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Megadose CD34+ Hemopoietic Stem Cell Transplantation for Patients with High Risk Acute Myeloid Leukemia Who Have No HLA Matched Donor

- A Pilot Study of a Full Haplotype Mismatch Transplantation -

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Background : Haploidentical transplantation has become a popular modality of treatment for acute myeloid leukemia (AML) patients lacking donors with matching HLA. We attempted to assess the success rate and ramifications of full haplotype mismatch transplantation.

Methods : Four patients received stem cell transplantation from their full haplotype mismatched family donors. The conditioning regimen included total-body irradiation, intravenous busulfan, antithymocyte globulin, and fludarabine. Megadose CD34+ stem cell transplants were performed, in a dosage range between 10.9×10^6/kg and 20.6×10^6/kg. Neither GvHD prophylaxis nor post-transplant G-CSF were administered. We monitored patients' bone marrow cellularity and peripheral blood chimerism using real-time PCR.

Results : All patients evidenced stable engraftment. The most frequent side effect was severe mucositis, but all patients recovered successfully, without early death. No patients exhibited acute GvHD. Two refractory patients relapsed soon after transplantation. The other 2 patients have remained in good clinical condition, with a follow-up duration of 1～4 months.

Conclusion : Using a newly-developed conditioning regimen, we were able to circumvent GvHD and graft failure, which are the main limitations associated with full haplotype mismatch transplantation. According to our analysis of the relevant literature, it appears that this is the first report of such a conditioning regimen.

Key Words : Full haplotype mismatch transplantation, High risk AML, GvHD, Graft failure

INTRODUCTION

Since the world-first allogeneic hematopoietic stem cell transplantation (allo-HSCT) was successfully performed in late 1960s, such transplantation could be conducted for malignant lymphohematopoietic diseases only when the human leukocyte antigens (HLA) were fully matched with respect to the major histocompatibility complex (MHC). The therapeutic principle most normally applied for adult AML patients with either unfavorable- or standard-risk cytogenetic characteristics is intensified therapy performed after a first remission induction, a method for which allo-HSCT is most effective with respect to long-term survival. However, in reality, the probability of finding a sibling donor whose MHC perfectly matches that of the patient is only 20～30%, and may be becoming even less likely, due to the recent trend towards nuclear families. In addition, it

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Table 1. Clinical characteristics of the study population

	Sex/Age Patient	Sex/Age Donor	Relation, HLA mismatch	Diagnosis, Status at transplant*	Cytogenetics, FISH†	IP‡	NK Allore-activity
UPN 1730	M/17	M/44	Father, Full Haplotype	AML-M5a Refractory	46 XY, t(16;21)	CD34+	+
UPN 1760	F/30	M/27	Brother, Full Haplotype	AML-M2 Refractory	46 XX, t(8;21) AML/ETO+	CD34+	-
UPN 1878	M/39	M/38	Brother, Full Haplotype	AML-M2 Refractory	46 XY, t(9;22), t(6;9), -7, +9, del(11)	CD34+	-
UPN 1940	M/44	M/14	Son, Full Haplotype	AML-M4 CR2	46 XY, t(7;11)	CD34+	+

* : CR2=second complete remission after relapse
† : fluorescent in situ hybridization
‡ : immunophenotype

has been estimated that at least 50% of domestic patients fail to find non-family donors, even when various kinds of domestic & foreign donor searching programs are employed. This sort of search requires a great deal of time, and some of the patients in remission suffer a recurrence or die while waiting for a suitable transplant donor. Also, some donors are reluctant to donate and some completely refuse to become donors at all. Meanwhile, allogeneic transplantation using cord blood is being studied in child patients, but several limitations are associated with this practice: the relevant cell amount is insufficient for adults, the engraftment requires a great deal of time after transplantation, and there remains a dearth of information regarding graft-versus-leukemia specificity.

Since 1994, some transplantation centers, most notably Perugia University in Italy, have reported that allo-HSCT in which the MHC exhibits a full haplotype mismatch, results in a fairly stable engraftment rate (97% in early stages) and a long-term disease-free survival rate[1-3]. This method is currently being used worldwide[4], and is expected to serve as an important treatment for those with unfavorable prognoses, and those who have failed to find donors with concordant MHC, despite the necessity of allo-HSCT. However, unlike conventional allogeneic transplantation, this method requires a powerful and well-designed conditioning regimen, highly-qualified T-cell depletion (TCD) in the donors, thorough prevention of infectious agents coupled with early diagnosis techniques, and a variety of new intensified immunological therapies, in order to transcend the likely severe immunologic obstacles involved[5, 6]. Most of the patients will then be able to find donors among their family members: the donors will ideally be normal family members, including parents, children, and siblings, and share haplotype with the patient, exhibiting concordance with respect to MHC phenotype and genotype. Also, 1~3 HLA should not coincide in the other unshared halotype between the patient and the donor. Therefore, patients who fail to find donors with concordant HLA profiles may receive various stem cell sources, from HLA 1 antigen mismatches to full haplotype mismatches, in order to achieve a cure.

In this study, the authors evaluated the results and problems associated with 4 cases of full haplotype mismatched transplantations in which the patients, all with high-risk adult AML, underwent our newly-designed conditioning regimen, and considered the future development of this method.

MATERIALS AND METHODS

Subjects

In this study, 4 patients with CD34+ adult AML were selected as subjects. Three of them were relapsing and refractory, and the last relapsed early after remission, but was successful in the induction of secondary remission. The median age of both the patients and donors were 34.5 (16~44) and 32.5 (14~44), respectively, and three of the patients were males and the last was female. As to the donors, one was a father, one was a son, and the remaining two were siblings of the respective patients. The HLA profiles of the patients and the donors were full haplotype mismatch transplantation in HLA-A, B, DR, in all four cases. Meanwhile, two of the cases were expected to exhibit natural killer cell alloreactivity (NK alloreactivity)[4] with regard to donor-versus patient vector (Table 1).

Conditioning regimen

All four cases underwent total body irradiation (TBI) (fractionated

Table 2. Conditioning regimens, GvHD prophylaxis, G-CSF usage and outcome of patients enrolled in this study

	Cond Regim*	GvHD† prophylaxis	G-CSF‡	RRT/ TRM§	Engraft‖	Acute GvHD	Chronic GvHD	DFS¶, Month	Survival**
UPN 1730	T12+B2+ F5+A5	-	-	mucositis 3	complete	-	NA	2	dead, relapse
UPN 1760	T12+B3+ F4+A4	-	-	mucositis 4	complete	-	-	4	dead, relapse
UPN 1878	T12+B3+F4+A4	-	-	mucositis 3	complete	-	-	>4	alive in CR
UPN 1940	T12+B3+F4+A4	-	-	mucositis 3	complete	-	NA	>1	alive in CR

*, conditioning regimen; A, antithymocyte globulin (ATG;Sangstat), A4=ATG 1.25 mg/kg/day i.v. for 4 days; B, busulfex, intravenous busulfan, B3=busulfex 3.2 mg/kg/day i.v. for 3 days; C, cyclophosphamide, C_2=cyclophosphamide 60 mg/kg/day i.v. for 2 days; F, fludarabine, F4=fludarabine i.v. 40 mg/kg/day for 4 days; M, melphalan, M_2=melphalan i.v. 70 mg/kg/day for 2 days; T, total body irradiation (TBI), $T_{13.2}$=fractionated TBI 1,320 cGy for 4 days, T_{12}=fractionated TBI 1,200 cGy for 3 days; †, graft-versus-host disease; ‡, granulocyte-colony stimulating factor; §, regimen-related toxicity/transplant-related mortality; ‖, engraftment; ¶, disease-free survival; **, complete remission

irradiation) and received an intravenous combination of busulfan (Busulfex™, Jeil-Kirin Pharm, Inc), fludarabine, rabbit antithymocyte globulin (Sangstat, France) (Table 2).

Transplantation of high-dose CD34+ hematopoietic stem cells

Recombinant human G-CSF (Grasin®, Jeil Pharm, Inc) was subcutaneously injected into donors at a concentration of 16 ug/kg in order to obtain a large number of mobilized peripheral blood mononuclear cells. The obtained cells were separated from highly purified CD34+ cells using the Clini-MACS® system (Miltenyi Biotec Co., Germany) and sufficient T-cells were removed. The median number of injected CD34+ cells was 16.5 $\times 10^6$/kg (10.9~20.6), while the median number of CD3+ cells was 1.4×10^4/kg (1.0~2.0) (Table 3, Figure 1).

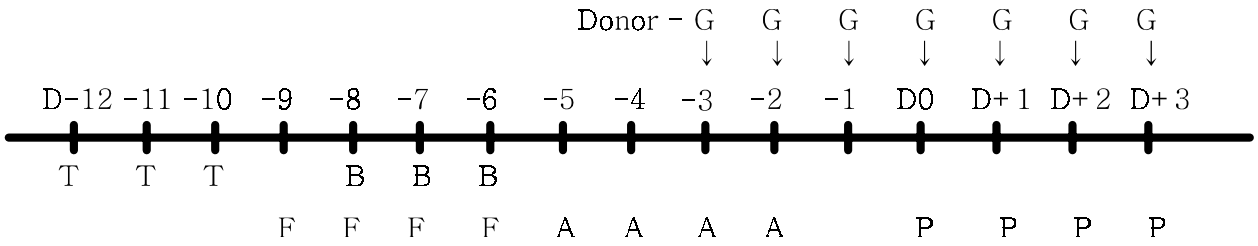
Prevention and adjuvant therapy of graft-versus- host disease

The hematopoietic stem cells were injected 24 hours after the conditioning regimen for transplantation, and the day of the infusion was designated as D0. None of the patients had been treated with any agents for preventing graft-versus-host disease (GvHD), nor were they treated with any growth factors, including G-CSF. During transplantation, the patients were managed in a laminar air flow room with a HEPA filter, given sterilized food and oral antibiotics for selective gut decontamination, and treated with antiviral and antifungal agents of the Center standard, for prevention. Beginning on the 7th day after transplantation, all patients were administered i.v. immunoglobulin at weekly intervals. On the 21st day after transplantation, the patients were examined for chimerism in the peripheral blood (PB) of both donor and patient cells for 16 human-specific gene probes, using the findings of bone marrow aspirate smears & histological examinations, and real-time polymerase chain reaction.

Table 3. Graft engineering data for patients who needed depletion of donor T-cells before transplantation

	MNCs*, $\times 10^8$/kg	CD34+ cells, $\times 10^6$/kg	CD3+ cells, $\times 10^4$/kg	CMV† Antigenemia Assay	Recovery of ANC‡ >500/uL	Recovery of platelet, >20,000/uL
UPN 1730	25.4	20.6	1.6	+	D+12	D+13
UPN 1760	28.4	17.6	1.6	+	D+11	D+13
UPN 1878	23.6	15.4	1.2	+/-	D+12	D+15
UPN 1940	17.8	10.9	2	+/-	D+10	D+13

*, mononuclear cells
†, cytomegalovirus
‡, absolute neutrophil count



A, ATG (rabbit, Sangstat), 1.25 mg/kg/d for 4 days
B, intravenous busulfan, 3.2 mg/kg/day for 3 days
F, 40 mg/m^2 IV for 4 days (total 160 mg/m^2)
G, G-CSF subcutaneously (16 ug/kg/day for 7 days) to graft donor and then processed MNCs using Clini-MACS^R system to purify CD34+ cells
P, mobilized peripheral blood CD34+ cells
T, total 12 Gy for 3 days, fractionated with lung shielding

Figure 1. One representative pretransplantation conditioning regimen and donor stem cell infusion after processing using CD34+ cell selection system

RESULTS

Engraftment

All the patients exhibited stable engraftment upon marrow aspiration tests and biopsy performed on the 21st day after transplantation, and around 96% of donor chimera was confirmed by multiplex fluorescent short tandem repeat (STR) analysis, using real-time polymerase chain reaction (Table 2). The median day at which the number of peripheral blood neutrophils reached at least 500/uL was the 11.5th day after transplantation (10～12 days), while the median day at which the number of platelets reached at least 20,000/uL for three consecutive days without transfusion was the 13th day after transplantation (12～15 days).

GvHD

None of the patients in this study exhibited acute GvHD, and the two patients who could be followed up for more than three months since the transplantation have not evidenced chronic GvHD.

Complications associated with transplantation

According to monitoring of infections by various tools based on our Center's protocol for standard infection prevention after transplantation, three cases were found to be positive on a CMV antigenemia assay, in which PB leukocytes were the designated targets, two cases received i.v. ganciclovir, and the remaining patient was treated with typical CMV specific immunoglobulin after transplantation, and exhibited no increases in antigenicity (Table 3). The two cases receiving therapeutic medication were converted to negative at least two consecutive times after administration for 2～3 weeks, and were then discharged and followed up. All four subjects exhibited early favorable recovery of PB cells, without severe infectious complications, and were discharged on the median 35th day (32～41). Mucositis was the most common early complication associated with transplantation, and all four subjects developed either severe oral pharyngolaryngitis (at least grade 3) or enteritis, which were successfully treated by the standard supportive therapies of the Center.

Results of Transplantation

As of the median 94th day (26～131), two out of the four patients had survived. The two patients with relapse high-risk & refractory AML who had undergone full haplotype mismatch transplantation finally relapsed at the 54th and 89th days after transplantation, respectively. There were two cases suspected to be positive with regard to NK alloreactivity, and one of them was refractory, before the transplantation evidenced recurrence. Presently, one of these transplantation patients is in a second complete remission, and is expected to be positive with regard to NK alloreactivity. The other, who is negative with regard to NK alloreactivity, and was transplanted while in incomplete remission, are now in total remission and clinically stable without transfusion, and the chimera with donors' cells after transplantation was measured to be 97.7% and 99%, respectively.

DISCUSSION

In this study, the authors successfully performed a full haplotype mismatch transplantation in order to evaluate the merits and demerits of the transplantation, and to design an active method of transplantation for cases of high-risk AML.

Because thiotepa agent production, which was considered essential for the performance of the necessary intensified conditioning regimen, was halted worldwide, it was necessary to adjust the existing conditioning regimen. Also, the method of single dose highly-intensified TBI (800～850 cGy) was harder to perform in domestic circumstances than the old method of fractionated irradiation and, therefore, considerable complementation & review were required. Therefore, it became necessary to establish a conditioning regimen for high-level full haplotype mismatch transplantation specific to domestic patients. The authors fractionally irradiated 1,200 cGy as TBI for three days, and administered the newly-developed i.v. busulfan for 2～3 days, instead of thiotepa, in order to remove residual AML cells. Fludarabine and rabbit antithymocyte globulin (Sangstat) were used for the essential inhibition of immunological functions (Figure 1). The transplantation of CD34+ cells (median: 16.5×10^6/kg) in amounts far greater than in conventional transplantation, and the removal of T-cells, were successfully performed. All four of the patients exhibited perfect primary engraftment, and no acute GvHD, including fatal initial infection, was observed.

Generally speaking, the problems expected in high-level HLA mismatch transplantations included (1) graft failure by rejection in the early stages of transplantation (2) severe acute graft-versus-host response and (3) major infections in severe immunosuppressive states after engraftment. As to graft failure, the study in Perugia reported that the rate of primary engraftment was only 80%, and then many of the subjects required additional transplantation of hematopoietic stem cells before 1995[1]. A method for the transplantation of CD34+ cells and the removal of T-cells has been developed, and, since 1998, the existing method of Clini-MACS^R purified CD34+ cells has been used widely[2, 3]. Considering the results of the studies by Perugia researchers and by Reisner, the role of veto cells included in the transplantation of high-dose CD34+ cells in order to overcome HLAbecame the center of researchers efforts, due to its expectation as an obstacle[5]. Some studies reported that the necessary amount of CD34+ cells in this method was increased by ten times that of typical allogeneic transplantation between siblings[6]. Meanwhile, this method required the removal of T-cells, even at concentrations of 1×10^4/kg or less, in order to sufficiently guard against graft-versus-host response. Fortunately, when the Clini-MACS^R or Isolex^R systems were used, CD34+ cells sufficient for successful transplantation were obtained, and a considerably small amount of T-cells were transplanted, via a one or two-step procedure. Recent studies reported by the above-mentioned researchers on ISH 2002[7] and IBMTR 2003[8] were remarkably hopeful: the primary engraftment rate was mostly 96% or over. In this study, we performed cell processing using Clini-MACS^R for four consecutive days, in order to obtain the PB CD34+ cells of the donors mobilized and stimulated by G-CSF through the same method, and were able to transplant 16.5×10^6/kg (median value), and therefore, all subjects exhibited stable primary engraftment. Due to insufficient experience, the authors established the above-mentioned number of CD34+ cells for stable engraftment as the primary aim. The initial results obtained, despite the economical charge, might be superior to those obtained by existing foreign studies with respect to primary engraftment and acute GvHD rates, indicating the need for further studies on a larger number of subjects.

In allogeneic hematopoietic stem cell transplantation between siblings or strangers, the more the mismatch of HLA is, the more the expected rate of acute GvHD increases. Therefore, existing protocols for HLA full haplotype mismatch transplantation cannot succeed with only conventional immunosuppressive therapy and conditioning regimens. Reisner et al. attempted to transcend these limitations through animal transplantation in the early 1990s, and demonstrated successful results using high-dose CD34+ cells and a highly-intensified regimen of conditioning & immunosuppressants[9]. The researchers at Perugia University have suggested the possibility of clinical introduction of this method in several studies since 1993. Other western researchers have also attempted this method, and in 2000, they came to a consensus on the method. Recently, researchers all over the world are investigating the possibility of clinical application of this method[4]. Existing reports have indicated four-agent therapy as a suitable choice for the intensified conditioning regimen, including one high-dose TBI (800～850 cGy), cyclophosphamide (100 mg/kg), thiotepa (10 mg/kg), and antithymocyte globulin (Fresenius) (5 mg/kg/day), but for adult patients, fludarabine has been replaced with cyclophosphamide, since 1995. For children, German researchers reported a conditioning regimen in which only OKT3 and drugs were used, without irradiation, and the results were similar to those of adults[6] According to those reports, the rate of acute GvHD of at least grade 2 was within only 10%, and the rate of chronic GvHD was similar to the former. Recently, with regard to high-risk acute leukemia, it may be that the aggressive prevention of GvHD can induce a decline in graft-versus-leukemia complications. In our experience, none of the patients on whom 2- & 3-loci mismatch transplantation were performed exhibited acute GvHD, and the two cases with refractory AML evidenced no primary acute GvHD, with the exception of secondary acute GvHD via the transfusion of donor's lymphocytes, though the follow-up period after transplantation was short. Therefore, our noble conditioning regimen, in which genetic purification and racial specificity were considered, will be necessary to prospective studies in the future, as well as clinical experiences with large sample numbers.

The most severe and unsolved problem is that of the various complications induced by the dysfunctional recovery of immunologic functions, which can be excessively delayed by intensified immunosuppressive therapy in high-level full haplotype mismatch transplantation. While the number of PB CD3+ cells of children was recovered, achieving levels of 200/uL and over 3~4 months after transplantation, that of adult patients showed long-term recovery failure (6 months~1 year). Such delays led to extremely severe infectious complications after transplantation, and about half of the fatality associated with transplantation was induced by severe fungal or cytomegaloviral infectious complications, with the exception of recurrence[2]. Also, most of the dead were refractory & relapsing acute leukemics, and fatal results ensued when their immunologic functions were disabled for long periods of time, due to major organ complications associated with intensive chemical treatment. The follow-up for recovery of CD3/CD4+ cells after transplantation revealed that all patients were delayed in terms of numerical recovery in the cells between 50 and 100/uL, three months after transplantation (the results of the follow-up were not presented in this study). Therefore, in this study, all the patients required infective prevention after transplantation, based on Center standards, and fortunately, exhibited no severe fungal or viral infectious complications. Further studies are necessary in order to develop an immunoregulatory therapy which intensifies humoral immunologic functions, and a non-specific but essential and thymic education course during immunologic reorganization after allogeneic transplantation. Researchers are now studying the intensification of immunologic function via the use of dendritic cells or methods for enhancing immune reconstitution[10, 11] by infection prophylaxis, interleukin-7, or keratinocyte growth factor. The complications induced by the abnormal proliferation of B-cells, in turn caused by the removal of intensified T-cells and the concomitant delay in the recovery of immunologic function may occur, but the onset of complications is reported to be rare, because a sufficient amount of B-cells and antigen- presenting cells are also removed when the T-cells are removed with Clini-MACS^{R} system[1, 2].

The donor-derived graft-versus-leukemia effect expected in HLA mismatch transplantations between family members is reported to be dependent on the tolerance between donor and patient cells of the killer cell immunoglobulin-like receptor (KIR) originated from the donor. This is the most important immunological mechanism for the irradiation of leukemia mainly studied by the researchers at Perugia University[12, 13]. The authors in this study attempted to use donor-oriented NK alloreactivity, but failed to remove the refractory AML cell clones in UPN1730, despite the presence of an apparent positive response. Therefore, complete engraftment was confirmed in the first marrow examination on the 21st day after transplantation, but disease recurred on the 54th day. Based on these results, further studies should focus on comparative analyses of NK alloreactivity, as well as the early sufficient recovery of donor-oriented T-cell immunological functions, and diversified molecular research regarding the immune escape mechanism exploited by leukemia cells.

Meanwhile, the results of cytogenetic tests on the four subjects with AML in this study revealed that one patient exhibited typically favorable-risk characteristics, having t(8;21)[14]. The patient underwent HLA full haplotype mismatch transplantation, but died of recurrence on the 89th day after short-term complete remission. The other two patients were intermediate-risk leukemia patients, based on the assessment of cytogenetic abnormality observed in each patient, as seen in Table 1, were combined as UPN 1878, and had extremely unfavorable prognoses. However, they were considered to be hopeful results, as they remained generally favorable for four months after transplantation, showed stable engraftment, and were remarkably low in terms of targets in the molecular genetic analysis of quantitative residual leukemia on the bcr-abl gene.

Despite the small subject number, the results of this study demonstrated some improvements when compared to those of studies reported when high-level MHC mismatch transplantations were rarely considered, and constitute a good example of clinical treatment for patients with AML who need allogeneic transplantation but are unable to receive active treatment, due to the absence of donors.

In conclusion, the merits of genetic mismatch transplantation between family members, as a diversified development in modern medical science in the 21st century, are in the limelight, and high-level MHC mismatch transplantation may yet become a widely applied clinical modality if some severe demerits, such as the delayed recovery of immunologic function, can be prospectively improved. Such improved transplantation methods can be widely adopted, as most families, despite the trend towards the nuclear family, are still composed of at least parents and siblings, and parents and children can be donors for each other. Therefore, further prospective studies are necessary in order to delineate appropriate transplantation periods, based on various diseases and clinical factors, while clinical trials are needed in the investigation of congenital juvenile diseases, and other lymphohematopoietic malignant diseases, as well as high-risk AML.

REFERENCES

1) Aversa F, Tabilio A, Terenzi A, Velardi A, Falzetti F, Giannoni C, Iacucci R, Zei T, Martelli MP, Gambelunghe C, Rossetti M, Caputo

P, Latini P, Aristei C, Raymondi C, Reisner Y, Martelli M. *Successful engraftment of T-cell-depleted haploidentical Three-loci incompatible transplants in leukemia patients by addition of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells to bone marrow inoculum. Blood 84:3948-3955, 1994*

2) Aversa F, Tabilio A, Velardi A, Cunningham I, Terenzi A, Falzetti F, Ruggeri L, Barbabietola G, Aristei C, Latini P, Reisner Y, Martelli M. *Treatment of high-risk acute leukemia with T-cell-depleted stem cells from relapsed donors with one fully mismatched HLA haplotype. N Engl J Med 339:1186-1193, 1998*
3) Aversa F, Terenzi A, Carotti A, Felicini R, Jacucci R, Zei T, Latini P, Aristei C, Santucci A, Martelli MP, Cunningham I, Reisner Y, Martelli MF. *Improved outcome with T-cell-depleted bone marrow transplantation for acute leukemia. J Clin Oncol 17:1545-1550, 1999*
4) Martelli MF, Reisner Y. *Haploidentical 'megadose' CD34+ cell transplants for patients with acute leukemia. Leukemia 16:404-405, 2002*
5) Rachamim N, Gan J, Segall H, Krauthgamer R, Marcus H, Berrebi A, Martelli M, Reisner Y. *Tolerance induction by "megadose" hematopoietic transplants. Transplantation 65:1386-1393, 1998*
6) Handgretinger R, Schumm M, Lang P, Greil J, Reiter A, Bader P, Niethammer D, Klingebiel T. *Transplantation of megadoses of purified haploidentical stem cells. Ann N Y Acad Sci 872:351-361, 1999*
7) Aversa F, Terenzi A, Felicini R, Carotti A, Falcinelli F, Tabilio A, Velardi A, Martelli MF. *Haploidentical stem cell transplantation for acute leukemia. Int J Hematol 76(Suppl I):165-168, 2002*
8) Aversa F, Reisner Y, Martelli MF. *Hematopoietic stem cell transplantation from alternative sources in adults with high-risk acute leukemia. Blood Cells Mol Dis 33:294-302, 2004*
9) Bachar-Lustig E, Rachamim N, Li HW, Lan F, Reisner Y. *Megadose of T cell-depleted bone marrow overcomes MHC barriers in sublethally irradiated mice. Nat Med 1:1268-1273, 1995*
10) Fry TJ, Mackall CL. *Interleukin-7: from bench to clinic. Blood 99:3892-3904, 2002*
11) Min D, Taylor PA, Panoskaltsis-Mortari A, Chung B, Danilenko DM, Farrell C, Lacey DL, Blazar BR, Weinberg KI. *Protection from thymic epithelial cell injury by keratinocyte growth factor: a new approach to improve thymic and peripheral T-cell reconstitution after bone marrow transplantation. Blood 99:4592-4600, 2002*
12) Ruggeri L, Capanni M, Volpi I, Tosti A, Perruccio K, Urbani E, Negrin RS, Martelli MF, Velardi A. *Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood 94:333-339, 1999*
13) Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, Posati S, Rogaia D, Frassoni F, Aversa F, Martelli MF, Velardi A. *Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science 295:2097-2100, 2002*
14) Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, Mohamed A, Paietta E, Willman CL, Head DR, Rowe JM, Forman SJ, Appelbaum FR. *Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group study. Blood 96:4075-4083, 2000*