

Stem Cells Micro-transplantation in Elderly Patients Aged Over 70 With Acute Myeloid Leukemia: a Multicenter, Prospective, Non-interventional Study

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Abstract

Background

The treatment outcomes of elderly patients aged over 70 with acute myeloid leukemia (AML) have been very disappointing. In comparison, our designed HLA-mismatched hematopoietic stem cell micro-transplantation (MST) has achieved such encouraging treatment results in AML patients as might warrant further investigations of the outcomes of MST for the above mentioned patients.

Methods

One hundred and eleven patients aged 70-88 years were enrolled. Eighty patients were assigned to the high-risk MST or standard MST group according to high-risk prognostic factors. The other thirty-one patients were assigned to either the chemotherapy group or support group. After receiving induction chemotherapy with cytarabine and anthracycline, patients who achieved complete remission (CR) were given another 2 cycles of post-remission therapy with cytarabine. Each chemotherapy regimen was followed by donor stem cell infusion in the MST groups.

Result

MST achieved an encouragingly high CR rate in patients (63.8%), even in high-risk patients (54%). It was significantly higher than that in the chemotherapy alone group. The 1-year overall survival (OS) of MST patients was 57.7% and was 68.6% in the high-risk and standard group, respectively, whereas the OS was only 37.3% in the chemotherapy group. The severe infection rate was 36% and 54% in MST and chemotherapy group. No GVHD was observed in MST patients. A larger updated T cell clones was observed in MST patients by T cell receptor repertoire analysis with a Next Generation Sequencing methodology.

Conclusions

These results suggested that MST is a safe and practical treatment regimen conducive to a longer-term survival for AML patients at a highly advanced age.

Background

The median age of AML at diagnosis is 69 years, with approximately one-third of patients aged 75 years or older. Treatment outcome in patients with AML is known to continuously deteriorate with progressively increasing age.(1) Although old age is not a defining characteristic of feature, it is of significant clinical relevance, in that it exerts a profound prognostic impact on treatment outcome. In contrast with younger patients older patients are more prone to have high-risk cytogenetic and hematologic disorders, or secondary AML, or higher expression of genes leading to drug resistance.(2, 3)Currently, the rate of complete response (CR) is reportedly between 30% and 50% in elderly patients, with a mean survival from just 8 to 12 months, The data from European Oncology Working Group including over 6000 cases showed the 1-year overall survival (OS) was about 50% and 30% for AML patients aged 55-65 and 65 to 75years, separately. The 3-year OS was only about 26.8% and 12.8% for these patients.(4)In particular, the benefits of receiving first-line therapy in patients over 70 years with AML remain controversial.(5, 6) Elderly patients with AML(EAML) often have poor physical status, organ dysfunction and poor hematopoiesis and immune recovery. Although EAML can also receive reduced intensity conditioning of allogeneic hematopoietic stem cell transplantation(allo-HSCT), high transplant-related mortality (TRM), graft-versus-host disease (GVHD) and other side effects limit the application of allo-SCT in such patients. Recent development of immunotherapy and molecular targeted therapy, such as FLT3, IDH1/2, BCL-2 inhibitors combined with demethylating agents decitabine or

azacytosis, has improved remission and survival rates of EAML patients. However, the mean OS of these patients is only 14 to 18 months, and the effects were worse for the patients aged over 70.(7–9)

Recently, primary clinical studies have shown that infusion of HLA-mismatched donor granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cells (GPBSCs) combined with chemotherapy (micro-transplantation, [MST]) increased the CR rate, improved survival, and avoided GVHD in patients with AML.(10, 11) However, studies involving patients aged over 70 years treated with MST are scarce. In this study, we focused on patients aged 70 to 88 years with AML undergoing HLA-mismatched MST. The primary objectives of this study were the CR rate, leukemia-free survival (LFS), and OS. Other than those objectives, hematopoietic recovery, recovery of T cell receptor (TCR) clones, GVHD, the relapse rate, NRM, and toxicities were also included in the investigation. Furthermore, we observed the difference in treatment outcomes between elderly patients with high-risk AML and those with standard-risk AML undergoing MST regardless of age. In addition, the efficacy and complications of conventional chemotherapy and MST in elderly patients were analyzed and compared.

Materials And Methods

Patients and Donors

From May 2012 to July 2020, patients aged 70 to 88 years with de novo AML were included in the study. The diagnoses were defined according to the French American British (FAB) and World Health Organization (WHO) criteria, and the prognostic risk groups were defined according to the 2016 European Leukemia Net (ELN) classification.(12) Molecular markers such as AML1-ETO, PML-RARa, NPM1, and FLT3-ITD were also analyzed. A total of 111 elderly patients were enrolled, of whom 80 selected MST, 15 conventional chemotherapy, and 16 support therapy. The 80 patients treated by MST were further divided into high risk group (n=50) and standard risk group (n=30) based on the presence of high-risk prognostic factors other than age.(13, 14)

Treatment Design

Induction therapy

Induction therapy consisted mainly of daunorubicin (45–60 mg/m²) or mitoxantrone (8–10 mg/m²) or idarubicin (8–10 mg/m²) for 3 days in combination with cytarabine 100–150 mg/m² for 5 days (DA or MA or IA), where a tiny number of patients received an alternative induction therapy combined with decitabine 20 mg/m² for 5 days, followed by an infusion of GPBSCs 24 hours (day 0) after the completion of cytarabine in MST group. Other induction therapies included decitabine 10 mg/m² for 5 days, cytarabine 10 mg/m² every 12 hours for 14 days, aclarubicin 14 mg/m² for 4 days and additional G-CSF 200 µg/m² for 14 days (DAAG) followed by an infusion of GPBSCs 24 hours (day 0) after the completion of cytarabine. In the case where the patients failed to achieve CR after the first cycle of induction therapy, a second cycle of the same induction therapy was given. Patients in routine chemotherapy group received the same chemotherapy regimen but without donor cell infusion. Patients in the support treatment group were given only oral hydroxyurea, blood transfusion, anti-infection, and nutrition supplementation.

Post-remission therapy: Patients who achieved CR received two courses of MST as post-remission therapy, which consisted of intermediate-dose cytarabine (1.0 g/m² for 6 doses) or DAAG chemotherapy followed by an infusion of GPBSCs after cytarabine chemotherapy with up to 10-week intervals between the courses in MST group. Supplementary MST consolidation treatments were administered if minimum residual disease (MRD) continued to be positive. None of the patients received any GVHD prophylaxis or further maintenance therapy. The recommended dose adjustment was as follows: for patients aged over 75, length of the administration of anthracycline and cytarabine of

DA, MA and IA were shortened to 2 days and 5 days, respectively, during induction therapy, and the dose of cytarabine was reduced to 500 mg/m² for 6 doses during post-remission therapy. Patients in the conventional chemotherapy group with CR were given the same consolidation treatment regimen as patients in the MST group.

Mobilization and Apheresis of Donor Peripheral Mononuclear Cells

Apheresis and mobilization of HLA-mismatched donor peripheral mononuclear cells were performed as previously described. After apheresis, the donor cells were aliquoted and cryopreserved in liquid nitrogen; however, fresh donor cells were used in the first course of treatment. Cell infusion was performed 24 hours following the chemotherapy course. The median numbers (range) of mononuclear, CD34+, CD3+, and natural killer (NK) cells infused per course were, respectively, 3.6 (2.7–4.5) × 10⁸, 2.2 (1.6–3.3) × 10⁶, 1.1 (0.8–1.6) × 10⁸, and 0.5 (0.2–0.8) × 10⁸ cells per kilogram.

Detection of Donor Chimerism

Peripheral-blood cells or bone marrow cells from all patients were tested for hematopoietic donor chimerism by a standard cytogenetic analysis and a semi-quantitative PCR-based analysis of the short tandem repeats as previously described.

Response Criteria and Outcome Evaluation

Patient responses including CR, LFS, OS, and NRM were determined according to the revised recommendations of the International Working Group for Diagnosis in Acute Myeloid Leukemia. Acute GVHD and chronic GVHD were defined according to published criteria. Death within 4 weeks after initiation of induction therapy was defined as early death.

T cell receptor (TCR) repertoire analysis by a CDR3 Next Generation Sequencing (NGS) methodology

The V, D, J genes were designated according to the nomenclature provided by the international Im Muno Gene Tics information system (IMGT). The purified products were sequenced by Illumina high-throughput sequencing platform, with a paired-end sequencing of 150 bp and a sequencing data volume of 2G. Length distribution analysis, known as CDR3 spectra typing, was performed in accordance with the addition of non-template nucleotides in V-(D)-J region, and applied to evaluating T cell clonality and diversity. Clonality values were calculated from entropy of the TCR Vβ CDR3 frequency distribution and subsequently normalized by logarithm (the number of unique TCR Vβ CDR3).

With respect to bioinformatics analysis, BCL2FASTQ software was adopted to convert NGS offline data into FASTQ format, while the numerical quality was filtered and counted with the aid of FASTP software. Next, the data was cleaned to conduct comparison, assembly, and clone subtype analysis using mixcr software, followed by statistics examination and calculation of subclones (length distribution of CDR3 and D genes, and utilization rate of V and J genes) with the help of vdjtools software. Finally, Python language was written to construct the process, where 3D map of V/J gene utilization rate and VJ combination utilization rate, statistics and output of VJ combination utilization rate result were drawn for quality control. Shannon entropy and Simpson inverse results were calculated by Perl language, and D50 was obtained. Notably, D50 reaching the maximum value of 0.5 means that all CDR3 sequences are equally proportioned, suggesting a satisfactory diversity; in contrast, a zero D50 value indicates only one CDR3 sequence (i.e. poor diversity). For statistical analysis, R software (version 3.6) was used to draw the length distribution map of CDR3 and the rectangular stacked charts. (15–18) TCR dynamic monitoring was performed on 4 patients in the MST and chemotherapy groups and their TCR clone recovery was analyzed and compared.

Statistical Analyses

Survival data were analyzed by the log-rank test, and survival curves were prepared using the Kaplan-Meier method. Statistical significance was defined as $P < 0.05$. SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was used in all of the statistical analyses.

Results

The characteristics of patients and donors

The median age of the patients was 74 (range, 70–88) years. In total, 32% of patients were above 75 years of age in the high-risk MST group and, in the standard-risk MST group, 43.3% of patients were over 75 years of age. In the high-risk MST group, 6% of patients were above 80 years of age, and in the standard MST group, 20% of patients were above 80 years of age. In the chemotherapy group, 20% of patients were above 75 years of age, and in the support group, 43.8% of patients were above 75 years of age. There was no difference in the sex ratio or FAB subtyping classification in the four groups. In the high-risk MST group, 34% of patients had a history of MDS. Of the 80 patient/donor pairs, 6 were matched at 0 of 10 HLA loci, 12 were matched at 1–4 of 10 loci, 54 were matched at 5 of 10 loci, and 8 were matched at 6–7 of 10 HLA loci. In terms of patient/donor relationships, of the 80 patient/donor pairs, 32 donors were the sons of the patients, 38 donors were the daughters of the patients, 3 donors were the grandchildren of the patients, 4 donors were otherwise relatives of the patients, and 3 donors were unrelated to the patients. (Table 1)

Response to Induction Chemotherapy

The overall CR rate was 63.8% in the two MST groups. The CR rate was higher than that in the chemotherapy group (63.8% vs. 46.7%; $p=0.04$). No statistical differences in CR rate were observed between the two MST groups (54% vs. 80%; $p=0.19$) (Table 2).

OS and LFS

Of the 80 patients in MST groups, 48 (60%) finished three courses of MST, including 28 (56%) patients in the high-risk MST group and 20 (66.7%) patients in the standard MST group. Of the 15 patients in the chemotherapy group, only 6 (40%) finished 3 courses of chemotherapy because of lower CR rate and higher complications rate compared with MST patients. The mean LFS time of total patients was 10 ± 1.1 months and 5 ± 2.8 months in all MST groups and chemotherapy group. The mean OS time was 13 ± 0.7 months and 6 ± 2.6 months in the all MST groups and chemotherapy group. The 1-year probability of LFS of patients was no statistically different between in MST groups and in chemotherapy group (37.8% vs. 16.7%, $p=0.36$). Furthermore, there was no significant difference in the 1-year probability of LFS between in the high-risk MST group and the standard MST group (36.1% vs. 40.1%; $p=0.78$). The 1-year probability of OS in MST groups was higher than that in chemotherapy group (57.7% vs. 37.3%, $P=0.04$), and the OS values were 55.9% and 68.6% in the high-risk MST group and the standard MST group, respectively ($p=0.23$). The 2-year OS was 22%, 35.9% and 18.7% in the high-risk MST, the standard MST, and chemotherapy group, respectively. (Figure 1)

NRM and Relapse

The relapse rate was 48.1%, 41.7% and 85.7% in the high-risk MST, standard MST groups and chemotherapy group, which pointed to a significant difference between the MST groups and the chemotherapy group ($p=0.01$). The mean relapse time was 10 ± 1.7 , 11 ± 3.1 and 6 ± 2.9 months in three groups. The 1-year cumulative incidences of relapse were 58.1% and 56% and 83.3% in three groups, respectively ($p=0.2$). The NRM was 10%, 20% and 26.7% in the three groups ($p=0.19$).

Hematopoietic Recovery

The median time needed for neutrophil recovery in the high-risk MST, standard MST groups and chemotherapy group was 12, 11 and 12 days, respectively, and the median time necessary for platelet recovery was 14, 12 and 16 days after induction therapy, respectively, which suggested no significant difference between these groups.

Donor Chimerism

Of the 80 patients treated by MST, none demonstrated stable full donor chimerism. About 40% of the patients had transient fever within 24 hours after cell infusion, where the body temperature was able to return to normal after symptomatic treatment.

CDR3 sequence analysis of TCR

TCR of patients showed polyclonal expression and relatively uniform expression in half a year and one year following MST treatment, indicating a rapid recovery of immune function. (figure 2) The subcloning data of top50 VJ genes showed that the VJ gene clustering was close half a year following and before treatment in the chemotherapy group, while the VJ gene in the MST group one and a half year subsequent to the treatment was contrastingly closer, demonstrating a greater number of neonatal VJ clones in the MST group. Moreover, as is visible from the left cluster lines, the expression of VJ gene cloning varied dramatically between the MST group and chemotherapy only group, which difference indicated that the infusion of donor cells may affect the expression of VJ cloning of the recipients. (Figure 3) Results from diagram of subclonals update frequency showed that 90.4% and 92.3% of the subclonals of the MST-treated groups were updated within half a year following the treatment, which was remarkably higher than that before MST. Moreover, in contrast to half a year following the treatment, 80.6% and 90.3% of the clones kept on updating 1 year after the treatment, whereas only 75% of the clones in the chemotherapy group were updated half a year following the treatment. There was significant difference between the MST and chemotherapy group, $t=0.0046$, $P<0.05$. (Figure 4)

GVHD and severe adverse events

No definite clinical acute or chronic GVHD was observed in any patients. Five patients developed transient skin rash, two exhibited renal insufficiency, 6 patients had heart disease, 8 intestinal infections, 21 lung infections, 13 sepsis, 2 cerebral hemorrhage, and 2 hepatic diseases, with no CMV or EB infections during MST treatment. The overall rate of severe infection was about 16% in the MST group, which was markedly lower than that in the chemotherapy group (46.7%). Eight patients (10%) with advanced disease developed multiple organ failure (MOF) in the MST groups, while the chemotherapy group demonstrated a higher MOF rate (Table3).

Discussion

For elderly patients with AML, despite the recommended guidelines, some small-sample clinical trials suggest that appropriately intensive chemotherapy regimens can improve survival compared to palliative and low-dose chemotherapy. (1, 5) However, for patients aged 70 and above, most centers believe that intensive chemotherapy leads to hematopoietic depression, severe complications, and even a higher risk of death, which accounts for the general reluctance to try intensive chemotherapy. Most centers prefer to give low-dose demethylated drugs, such as decitabine, azacytidine, venetoclax or a half-dose DCAG regimen, of which the results regarding the CR rate and OS are limited due to the lack of sufficient killing to the leukemia clones, (6) although they are instrumental in reducing tumor cell load. The age of the 111 elderly patients in this study was from 70 to 88 years old, with an average of 74. The kappa score was greater than 60 points, and there were no severe cardiopulmonary diseases. The data showed that the

mean of OS of these patients treated by MST were longer than that in the elderly patients receiving chemotherapy alone(4). The 1-year and 2-year OS of these patients was 57.7% and 22.8%, which were roughly the same with the published results of patients aged 55 to 65 treated by chemotherapy alone.

There are several key factors involved in improving the survival of elderly patients by MST. For a start, the CR is the primary observation index, because only by increasing the CR is it possible to prolong disease-free survival. However, with increasing age, the occurrence of malignant genes and clonal leukemia mutations obviously increases, as such maintaining even an intensity of chemotherapy to give leukemia clones sufficient killing can help in obtaining a relatively high CR rate. The induction regimen in this study was basically a chemotherapy regimen, such as anthracycline combined with cytarabine. For patients above 75 years of age, anthracycline drug dose was correspondingly reduced. In addition, the GPBSCs used by MST also played an important role in the induction protocol. The input cells contained a large number of allogeneic lymphocytes, including CD4, CD8, NK, and DC cells, where the number of CD3 cells in a single infusion was close to $1 \times 10^8/\text{kg}$, the total number of multiple CD3 cell transfusions $3-5 \times 10^8/\text{kg}$. However the number of CD3 cells in other published lymphocyte immunotherapies is generally $10^5/\text{kg}$.(19, 20) As a result, a synergy derived from chemotherapy and allogeneic hematopoietic stem cell infusion is capable of both maximizing the killing effect on leukemia cells and ensuring safety. In this study, the overall CR rate was higher than 50%, close to that for young patients.

Intensive chemotherapy administered to elderly patients results in a lengthy period of hematopoietic depression and serious infection complications, which is another side effect problem difficult to tackle. The cells used in the MST protocol were peripheral hematopoietic stem cells that had been activated by G-CSF. These cells contain a large number of hematopoietic stem progenitor cells, such as CD34 and CD38 cells. Although these cells are not stably implanted after infusion, they release a large number of hematopoietic differentiation-related stimulating factors, promoting the rapid recovery of the recipient's own hematopoiesis. (21, 22)The specific mechanism has been investigated and accounted for in relevant articles published.(19) In this study, white blood cells and platelets generally recovered in approximately 2 weeks, which result was even applicable to patients above 70years of age. Another important facilitator for promoting hematopoietic recovery is a sufficient intensity of chemotherapy to remove malignant cells for achieving complete remission, which in turn accelerates the recovery of normal hematopoietic stem cells. The prevention and management of severe infection are also inseparably connected with the above two factors mentioned above. The cells used in MST contain a large number of allogeneic lymphocytes, including CD4, CD8, NK and DC cells, by means of which the incidence of severe infection can be significantly reduced. Our center has repeatedly made use of such cells combined with antibiotics to rescue patients with highly refractory, pan resistant bacterial infections, achieving results better than expected. (23). Although nearly 36% of the patients in this study experienced various infection-related complications during treatment, most of the patients were effectively cured, and mortality was predictably attributable to various complications following leukemia relapse.

How to prolong the disease-free survival of elderly patients is another area of interest. In this study, after improving the CR rate in elderly patients, 2 courses of MST were given as consolidation and intensive therapy, which was composed of moderate-dose chemotherapy and multiple stem cell infusions. The consolidation MST proved to be effective in further reducing leukemia load and decreasing MRD values, as well. Considering the physical tolerance of chemotherapy by elderly patients, this study administered 2 courses of intensive treatment. It entails an analysis of a larger number of cases to ascertain whether 3 or more doses of chemotherapy are more effective than 2 doses.

GPBSCs generally exercise two functions in consolidation and intensive therapy. First, repeated infusions of GPBSCs provide allogeneic lymphocytes which can constantly produce GVL effects.(20, 21, 24) Second, repeated infusion of heterologous lymphocytes continuously stimulates and activates the recipient's immune system, and upregulates the

recipient's immune status, resulting in the killing of leukemia cells.⁽²²⁾TCR results showed that nearly 90% of the new clones were produced in half and one year after MST treatment by a Next Generation Sequencing methodology. These clones were different from those of the patients before treatment and from the existing clones of the donors. It would be of clinical significance to further determine whether those were previously unexpressed clones in the TCR bank of patients or newborn ones carrying over information from both the recipient and donor. Regardless of the sources of the clones, the conclusion that can be reached here is that these changes are the equivalent of immune T cell rejuvenation for older patients with leukemia at an average age of 75, suggesting that MST is of significant clinical value to the recovery of immune function in elderly patients.

In this study, the impacts of high-risk factors on CR and OS were compared. The data show that MST was capable of enabling patients to maintain a high CR rate even in high-risk patients, despite a higher relapse rate of leukemia in the high-risk MST group. Therefore, it is advisable that newly diagnosed patients with high risk factors adopt a positive attitude toward treatment prolonging survival time. On the other hand, the therapeutic efficacy was limited in patients with high risk factors, where MST was only able to delay the recurrence of the disease, but unable to produce a permanent solution to the problem. Further studies are in consequence advised to focus on the ways in which MST can be combined with more effective targeted drugs, as well as improvement of techniques of modified stem cells for elderly patients to prolong survival.^(25, 26)

A large number of allogeneic hematopoietic stem cells were repeatedly infused, where no immunosuppressants were used before and after MST treatment. Therefore, it is particularly important to observe and identify GVHD, although the most of patients did not have a stable and high percentage of chimerism of donor cells. The following procedures are a prerequisite for screening the donor and recipient: an evaluation of immune function in recipients needs to be performed before treatment, including tests of humoral immunity and T cell function tests; determination of the patients' previous immune disorder-related diseases, such as rheumatic immune system diseases, or severe liver diseases needs to be determined; confirmation of previous long-term use of immune-damaging drugs, especially recent use of the fludarabine; and the carrying out of HLA typing of the donor and recipient. The results of HLA typing of donor and recipient are critically important and may contraindicate MST in the case where donors or patients have more than 2 homozygous HLA loci.

It is difficult to determine whether GVHD or severe infection is responsible for symptoms such as persistent fever in the initial stage following MST, which are accompanied by skin congestion, rash, an elevated liver enzyme profile and occasionally mild diarrhea. Although the presence of donor chimerism is likely to make pinpointing the causes much easier, in the early stage of cell transfusion, the above symptoms appear within 2 weeks, which are particularly noticeable in donors having a low proportion of mixed chimerism. As a result, differential diagnosis needs to be taken into consideration in view of changes in inflammatory factors and the liver and myocardial enzyme spectrum.⁽²⁴⁾

The allogeneic hematopoietic stem cells collected after G-CSF mobilization in MST are fundamentally different from those used by previous cellular therapies, such as donor lymphocyte infusion and umbilical cord blood transfusion. First, the peripheral blood cells present after G-CSF mobilization contain not only a large number of hematopoietic stem and progenitor cells, but also immune-related cell subsets and some pre-T cells that are still in the developmental stage.⁽²⁷⁾Second, MST uses cells from healthy HLA-incompatible allogeneic donors, whereas classical donor lymphocyte infusion uses mature and terminal-stage lymphocytes or lymphocytes from patients with autoimmune disorders. These differences may account for the unique role played by MST in the treatment of elderly patients with AML.

Conclusion

the results from the present study show that MST for elderly patients with acute myeloid leukemia is a safe, effective and repeatable treatment, which justifies a more positive attitude towards this treatment for elderly AML patients even above the age of 70.

Abbreviations

AML: acute myeloid leukemia

MST: micro-transplantation

CR: complete remission

OS: overall survival

EAML: elder with acute myeloid leukemia

allo-HSCT: allogeneic hematopoietic stem cell transplantation

TRM: transplant-related mortality

GVHD: graft-versus-host disease

G-CSF: granulocyte colony-stimulating factor

GPBSCs: granulocyte colony-stimulating factor -mobilized peripheral blood stem cells LFS: leukemia-free survival

TCR: T cell receptor

FAB: French American British

WHO: World Health Organization

ELN: European Leukemia Net

DA or MA or IA: daunorubicin or mitoxantrone or idarubicin combination with cytarabine

DCAG: decitabine, cytarabine, aclarubicin and additional G-CSF

MRD: minimum residual disease

NK: natural killer

NGS: Next Generation Sequencing

IMGT: Im Muno Gene Tics information system

MOF: multiple organ failure

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Human Ethics Committee at center and was conducted in accordance with the Declaration of Helsinki. All patients and donors gave written informed consent before enrollment for the study.

Consent for publication

All authors have reviewed and approved the manuscript for submission.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict-of-interests disclosures

The authors declare no competing financial interests.

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Authors' contributions

HSA, KXH designed and analyzed the experiments and wrote the manuscript. KXH, MG, CLY, JHQ, QYS, HLZ, Bo Cai, XRZ, ZD, YJH, YW, XNT, ZZZ performed and analyzed the experiments and prepared the figures. All authors have read and approved the final manuscript. All authors have read and approved the final manuscript.

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Tables

Table 1. Characteristics of Study Group

Group(n)	MST(80)		non-MST(31)	
	high risk	standard risk	chemo	support care
number(%)	50(100%)	30(100%)	15	16
midian age(range)	73(70-82)	74(70-88)	73(70-85)	75
age over 75 years	16(32%)	13(43.3%)	3(20%)	7(43.8%)
age over 80 years	3(6%)	6(20%)	1(6%)	0
sex				
female	27(54%)	9(30%)	7(46.7%)	4(25%)
male	23(46%)	21(70%)	8(63.3%)	12(75%)
FAB subtype				
M1	2(4%)	1(3.3%)	0	0
M2	12(24%)	10(33.3%)	2(13.3%)	2(12.5%)
M4	10(20%)	14(46.7%)	5(33.3%)	6(37.5%)
M5	10(20%)	5(16.7%)	3(20%)	4(25%)
M6	2(4%)	0	2(13.3%)	0
T-AML	5(10%)	0	1(6%)	0
MDS-AML	17(34%)	0	3(20%)	4(25%)
Cytogenetics				
good[inv(16),t(8;21),t(16;16)]	0	3(10%)	0	0
intermediate (nomal,+8,others)	37(74%)	28(93.3%)	11(73.3%)	13(81.3%)
poor (complex,-5,-7,5q-,7q-,11q23,et al)	12(24%)	0	4(26.7%)	3(18.7%)
Donor				
daughter/son/grandchild/other relative/unrelated	20/24/2/2/2	12/14/1/2/1	0	0

Table 2. Response Data by the modified IWG Criteria

	total	high risk	standard risk	P1	chemo	support care	P2
number(%)	80(100%)	50(100%)	30(100%)		15	16	NA
treated over 3 courses	48(60%)	28(56%)	20(66.7%)	0.11	6(40%)	0	0.02#
treated 2 course	17(21.3%)	9(18%)	8(26.7%)	0.31	3(20%)	0	0.15
treated 1 course	15(18.8%)	11(22%)	4(13.3%)	0.25	6(40%)	0	0.16
CR,no.%	51(63.8%)	27(54%)	24(80%)	0.19	7(46.7%)	0	0.04#
partial remission	3(3.8%)	3(6%)	1(3.3%)	0.36	0	0	NA
relapse	23/51(45.1%)	13/27(48.1%)	10/24(41.7%)	0.27	6/7(85.7%)	0	0.01#
no response	23(28.8%)	20/50(40%)	3/30(10%)	0.26	3(20%)	0	0.1
NRM	11/80(13.8%)	5/50(10%)	6/30(20%)	0.34	4/15(26.7%)	16	0.19
early death	10/80(12.6%)	7/50(14%)	3/30(10%)	0.34	8/15(53.3%)	NA	0.19
12 months LFS	37.80%	36.10%	40.1%	0.78	16.70%		0.36
12 months OS	57.70%	55.90%	68.60%	0.23	37.30%		0.04#
24 months LFS	24.50%	24.00%	25.1%	0.78	NA		0.36
24 months OS	22.80%	22.00%	35.90%	0.23	18.70%		0.04#
36 months LFS	12.20%	12.00%	25.10%	0.78	NA		
36 months OS	18.30%	14.70%	23.90%	0.23	NA		
12 months RL	57.10%	58.10%	56.00%	0.83	83.30%		0.22
24 months RL	70.50%	71.40%	68.60%	0.83	NA		NA
36 months RL	85.30%	85.70%	68.60%	0.83	NA		NA
granulocyte recovery	12	12	11	0.8	12		NA
platelet recovery	14	14	12	0.7	16		NA

Abbreviaton: CR,complete remission; NRM,nonrelapse mortality; LFS:Leukemia free survival; OS,overall survival; RL,relapse; early death,os<3months; granulocyte recovery=median days to granulocyte recovery to

1x10⁹/L platelet recovery median days to platelet recovery to 50x10⁹/L P1,high risk MST VS standard risk MST;P2 total MST VS Chemotherapy;#,P<0.05.

Table3. Most Frequent Adverse Events During therapy

adverse event	No. of patients					
	high risk (n=50)	Total %	standard risk (n=30)	Total %	Chemo (n=15)	Total %
Cardic	3	6%	3	10%	6	40%
rash	3	5%	2	6.70%	0	0
Hemorrhage/bleeding	2	4%	2	6.70%	3	20%
Hepatic	2	4%	0	0	0	0
Neurologic	0	0	0	0	0	0
Renal	0	0	2	6.70%	1	6.60%
intestinal infection	5	10%	3	10%	5	33.30%
respiratory infection	13	26%	8	26.70%	7	21.70%
septicemia	8	16%	5	16.70%	6	46.70%
thrombosis	0	0	0	0	0	0
Graft versus host disease	0	0	0	0	0	0
cytomegalovirus/EB virus	0	0	0	0	0	0
multiple organ failure	5	10%	3	10%	6	40%

Abbreviation: EB, Epstein Barr.

Figures

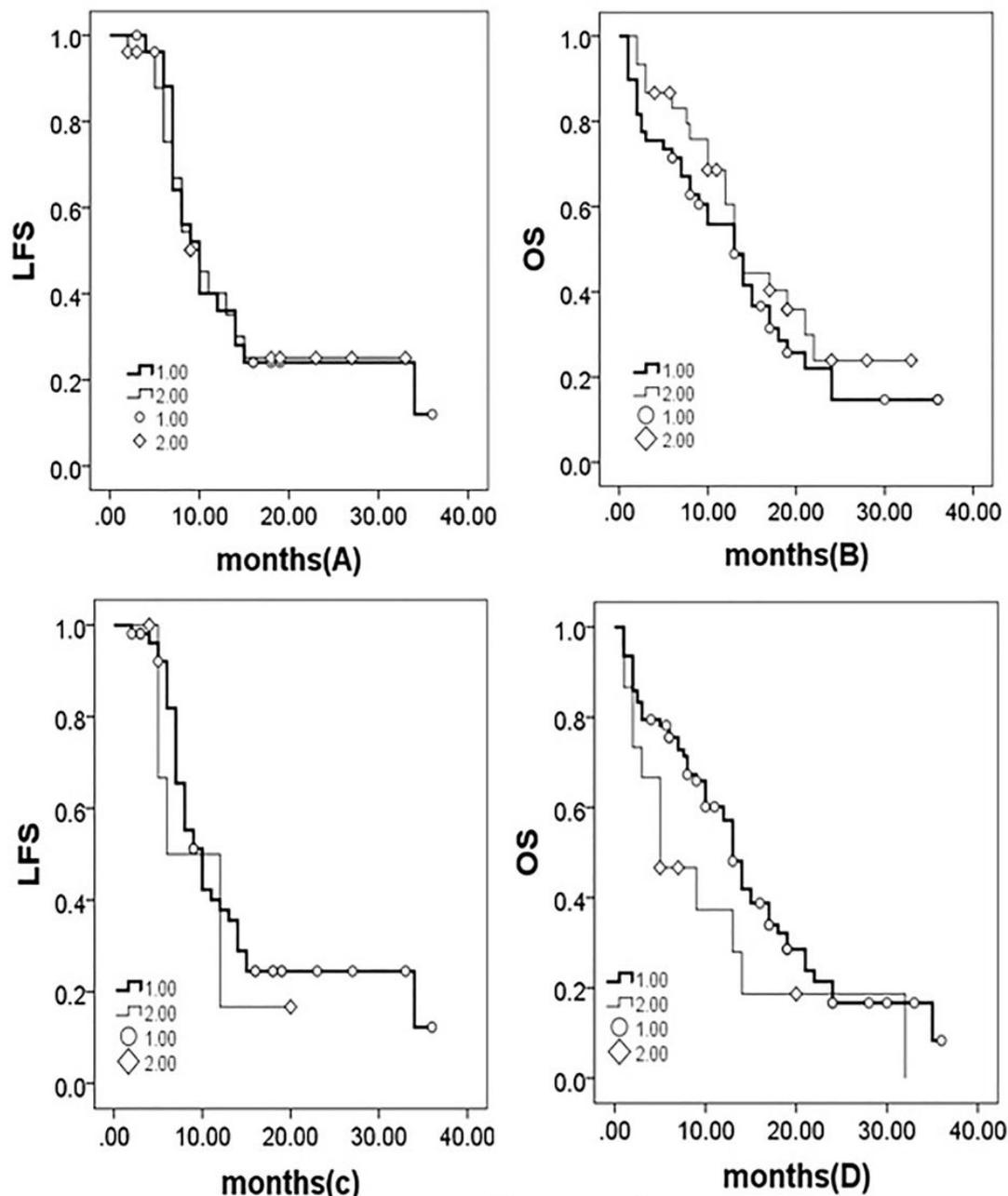
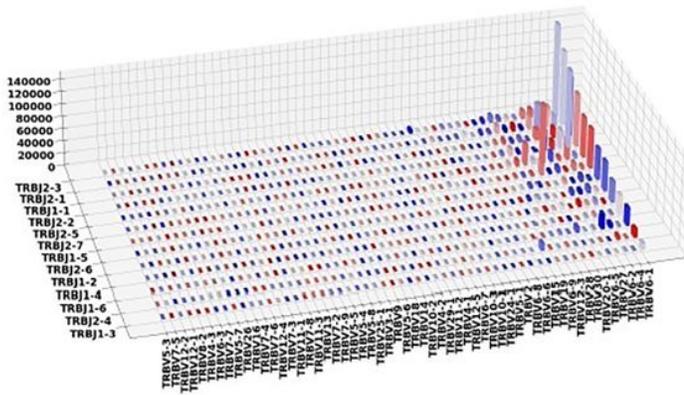


figure 1

Figure 1

(A,B) The probabilities of LFS and OS in the high risk group compared with standard MST group. 1. Thick line, high risk group; 2. Thin line, standard group. The mean months: LFS, 10 ± 1.2 vs. 10 ± 1.5 months, $p = 0.78$; OS, 13 ± 2 months vs. 13 ± 1.2 months, $p = 0.22$. (C,D) The probabilities of LFS and OS in the all MST patients compared with routine chemotherapy patients. 1. Thick line, all MST group; 2. Thin line, the chemotherapy group. The mean months: LFS, 10 ± 1.1 vs. 5 ± 2.8 months, $p = 0.36$; OS, 13 ± 0.7 months vs. 6 ± 2.6 months, $p = 0.04$.

MST1-Pre-Operation



MST1-Y

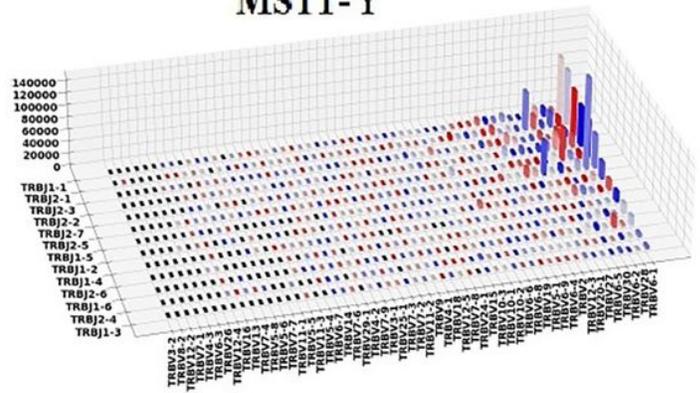


figure 2

Figure 2

Subclonals changing of VJ gene combination before and after micro-transplantation (MST). The X axis represents the type of V gene, Y axis represents the type of J gene, and Z axis represents the number of gene expression, and each column represents the expression of a combination of VJ gene. MST1-pre, results of TCR subclonals of case 1 before MST; MST1-Y, results of TCR subclonals of case 1 one year after MST treatment. The color clone in the figure represents the same clone as that before treatment, where black indicates the newborn clone. According to the figure, TCR of patients showed polyclonal expression and relatively uniform expression in half a year and one year after MST treatment, indicating a rapid recovery of immune function.

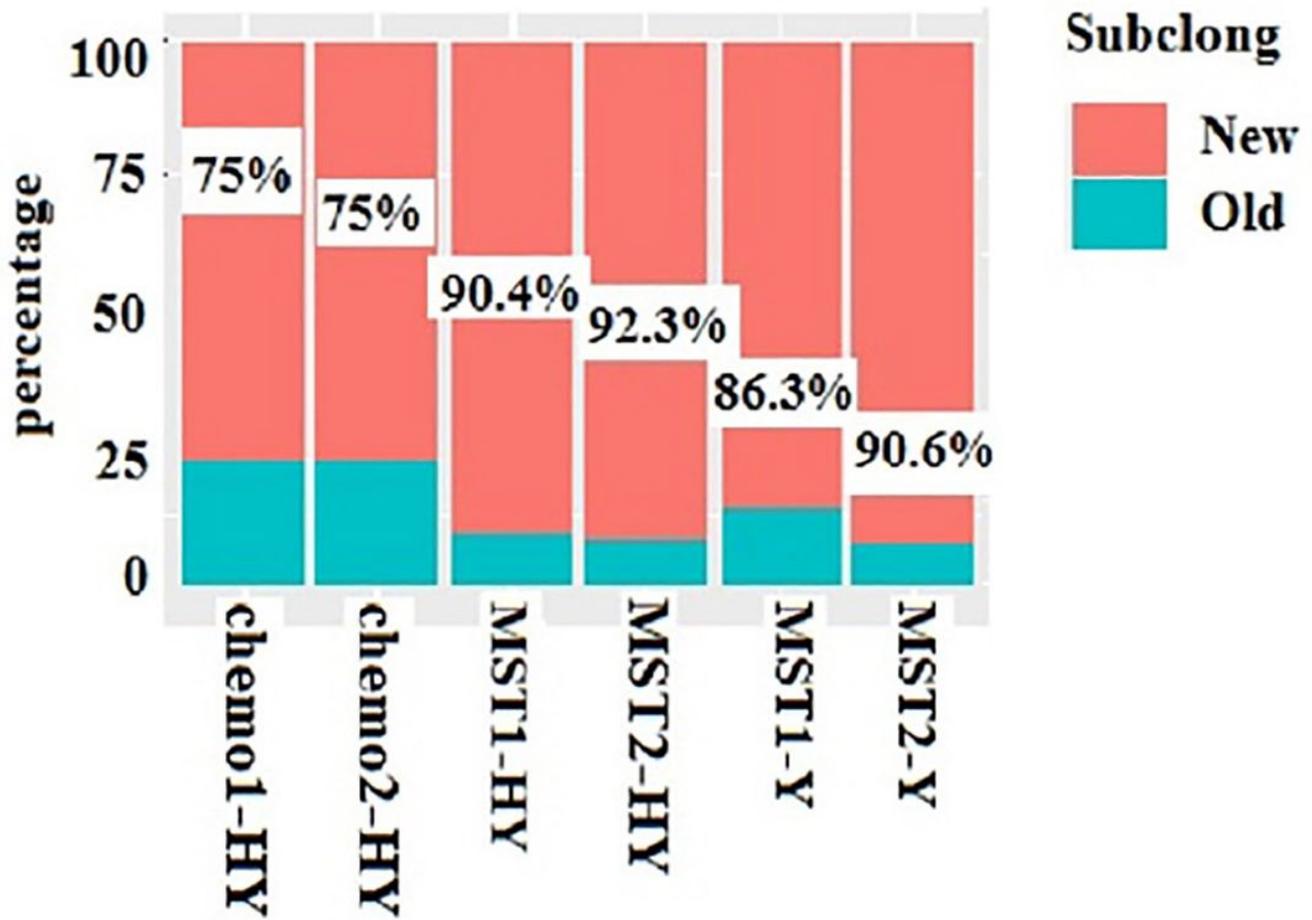


figure 4

Figure 4

Comparison diagram of subclonals update frequency. Unlike the method of observing at the VJ gene combination only, the software classification method for subcloning (VDJ gene combination + CDR3 nucleic acid sequence classification) was adopted to enhance the detection of the number of subclonals, with each sample reading about 10,000 subclonals. Results showed that 90.4% and 92.3% subclonals of two cases were updated half a year following the treatment, as compared with the number before MST. Moreover, the 80.6% and 90.3% of the clones kept on updating 1 year following the treatment, as compared with the result half a year after treatment. For the two cases in the chemotherapy group, only 75% of the clones were updated half a year after the treatment, as compared with the number previous to the treatment. A significant difference was detected between the two groups, $t=0.0046$ $P<0.05$.