheresis products. At the same time, we suspended post-transplant G-CSF administration to the recipients, which had been given to speed-up the neutrophil recovery, because reports on experimental models and in our own preliminary observations have shown it is immunosuppressive [11,12].

All these procedures yielded very large numbers of CD34⁺ cells (median 10×10^6 /kg, range 5.8-25), but when we started using immunoselected CD34⁺ cells we reduced the number of T lymphocytes in the graft by one log, from a median of 2×10^5 /kg (range 1.1-7) to a median of 1×10^4 /kg (range 0.1-6.2). None of these modifications compromised engraftment which was primary and sustained in 91 (94%) of the 97 acute leukemia patients who received an immunoselected CD34⁺ cell transplant between October 1995 and June 2002. All patients achieved and sustained a full donor-type chimerism in the peripheral blood and the bone marrow. Four patients developed acute GvHD (grade II=2, grade III-IV=2) which progressed to chronic in one. The extra-hematological toxicity of this innovative conditioning regimen was minimal even in these advanced stage, heavily pretreated patients. We had no case of veno-occlusive disease of the liver. Severe oral mucositis was minimal. Two of the first 16 patients died from toxic acute pulmonary decompensation soon after transplant. After we reduced the dose of total lung radiation from 6 to 4 Gy no other case of acute lung toxicity was observed.

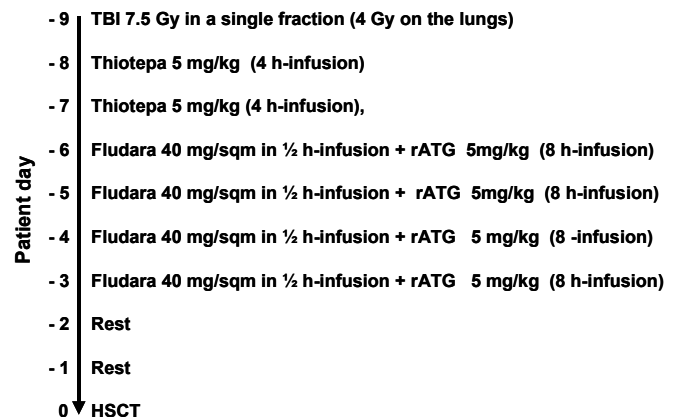


Figure 1. Conditioning regimen.

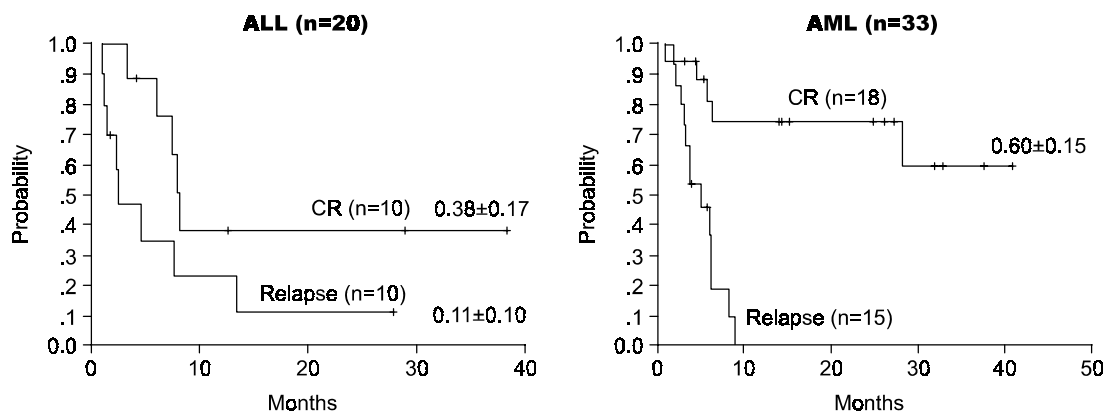


Figure 2. Event-free survival.

Work in progress: We report here outcomes in 53 high-risk acute leukemia patients, all of whom were transplanted under our modified protocol. Ages ranged from 9 to 62 years with a median of 38 years for the 33 patients with AML and 23 for the 20 with ALL. All were at high risk for post-transplant relapse because 25 were actually in relapse at transplant, 16 were in second or later CR and even the 12 patients in first hematological remission were at high risk because of the unfavourable prognostic features: complex karyotypes x 2, t(8;12), monosomy 7, secondary leukemia, t(9;22) x 3, t(4;11), inversion of chromosome 13, lymphoma-leukemia pattern and slow first remission.

After receiving the TBI-fludarabine-based conditioning (Figure 1), patients received a median of 12×10^6 CD34⁺ cells/kg and 1.5×10^4 CD3⁺ cells/kg. 49 patients achieved primary engraftment. The 4 who rejected the first transplant were given second transplants from different haploidentical donors and three engrafted after a second cycle of conditioning with fludarabine, ATG and CY. So overall 52/53 patients (98%) engrafted. Hematopoietic recovery was extremely rapid with neutrophil counts reaching $1 \times 10^9/l$ and platelet counts $25 \times 10^9/l$ at a median of 13 days (range 8-19) and 18 days (range 12-84), respectively.

Even though no post-transplant immunosuppressive therapy was given, acute GvHD occurred in 4 cases (grade II=2, grade III-IV=2) and only one progressed to chronic GvHD.

Overall, 16 patients (30%) have died of non-leukemic causes, 10 from infections and 6 from other causes (GvHD x 2, leukoencephalopathy, MOF, rejection and idiopathic interstitial pneumonia). Interestingly, the probability of transplant-related mortality was low (12%) in the 18 AML patients transplanted in remission who did not receive G-CSF as compared to the 58% TRM of the 15 patients in CR who received G-CSF. As we have observed a significant improvement in the immunological reconstitution, as shown by CD4⁺ cell recovery, after we stopped administering G-CSF post transplant, it could have contributed to the very low probability of TRM in these patients [12].

Leukemia relapses occurred mainly in patients who were already in relapse at transplant (12/25). Only 3 of the 28 who were in any CR at transplant have so far relapsed. As our group has already shown, donor versus recipient NK cell alloreactivity exerts a specific graft-vs-AML effect in the absence of GvHD [13,14]. In fact, leukemia relapse was largely controlled in AML recipients whose donor was NK alloreactive, with only 2 out of 16 relapsing.

To date, 13 of 18 AML (72%) and 5 of 10 ALL (50%) who were in any CR at transplant, survive disease-free while only 4 of the 15 patients (16%) in relapse at transplant survive. The 3-year probability of EFS is 53% for patients who, at transplant, were in any CR and only 5% for those in relapse. The probability of event-free survival for patients transplanted in CR is 60% in the 18 AML patients and 38% in the 10 ALL (Figure 2). The probability of EFS was significantly better in the 16 AML patients whose transplant included donor vs recipient NK cell alloreactivity than in those whose transplant did not (70% vs 7%).

In conclusion, our experience in this field over the past 9 years demonstrates that a high rate of engraftment can be achieved without severe GvHD and with low regimen-related toxicity and mortality. Today, T-cell-depleted stem cell transplantation from full-haplotype mismatched donors can be offered, not as a last resort, but as a viable option in the early stages of the disease to high risk acute leukemia patients without a matched donor.

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