Annals of Hematology (2019) 98:997–1007
https://doi.org/10.1007/s00277-018-3584-2

ORIGINAL ARTICLE

Allogeneic hematopoietic stem cells transplantation improves the survival of intermediate-risk acute myeloid leukemia patients aged less than 60 years

Ying Zhang[1] · Yimin Zhang[1] · Qi Chen[2] · Gusheng Tang[1] · Weiping Zhang[1] · Jianmin Yang[1] · Jianmin Wang[1] · Xiaoxia Hu[1]

Received: 29 July 2018 / Accepted: 11 December 2018 / Published online: 3 January 2019

Abstract

The prognosis of acute myeloid leukemia (AML) with normal karyotype is further determined by specific genetic alterations. The optimal post-remission therapy (PRT) in younger patients within this group after first complete remission (CR1) remains to be determined. We report a retrospective evaluation of PRT approaches in 223 patients under the age of 60 years old with intermediate-risk AML in CR1. Patients receiving allogenic hematopoietic stem cell transplantation (alloHSCT) obtained improved overall survival (OS) than patients who treated with chemotherapy (5-year 61.6 ± 5.2% versus 41.1 ± 5.3%, $p = 0.004$). AlloHSCT led to fewer cases of relapse (hazard ratio [HR] 0.14, $p < 0.001$) and increased the relapse-free survival (RFS, HR 0.45, $p < 0.001$). With alloHSCT, the outcome of patients who reached negative minimal residual disease after 2 cycles of consolidation could be further improved with an increased OS of 66% and RFS of 61%. Nucleophosmin-1 (NPM1) mutation negative, CCAAT/enhancer binding protein alpha (CEBPA) double mutation negative, and FLT-3 internal tandem duplication negative ($NPM1^{mut\text{-}neg}CEBPA^{dm\text{-}neg}FLT3\text{-}ITD^{neg}$) patients had a significantly longer RFS with alloHSCT. In conclusion, our results provide additional evidence that alloHSCT is preferential PRT in patients with intermediate-risk AML that are under the age of 60 years old in CR1.

Keywords Acute myeloid leukemia · Intermediate risk · Postremission therapy · Allogeneic hematopoietic stem cell transplantation · $NPM1^{mut\text{-}neg}CEBPA^{dm\text{-}neg}FLT3\text{-}ITD^{neg}$

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease that is characterized by impaired differentiation and increased proliferation of myeloid progenitors [1]. Most patients with AML receive induction chemotherapy to achieve complete remission (CR), followed by post-remission therapy (PRT). For younger patients (18–60 years old), 4–6 cycles of intermediate to high doses of cytarabine [2], combined chemotherapy [3], autologous HSCT (ASCT) [4], and allogeneic hematopoietic stem cell transplantation (alloHSCT) [5–7] are all recommended as PRT.

The majority of patients with AML present intermediate-risk cytogenetics. The European Leukemia Net (ELN) proposes three groups of standardized genetic prognostic system, which correlates cytogenetic and molecular profiles with clinical data [8]. Patients with normal karyotype belong to the intermediate-risk category, and their prognosis is further determined by specific genetic alterations, particularly Nucleophosmin-1 (NPM1) mutation, CCAAT/enhancer binding protein alpha (CEBPA) and FLT-3 internal tandem duplication (ITD) [9–11]. PRT with repeated cycles of intermediate or high-dose cytarabine may not improve the survival for

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00277-018-3584-2) contains supplementary material, which is available to authorized users.

✉ Xiaoxia Hu
hu_xiaoxia@126.com

[1] Department of Hematology, Institute of Hematology, Changhai Hospital, Shanghai 200433, China

[2] Department of Health Statistics, Second Military Medical University, No. 800 Xiangyin Road, Shanghai 200433, China

intermediate-risk AML, especially for patients harboring concurrent wild-type NPM1 and FLT-ITD [9, 12, 13]. Although a few studies have suggested that ASCT might improve event-free survival, randomized trials have not demonstrated any long-lasting benefits for ASCT in intermediate-risk AML [14–16]. AlloHSCT is an effective antileukemic therapy, but is compromised by non-relapse mortality (NRM). To balance the treatment-related toxic effects with the risk of relapse, clinical trials involving young adults have adopted alternative strategies, such as reduced intensity conditioning (RIC) [17]. Therefore, the optimal PRT for intermediate-risk AML, which accounts for nearly half of the cases of AML, remains to be controversial.

The "triple-negative" subgroup ($NPM1^{mut\text{-}neg}CEBPA^{dm\text{-}neg}FLT3\text{-}ITD^{neg}$) within intermediate-risk group is becoming to the focus of clinical research. Schlenk et al. demonstrated that the benefit of transplantation was limited for patients with FLT3-ITD and the triple-negative group [13]. The other retrospective studies reported a favorable OS after alloHSCT for the subgroup, and the survival was even similar to those of favorable-risk group by ELN risk classification [18, 19]. Therefore, the optimal PRT for triple-negative intermediate-risk AML remains to be determined.

In this study, we retrospectively evaluated the association of the PRT with the treatment outcome in patients with intermediate-risk AML. In addition to cytogenetic risk, the patients were also stratified by the minimal residual disease (MRD) detected after 2 cycles of consolidation. We found that $NPM1^{mut\text{-}neg}CEBPA^{dm\text{-}neg}FLT3\text{-}ITD^{neg}$ patients after alloHSCT in CR1 had a significant longer RFS rather than OS, when compared with patients treated with PR-CT. AlloHSCT is demonstrated as a preferred choice for younger patients with intermediate-risk AML, even with an early achievement of negative MRD.

Materials and methods

Patients

Between January 2010 and June 2017, 400 de novo AML patients (212 male and 188 female) who received induction chemotherapy and PRT in Changhai Hospital were included in this study. The last follow-up was July 2018. Patients with acute promyeloid leukemia were excluded. According to the ELN [8], 46 patients were classed with favorable risk (11.5%), 279 with intermediate risk (69.8%), and 75 with unfavorable risk (18.7%). Among these 400 patients, 338 (84.5%) achieved first complete remission (CR1) after 1 or 2 cycles of induction therapy. From the 279 intermediate-risk patients, 223 patients (79.9%) who were under 60 years old and obtained CR1 were subjected to further analysis (Fig. 1). Institutional databases were retrospectively reviewed;

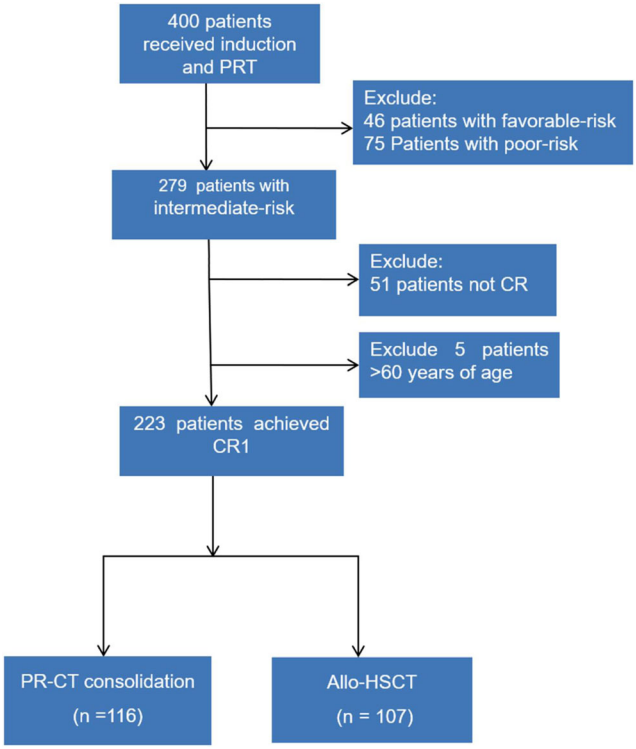

Fig. 1 Consort diagram outlining the analysis strategy of patients

demographic, clinical, and genetic information were extracted from the medical records. All procedures complied with the Helsinki Declaration standards and approved by the Changhai Hospital Institutional review board. The requirement for written informed consent was waived off, because this study used retrospective data from medical records, and there were no interventions in the patients.

Mutational analysis

NPM1 and FLT3-ITD mutations were analyzed on DNA as previously described [20, 21]. CEBPA mutational status was studied by PCR and direct sequencing as reported [22].

Treatment protocols

Induction chemotherapy consisted of DA (daunorubicin [DNR] 60 mg/m^2/day by intravenous infusion from day 1–3; cytarabine [Ara-C] 100–200 mg/m^2/day by intravenous infusion from day 1–7) or IDA (idarubicin [IDA] 10 mg/m$^{2/}$day by intravenous infusion from day 1–3; Ara-C 100–200 mg/m^2/day by intravenous infusion from day 1–7). The treatment included a maximum of two induction cycles. Patients who did not reach hematologic CR after 2 cycles of induction were not included in the study. Patients with CR received 4 cycles of consolidation with Ara-C (2 g/m^2, every 12 h, for 6 doses, PR-CT group) or proceeded to alloHSCT (alloHSCT group) after 2 cycles of consolidation followed by reduced intensity

conditioning (RIC) or myeloablative conditioning (MAC) conditioning if they had a matched sibling or ≥9/10 HLA allele fully matched unrelated donor. Patients with CNS-1(central nervous system) or CNS-2 received triple intrathecal infusions (methotrexate 10 mg, cytarabine 50 mg, dexamethasone 5 mg) twice a week until disappearance of blast cells in the cerebrospinal fluid (CNS-0).

Definitions

Hematological CR was defined as less than 5% marrow blasts, the absence of extramedullary disease, an absolute neutrophil count (ANC) > 1.0×10^9/L, a platelet count > 100×10^9/L and independence from red cell transfusions. Relapse was defined as ≥5% blasts in a bone marrow aspirate or peripheral blood or the presence of extramedullary disease. Overall survival (OS) was measured from the date of diagnosis until death or the last follow-up, and relapse-free survival (RFS) was measured from the date of CR1 until death, the first relapse or the last follow-up in continuous CR, respectively. In the computation of the cumulative incidence of leukemic relapse (CIR) and NRM, death and relapse were included as competing events and survival was counted as a censored event.

MRD analysis by flow cytometry

The MRD study was performed in bone marrow samples from patients in CR at pre-determined time points as previously described [23]. The flow cytometry strategy for MRD detection was based on the detection of cells expressing an aberrant phenotype, including asynchronous antigen expression, antigen overexpression, and abnormal light scatter patterns. Monoclonal antibody combinations used in all of the cases were as follows: CD34/CD117/CD45/CD56/CD2/CD7 and CD34/CD64/CD15/CD11c/CD45. These combinations included immature and myeloid markers commonly expressed in most AML cases. The acquisition and analyses were performed on a FACS Aria II flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). At least 1,000,000 events/cases were collected.

Negative MRD (MRD^{neg}) by flow cytometry was defined as < 10^{-3} blasts (< 0.1%) in bone marrow samples and positive MRD (MRD^{pos}) was defined as ≥ 10^{-3} blasts (≥ 0.1%) in bone marrow.

Transplant procedure

RIC-alloHSCT was developed to reduce the toxicity of conditioning regimens, decrease the incidence of graft versus host disease (GVHD), and preserve a curative antitumor effect corresponding to alloHSCT [24, 25]. To further optimize the outcome of RIC-alloHSCT for acute leukemia, our center designed a novel RIC regimen named FBA consisting of fludarabine, cytosine arabinoside, and busulfan [26]. The conditioning regimen for MAC-alloHSCT was classical BuCy regimen (Bu 0.8 mg/kg, day − 8 to − 5 and cyclophosphamide 60 mg/kg/day, day − 4 to − 3). If an unrelated donor was accepted, the patient would also receive antithymocyte globulin.

A total of 107 patients (47.9%) received alloHSCT. RIC-alloHSCT was applied in 47 patients (43.9%), whereas 60 (56.1%) received a MAC-alloHSCT. Of 107 patients, 82 intermediate-risk patients in CR1 were from the prospective, randomized phase II study (ChiCTR-TRC-08000102) [26]. These patients were 1:1 assigned to receive either RIC or MAC. For the remaining 25 patients, 6 patients were treated with RIC conditioning, and 19 patients with MAC conditioning (BuCy). Generally, patients who were fragile, and > 55 years old were preferred with RIC conditioning. All patients received cyclosporine, short-term methotrexate, and mycophenolate mofetil as GVHD prophylaxis [27]. Fluconazole, ganciclovir, and sulfamethoxazole were used as infection prophylaxis from day − 10 to − 1 before transplantation. After neutrophil and platelet engraftment, each drug was administered alone for 7 days in turn in the sequence of fluconazole, ganciclovir, and sulfamethoxazole until the tapering or cession of immunosuppression. Granulocyte colony-stimulating factor was administered starting on day +5 until neutrophil engraftment. All patients received blood products when necessary.

Statistics

The Kaplan–Meier method was used to estimate the survival probability for a defined time. The multivariate Cox model was applied to analyze the impact of PR-CT and alloHSCT on OS and RFS. We compared characteristics of two groups using the Mann-Whitney test, Kruskal-Wallis test, or Fisher exact test. The CIR was performed between groups using the method of Gray with Kalbfleisch and Prentice method for estimation [28]. Hazard ratios were given with 95% confidence intervals (95% CI). To exclude the bias that may result from involving high-risk patients who relapsed or died too early to receive alloHSCT in CR1, landmark analysis was taken when we compared the outcomes of patients receiving alloHSCT with those receiving chemotherapy [29, 30]. As the average time from CR1 to PRT was 1 month, a 1-month landmark was set for the Simon–Makuch plots for RFS. For the OS, a 3-month landmark was set as the average time from induction therapy to PRT in alloHSCT. A P value less than 0.05 was considered to be statistically significant. SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and R 3.4.2, were used for statistical analyses.

Table 1 Patient characteristics

Patients	PR-CT	AlloHSCT	Total	*p*
Number of patients, n	116	107	223	0.365
Age, years; median (range)	47 (17–60)	35 (17–58)	41.5 (17–60)	*< 0.001*
Female, *n* (%)	62 (48.8%)	56 (58.9%)	118 (52.9%)	0.643
Blood and laboratory values at diagnosis				
WBC, $\times 10^9$/L; median (range)	7.33 (0.54–264.1)	8.56 (0.9–276.3)	7.8 (0.54–276.3)	0.981
HB, g/L; median (range)	84 (39–157)	91 (24–131)	87 (24–157)	0.966
PLT, $\times 10^9$/L; median (range)	52 (5–268)	44 (2–307)	47.5 (2–307)	0.422
Blast in BM,%; median (range)	71 (21–96)	61.5 (23.5–98.5)	66.25 (21–98.5)	0.297
Flow cytometry-MRD before PRT, %; median (range)				
	0.074 (0.001–1.421)	0.077 (0.003–17)	0.076 (0.001–17)	*0.014*
FAB type				
M0	9	4	13	0.201
M1	12	18	30	0.157
M2	36	29	65	0.519
M4	30	27	57	0.914
M5	24	24	48	0.752
M6	3	5	8	0.403
M7	2	0	2	0.513
Karyotype *n* (%)				
CN-X-Y, *n* (%)	85 (72.3%)	80 (74.8%)	165 (74.0%)	0.800
Molecular classification (positive patient)				
$NPM1^{mut}FLT3\text{-}ITD^{high}$†	12 (10.3%)	20 (18.7%)	32 (14.3%)	0.071
$NPM1^{wt}$ without FLT3-ITD	10 (8.6%)	18 (16.8%)	28 (12.6%)	0.093
$NPM1^{wt}$ with $FLT3\text{-}ITD^{low}$†	8 (6.9%)	10 (9.3%)	18 (8.1%)	0.602
t(9;11)(p21.3;q23.3)	7 (6.1%)	4 (3.7%)	11 (4.9%)	0.516
$NPM1^{mut\text{-}neg}CEBPA^{dm\text{-}neg}FLT3\text{-}ITD^{neg}$	79 (68.1%)	55 (51.5%)	134 (60.1%)	*0.014*
CR reached after				
Cycle 1 (early CR)	94 (81%)	72 (67%)	166 (74%)	0.133
Cycle 2 (late CR)	22 (19%)	35 (33%)	57 (26%)	*0.024*
Relapse, *n* (%)	79 (68.1%)	34 (31.8%)	113 (50.7%)	*< 0.001*
NRM, % (at 5 years)	1.24 ± 0.02	18.08 ± 1.92	–	< 0.001
CIR, % (at 5 years)	74.41 ± 4.13	25.53 ± 2.50	–	*< 0.001*
Follow-up; median (range) (months)	41 (14–104)	46 (9–106)	44 (9–106)	0.075

Italics denotes statistically significant

PR-CT, post remission chemotherapy; *AlloHSCT*, allogeneic hematopoietic stem cell transplantation; *WBC*, white blood cell count; *HB*, hemoglobin; *PLT*, platelet; *BM*, bone marrow; Flow cytometry-MRD before PRT, the MRD study was performed in bone marrow samples from patients in CR by the flow cytometry before post-remission therapy. MRD, minimal residual disease before PR-CT or AlloHSCT; *PRT*, post-remission therapy, *FAB*, French-American British classification; *CN-X-Y*, cytogenetically normal or only loss of X or Y chromosome; *AML*, acute myeloid leukemia; *CR*, complete remission; *OS*, overall survival (with event death whatever the cause); *RFS*, relapse-free survival (with event death in first CR or relapse); *NRM*, non-relapse mortality (with event death in first CR and censored at relapse); *CIR*, cumulative incidence of relapse. †Low, low allelic ratio (< 0.5); high, high allelic ratio (≥ 0.5); semiquantitative assessment of FLT3-ITD allelic ratio (using DNA fragment analysis) is determined as ratio of the area under the curve "FLT3-ITD" divided by area under the curve "FLT3 wild-type"

Results

Patient characteristics

Patients' characteristics were presented in Table 1. The median age of the 223 patients at diagnosis was 41.5 years old (range 17–60 years). The median follow-up time for patients without death was 44 months (range 9–106 months). Among them, 105 (47.1%) were male and 118 (52.9%) were female. There was no significant difference in the white blood cell count, hemoglobin, platelet count and marrow blasts between the PR-CT and alloHSCT groups. The features concerning FAB type, karyotype, and molecular classification between the two groups were comparable. As expected, a higher percentage of patients with

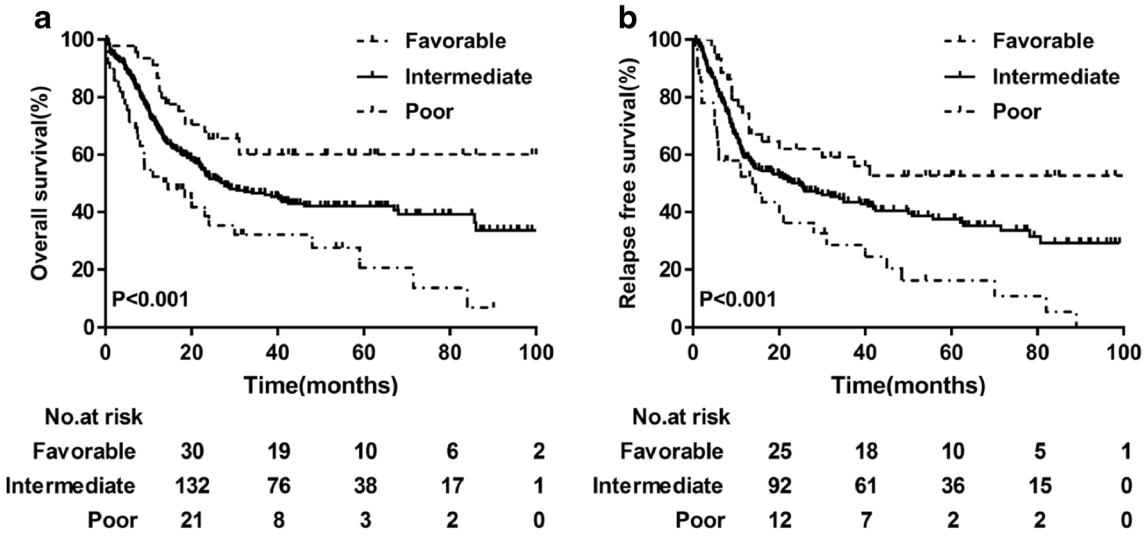

Fig. 2 Patient outcomes based on risk profiles. **a** OS and **b** RFS

2 cycles of induction was observed in the alloHSCT group ($p = 0.024$). The proportion of patients with NPM1$^{mut\text{-}neg}$CEBPA$^{dm\text{-}neg}$FLT3-ITD^{neg} genetic alteration was higher in PR-CT group (68.1 versus 51.5%, $p = 0.014$).

Survival by PRT in intermediate-risk AML patients

The outcome of 400 patients was categorized into favorable-risk, intermediate-risk, and adverse-risk groups. There was a significant difference in the OS and RFS between the three groups (all $p < 0.001$). The OS at 5 years were 60.1 ± 7.6% for favorable-risk patients, 41.9 ± 3.3% for intermediate-risk patients, and 19.8 ± 8.0% for adverse-risk patients (Fig. 2a). The RFS in each group were 52.7 ± 7.6%, 37.5 ± 3.3%, and 16.3 ± 7.1%, respectively (Fig. 2b).

For all patients, alloHSCT markedly improved the OS and RFS when compared with PR-CT (OS 55.8 ± 5.1% versus 37.1 ± 3.6% at 5 years, $p < 0.001$; RFS 48.6 ± 5.1% versus 26.8 ± 3.5% at 5 years, $p = 0.009$, Fig. 3). Subsequently, the OS and RFS of the intermediate-risk patients were further analyzed according to the types of PRT. AlloHSCT group had a significantly improved OS at 5 years as compared with PR-CT group (61.6 ± 5.2% versus 41.1 ± 5.3%, $p = 0.004$, Fig. 4a). A longer RFS was also found in patients receiving alloHSCT (alloHSCT group: 51.3 ± 5.3% versus PR-CT group: 27.4 ± 4.9% at 5 years, $p < 0.001$, Fig. 4b). After CR, 116 (52.0%) patients received chemotherapy, with a relapse rate of 68.1%, whereas 107 (48.0%) patients received alloHSCT, with a relapse rate of 31.8%. The difference in the relapse rates between the groups was significant ($p < 0.001$).

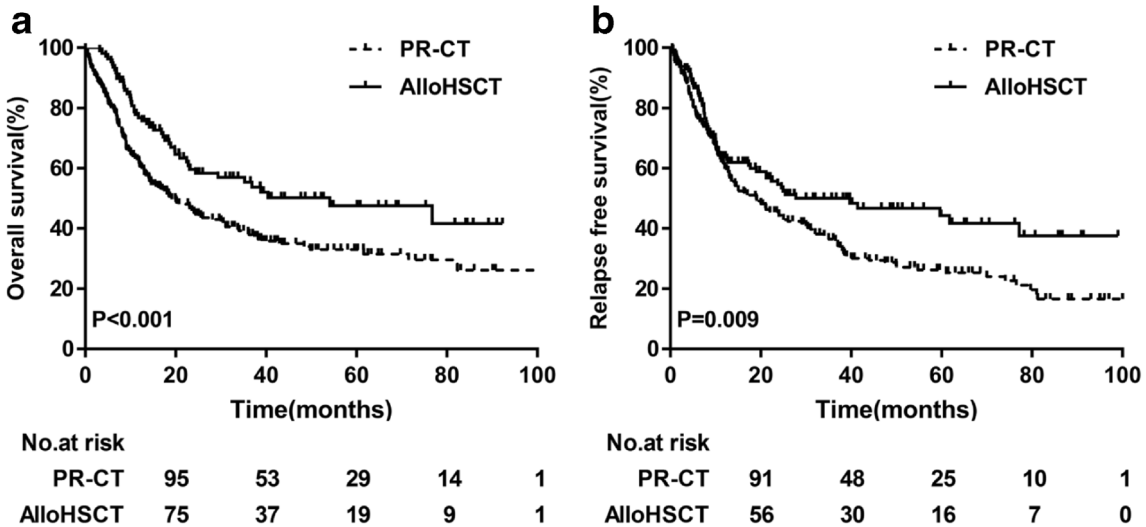

Fig. 3 Comparison of outcomes of PR-CT versus alloHSCT in all patients. **a** OS, **b** RFS. A landmark was set at 1 month for RFS and 3 months for overall survival

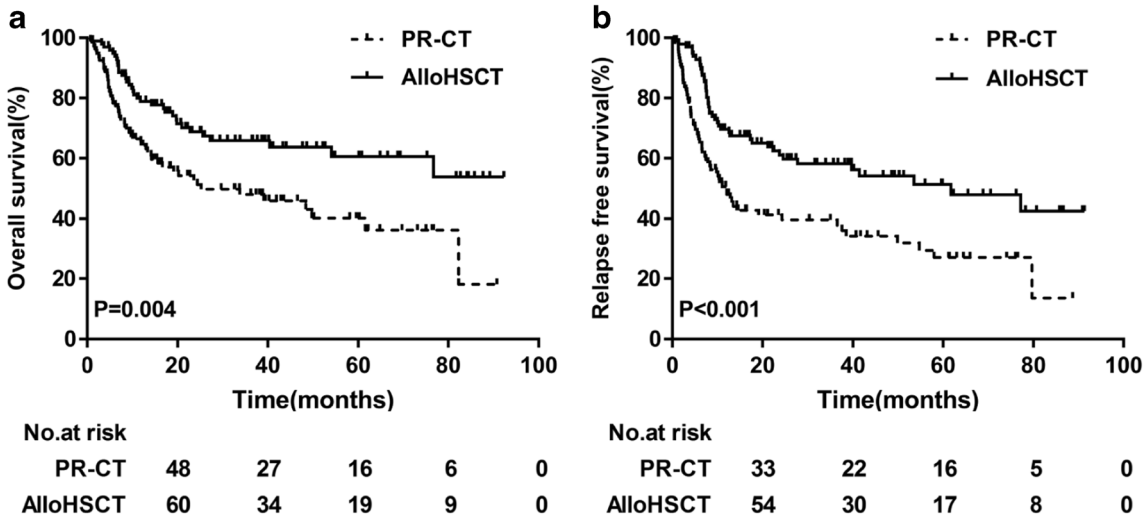

Fig. 4 Comparison of outcomes of PR-CT versus alloHSCT in intermediate risk patients with CR1. **a** OS, **b** RFS. A landmark was set at 1 month for RFS and 3 months for overall survival

Out of 223 intermediate-risk patients, 134 patients (60.1%) were with NPM1$^{mut\text{-}neg}$CEBPA$^{dm\text{-}neg}$FLT3-ITD^{neg} genetic alteration. Of those, 55 patients received alloHSCT, and 79 patients with PR-CT. The characteristics of the analyzable patients were shown in Table 2. The probability of OS at 5-year was 58.6 ± 9.0% with alloHSCT, compared with 33.3 ± 8.8% with PR-CT ($p = 0.024$, Fig. 5a). The probabilities of RFS at 5 years were 50.1 ± 9.9% for alloHSCT, and 30.0 ± 9.1% for PR-CT ($p = 0.012$, Fig. 5b). The CIR for patients treated with PR-CT was 59.3 ± 0.4% and 26.9 ± 0.3% with alloHSCT ($p = 0.001$, Fig. 5c).

Multivariable analysis with adjustments for sex, age, time from CR to PRT, and PRT type was performed (Table 3). Both OS and RFS were significantly improved following alloHSCT

Table 2 Characteristics of PR-CT and AlloHSCT in intermediate-risk AML patients with NPM1$^{mut\text{-}neg}$CEBPA$^{dm\text{-}neg}$FLT3-ITD^{neg} genetic alteration

	PR-CT ($n = 79$)	AlloHSCT ($n = 55$)	p
WBC, $\times 10^9$/L; median (range)	6.5 (0.6–264.1)	14 (0.9–276.3)	0.519
HB, g/L; median (range)	84 (40–148)	94.5 (52–131)	0.466
PLT, $\times 10^9$/L; median (range)	58 (14–268)	31 (5–265)	0.228
Blast in BM, %; median (range)	73.5 (21–96)	65.7 (23.5–95.5)	0.986
Age (years)			
Median (range)	48 (18–60)	36 (23–58)	0.044
CR reached after			
Cycle 1 (early CR)	54 (68%)	39 (71%)	0.645
Cycle 2 (late CR)	25 (32%)	16 (29%)	
MRD^{pos}*(≥ 0.1%)	14 (18%)	11 (20%)	*0.010*
MRD^{neg}*(< 0.1%)	37 (47%)	39 (71%)	
Miss	28 (35%)	5 (9%)	
Relapse, *n* (%)	45 (57%)	18 (33%)	*0.001*
NRM, % (at 5 years)	5.6 ± 0.2	20.4 ± 0.4	*0.018*
CIR, % (at 5 years)	59.3 ± 0.4	26.9 ± 0.3	*0.001*
Follow-up; median (range) (months)	35 (19–88)	36(7–84)	*0.469*

Italics denotes statistically significant

WBC, white blood cell count; *HB*, hemoglobin; *PLT*, platelet; *BM*, bone marrow; *CR*, complete remission; *MRD*, minimal residual disease; *AlloHSCT*, allogeneic hematopoietic stem cell transplantation; *OS*, overall survival (with event death whatever the cause); *RFS*, relapse-free survival (with event death in first complete remission (CR) or relapse); *NRM*, non-relapse mortality (with event death in first CR and censored at relapse); *CIR*, cumulative incidence of relapse

* MRD ^{neg}, MRD < 0.1%; MRD^{pos}, MRD ≥ 0.1%

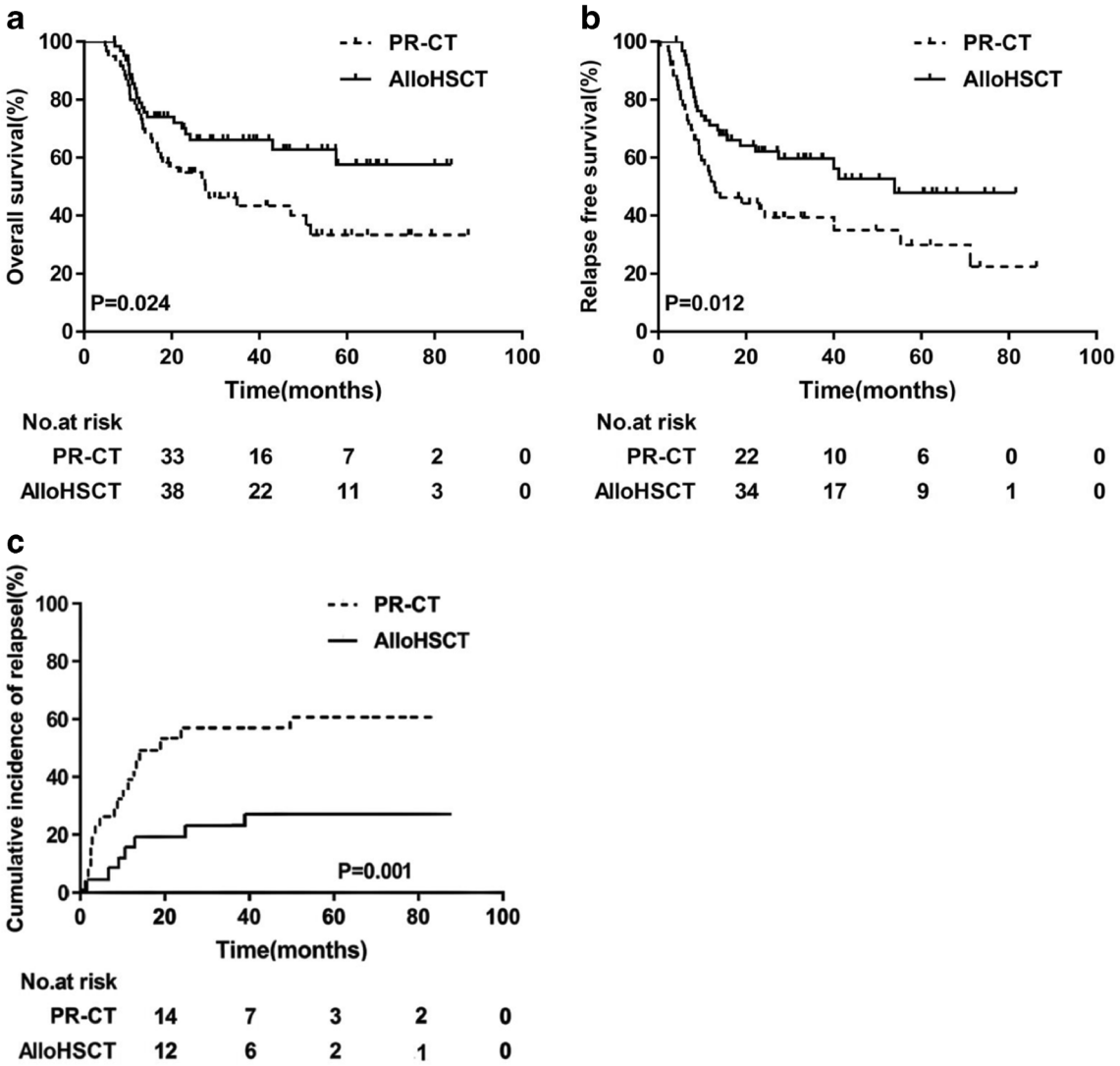

Fig. 5 Comparison of outcomes of NPM1$^{mut\text{-}neg}$CEBPA$^{dm\text{-}neg}$FLT3-ITD^{neg} genetic alteration in PR-CT and alloHSCT group. **a** OS, **b** RFS, **c** CIR. A landmark was set at 1 month for RFS and 3 months for overall survival

as compared to the rates in PR-CT group, with HRs of 0.51 (95% CI 0.34–0.77, $p = 0.001$), and 0.45 (95% CI: 0.29–0.26, $p < 0.001$), respectively. Moreover, the relapse risk in the alloHSCT group was significantly lower than that of PR-CT group (HR 0.14, 95% CI 0.05–0.37, $p < 0.001$).

Survival by MRD in intermediate-risk AML patients

MRD data after 2 cycles of consolidation were available in 144 out of 223 patients. Eighty-eight patients achieved MRD^{neg} after 2 cycles of consolidation. The overall characteristics of the analyzable patients were shown in Table 4. Forty-eight MRD^{neg} patients (55%) underwent alloHSCT and 40 patients (45%) with PR-CT. Patients in the two groups were comparable for most variables including WBC counts and ECOG status. As expected, the patients with MRD^{pos} underwent alloHSCT were younger than those in PR-CT group ($p = 0.001$). At last follow-up, for patients receiving alloHSCT, 8 MRD^{neg} patients (16.6%) experienced relapse, whereas 6 patients (12.5%) died due to non-relapse treatment-related complications. For patients received chemotherapy, 20 MRD^{neg} patients (50.0%) relapsed. The 4-year RFS for MRD^{neg} patients with alloHSCT was better than that with PR-CT. (61.1 versus 33.4%, $p = 0.035$), although it did not have a significant effect on OS (Table 4, Fig. 6).

Discussion

PRT in patients with AML includes continuing chemotherapy and autologous or allogeneic HSCT. Favorable-risk patients generally receive chemotherapeutic consolidation [31]. Although alloHSCT is considered to be the preferred PRT for poor-risk AML, the effect of alloHSCT in intermediate-

Table 3 Results of multivariable analysis for OS and RFS

	OS			RFS			Relapse			NRM		
	HR§	95% CI	*p*	HR§	95% CI	*p*	HR§	95% CI	*p*	HR§	95% CI	*p*
Transplantation type												
RIC vs. MAC	1.24	0.47–3.27	0.61	1.41	0.54–3.67	0.48	0.84	0.45–1.46	0.54	1.78	0.43–7.29	0.42
MRD^{neg}* vs. MRD^{pos}*	2.41	1.35–4.31	*0.003*	2.45	1.46–4.11	*0.001*	1.39	0.80–2.39	0.23	1.03	0.09–11.62	0.97
Post-remission treatment												
PR-CT vs. alloHSCT	0.51	0.34–0.77	*0.001*	0.45	0.29–0.62	*< 0.001*	0.14	0.05–0.37	*< 0.001*	4.77	0.14–10.38	0.37
Sex (male vs. female)	0.93	0.53–1.61	0.79	1.04	0.64–1.71	0.85	0.94	0.55–1.60	0.52	1.17	0.25–5.29	0.83
Age†	1.62	0.87–2.98	0.12	1.23	0.72–2.11	0.44	1.02	0.59–1.75	0.92	1.76	0.74–4.19	0.11
CR (late vs. early)	0.80	0.31–2.02	0.64	0.96	0.43–1.71	0.92	1.46	0.63–3.39	0.36	1.84	0.05–4.59	0.73

Italics denotes statistically significant

OS, overall survival (with event death whatever the cause); *RFS*, relapse-free survival (with event death in first complete remission (CR) or relapse); *NRM*, non-relapse mortality (with event death in first CR and censored at relapse); *RIC*, reduced intensity conditioning allogeneic hematopoietic stem cell transplantation; *MAC*, myeloablative conditioning allogeneic hematopoietic stem cell transplantation; *HR*, hazard ratio; *CI*, confidence interval; *AlloHSCT*, allogeneic hematopoietic stem cell transplantation; *vs*., versus; *PR-CT*, post remission chemotherapy; *CR*, complete remission

§ The HRs are the estimates of the effect of covariates for each outcome parameter, stratified by leukemia risk and adjusted for sex, age, CR (late vs. early), and type of post-remission treatment

† Linear with estimates of HRs for 7 years difference

* MRD^{neg}, MRD < 0.1%; MRD^{pos}, MRD ≥ 0.1%

risk AML remains controversial [25, 31]. In the present study, we observed improved survival through alloHSCT in CR1 patients who were less than 60 years of age. Our findings suggest that alloHSCT is a preferred PRT for younger patients with intermediate-risk AML.

AlloHSCT is a potentially curative approach for AML patients. However, the high NRM limits its further application [7]. In our previous study, we demonstrated that the OS, event-free survival and NRM at 3 years between RIC and MAC group were not significantly different. AlloHSCT with RIC conditioning achieves higher graft-versus-host disease and relapse-free survival (GRFS) than the MAC regimen, and is associated with fewer early transplant-related complications [26]. AlloHSCT following MAC or RIC conditioning resulted in comparable outcome with respect to OS, RFS, and CIR (see details in supplemental Table 1). ASCT in AML has been

Table 4 Characteristics of patients in according to MRD

	MRD^{neg} ($n = 88$)			MRD^{pos} ($n = 56$)		
	PR-CT	AlloHSCT	*p*	PR-CT	AlloHSCT	*p*
Number of patients, n	40	48	0.080	27	29	0.753
Age, years; median (range)	46 (18–60)	39 (18–60)	0.126	48 (19–59)	34 (20–60)	*0.001*
Female, *n* (%)	21 (52.5%)	24 (50%)	0.818	12 (44.4%)	13 (44.8%)	0.978
Minimal residual disease,%; median (range) after 2 cycles of consolidation						
	0.043 (0.001–0.098)	0.05 (0.003–0.097)	0.281	0.202 (0.1–1.421)	0.9 (0.104–7.1)	*0.002*
OS, % (at 4 years)	45.1 ± 0.8	66.8 ± 7.1	0.486	28.3 ± 9.4	41.0 ± 9.1	*0.011*
RFS, % (at 4 years)	33.4 ± 0.9	61.1 ± 7.9	*0.035*	10.3 ± 6.3	35.1 ± 9.7	*0.002*
Relapse, *n* (%)	20 (50%)	8 (16.6%)	*0.001*	22 (81.5%)	11 (37.9%)	*< 0.001*
NRM, % (at 4 years)	2.5 ± 0.6	14.1 ± 0.3	*0.005*	4.0 ± 0.2	8.1 ± 0.3	0.234
CIR, % (at 4 years)	61.1 ± 0.8	33.4 ± 0.2	*0.001*	85.3 ± 0.6	50.9 ± 0.3	*0.001*
Follow-up; median (range) (months)	27 (7–90)	35(9–83)	0.450	26 (5–85)	32 (5–87)	0.350

Italics denotes statistically significant

MRD, minimal residual disease; MRD^{neg}, MRD negative, MRD < 0.1%; MRD^{pos}, MRD positive, MRD ≥ 0.1%; *PR-CT*, post remission chemotherapy; *AlloHSCT*, allogeneic hematopoietic stem cell transplantation; *OS*, overall survival (with event death whatever the cause); *RFS*, relapse-free survival (with event death in first complete remission (CR) or relapse); *NRM*, non-relapse mortality (with event death in first CR and censored at relapse); *CIR*, cumulative incidence of relapse

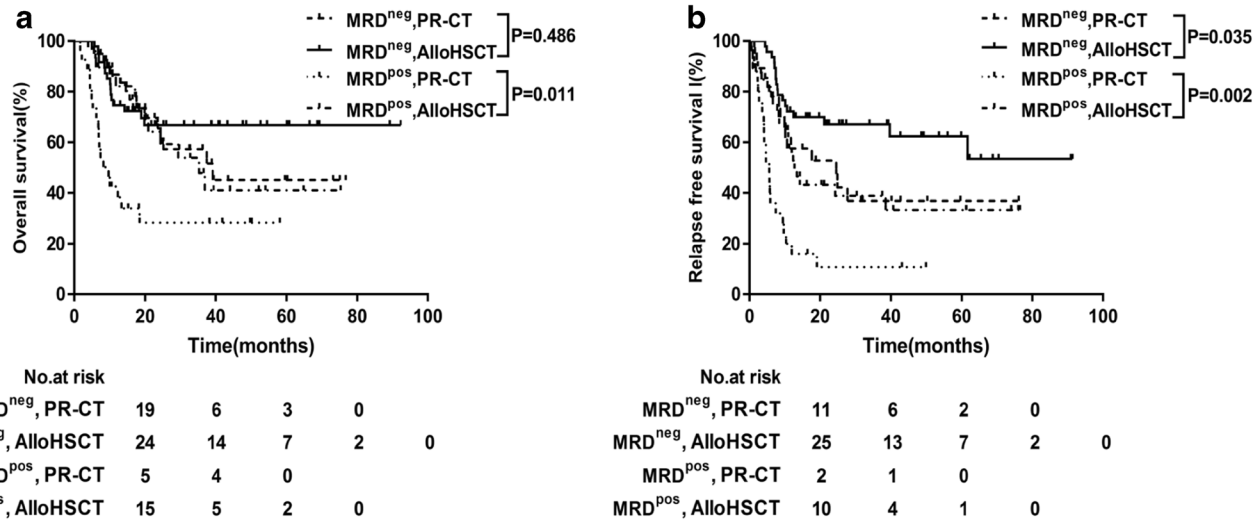

Fig. 6 Comparison of outcomes of PR-CT versus alloHSCT in MRD^{neg} and MRD^{pos} group. **a** OS, **b** RFS. A landmark was set at 1 month for RFS and 3 months for overall survival

recently revisited [31, 32] taking into account recent improvements resulting mainly from better selection of patients in the good and intermediate risk. Relapse is still the main concern after ASCT. With BuCy conditioning regimen, the 5-year relapse rate and OS were 50% and 56%, respectively. The lower NRM in ASCT contributed to OS after ASCT.

In terms of relapse risk, alloHSCT is the most effective antileukemic treatment option. Patients with FLT3-ITD profoundly benefit from alloHSCT [13]. Data comparing the outcomes of alloHSCT and PR-CT for $NPM1^{mut\text{-}neg}CEBPA^{dm\text{-}neg}FLT3\text{-}ITD^{neg}$ AML is few. Heidrich et al. demonstrated that patients with intermediate-risk $NPM1^{mut\text{-}neg}CEBPA^{dm\text{-}neg}FLT3\text{-}ITD^{neg}$ AML benefited from matched sibling alloHSCT in CR1 in terms of RFS (HR 0.5, $p = 0.02$) compared with PR-CT [19]. In the present analysis, we have indicated a 5-year OS close to 59% for $NPM1^{mut\text{-}neg}CEBPA^{dm\text{-}neg}FLT3\text{-}ITD^{neg}$ patients in alloHSCT group, whereas 33% in PR-CT. The improvement in survival was due to a lower risk of relapse after alloHSCT. Ahn et al. reported a OS of 61% with alloHSCT in 27 patients, which are comparable to our results [18].

Combining MRD into a pretreatment risk-directed treatment algorithm would contribute to favorable clinical outcomes [33]. Although negative MRD after 2 cycles of consolidation may be a predictor for better outcomes, our results showed that relapses still occurred in 50% of MRD^{neg} cases, and long-term survival is less than 50% with PR-CT. With alloHSCT, the outcome of MRD^{neg} intermediate-risk AML would be further improved with an increased OS of 66% and RFS of 61%. Of note, for patients with alloHSCT, 71% patients were MRD^{neg} (Table 2). For MRD^{neg} patients, the OS was comparable between PR-CT and alloHSCT group, while CIR were significantly higher in MRD^{neg} patients who received PR-CT (Table 4). The data implied that for intermediate-risk AML patients, alloHSCT would improve survival with the reduction of relapse, even for patients who already achieved negative MRD. Multivariate analysis further demonstrated that MRD after 2 cycles of consolidation was an independent prognostic factor for OS and RFS. The prognosis of MRD^{neg} and MRD^{pos} AML patients is related to age and conditioning regimen [34]. In a retrospective study by European Society for Blood and Marrow Transplantation registry, RIC was only inferior to MAC for patients in the < 50 years MRD^{pos} group, with worse relapse rate (HR 1.71) and RFS (HR 1.554). New approaches are needed for ≥ 50 years AML CR1 MRD^{pos} [33]. Given the promising results reported in recent retrospective studies of ASCT, prospective studies may also usefully address whether ASCT might be at least equivalent to alloHSCT in patients with rigorously confirmed AML CR1 MRD^{neg}, at least in the lower risk categories of AML [33, 35].

A limitation of our work is mainly related to the retrospective nature of this study. These include missing MRD data and molecular profile in a proportion of patients, and different conditioning intensity. Thus, caution should be taken when interpreting our data since this was a single-center, retrospective study with a small number of heterogeneous patients.

In summary, our results suggest that alloHSCT is a preferred choice of PRT over chemotherapy for patients with intermediate-risk AML who are less than 60 years of age, even for patients who achieved negative MRD after 2 cycles of consolidation. Additionally, we demonstrate that alloHSCT should be preferred PRT in $NPM1^{mut\text{-}neg}CEBPA^{dm\text{-}neg}FLT3\text{-}ITD^{neg}$ intermediate-risk patients in CR1.

Authors' contribution Y.Z. collected and reviewed patient information, analyzed and interpreted the data, and wrote the manuscript; Y.M.Z. and Q.C. performed the statistical analysis; G.T. performed the molecular analysis; W.Z. and J.Y. performed the diagnosis and treatment for patients; J.W. and X.H. designed the study, interpreted the data, and

critically reviewed the manuscript. All authors reviewed and approved the manuscript.

Funding This work was supported by the National Natural Science Foundation of China (NSFC; 81770160, 81470321, 81270567, 81530047). X.H. was sponsored by Municipal Human Resources Development Program for Outstanding Leaders in Medical Disciplines in Shanghai (2017BR012).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent For this retrospective study, formal content is not required.

References

1. Dohner H, Estey EH, Amadori S, Appelbaum FR, Buchner T, Burnett AK, Dombret H, Fenaux P, Grimwade D, Larson RA, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz MA, Sierra J, Tallman MS, Lowenberg B, Bloomfield CD (2010) Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood 115(3):453–474
2. Burnett AK, Russell NH, Hills RK, Hunter AE, Kjeldsen L, Yin J, Gibson BE, Wheatley K, Milligan D (2013) Optimization of chemotherapy for younger patients with acute myeloid leukemia: results of the medical research council AML15 trial. J Clin Oncol 31(27):3360–3368
3. Weick JK, Kopecky KJ, Appelbaum FR, Head DR, Kingsbury LL, Balcerzak SP, Bickers JN, Hynes HE, Welborn JL, Simon SR, Grever M (1996) A randomized investigation of high-dose versus standard-dose cytosine arabinoside with daunorubicin in patients with previously untreated acute myeloid leukemia: a Southwest Oncology Group study. Blood 88(8):2841–2851
4. Kern W, Estey EH (2006) High-dose cytosine arabinoside in the treatment of acute myeloid leukemia: review of three randomized trials. Cancer 107(1):116–124
5. Herr AL, Labopin M, Blaise D, Milpied N, Potter M, Michallet M, Heit W, Ferrara F, Esteve J, Arcese W, Ehninger G, Rowe JM, Kobbe G, Rosselet A, Bunjes D, Rio B, Brune M, Nagler A, Gorin NC, Frassoni F, Rocha V (2007) HLA-identical sibling allogeneic peripheral blood stem cell transplantation with reduced intensity conditioning compared to autologous peripheral blood stem cell transplantation for elderly patients with de novo acute myeloid leukemia. Leukemia 21(1):129–135
6. Rollig C, Bornhauser M, Thiede C, Taube F, Kramer M, Mohr B, Aulitzky W, Bodenstein H, Tischler HJ, Stuhlmann R, Schuler U, Stolzel F, von Bonin M, Wandt H, Schafer-Eckart K, Schaich M, Ehninger G (2011) Long-term prognosis of acute myeloid leukemia according to the new genetic risk classification of the European LeukemiaNet recommendations: evaluation of the proposed reporting system. J Clin Oncol 29(20):2758–2765
7. Devine SM, Owzar K, Blum W, Mulkey F, Stone RM, Hsu JW, Champlin RE, Chen YB, Vij R, Slack J, Soiffer RJ, Larson RA, Shea TC, Hars V, Sibley AB, Giralt S, Carter S, Horowitz MM, Linker C, Alyea EP (2015) Phase II study of allogeneic transplantation for older patients with acute myeloid leukemia in first complete remission using a reduced-intensity conditioning regimen: results from cancer and leukemia group B 100103 (alliance for clinical trials in oncology)/blood and marrow transplant clinical trial network 0502. J Clin Oncol 33(35):4167–4175
8. Dohner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Buchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, Levine RL, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz M, Sierra J, Tallman MS, Tien HF, Wei AH, Lowenberg B, Bloomfield CD (2017) Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 129(4):424–447
9. Frohling S, Schlenk RF, Breitruck J, Benner A, Kreitmeier S, Tobis K, Dohner H, Dohner K, leukemia AMLSGUAm (2002) Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. Blood 100(13):4372–4380
10. Patel JP, Gonen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J, Van Vlierberghe P, Dolgalev I, Thomas S, Aminova O, Huberman K, Cheng J, Viale A, Socci ND, Heguy A, Cherry A, Vance G, Higgins RR, Ketterling RP, Gallagher RE, Litzow M, van den Brink MR, Lazarus HM, Rowe JM, Luger S, Ferrando A, Paietta E, Tallman MS, Melnick A, Abdel-Wahab O, Levine RL (2012) Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med 366(12):1079–1089
11. Ostronoff F, Othus M, Lazenby M, Estey E, Appelbaum FR, Evans A, Godwin J, Gilkes A, Kopecky KJ, Burnett A, List AF, Fang M, Oehler VG, Petersdorf SH, Pogosova-Agadjanyan EL, Radich JP, Willman CL, Meshinchi S, Stirewalt DL (2015) Prognostic significance of NPM1 mutations in the absence of FLT3-internal tandem duplication in older patients with acute myeloid leukemia: a SWOG and UK National Cancer Research Institute/Medical Research Council report. J Clin Oncol 33(10):1157–1164
12. Estey E, Dohner H (2006) Acute myeloid leukaemia. Lancet 368(9550):1894–1907
13. Schlenk RF, Dohner K, Krauter J, Frohling S, Corbacioglu A, Bullinger L, Habdank M, Spath D, Morgan M, Benner A, Schlegelberger B, Heil G, Ganser A, Dohner H, German-Austrian Acute Myeloid Leukemia Study G (2008) Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med 358(18):1909–1918
14. Baldus CD, Thiede C, Soucek S, Bloomfield CD, Thiel E, Ehninger G (2006) BAALC expression and FLT3 internal tandem duplication mutations in acute myeloid leukemia patients with normal cytogenetics: prognostic implications. J Clin Oncol 24(5):790–797
15. Stirewalt DL, Kopecky KJ, Meshinchi S, Engel JH, Pogosova-Agadjanyan EL, Linsley J, Slovak ML, Willman CL, Radich JP (2006) Size of FLT3 internal tandem duplication has prognostic significance in patients with acute myeloid leukemia. Blood 107(9):3724–3726
16. Cornelissen JJ, van Putten WL, Verdonck LF, Theobald M, Jacky E, Daenen SM, van Marwijk Kooy M, Wijermans P, Schouten H, Huijgens PC, van der Lelie H, Fey M, Ferrant A, Maertens J, Gratwohl A, Lowenberg B (2007) Results of a HOVON/SAKK donor versus no-donor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle-aged adults: benefits for whom? Blood 109(9):3658–3666
17. Rambaldi A, Grassi A, Masciulli A, Boschini C, Mico MC, Busca A, Bruno B, Cavattoni I, Santarone S, Raimondi R, Montanari M, Milone G, Chiusolo P, Pastore D, Guidi S, Patriarca F, Risitano AM, Saporiti G, Pini M, Terruzzi E, Arcese W, Marotta G, Carella AM, Nagler A, Russo D, Corradini P, Alessandrino EP,

Torelli GF, Scime R, Mordini N, Oldani E, Marfisi RM, Bacigalupo A, Bosi A (2015) Busulfan plus cyclophosphamide versus busulfan plus fludarabine as a preparative regimen for allogeneic haemopoietic stem-cell transplantation in patients with acute myeloid leukaemia: an open-label, multicentre, randomised, phase 3 trial. Lancet Oncol 16(15):1525–1536

18. Ahn JS, Kim HJ, Kim YK, Jung SH, Yang DH, Lee JJ, Kim NY, Choi SH, Jung CW, Jang JH, Kim HJ, Moon JH, Sohn SK, Won JH, Kim SH, Kim DD (2016) Transplant outcomes of the triple-negative NPM1/FLT3-ITD/CEBPA mutation subgroup are equivalent to those of the favourable ELN risk group, but significantly better than the intermediate-I risk group after allogeneic transplant in normal-karyotype AML. Ann Hematol 95(4):625–635
19. Heidrich K, Thiede C, Schafer-Eckart K, Schmitz N, Aulitzky WE, Kramer A, Rosler W, Hanel M, Einsele H, Baldus CD, Trappe RU, Stolzel F, Middeke JM, Rollig C, Taube F, Kramer M, Serve H, Berdel WE, Ehninger G, Bornhauser M, Schetelig J, Study Alliance L (2017) Allogeneic hematopoietic cell transplantation in intermediate risk acute myeloid leukemia negative for FLT3-ITD, NPM1- or biallelic CEBPA mutations. Ann Oncol 28(11):2793–2798
20. Boissel N, Renneville A, Biggio V, Philippe N, Thomas X, Cayuela JM, Terre C, Tigaud I, Castaigne S, Raffoux E, De Botton S, Fenaux P, Dombret H, Preudhomme C (2005) Prevalence, clinical profile, and prognosis of NPM mutations in AML with normal karyotype. Blood 106(10):3618–3620
21. Thiede C, Steudel C, Mohr B, Schaich M, Schakel U, Platzbecker U, Wermke M, Bornhauser M, Ritter M, Neubauer A, Ehninger G, Illmer T (2002) Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood 99(12):4326–4335
22. Frohling S, Schlenk RF, Stolze I, Bihlmayr J, Benner A, Kreitmeier S, Tobis K, Dohner H, Dohner K (2004) CEBPA mutations in younger adults with acute myeloid leukemia and normal cytogenetics: prognostic relevance and analysis of cooperating mutations. J Clin Oncol 22(4):624–633
23. Wang L, Gao L, Xu S, Gong S, Chen L, Lu S, Chen J, Qiu H, Xu X, Ni X, Song X, Zhang W, Yang J, Liu M, Hu X, Wang J (2013) FISH+CD34+CD38- cells detected in newly diagnosed acute myeloid leukemia patients can predict the clinical outcome. J Hematol Oncol 6(1):85
24. Ringden O, Labopin M, Ehninger G, Niederwieser D, Olsson R, Basara N, Finke J, Schwerdtfeger R, Eder M, Bunjes D, Gorin NC, Mohty M, Rocha V (2009) Reduced intensity conditioning compared with myeloablative conditioning using unrelated donor transplants in patients with acute myeloid leukemia. J Clin Oncol 27(27):4570–4577
25. Cornelissen JJ, Versluis J, Passweg JR, van Putten WL, Manz MG, Maertens J, Beverloo HB, Valk PJ, van Marwijk Kooy M, Wijermans PW, Schaafsma MR, Biemond BJ, Vekemans MC, Breems DA, Verdonck LF, Fey MF, Jongen-Lavrencic M, Janssen JJ, Huls G, Kuball J, Pabst T, Graux C, Schouten HC, Gratwohl A, Vellenga E, Ossenkoppele G, Lowenberg B (2015) Comparative therapeutic value of post-remission approaches in patients with acute myeloid leukemia aged 40-60 years. Leukemia 29(5):1041–1050
26. Zhang WP, Wang ZW, Hu XX, Chen J, Yang D, Song XM, Gao L, Ni X, Chen L, Xia XX, Zhou H, Tang GS, Cheng H, Luo YR, Li HM, Yang JM, Wang JM (2018) Preconditioning with fludarabine, busulfan and cytarabine versus standard BuCy2 for patients with acute myeloid leukemia: a prospective, randomized phase II study. Bone Marrow Transplant. https://doi.org/10.1038/s41409-018-0356-5
27. Zhang WP, Yang D, Song XM, Ni X, Chen J, Chen L, Yang JM, Zhou H, Cheng H, Liu BH, Li HM, Wang JM (2013) Allogeneic peripheral blood stem cell transplantation is a promising and safe choice for the treatment of refractory/relapsed acute myelogenous leukemia, even with a higher leukemia burden. Biol Blood Marrow Transplant 19(4):653–660
28. Lin DY (1997) Non-parametric inference for cumulative incidence functions in competing risks studies. Stat Med 16(8):901–910
29. Zhu HH, Zhang XH, Qin YZ, Liu DH, Jiang H, Chen H, Jiang Q, Xu LP, Lu J, Han W, Bao L, Wang Y, Chen YH, Wang JZ, Wang FR, Lai YY, Chai JY, Wang LR, Liu YR, Liu KY, Jiang B, Huang XJ (2013) MRD-directed risk stratification treatment may improve outcomes of t(8;21) AML in the first complete remission: results from the AML05 multicenter trial. Blood 121(20):4056–4062
30. Anderson JR, Cain KC, Gelber RD (1983) Analysis of survival by tumor response. J Clin Oncol 1(11):710–719. https://doi.org/10.1200/JCO.1983.1.11.710
31. Cornelissen JJ, Blaise D (2016) Hematopoietic stem cell transplantation for patients with AML in first complete remission. Blood 127(1):62–70
32. Gorin NC, Giebel S, Labopin M, Savani BN, Mohty M, Nagler A (2015) Autologous stem cell transplantation for adult acute leukemia in 2015: time to rethink? Present status and future prospects. Bone Marrow Transplant 50(12):1495–1502
33. Gilleece MH, Labopin M, Yakoub-Agha I, Volin L, Socié G, Ljungman P, Huynh A, Deconinck E, Wu D, Bourhis JH, Cahn JY, Polge E, Mohty M, Savani BN, Nagler A. Measurable residual disease, conditioning regimen intensity and age predict outcome of allogeneic hematopoietic cell transplantation for acute myeloid leukemia in first remission:a registry analysis of 2292 patients by the Acute Leukemia Working Party European ociety of Blood and Marrow Transplantation. Am J Hematol 93(9):1142–1152
34. Vidriales MB, Perez-Lopez E, Pegenaute C, Castellanos M, Perez JJ, Chandia M, Diaz-Mediavilla J, Rayon C, de Las Heras N, Fernandez-Abellan P, Cabezudo M, de Coca AG, Alonso JM, Olivier C, Hernandez-Rivas JM, Montesinos P, Fernandez R, Garcia-Suarez J, Garcia M, Sayas MJ, Paiva B, Gonzalez M, Orfao A, San Miguel JF (2016) Minimal residual disease evaluation by flow cytometry is a complementary tool to cytogenetics for treatment decisions in acute myeloid leukaemia. Leuk Res 40:1–9
35. Gorin NC, Labopin M, Pabst T, Remenyi P, Wu D, Huynh A, Volin L, Cahn JY, Yakoub-Agha I, Mercier M, Houhou M, Mohty M, Nagler A (2017) Unrelated matched versus autologous transplantation in adult patients with good and intermediate risk acute myelogenous leukemia in first molecular remission. Am J Hematol 92(12):1318–1323