Supplementary Materials

# The criteria of unfit for intensive chemotherapy

Patients who were unfit for allogeneic stem cell transplantation should meet at least one of the following criterion: older than 70y; 2 to 3 status of Eastern Cooperative Oncology Group (ECOG) performance; ejection fraction ≤50% or congestive heart failure requiring treatment or chronic stable angina; reduced diffusion capacity of the lung with carbon monoxide ≤65%; creatinine clearance <45 mL/min; hepatic impairment with total bilirubin ＞1.5 × upper limit of normal; or any other comorbidity that was judged by physician to be incompatible with conventional intensive chemotherapy.

# Microchimerism detection

The microchimerism detection is based on real-time quantitative RT-PCR technology (RQ-PCR). We detect the InDel (Insertion or Deletion Polymorphisms) loci on 15 autosome chromosomes, they are N1-1, N1-2, N1-3, N2-1, N5-1, N5-4, N7-1, N9-1, N11-1, N11-2, N13-1, N13-2, N14-1, N16-2 and N21-1; and a sex chromosome site SRY.

# The sample size estimation

We used the web tool "StatBox" (https://www.cnstat.org/statbox) to estimate sample size (1), and SAS9.4 and PASS15 verify the reliability of the results. A significant difference test was used to estimate the number of cases to be enrolled in this study. For elderly *de novo* AML patients, the CR rate was approximately 35% for patients treated with DA protocol as induction (daunorubicin 45 mg/m2 for 3 consecutive days and cytarabine 200 mg/m2 for 7 consecutive days) (2). And in a multicenter analysis, the CR rate for those used allo-TLI plus IA, MA, or DAC+CAG as induction and consolidation with high-dose cytarabine was 75% (3). While for MDS patients with int-2/high risk, the CR rate for those treated with allo-TLI+ decitabine and cytarabine was 52.4% (4), and the CR rate for those treated with decitabine alone or combination with other chemotherapy agents was approximately 35% (5). Therefore, we hypothesized that the treatment strategy in our study could achieve a CR rate of 75% in *de novo* AML and 52% in MDS patients with int-2/high risk, which were simultaneously statistically different from that of treatment protocol without allo-TLI. The test level (α) was defined as 0.05 and the test efficiency (1-β) was defined as 0.80. Number of samples in historical control cohort: AML =2:1. According to Person Chi-square test, the sample sizes of AML patients and the historical control cohort to be included in this study were 15 and 30 (power = 0.8058). Furthermore, based on the variance of null hypothesis estimation and the standard approximation method, the sample sizes of MDS patients to be included in this study were 64 (power = 0.8026). The sample size of AML patients but not MDS patients included in this study met the estimated sample size, and 30 *de novo* AML patients treated with IA (3+7) protocol served as historical controls.

# Supplementary Figures and Tables

## Supplementary Figures

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**Supplementary Figure 1.** Mean CD4+ and CD8+ T-cell numbers per unit volume in peripheral blood of patients from pre- to post-allo-TLI (day 7), analyzed by mixed model repeated measures. \**P*<0.05; \*\**P*<0.01; \*\*\**P*<0.001 compared with D0. All data shown as estimated mean with 95% confidence intervals. D0: day of donor T-cell infusion.



**Supplementary Figure 2.** Changes in CD3+ T cells in peripheral blood from pre- to post-allo-TLI (day 7) in patients with different absolute neutrophil (ANC) recovery times. Shorter ANC recovery time: less than the median ANC recovery time.

## Supplementary Tables

**Supplementary Table 1.** Clinical features of patient with graft versus host disease and other patients.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **aGvHD Patient** | **Other MDS patients** | **All Other Patients** |
| Patients |  | 7 | 24 |
| Patient age |  |  |  |
| Median, IQR | 65 | 64(61-72) | 65(62-68) |
| Donor age |  |  |  |
| Median, IQR | 42 | 34(29-41) | 34(28-39) |
| Patient sex |  |  |  |
| Male |  | 5 | 17 |
| Female | Y | 2 | 8 |
| Donor sex |  |  |  |
| Male | Y | 6 | 19 |
| Female |  | 1 | 6 |
| MDS prognostic stratification (IPSS)a |  |  |  |
| Intermediate-2 | 1 | 4 | 4 |
| High risk |  | 3 | 3 |
| Median number of stem cells infused (IQR) | |  |  |
| MNC, 108/kgb | 3.60 | 3.02(1.94-3.49) | 2.58(2.25-3.49) |
| CD3+, 108/kgb | 0.57 | 0.40(0.24-0.56) | 0.42(0.35-0.60) |

*aGvHD, acute graft versus host disease; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; HLA, human leukocyte antigen; IPSS, International Prognostic Score System; MNC, mononuclear cells; NK, natural killer cells.*

*aPrognostic risk groups defined by NCCN guidelines, version 2019.*

*bRecipient/patient weight.*

*This patient developed GvHD.*

**Supplementary Table 2.** The relationship between donor and recipient.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Patients | | | Donors | | Relationship |
| Gender | Age | Diagnosis | Gender | Age |
| male | 71 | AML | male | 34 | son |
| female | 66 | AML | male | 21 | grandson |
| female | 64 | AML | male | 40 | son |
| male | 58 | AML | female | 33 | daughter |
| male | 55 | AML | male | 38 | son |
| male | 62 | AML | male | 19 | grandson |
| male | 63 | AML | male | 35 | son |
| male | 66 | AML | male | 39 | son |
| female | 71 | AML | male | 42 | son |
| male | 63 | AML | female | 31 | daughter |
| male | 62 | AML | male | 38 | son |
| male | 62 | MDS | male | 41 | nephew |
| female | 65 | MDS | male | 42 | son |
| female | 64 | MDS | male | 38 | son |
| male | 66 | MDS | male | 33 | son |
| male | 61 | MDS | female | 34 | daughter |
| male | 72 | MDS | male | 23 | grandson |
| male | 56 | MDS | male | 29 | son |
| male | 69 | AML | female | 23 | niece |
| female | 76 | AML | female | 20 | granddaughter |
| female | 73 | MDS | male | 42 | son |
| male | 67 | AML | male | 42 | son |
| male | 67 | AML | male | 27 | nephew |
| female | 67 | AML | male | 35 | Son |
| male | 59 | AML | female | 29 | daughter-in-law |

**Supplementary Table 3.** Chimerism detection of peripheral blood T cells in one patient.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Patient Pre-allo-TLI** | **Donor** | **Patient Post-allo-TLIb** |
| STR locia | | | |
| D8S1179 | 12 , 14 | 12 , 16 | 12 , 14 |
| D21S11 | 29 , 32.2 | 32.2 , 33.2 | 29 , 32.2 |
| D7S820 | 11 , 12 | 11 , 12 | 11 , 12 |
| CSF1PO | 10 , 12 | 10 , 12 | 10 , 12 |
| D3S1358 | 15 , 16 | 15 , 16 | 15 , 16 |
| D5S818 | 11 , 13 | 13 | 11 , 13 |
| D13S317 | 8 | 8 , 10 | 8 |
| D16S519 | 9 , 11 | 9 , 11 | 9 , 11 |
| D2S1338 | 19 , 24 | 24 | 19 , 24 |
| D19S433 | 14 , 14.2 | 14 , 14.2 | 14 , 14.2 |
| VMA | 14 , 17 | 16 , 17 | 14 , 17 |
| D12S391 | 15 , 19 | 19 , 20 | 15 , 19 |
| D18S51 | 14 , 15 | 15 | 14 , 15 |
| Amel | X , Y | X | X , Y |
| D6S1043 | 19 | 13 , 19 | 19 |
| FGA | 19 , 22 | 19 , 23 | 19 , 22 |

*STR, short tandem repeat; TLI, T-cell infusion.*

*aDonor chimerism was performed on STRs using semi-quantitative polymerase chain reaction.*

*bDay 6 after HLA-mismatched allo-TLI.*

**Supplementary Table 4.** Mixed model repeated measure analysis for CD3+T cells.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Mean of CD3+T cells(109/L)** | **Sd** | ***P*** | **95%CI** | | |
| Age | | | | | |
| ≥63y | 0.746 | 0.030 |  | 0.687 | 0.805 | |
| <63y | 0.849 | 0.031 | 0.011 | 0.787 | 0.910 | |
| Allo-TLI cycle | | | | | |
| 1 st cycle | 0.667 | 0.036 | x | 0.595 | 0.738 | |
| 2 nd cycle | 0.734 | 0.036 | 0.157 | 0.664 | 0.804 | |
| 3 rd cycle | 0.823 | 0.037 | 0.001 | 0.75 | 0.897 | |
| 4 th cycle | 0.965 | 0.052 | <0.001 | 0.863 | 1.066 | |
| ANC recovery timea | | | | | |
| Longer recovery time | 0.798 | 0.028 |  | 0.744 | 0.852 | |
| Shorter recovery time | 0.796 | 0.035 | 0.967 | 0.728 | 0.865 | |
| PLT recovery timeb | | | | | |
| Longer recovery time | 0.664 | 0.029 |  | 0.607 | 0.722 | |
| Shorter recovery time | 0.930 | 0.034 | <0.001 | 0.862 | 0.998 | |

*aANC recovery time: longer recovery time (more than the median recovery time, ≥13 days for AML or ≥7 days for MDS); shorter recovery time (less than the median recovery time, <13 days for AML or <7 days for MDS).*

*bPLT recovery time: longer recovery time (more than the median recovery time, ≥10 days for AML or ≥8 days for MDS); shorter recovery time (less than the median recovery time, <10 days for AML or <8 days for MDS).*

*TLI: T-cell infusion; ANC: absolute neutrophil count; PLT: platelet; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome.*

*x: Other groups compared with this group.*

**Supplementary Table 5.** The follow-up time of the historical control group.

|  |  |  |
| --- | --- | --- |
| Patients | Follow-up time（months） | Status |
| 1 | 0.2 | Died |
| 2 | 0.4 | Died |
| 3 | 0.4 | Died |
| 4 | 0.7 | Died |
| 5 | 1.3 | Died |
| 6 | 1.4 | Died |
| 7 | 3.0 | Died |
| 8 | 3.5 | Died |
| 9 | 3.6 | Died |
| 10 | 3.9 | Died |
| 11 | 4.2 | Died |
| 12 | 4.9 | Died |
| 13 | 6.0 | Died |
| 14 | 7.2 | Died |
| 15 | 7.4 | Died |
| 16 | 7.7 | Died |
| 17 | 8.0 | Died |
| 18 | 8.4 | Died |
| 19 | 9.9 | Died |
| 20 | 10.8 | Died |
| 21 | 11.4 | Died |
| 22 | 11.8 | Died |
| 23 | 13.8 | Died |
| 24 | 31.0 | Died |
| 25 | 34.1 | Died |
| 26 | 40.7 | Died |
| 27 | 40.8 | Died |
| 28 | 56.5 | Alive |
| 29 | 61.2 | Alive |
| 30 | 62.2 | Alive |

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