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Allografting

Haplotype mismatched transplantation using high doses of peripheral blood CD34+ cells together with stratified conditioning regimens for high-risk adult acute myeloid leukemia patients: a pilot study in a single Korean institution

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Summary:

A total of 11 high-risk Korean acute myeloid leukemia (AML) patients received stem cell transplantation from human leukocyte antigen (HLA) haploidentical donors. Specifically, for eight patients with 2–3 mismatched antigens and bidirectional vectors, we used a newly designed conditioning regimen that consists of total body irradiation, busulfex, ATG, and fludarabine. The median number of CD34+ cells infused was 15.4×10^6/kg (range, 8–21.2). These patients received neither graft-versus-host disease (GvHD) prophylaxis nor post transplantation G-CSF. All of the patients who were followed up for a median of 6 months (range, 17 days–28 months) showed stable primary engraftment and had no acute GvHD or transplant-related mortality for 100 days post transplant. Three patients with high-risk or refractory disease eventually died in relapse, even with GvH-directed NK alloreactivity. However, the patients in complete remission (CR), with the exception of one patient who is alive at 18 months EFS, died at 4, 6, and 8 months post transplantation due to infections that were associated with delayed immune recovery. Our findings suggest that haploidentical transplantation represents a feasible treatment for patients with high-risk AML in CR, provided that a plan for the enhancement of immune recovery is implemented.
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Ever since the first allogeneic hematopoietic stem cell transplant (allo-HSCT), performed successfully in the late 1960s, transplantation for malignant lymphohematopoietic diseases has been conducted only when the human leukocyte antigens (HLA) of donor and recipient show a complete match at the level of the major histocompatibility complex (MHC). The most widely used therapeutic strategy for adult acute myeloid leukemia (AML) patients, with cytogenetically unfavorable or standard risk characteristics, is intensive therapy after first remission induction, for which allo-HSCT confers the best potential for long-term survival. However, in reality, the probability of finding a sibling donor whose MHC totally matches that of the patient is only 20–30%, and may be lower as a result of the modern trend towards the nuclear family. In addition, it has been estimated that at least 50% of Korean patients fail to find non-family donors, even when various domestic and foreign donors searching programs are utilized. These searches require a lot of time, and some patients in remission relapse while waiting for transplantation, while some donors are reluctant to donate at the optimal time for treatment, or refuse to donate at all. Although allogeneic transplantation using cord blood is being studied for both children and adult patients,[1,2] several major obstacles, including the low number of cells available for the treatment of adults, the amount of time needed for engraftment after transplantation, and the lack of large, well-controlled comparative studies on graft-versus-leukemia specificity, need to be overcome.

Since 1994, some transplantation centers, including that at Perugia University in Italy, have reported that allo-HSCT, in which the MHC shows a full haplotype mismatch, gives stable engraftment rates of 97% in the early stages, as well as long-term disease-free survival rates.[3–5] This method is currently used worldwide[6] and, despite the requirement for allo-HSCT, is an important strategy for patients with an unfavorable prognosis who fail to find a donor with concordant MHC characteristics. However, unlike conventional allogeneic transplantation, this method requires a powerful, well-designed conditioning regimen, and high-efficiency T-cell depletion (TCD) of the donor. In addition, stringent infection prevention, with early diagnosis and intensive immunological therapies to overcome the expected severe immunological obstacles, is essential.[7,8] In this system, most patients should be able to

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find donors among their family members; the donor could be a parent, child, or sibling who shares the haplotype with concordance of the MHC phenotype and genotype, while 1–3 HLA antigens usually do not coincide in the other unshared haplotype between the patient and the donor. Therefore, patients who fail to find a donor with concordant HLA may benefit from stem cell sources that range from HLA 1 antigen mismatch to full haplotype mismatch.

In this study, we investigated 11 cases of haplotype mismatched transplantation, in which patients with high-risk AML were subjected to a newly designed conditioning regimen.

Patients and methods

Subjects

In this study, the following 11 adult AML patients were selected: four patients with relapsing and refractory disease, one patient who relapsed after allogeneic sibling-matched transplantation, two patients who relapsed after autologous transplantation but who reached second complete remission (CR), one patient who relapsed early after remission but was successful in attaining second CR, and three patients who were in first CR. Median ages of patients and donors were 34 (16–47) and 27 (12–44) years, respectively, with seven male and four female patients. As for the donors, one was the father, two were sons, and the remaining eight donors were siblings. ABO types of patients and donors were as follows: 9/11 pairs were matched, and the other two pairs were either minor mismatches or major and minor mismatches. In this study, family members were assessed for HLA compatibility by serologic typing. DNA typing has not been performed routinely in this familial donor/recipient pair in the transplant setting. Only the patients were DNA typed for the purpose of searching for HLA matched unrelated donor before enrolment in the haplotype mismatch transplantation program. The HLA type of patients and donors was full haplotype mismatches at HLA-A, B, C, and DR in six cases, two antigen mismatched in two cases, and one antigen mismatched in three cases. Host-versus-graft and graft-versus-host vector directions are shown in Table 1. Four cases were expected to have natural killer cell alloreactivity (NK alloreactivity),[6] having donor versus patient vector (Table 1). Informed consent for donation was obtained from all the donors enrolled in this study, and parents signed the donor consent form if the donor was a child. Donors UPN 1940 (Table 1) and UPN 1908 were chosen from family members due to the presence of NK alloreactivity and the absence of relatives for the recipient, respectively.

Conditioning regimens

Six out of eight cases with more than two mismatched antigens were transplanted with fractionated total body irradiation (TBI) at 1200 cGy for 3 days (D-12–D-10), together with 3.2 mg/kg/day busulfan (Busulfex; Jeil-Kirin Pharmaceutical Inc., Tokyo, Japan) given intravenously (i.v.) for 2–3 days (D-8–D-6), 40 mg/m^2 fludarabine i.v. for 4–5 days (D-9–D-5), and 1.25 mg/kg/day rabbit antithymocyte globulin (SangStat, Lyon, France) i.v. for 4 days (D-5–D-2). The other two cases received 70 mg/kg/day melphalan i.v. for 2 days (D-8, D-7) instead of TBI. In these cases, busulfex was administered between D-12 and D-10. High-risk patients who had received single antigen mismatched transplants received either a TBI regimen that comprised 1320 cGy irradiation or a non-TBI regimen, depending on the specific clinical conditions. Two patients (UPN 1752 and UPN 1988) and one other patient (UPN 1735) received the non-TBI regimen, due to previous exposure to TBI conditioning at the time of their first autologous or allogeneic transplant, respectively. Specifically, most of the patients enrolled in this study, starting with UPN 1760, received the basic therapy of 4 days fludarabine, instead of the 5-day schedule, based on our previous experience of patients who had received trans-

Table 1 Characteristics of the patients and donors, and outcomes of haploidentical transplantation

	Subtype/status	*Sex/age (patient/donor)*	*ABO type: match or mismatch*	*HLA mismatch: HVG/GVH directions*	*Chromosome*	*NK AlloR*	*GvHD*	*Outcome*	*DFS months*
UPN1727	M1,CR1	F/34 M/24	Match	1/0	46 XX,r(p22q36)	—	Chronic	Alive CCR	28
UPN1752	M1,CR2	M/21 M/23	Major and minor	0/1	47 XY, + 21	—	—	Alive CCR	25
UPN1871	M2,Refr	F/47 M/43	Match	1/1	46 XX,t(6;9)	—	—	Died relapse	3
UPN1735	M2,Rel	M/32 M/36	Match	2/2	46 XY	—	NA	Died TRM	1
UPN1908	M4,CR1	F/36 M/12	Minor	2/2	47 XX, + 8	—	—	Alive CCR	20
UPN1730	M5,Refr	M/17 M/44	Match	3/2	46 XY,t(16;21)	+	—	Died relapse	2
UPN1760	M2,Refr	F/30 M/27	Match	3/2	46 XX,t(8;21)	—	—	Died relapse	4
UPN1878	M2,Refr	M/39 M/38	Match	3/3	46 XY,t(9;22), t(6;9),-7, + 9,del(11)	—	—	Died sepsis	8
UPN1940	M4,CR2	M/44 M/14	Match	2/3	46 XY,t(7;11)	+	Chronic	Died NSIP	6
UPN1987	M4,CR1	M/22 M/23	Match	3/3	46 XY,dup(9)	+	—	Alive CCR	5
UPN1988	M2,CR3	M/33 F/27	Match	3/3	46 XY	+	—	Died ARDS	4

HVG = host-versus-graft; GVH = graft-versus-host; NK AlloR = natural killer cell alloreactivity; DFS = disease-free survival; CCR = continuous complete remission; TRM = transplant-related mortality; NSIP = nonspecific interstitial pneumonia; ARDS = adult-respiratory distress syndrome; CR1 = 1st complete remission; CR2 = 2nd complete remission; Refr = refractory; Rel = relapse.

Table 2 Conditioning regimens and GvHD prophylaxis according to the extent of HLA mismatch

HLA mismatch	*Conditioning regimen*	*GvHD prophylaxis*	*G-CSF*
1 antigen			
UPN1727	TBI13.2 + CY2	FK-506 + methotrexate	Yes
UPN1752	Bu4 + CY2	FK-506 + methotrexate	Yes
UPN1871	TBI13.2 + Bu2	FK-506 + methotrexate	Yes
2–3 antigens			
UPN1735	Bu3 + Mel2 + F5 + A4	None	None
UPN1908	TBI12 + Bu3 + F4 + A4	None	None
UPN1730	TBI12 + Bu2 + F5 + A4	None	None
UPN1760,1878,1940,1987	TBI12 + Bu3 + F4 + A4	None	None
UPN1988	Bu3 + Mel2 + F4 + A4	None	None

A = antithymocyte globulin (ATG; Sangstat), A4 = ATG 1.25 mg/kg/day i.v. for 4 days; Bu = busulfex, intravenous busulfan, Bu_3 = busulfex 3.2 mg/kg/day i.v. for 3 days; CY = cyclophosphamide, CY2 = cyclophosphamide 60 mg/kg/day i.v. for 2 days; F = fludarabine, F5 = fludarabine i.v. 40 mg/day for 5 days, F4 = fludarabine i.v. 40 mg/kg/day for 4 days; Mel_2 = melphalan i.v. 70 mg/kg/day for 2 days; TBI = total body irradiation, TBI13.2 = fractionated TBI 1320 cGy for 4 days, TBI12 = fractionated TBI 1200 cGy for 3 days.

plants from donors with more than two mismatched antigens. Table 2 shows the details of the regimens given to each patient according to the different clinical conditions.

Transplantation with high doses of CD34+ hematopoietic stem cells

For patients who had received transplantation with 2–3 antigens mismatched, 10–16 μg/kg recombinant human G-CSF (Grasin; Jeil-Kirin Pharmaceutical) was injected subcutaneously into the donors, in order to obtain high numbers of mobilized peripheral blood mononuclear cells. Highly purified CD34+ cells were prepared using Clini-MACS (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), which removed the T cells. Leukapheresis and Clini-MACS processing were repeated for four consecutive days using the same graft engineering method.

A different method was adopted for patients who had received transplants with one antigen mismatched. Based on our previous experience[9] of high-dose stem cell transplantation, these patients received G-CSF-primed unmanipulated bone marrow (BM) plus peripheral blood CD34+ stem cells (PBSC). Briefly, G-CSF (10 μg/kg/day) was administered subcutaneously to the donors. Two rounds of leukapheresis were performed on days 5 and 6 of G-CSF administration. PBSC preparations were depleted of T lymphocytes using the Clini-MACS system. BM harvesting was performed 48 h after the last leukapheresis at a volume of 15 ml/kg body weight of the recipient. Taking into account the insufficient number of CD34+ cells obtained by this method, we collected the back-up peripheral blood stem cells (PBSCs) during consolidation chemotherapy in all the CR patients.

Graft-versus-host disease prevention, adjuvant therapy, and chimerism

Hematopoietic stem cells were injected for transplantation 24 h after the conditioning regimen, and the day of infusion was designated as D0. None of the eight cases transplanted with more than two mismatched antigens received any agent for the prevention of graft-versus-host disease (GvHD) or any growth factor (including G-CSF). In contrast, the policy of our Center was to use G-CSF and GvHD prophylaxis post transplantation for patients who had received transplants with one mismatched antigen, as shown in Table 2. During transplantation, patients were managed in a laminar air flow room with a HEPA filter; they received sterilized food and oral antibiotics for selective gut decontamination, and they also received prophylactic antiviral and antifungal agents. From the 7th day of transplantation, all patients were given i.v. immunoglobulin at intervals of a week. On the 21st day of transplantation, the patients underwent bone marrow aspirate and trephine biopsy and chimerism of donor and recipient cells in peripheral blood (PB) was assessed for 16 human-specific gene probes, using the total DNA preparation method and real-time polymerase chain reaction (RQ-PCR).

Results

Infused cell dosages and engraftment

The median number of injected CD34+ cells was 15.4×10^6/kg (8–21.2) for all patients, while the median number of CD3+ cells was 1.2×10^4/kg (1.0–2.0) for patients who had received transplants with more than two mismatched antigens, and 7.0×10^7/kg (4.7–8.5) for patients who had received transplants with one mismatched antigen. There were no significant differences in body weight between donors and recipients, except in the two instances where child donors had been used. The final numbers of CD34+ cells from these children were 10.8×10^6/kg and 10.9×10^6/kg, respectively. None of the donors experienced any toxic complications during graft engineering or harvesting. Although two donors showed decreased platelet counts in the range of 50×10^9/l and 100×10^9/l, they successfully completed the protocol without transfusion support.

One patient (UPN 1735) died early at day 17 post transplantation, while the remaining 10 patients showed

stable engraftment on marrow aspirate and trephine biopsies performed on the 21st day of the transplantation. These patients were confirmed to have approximately 96% donor chimerism in the multiplex fluorescent short tandem repeat (STR) analysis using RQ-PCR. The median day at which the number of peripheral blood neutrophils reached a minimum of 500/μl was the 12th day of transplantation (range, 8–16 days), while the median day at which the number of platelets reached at least 20 000/μl for three consecutive days without transfusion was the 13th day of transplantation (range, 10–19 days). The median percentage of donor chimerism 100 days after transplantation was 98% (range, 97.7–99%) by RQ-PCR. To date, all of the surviving patients have approximately 99% donor chimerism. No engraftment failures occurred in this study.

GvHD

None of the patients in this study showed acute GvHD, and eight of the nine patients who were followed up for more than 3 months after transplantation showed no chronic GvHD. One patient (UPN 1727), who had received an HLA-DR 1 antigen mismatch transplant and developed chronic hepatic GvHD 6 months post transplantation, responded very well to conventional corticosteroid-pulse therapy.

Early complications associated with transplantation

All 10 subjects showed favorable PB cell recovery at an early stage, without severe infectious complications, and were discharged after a median stay of 35 days (range, 28–69 days). However, infection monitoring, based on standard infection prevention protocols after transplantation in our Center, showed that nine patients were positive in the CMV antigenemia assay, in which PB leukocytes were considered the targets, at a median of 34 days (range, 14–69). All of these patients received ganciclovir i.v. and/or typical CMV-specific immunoglobulin i.v. weekly (for patients who received transplants with 2–3 mismatched antigens) after transplantation, and were observed without increases in antigenicity. Two patients (UPN 1727 and UPN 1878), who converted on at least two consecutive occasions to negative status after treatment for 2–3 weeks, were discharged and followed up. The most common early complication associated with transplantation was mucositis, and all (10/10, 100%) subjects showed severe pharyngolaryngitis or enteritis (grade 3 or higher), which was successfully treated by the standard supportive therapies of the Center. All of the patients, with the exception of one patient who died early at day 17 post transplantation from complications of old pneumonic problems and poor performance status, tolerated the transplantation procedure. We did not experience any regimen-related complications, such as veno-occlusive disease or toxic organ dysfunction.

Outcome of transplantation

At a median of 6 months (range, 17 days–28 months), four out of the 11 patients were alive. Three out of seven

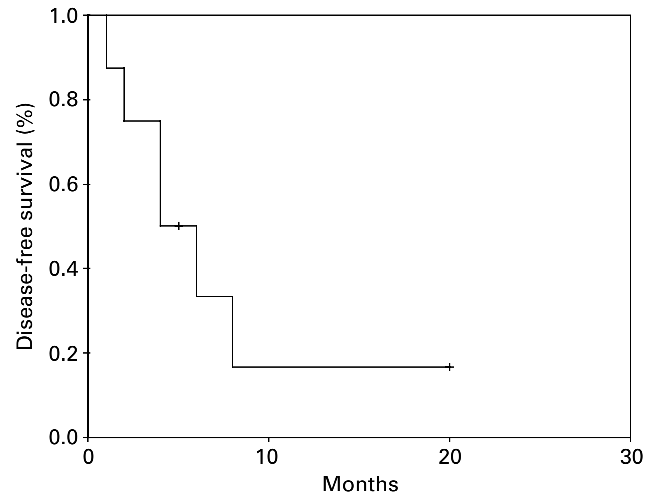

Figure 1 Kaplan–Meier estimates of survival probability for all patients in the 2–3 mismatched antigens transplantation group (17% at 8 months, 95% CI 2–32). The marks on the survival curves represent censoring points.

patients with relapsed high-risk and refractory AML who had received haplotype mismatched transplants finally relapsed on the 54th (UPN 1730), 89th (UPN 1871), and 114th (UPN 1760) days after transplantation. Four cases who were expected to be positive for NK alloreactivity, one of whom was refractory before transplantation, still experienced recurrence. Patient UPN 1940, who was transplanted in second complete remission and who was expected to be positive for NK alloreactivity; patient UPN 1878, who was negative for NK alloreactivity and transplanted during partial remission; and patient UPN 1988, who was positive for NK alloreactivity and who was transplanted in CR3, were all in complete remission at the median time point of 5 months (range, 4–25 months) post transplantation, and showed stable engraftment without transfusion. In brief, 2/8 patients survived who had received transplants with two or three mismatched antigens, and the estimated disease-free survival rate at 8 months was 17% (Figure 1). Of the remaining patients who had received transplants with one mismatched antigen, 2/3 survived (no survival curve due to insufficient sample size).

Discussion

In this study, we successfully performed haplotype mismatched transplantation, including full haplotype mismatched transplantation, using our modified conditioning regimens, for high-risk AML patients. Three patients underwent transplantation with one mismatched antigen using a totally different scheme. They received conditioning with a combination of GvHD prophylaxis and post transplantation G-CSF. As mentioned before, they received high doses of CD34+ cells after partial depletion of T cells, achieved by infusion of G-CSF-primed unmanipulated BM cells, together with Clini-MACS-processed peripheral blood CD34+ cells.[9] In contrast, we designed a new conditioning regimen for patients who received 2–3 antigens mismatched transplants. Most importantly, frac-

tionated TBI (1200 cGy in total) was given for 3 days, and busulfan was administered i.v. for 2–3 days. A combination of fludarabine and rabbit antithymocyte globulin was used for the crucial inhibition of immunological function. The transplantation of CD34 + cells occurred at a concentration (median: 15.4×10^6/kg) much higher than that used in conventional transplantation, and T-cell removal was performed successfully using Clini-MACS. Not only those patients who had received HLA 1 antigen mismatch transplantation but also those patients who had received transplants with 2–3 mismatched antigens, which includes all 10 evaluable patients, showed perfect primary engraftment, no acute GvHD, and no transplant-related mortality, including fatal infections, within 100 days of transplantation. In this small study, the estimated disease-free survival rate for the latter group of patients ($N = 8$) was 17%. Therefore, although this study contained few subjects, we believe that cautious enrolment of high-risk patients is warranted in the future.

As for graft failure, the initial study in Perugia reported that the rate of primary engraftment was only 80%, and that many of the subjects needed additional transplantation of hematopoietic stem cells prior to 1995.[3] Since 1998, the Clini-MACS system has been used successfully and widely.[4,5] Following the results of the Perugia researchers and those of Reisner, the role of veto cells, which are included in the transplantation of high-dose CD34 + cells in order to overcome HLA problems, has attracted considerable interest.[7] Some studies have reported that the amount of CD34 + cells required for this method is 10-fold higher than that used, typically, in allogeneic transplantation between siblings.[8] Moreover, the method requires the reduction of T-cell numbers to $< 1 \times 10^4$/kg, in order to prevent GvHD. Recent studies by the above-mentioned researchers on ISH 2002[10] and IBMTR 2003[11] show remarkable potential: the rate of primary engraftment was 96% or higher. In addition, the rate of acute GvHD of grade 2 or higher was only 10%, with a similar rate for chronic GvHD. With respect to high-risk acute leukemia, we believe that the excessive suppression of GvHD may reduce the effect of graft-versus-leukemia. In our studies, none of the patients who had received two- and three-loci mismatch transplants developed acute GvHD, and the two cases with refractory AML showed no primary acute GvHD. They did develop secondary acute GvHD, because of donor lymphocyte transfusion. In this study, we performed PB cell processing using Clini-MACS for four consecutive days, in order to obtain high doses of mobilized PB CD34 + cells from donors who had been stimulated with G-CSF. Thus, we were able to transplant a median of 15.4×10^6 cells/kg, and all the subjects showed stable primary engraftment. Owing to our lack of experience, we established the above-mentioned high number of CD34 + cells as the primary goal for stable engraftment. Despite the high cost, our initial results suggest that our strategy is superior to existing and previously employed approaches, in terms of the rates of primary engraftment and acute GvHD.

The success of our strategy may be ascribed to the potentially greater homogeneity of minor histocompatibility antigens among Koreans, as compared to other Asian populations, if we consider the presence of ethnic variations. Therefore, the data in this study, showing a relatively lower rate of acute GvHD after haplotype mismatch transplantation, form a basis for population genetics and disease association studies in Korea.[12] Therefore, conditioning regimens in which consideration is given to genetic differences and racial specificity should be evaluated in a larger patient population.

The most serious and, as yet, unsolved problems in high-level full haplotype mismatch transplantation involve the various complications that are induced by dysfunctional recovery of immunological function. While the number of PB CD3 + cells recovered from children reached 200/μl over 3–4 months post transplantation, the corresponding number recovered from adult patients showed long-term recovery failure (6 months to more than 1 year). This type of delay led to extremely severe infectious complications after transplantation, and about 50% of the deaths associated with transplantation were due to severe fungal or CMV infections.[4] In addition, most of the deaths occurred in refractory and relapsed patients suffering from long-term immunological dysfunction. Follow-up evaluation of CD3/CD4 + cell recovery post transplantation showed that all patients had delayed recovery of lymphocytes, with between 50 and 100 cells/μl 3 months after transplantation (the results of the follow-up are not presented in this study). We therefore recommend that all transplant patients receive stringent infection prevention measures (such as those used at our Center) after transplantation. Fortunately, in this study, there were no severe complications linked to fungal or viral infections within 100 days of transplantation. Studies concentrating on accelerating restoration of immunological function using dendritic cells, and methods that enhance immune reconstitution,[13,14] involving interleukin-7, or keratinocyte growth factor, are under way in several laboratories. The donor-derived graft-versus-leukemia effect, which is expected in HLA mismatch transplantation between family members, is reported to be largely dependent on tolerance between donor and patient cells with respect to the killer cell immunoglobulin-like receptor (KIR) in the context of GvHD-directed allogeneic donor NK cells. This important immunological mechanism for the eradication of leukemia is being studied by researchers at Perugia University.[15,16] We have also attempted to use donor-derived NK alloreactivity (based on the guidelines of the Perugia group), which determines the type of NK alloreaction, based on the receptor-ligand model.[6,15,16] However, we failed to remove the clones of refractory AML cells in patient UPN 1730, despite an apparently positive response. Interestingly, although complete primary engraftment was confirmed on the first marrow examination on the 21st day of transplantation, the disease recurred on the 54th day in this patient. In this study, we found only one case of Bw4 mismatch and three cases of C-locus mismatch in the donor-versus-host direction. Based on the above results, further studies should focus on comparative analysis of NK alloreactivity, as well as on the early and efficient recovery of donor-derived T-cell numbers and their immunological functions, which would further molecular research on the immune escape mechanisms of leukemia cells.

Results of cytogenetic testing on the 11 subjects with AML show that one patient had typically favorable-risk characteristics with t(8;21).[17] This patient received HLA full haplotype mismatch transplantation in relapse but died of recurrence 4 months after short-term CR. Furthermore, other patients who were categorized as intermediate-risk or extremely-unfavorable-risk leukemia cases (UPN 1871 and UPN 1878) showed promising results, as they were generally in CR for 5–6 months post transplantation, showed stable engraftment, and had remarkably low levels of markers for quantitative residual leukemia, for example, the bcr-abl gene, specifically for UPN 1878. Concerning the significance of the noninherited maternal HLA antigen (NIMA) or feto-maternal microchimerism in haplotype mismatch transplantation,[18,19] we did not perform PCR-SSP to reveal the presence or absence of NIMA mismatch between donors and recipients. However, based on the HLA serotyping results, there were two NIMA mismatches in the patients who had received transplants with more than two mismatched antigens. Thus, further studies involving more accurate analyses and greater data accumulation are needed to interpret the association with outcome.

In conclusion, the merits of genetically mismatched transplantation between family members have been highlighted. High-level MHC mismatch transplantation may be applied widely in clinical practice, provided that some of the drawbacks, such as delayed recovery of immunological function, are overcome. This improved transplantation method could be used widely, as most families include parents and siblings, despite the trend toward the nuclear family, and parents and children could act as donors for each other. Therefore, prospective studies into the appropriate timing of transplantation based on various disease and clinical factors, and clinical trials to investigate the most appropriate schedules for different races, are needed for high-risk AML as well as for other lymphohematopoietic malignant diseases.

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