Regular Article

CLINICAL TRIALS AND OBSERVATIONS

MRD-directed risk stratification treatment may improve outcomes of t(8;21) AML in the first complete remission: results from the AML05 multicenter trial

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Key Points

- Risk stratification treatment of t(8;21) acute myeloid leukemia may decrease relapse and improve long-term survival.
- Allo-HSCT benefited high-risk patients, but impaired the survival of low-risk patients.

We aimed to improve the outcome of t(8;21) acute myeloid leukemia (AML) in the first complete remission (CR1) by applying risk-directed therapy based on minimal residual disease (MRD) determined by *RUNX1/RUNX1T1* transcript levels. Risk-directed therapy included recommending allogeneic hematopoietic stem cell transplantation (allo-HSCT) for high-risk patients and chemotherapy/autologous-HSCT (auto-HSCT) for low-risk patients. Among 116 eligible patients, MRD status after the second consolidation rather than induction or first consolidation could discriminate high-risk relapse patients ($P = .001$). Allo-HSCT could reduce relapse and improve survival compared with chemotherapy for high-risk patients (cumulative incidence of relapse [CIR]: 22.1% vs 78.9%, $P < .0001$; disease-free survival [DFS]: 61.7% vs 19.6%, $P = .001$), whereas chemotherapy/auto-HSCT achieved a low relapse rate (5.3%) and high DFS (94.7%) for low-risk patients. Multivariate analysis revealed that MRD status and treatment choice were independent prognostic factors for relapse, DFS, and OS. We concluded that MRD status after the second consolidation may be the best timing for treatment choice. MRD-directed risk stratification treatment may improve the outcome of t(8;21) AML in CR1. This trial was registered at http://www.chictr.org as #ChiCTR-OCH-12002406. (*Blood*. 2013;121(20):4056-4062)

Introduction

Although acute myeloid leukemia (AML) with t(8;21) generally has a favorable prognosis, relapse occurs in ~40% of cases, and long-term survival is <50%.[1-9] Patients with a KIT mutation exhibit an even higher relapse rate (up to 70%) and poor survival rates.[10-12] Once patients experience a relapse, their outcomes are extremely poor even after receiving allogeneic hematopoietic stem cell transplantation (allo-HSCT).[4-6] Therefore, rapid identification of high-risk relapse patients and preemptively treating them with more aggressive therapy, such as HSCT, may decrease relapse and improve survival.

Cytogenetics is the most powerful predictor of prognosis in AML, and integrating additional genetic data may further improve prediction.[13-15] However, minimal residual disease (MRD) monitoring during treatment remains an efficient tool to estimate an individual leukemia's susceptibility to chemotherapy and enhance the delivery of risk-directed therapies.[16,17] More recently, a trial of a prospective MRD-directed risk stratification strategy successfully led to improvement in the outcomes of high-risk pediatric AML patients.[18]

AML with t(8;21) harbors a specific fusion gene, *RUNX1/RUNX1T1*, that provides an ideal target for MRD monitoring. Several small, retrospective cohort studies have suggested that monitoring MRD using real-time quantitative reverse transcriptase-polymerase chain reaction (RQ-PCR) may identify patients at high risk for relapse.[19-24] Two studies found that a reduction in MRD (*RUNX1/RUNX1T1* transcript levels) of <3 logs compared with pretreatment levels was a significant predictor of relapse after 2 courses of consolidation or within 3 to 4 months after achieving complete remission (CR).[19,21] Because HSCT exhibits the strongest anti-leukemia effects, we hypothesize that HSCT can decrease the relapse rate and improve the outcomes of patients with t(8;21) AML who are classified as high risk according to their MRD status. Therefore, we performed a prospective multicenter cohort study to determine whether risk-directed treatment according to MRD status could decrease relapse rates and improve long-term survival of t(8;21) AML patients. We also investigated whether allo-HSCT could improve the outcomes of high-risk and KIT-mutated patients with t(8;21) AML.

Figure 1. AML05 treatment scheme. Con, consolidation; Ind, induction.
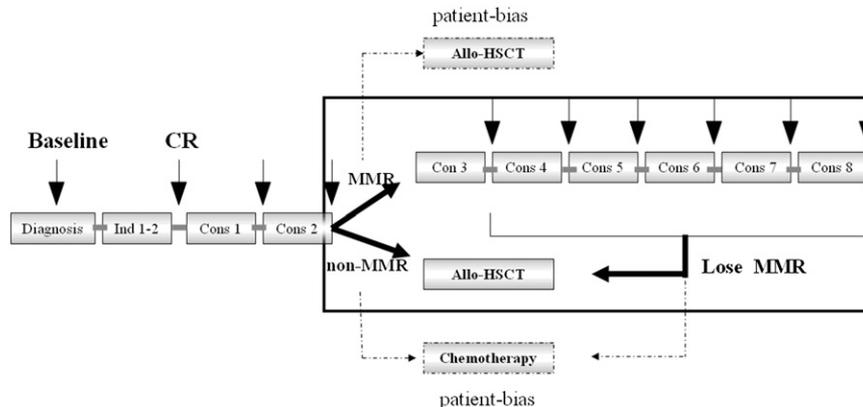
Methods

Patients

One hundred thirty-seven newly diagnosed AML patients with t(8;21) were enrolled at 3 centers between June 2005 and December 2011. The following inclusion criteria were applied: (1) 14 to 60 years old; (2) newly diagnosed AML with t(8;21) and/or *RUNX1/RUNX1T1* transcripts and had achieved CR after 2 induction cycles; and (3) no contraindications to consolidation chemotherapy or HSCT. Figures 1 and 2 present the treatment scheme and study protocol. This protocol was approved by the institutional review boards at each center. Written informed consent was obtained from all patients or guardians before entry into the study in accordance with the Declaration of Helsinki.

MRD monitoring and KIT mutation screening

Bone marrow samples were collected from patients at the time of diagnosis, after induction therapy, after every consolidation chemotherapy, and then at 3-month intervals for 1 year. In total, 978 bone marrow samples (median, 8 samples per patient) were collected. MRD was monitored using RQ-PCR to quantify the level of *RUNX1/RUNX1T1* transcripts. Total RNA was extracted with TRIzol reagent (Invitrogen) according to the manufacturer's instructions. PCR reaction conditions have been previously published.[25] Each RQ-PCR reaction and analysis were performed on an AB 7500 platform. Primers and probes for the *RUNX1/RUNX1T1* gene and the control gene *ABL* have been previously described.[25] c-KIT mutations in exons 17 and 8 were screened using the direct sequencing method. We did not include the c-KIT mutation screening in the initial protocol. After the prognostic value of the c-KIT mutation was confirmed in 2006, we only added to detect the c-KIT mutation without modifying the treatment protocol according to KIT status. In total, the pretreatment bone marrow samples for 84 patients were successfully sequenced, whereas the KIT mutational status of 32 patients was undetermined because of unavailable or poor-quality samples.

Diagnosis and treatment response assessment

The diagnosis of AML was performed as previously described.[26] CR was defined as <5% bone marrow blasts, the absence of blasts with Auer rods, the absence of extramedullary disease, an absolute neutrophil count >1.0 × 10^9/L, and a platelet count >100 × 10^9/L with no red cell transfusions. Relapse was defined as the recurrence of ≥5% bone marrow blasts and the reappearance of blasts in the blood or the development of extramedullary disease infiltrates at any site.

Definitions of MMR and risk groups

Major molecular remission (MMR) was defined as a >3-log reduction in *RUNX1/RUNX1T1* transcripts (<0.4%) compared with the pretreatment baseline of 388% in our center, as previously described.[25] Loss of MMR

was defined as *RUNX1/RUNX1T1* transcript levels >0.4% in MMR patients. The high-risk group was defined as those patients not achieving MMR after the second consolidation therapy[19,21] or those exhibiting the loss of MMR within 6 months of achieving MMR. The low-risk group was defined as patients who achieved MMR after the second consolidation therapy and maintained MMR for 6 months thereafter.

Treatment protocols

Induction chemotherapy was composed of 1 to 2 cycles of induction with an anthracycline (either daunorubicin at 45 mg/m^2 or idarubicin at 8-10 mg/m^2 for 3 days) in combination with cytarabine at 100 mg/m^2 for 7 days. The first and second consolidation therapies included intermediate-dose cytarabine (IDAC; 1-2 g/m^2 every 12 hours for 3 days) with or without an anthracycline (daunorubicin at 45 mg/m^2 or mitoxantrone at 8 mg/m^2 for 3 days). High-risk patients defined as above were recommended for allo-HSCT, and low-risk patients were recommended to continue 6 cycles of chemotherapy, including IDAC (1-2 g/m^2 every 12 hours for 3 days) for
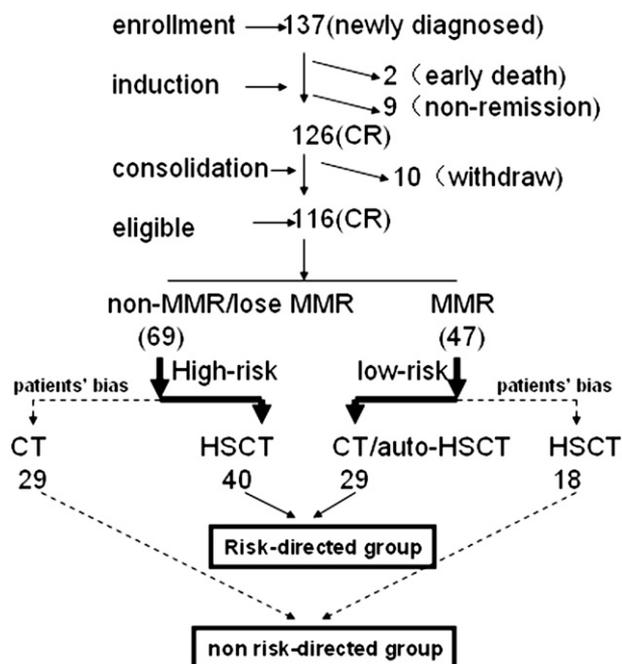

Figure 2. Trial design and patient accrual flowchart. Eighty-two and 34 patients achieved CR after 1 and 2 induction cycles. Sixty-three of 116 patients received anthracycline during consolidation cycles 1 and 2, including 45 mg/m^2 daunorubicin or 8 mg/m^2 mitoxantrone for 3 days. CT, chemotherapy.

Table 1. Characteristics of t(8;21) AML

	Risk directed			Not risk directed			
Characteristic	Low risk—chemo (n = 29)	High risk—HSCT (n = 40)	Total	Low risk—HSCT (n = 18)	High risk—Chemo (n = 29)	Total	P
Age (years)	28 (15-56)	37 (15-54)	33 (15-56)	35 (16-54)	37 (15-60)	36 (15-60)	NS
Gender (male/female)	16/16	26/14	39/30	12/6	16/13	28/19	NS
WBC (10⁹/L)	5.4 (1.4-79)	9.0 (1.9-59)	8.1 (1.4-79)	6.0 (1.2-30)	8.0 (2.5-80.5)	7.2 (1.2-80.5)	NS
HGB (g/L)	83 (34-136)	81 (49-133)	81(45-136)	95 (41-123)	74 (34-135)	78 (34-135)	NS
PLT (10⁹/L)	37 (5-286)	27 (4-108)	32.5 (4-286)	33 (2-106)	35 (7-225)	34 (2-225)	NS
Blasts in BM (%)	52 (26-89)	51 (20-86)	52(20-89)	56 (21-82)	39 (24-92)	46 (21-92)	NS
RUNX1-RUNX1T1 (%)	506 (353-993)	411(97-1082)	439 (35-1082)	422 (98-848)	358(52-1163)	380 (43-1163)	NS
KIT mutation (%)	17.6(3/17)	54.8(17/31)	54.8(20/48)	50(5/10)	34.6(9/26)	38.9(14/36)	NS
Comorbidity score (0/1-2/3)*	23/17/0	18/11/0	41/28/0	16/13/0	11/7/0	27/20/0	NS

There are no differences in the characteristics between the low risk—chemotherapy group and the low risk—HSCT group and between the high risk—chemotherapy group and the high risk—HSCT group. HGB, hemoglobin; PLT, platelet; BM, bone marrow; NS, not significant.

*According to the Charlson Comorbidity Index.

2 cycles and then 45 mg/m² daunorubicin or 8 to 10 mg/m² idarubicin for 3 days plus 100 mg/m² cytarabine for 7 days; 2 mg/m² homoharringtonine for 7 days plus 100 mg/m² cytarabine for 7 days; 8 mg/m² mitoxantrone for 3 days plus 100 mg/m² cytarabine for 7 days; and 20 mg/d aclamycin for 7 days plus 100 mg/m² cytarabine for 7 days. Autologous-HSCT (auto-HSCT) was permitted in low-risk patients after 4 courses of consolidation. Patients who lost MMR within 6 months of the second consolidation were redefined as high-risk patients and recommended for allo-HSCT (Figures 1 and 2). Allo-HSCT required an HLA-matched sibling donor (MSD), a matched unrelated donor (MUD), or a haploidentical related donor (HRD) HSCT. For MSD and HRD HSCT patients, granulocyte-colony-stimulating factor–mobilized bone marrow and peripheral blood stem cells were used as a graft source. For MUD HSCT patients, only granulocyte-colony-stimulating factor–mobilized peripheral blood stem cells were used as a graft source. The HSCT conditioning regimen and graft-versus-host disease prophylaxis were previously described.[27]

Patients who could not comply with the risk-directed treatment recommendations described above due to patient bias, which meant that low-risk patients preferred to undergo allo-HSCT and high-risk patients preferred to undergo chemotherapy as postremission treatment, were categorized into the non–risk-directed therapy group (Figure 2).

End points and statistical methods

The primary end points studied were relapse and disease-free survival (DFS). The secondary end point was overall survival (OS). DFS was measured from the date when CR was achieved. Events for DFS included death in first CR (CR1) or relapse. The event for OS was death (regardless of the cause), and patients were queried at the date of last follow-up to determine whether they were still alive. At a significant level of 5%, the study included 106 patients, providing an overall power of 80% for a 2-sided test to detect a difference of 25% between the 2 groups, assuming that 1 group had a relapse rate of 45%.

Survival functions were estimated using the Kaplan-Meier method and compared using the log-rank test. To exclude bias that may arise from including patients who relapsed or died too early to receive allo-HSCT in CR1 in high-risk patients, landmark analysis was used when comparing the outcomes of patients receiving allo-HSCT with those receiving chemotherapy/auto-HSCT.[28] In our study, the median time from CR achievement to allo-HSCT was 4 months. Thus, we defined 4 months after achieving CR as a landmark for this analysis.

Cox regression model was also used to identify prognostic variables for the patients, which included c-KIT status (mutation or no mutation), treatment choice (risk directed or not risk directed), white blood cell (WBC) count at diagnosis (<20 or ≥20 × 10⁹/L), CD56 (positive or negative), additional chromosome abnormalities (yes or no), and the number of courses required to achieve CR1 (1 or >1 course). The independence of categorical parameters was calculated using either a χ² or Fisher's exact test. The distribution of the continuous variables was calculated using

a Mann-Whitney U test. Any probability mentioned in this study is the probability at 5 years.

Results

Patient characteristics

A total of 137 newly diagnosed patients with t(8;21) were enrolled in this study (Figure 1). Twenty-one of these patients were excluded from the final analysis due to death during induction (n = 2), failure to achieve CR after 2 courses of induction chemotherapy (n = 9), or withdrawal before the second consolidation treatment (n = 10; Figure 2). Eight-two patients and 34 patients achieved CR after 1 and 2 cycles of induction, respectively. Therefore, we analyzed 116 patients composed of 69 patients who received risk-directed treatment and 47 patients (18 low risk and 29 high risk) who received non–risk-directed treatment due to patient bias. Potential reasons for the 18 low-risk patients to be withdrawn from the trial were loss of Y or X chromosome (n = 10), KIT-mutation (n = 5), extramedullary disease (n = 2), and patient's persistence (n = 1). The potential reasons for the 29 high-risk patients to be withdrawn from the trial might result from complex factors such as fear of transplantation-related mortality, donors available, and consulting with other doctors about treatment options. There was no significant difference in the presenting features between the risk-directed and non–risk-directed groups (Table 1).

Fifty-eight patients received chemotherapy-based consolidation therapy (including 17 auto-HSCT), and 58 patients received allo-HSCT in CR1 (MSD, 30 patients; MUD, 5 patients; HRD, 23 patients). The similar outcomes of auto-HSCT and chemotherapy as consolidation therapy were confirmed (data were not shown). There were no significant differences in transplantation outcomes between the 23 patients who received HRD HSCT and the 35 patients who received MSD/MUD HSCT (supplemental Appendix on the *Blood* website).

Patient outcomes

The median follow-up time was 36 months (6-83 months) in surviving patients. Of the 116 patients analyzed, 29 died (18 of relapse; 11 of treatment-related mortality) during treatment and 87 survived; 33 patients experienced a relapse. For the entire study population, the cumulative incidence of relapse (CIR) was 32.8%. The DFS and OS rates were 58.8% and 69.3%, respectively.
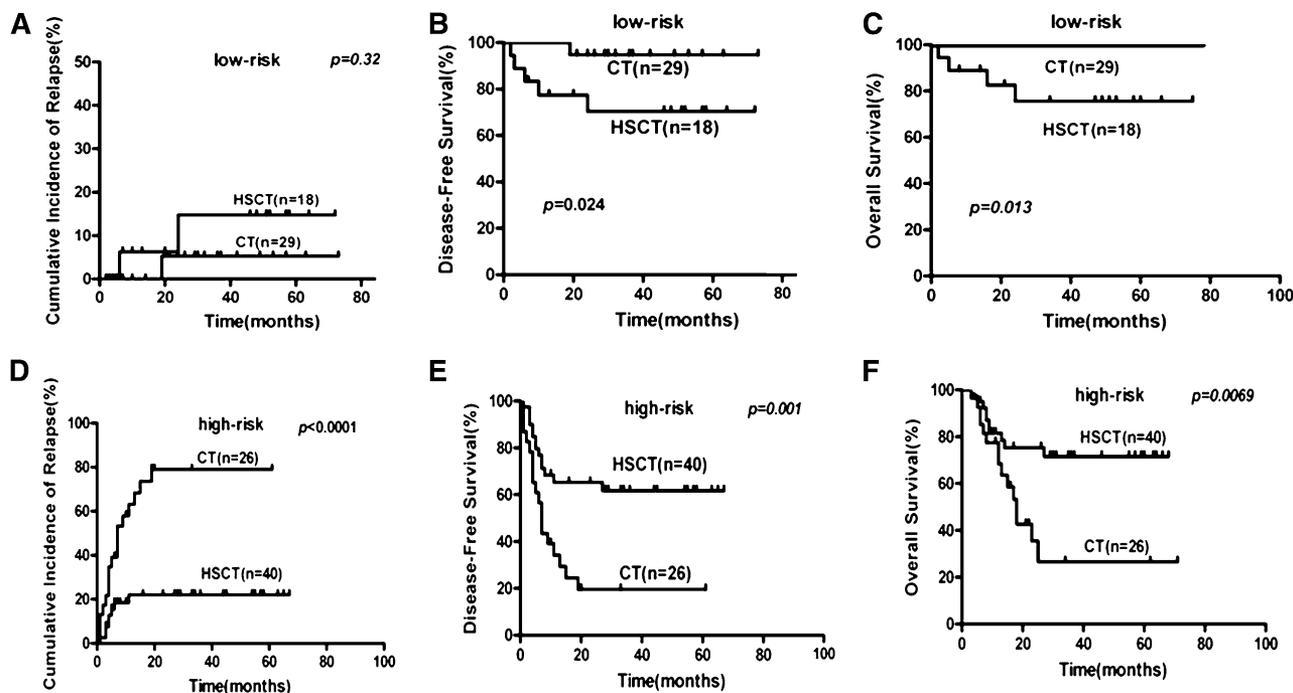
Figure 3. Subgroup analysis of risk stratification treatments and outcomes of t(8;21) AML. (A,D) Relapse. (B,E) DFS. (C,F) OS. HSCT, hematopoietic stem cell transplantation; CT, chemotherapy.

Impact of MRD on relapse at different checkpoints

MRD status (non-MMR vs MMR) at the end of induction could not discriminate high-risk relapse patients ($P = .87$ for CIR) and had a trend to discriminate agaist high-risk relapse patients ($P = .06$ for CIR) at the end of first consolidation. However, it could significantly discriminate high-risk relapse patients at the second consolidation (CIR, 46.9% vs 22.9%; $P = .001$).

Relapse

By subgroup analysis, allo-HSCT lowered the relapse rate compared with chemotherapy for high-risk patients using the landmark analysis (CIR, 22.1% vs 78.9%, $P < .0001$; Figure 3), even for high-risk patients without achieving MMR after second consolidation (CIR, 20.6% vs 100%; $P < .0001$; n = 47). Allo-HSCT did not lower the relapse rate compared with chemotherapy for low-risk patients (14.7% vs 5.3%; $P = .33$). Allo-HSCT had a trend to lower the relapse of KIT-mutated patients (CIR, 28% vs 70%; $P = .07$; Figure 4). When we took all patients that received traditional high-dose cytarabine-based consolidation (according to National Comprehensive Cancer Network [NCCN] guidelines) as a subgroup (n = 58), risk-directed therapy also decreased the relapse rate (CIR, 15.0% vs 54.6%; $P = .0066$). To avoid the influence of KIT mutation, we also analyzed the data in KIT wild-type patients (n = 50) and found that risk-directed therapy also may be superior to the traditional approach (CIR, 11.4% vs 50.1%; $P = .003$).

The 5-year CIR of risk-directed therapy was 15.0%. The CIR of high-risk patients receiving allo-HSCT was 22.1%, and low-risk patients receiving chemotherapy/auto-HSCT was 5.3%.

Based on multivariate analysis, MRD status (high risk vs low risk), treatment strategy (risk directed vs not risk directed), and KIT status (mutation vs no mutation) were all independent risk factors for relapse (Table 2). The hazard ratio for relapse was 8.85 (95% confidence interval [CI], 2.05-38.13; $P = .003$) for high-risk

patients, 0.26 (95% CI, 0.12-0.61; $P = .002$) for risk-directed treatment, and 2.12 (95% CI, 1.01-4.48; $P = .049$) for mutant KIT patients. Other variables such as age (<35 vs ≥35 years), high WBC count at diagnosis ($>20 \times 10^9$/L vs $\leq20 \times 10^9$/L), CD56 expression (positive vs negative), additional chromosome abnormalities (yes vs no), and the number of courses to achieve CR (1 vs >1) did not influence the risk of relapse.

Disease-free survival

Landmark analysis was used when comparing the DFS of patients receiving allo-HSCT with those receiving chemotherapy/auto-HSCT, which defined 4 months after achieving CR as a landmark. By subgroup analysis, allo-HSCT could improve DFS rate when compared with chemotherapy for high-risk patients (61.7% vs 19.6%, $P = .001$; Figure 3) and had a potential to improve DFS for KIT-mutated patients (62.0% vs 30.0%, $P = .19$; Figure 4) using the landmark analysis. Allo-HSCT also improved DFS rate when compared with chemotherapy for high-risk patients without achieving MMR after second consolidation (DFS, 60.5% vs 0%, $P < .0001$, n = 47). DFS decreased in low-risk patients when compared with allo-HSCT recipients (70.3% vs 94.7%, $P = .024$; Figure 4). When we took all patients that received traditional high-dose cytarabine-based consolidation (according to NCCN guidelines) as a subgroup to study (n = 58), risk-directed therapy (n = 69) also improved DFS (74.7% vs 53.1%, $P = .01$). To avoid the influence of KIT mutation, we also analyzed the data in KIT-wild type patients (n = 50) and found that risk-directed therapy might be superior to traditional approach (DFS 80.7% vs 46.7%, $P = .013$).

The 5 year DFS of risk-directed therapy was 74.7%. The DFS of high-risk patients receiving allo-HSCT was 61.7%, and low-risk patients receiving chemotherapy/auto-HSCT was 94.7%.

Multivariate analysis demonstrated that MRD status and treatment strategy were independent risk factors for DFS (Table 2). The hazard ratio for DFS was 9.32 (95% CI 2.21–39.3; $P = .002$) for high-risk
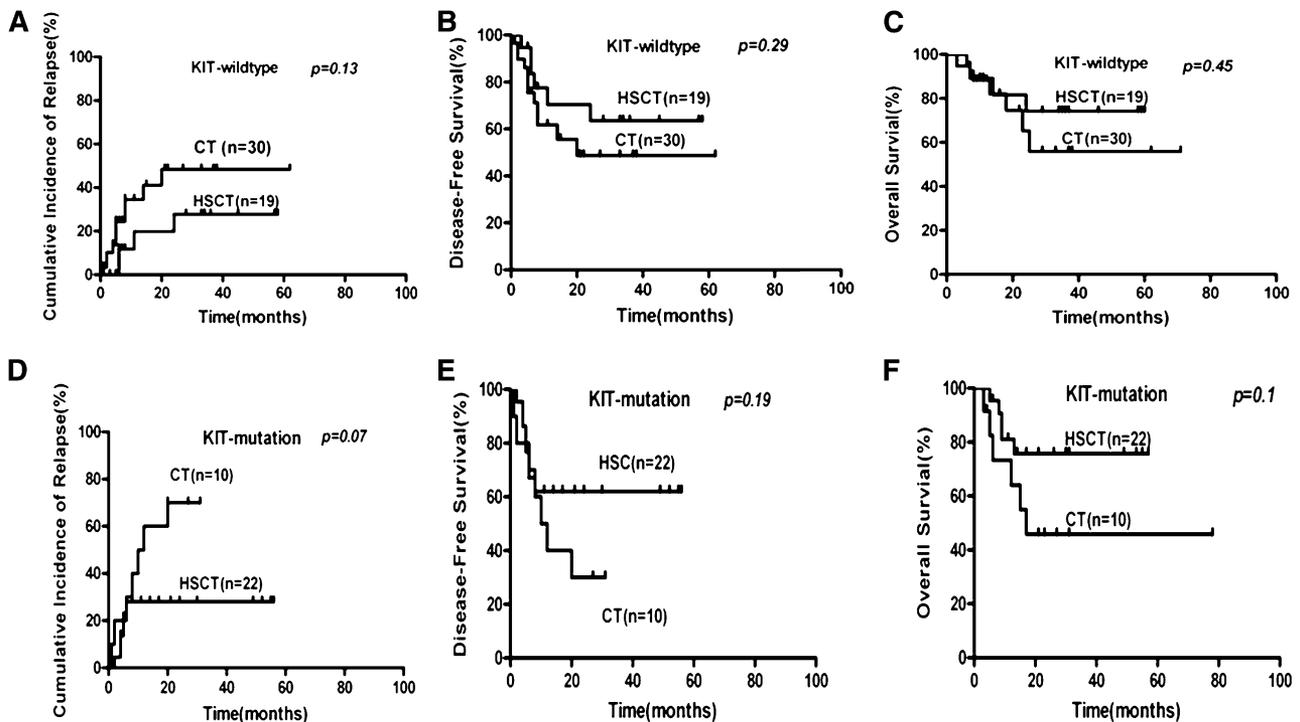
Figure 4. Outcome by KIT gene mutations and treatments of t (8;21) AML patients. The comparison of (A,D) relapse, (B,E) DFS, and (C,F) OS between patients receiving hematopoietic stem cell transplantation (HSCT) and chemotherapy (CT).

patients and 0.36 (95% CI 0.17–0.75, P = .007) for risk-directed treatment. Other variables, such as KIT mutational status, age, high WBC count, CD56 expression, additional chromosome abnormalities and the number of courses to achieve CR, did not influence the risk of DFS.

Overall survival

By subgroup analysis, allo-HSCT improved OS compared with chemotherapy for high-risk patients (71.6% vs 26.7%; P = .007) but decreased OS for low-risk patients (75.7% vs 100%; P = .013; Figure 3) using the landmark analysis. Allo-HSCT also improved OS compared with chemotherapy for high-risk patients without achieving MMR after second consolidation (OS, 69.95% vs 0%; P < .0002; n = 47). Allo-HSCT tended to improve OS compared with chemotherapy for KIT-mutated patients, but this trend did not achieve statistical significance (75.7% vs 45.8%; P = .1; Figure 4).

The 5-year OS of risk-directed therapy was 82.7%. The OS of high-risk patients receiving allo-HSCT was 71.6% and of low-risk patients receiving chemotherapy/auto-HSCT was 100%.

Table 2. Multivariable analysis of prognostic factors

Covariate	CIR P	DFS P	OS P
MRD (high vs low risk)	.0030	.0020	.020
Treatment (risk directed vs not risk directed)	.026	.036	.037
KIT (mutation vs wild type)	.049	NS	NS
BM blasts (≥50% vs <50%)	NS	NS	NS
WBC (≥20 vs <20)	NS	NS	NS
CD56 (positive vs negative)	NS	NS	NS
Age (<35 vs ≥35 years old)	NS	NS	NS
CR (1 vs >1 course)	NS	NS	NS
Cytogenetic (with vs without additional changes)	NS	NS	NS

BM, bone marrow.

When we took all patients that received traditional high-dose cytarabine-based consolidation (according to NCCN guidelines) as a subgroup to study (n = 58), risk-directed therapy (n = 69) tended to have improved OS (82.7% vs 65.1%; P = .07). To avoid the influence of the KIT mutation, we also analyzed the data in KIT wild-type patients (n = 50) and found that risk-directed therapy might be potentially superior to the traditional approach (OS, 85.7% vs 55.9%; P = .17).

Multivariate analysis demonstrated that MRD status and treatment strategy were independent risk factors for DFS (Table 2). The hazard ratio for DFS was 10.53 (95% CI, 1.41-78.83; P = .022) for high-risk patients and 0.37 (95% CI, 0.15-0.93, P = .035) for risk-directed treatment. Other variables, such as KIT mutation, age, high WBC count, CD56 expression, additional chromosome abnormalities, and the number of courses to achieve CR, did not influence the risk of OS.

Discussion

In this multicenter prospective cohort study, we demonstrated that allo-HSCT could reduce relapse and improve survival compared with chemotherapy/auto-HSCT for high-risk patients, and chemotherapy/auto-HSCT achieved a low relapse rate and high survival for low-risk patients. MRD-directed risk stratification treatment may improve the outcome of patients with AML with t(8;21) in CR1.

Combining pretreatment and posttreatment parameters into an optimal, risk-directed treatment algorithm likely contributed to our improved results. Our novel risk stratification system has significant power to identify patients at high risk for relapse. As demonstrated by our results, among patients who received chemotherapy as postremission therapy, high-risk patients had a very high CIR (78.9%) compared with low-risk patients (5.3%). Multivariate analysis further demonstrated that high-risk patient status is an independent prognostic factor for relapse. Our results agree with those of 2

earlier studies in patients with t(8;21).[19,21] These studies found that a reduction in the MRD of <3 logs compared with pretreatment levels was a significant predictor of relapse after 2 courses of consolidation or within 3 to 4 months after achieving CR.[19,21]

Identifying the best checkpoints for MRD examination is very important for applying MRD-directed risk stratification treatment. We found MRD status after second consolidation may be the best timing for MRD examination in our treatment protocol. The reasons why we chose the key checkpoint for MRD assessment after 2 courses of IDAC-based consolidation was according to 2 previous reports,[19,21] which suggested that MRD levels at this time may reflect the sensitivity of leukemia cells to high-dose cytarabine. However, we did not find the predictive value of MRD at earlier checkpoints (after induction and first consolidation) as reported by Liu Yin et al,[29] which might result from our relative low intense of induction (eg, daunorubicine dose, 45 mg/m^2) and consolidation compared with the United Kingdom Medical Research Council AML-15 protocol. Not only the MRD level but also the kinetics of MRD is reliable for the prediction of imminent relapse. Recently published results of the United Kingdom Medical Research Council AML-15 trial showed that not only the >3 log reduction of RUNX1/RUNX1T1 transcript levels at the time of achieving CR but also rising MRD levels on serial monitoring accurately predicted hematological relapse.[29] Therefore, combination monitoring of MRD levels and MRD kinetics translates into a timely, accurate prediction for relapse.

In our study, we could not only predict relapse accurately, but we could also identify who would benefit from allo-HSCT. We demonstrated that allo-HSCT could significantly lower the relapse rates of high-risk patients, translating into improved DFS and OS. Recently, Boissel et al[30] reported primary results in high-risk patients (<3 log reduction before second consolidation), demonstrating that 10 of 18 patients who did not receive allo-HSCT experienced relapse compared with only 1 of 9 patients receiving allo-HSCT. The results of Boissel et al were in agreement with our study. Moreover, we found for the first time that allo-HSCT impaired the outcome of low-risk patients. The reason for the negative effect of allo-HSCT in low-risk patients may be its inability to lower the risk of relapse combined with an increase in treatment-related mortality. Our results support the perspectives reported in the NCCN guidelines that t(8;21) AML is a favorable subgroup of AML and that high-dose cytarabine-based chemotherapy/auto-HSCT is recommended as postremission therapy for low-risk patients. We also found that allo-HSCT tended to lower the relapse rates of KIT-mutated patients and improved their survival. Not reaching statistical difference may result from relatively limited numbers of KIT-mutated patients in our study (supplemental Appendix) and needs to be confirmed by future studies with large samples. Our results suggest recommending allo-HSCT to high-risk, but not low-risk, patients to avoid under- or overtreatment of t(8;21) AML, which might be a complement to current NCCN guidelines.

The primary aim of risk-directed treatment is to improve the survival of the entire patient population. Our results suggested that risk-directed treatment might improve survival (5-year DFS and OS of 74.7% and 82.7%, respectively). This improved survival advantage in the risk-directed treatment group is better than those previous reported without risk-directed treatment, including the French AML Intergroup (5-year DFS and OS of 52% and 59%, respectively; n = 154),[3] the German AML Intergroup (3-year DFS and OS of 60% and 65%, respectively; n = 191),[4] the Cancer and Leukemia Group B Study (5-year OS of 46%; n = 139),[5] the Southwest Oncology Group/Eastern Cooperative Oncology Group/MD. Anderson Study (5-year DFS and OS of 45% and 45%, respectively; n = 174),[6] and the European Group for Blood and Marrow Transplantation Study (5-year DFS: 60% for allo-HSCT and 66% for auto-HSCT; n = 166).[8]

However, our study has some limitations. Forty percent (47/116) of the patients went off the trial. This made it difficult to compare the outcome of risk-directed treatment and non–risk-directed treatment directly, and a randomization controlled trial is needed in the future. Similarly, we compared the efficacy of allo-HSCT and chemotherapy in a nonrandomized condition, which also was a source of bias. Although best compared in a randomized trial, no such study is in progress or is being planned. The low incidence (~10%) of t(8;21) AML within AML makes a randomized trial enrolling >100 patients very difficult. Therefore, our study enrolling 137 patients is likely the best comparison of allo-HSCT and chemotherapy.

In summary, our results suggested that MRD-directed risk stratification treatment may improve the outcome of t(8;21) AML patients in CR1. Treatment of t(8;21) by risk stratification according to MRD status might be incorporated in clinical decision making.

Acknowledgments

Authorship

Contribution: H.-H.Z., X.-J.H., X.-H.Z., and Y.-Z.Q. designed the study; D.-H.L., H.J., H.C., Q.J., L.-P.X., J.L., W.H., L.B., Y.W., Y.-H.C., J.-Z.W., F.-R.W., Y.-Y.L., J.-Y.C., L.-R.W., and Y.-R.L. collected and assembled the data; K.-Y.L. and B.J. analyzed and interpreted the data; H.-H.Z. and X.-J.H. wrote the manuscript; and all authors gave final approval for the manuscript.

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References

1. Grimwade D, Walker H, Oliver F, et al; The Medical Research Council Adult and Children's Leukaemia Working Parties. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. *Blood.* 1998;92(7):2322-2333.

2. Byrd JC, Dodge RK, Carroll A, et al. Patients with t(8;21)(q22;q22) and acute myeloid leukemia have superior failure-free and overall survival when repetitive cycles of high-dose cytarabine are administered. *J Clin Oncol.* 1999;17(12):3767-3775.

3. Nguyen S, Leblanc T, Fenaux P, et al. A white blood cell index as the main prognostic factor in t(8;21) acute myeloid leukemia (AML): a survey of 161 cases from the French AML Intergroup. *Blood.* 2002;99(10):3517-3523.

4. Schlenk RF, Benner A, Krauter J, et al. Individual patient data-based meta-analysis of patients aged 16 to 60 years with core binding factor acute myeloid leukemia: a survey of the German Acute Myeloid Leukemia Intergroup. *J Clin Oncol.* 2004;22(18):3741-3750.

5. Marcucci G, Mrózek K, Ruppert AS, et al. Prognostic factors and outcome of core binding factor acute myeloid leukemia patients with t(8;21)

differ from those of patients with inv(16): a Cancer and Leukemia Group B study. *J Clin Oncol.* 2005; 23(24):5705-5717.

6. Appelbaum FR, Kopecky KJ, Tallman MS, et al. The clinical spectrum of adult acute myeloid leukaemia associated with core binding factor translocations. *Br J Haematol.* 2006;135(2): 165-173.

7. Schlenk RF, Pasquini MC, Pérez WS, et al; CIBMTR Acute Leukemia Working Committee. HLA-identical sibling allogeneic transplants versus chemotherapy in acute myelogenous leukemia with t(8;21) in first complete remission: collaborative study between the German AML Intergroup and CIBMTR. *Biol Blood Marrow Transplant.* 2008;14(2):187-196.

8. Gorin NC, Labopin M, Frassoni F, et al. Identical outcome after autologous or allogeneic genoidentical hematopoietic stem-cell transplantation in first remission of acute myelocytic leukemia carrying inversion 16 or t(8;21): a retrospective study from the European Cooperative Group for Blood and Marrow Transplantation. *J Clin Oncol.* 2008;26(19): 3183-3188.

9. Kuwatsuka Y, Miyamura K, Suzuki R, et al. Hematopoietic stem cell transplantation for core binding factor acute myeloid leukemia: t(8;21) and inv(16) represent different clinical outcomes. *Blood.* 2009;113(9):2096-2103.

10. Paschka P, Marcucci G, Ruppert AS, et al; Cancer and Leukemia Group B. Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B Study. *J Clin Oncol.* 2006;24(24):3904-3911.

11. Cairoli R, Beghini A, Grillo G, et al. Prognostic impact of c-KIT mutations in core binding factor leukemias: an Italian retrospective study. *Blood.* 2006;107(9):3463-3468.

12. Schnittger S, Kohl TM, Haferlach T, Kern W, Hiddemann W, Spiekermann K, Schoch C. KIT-D816 mutations in AML1-ETO-positive AML are associated with impaired event-free and overall survival. *Blood.* 2006;107(5):1791-1799.

13. Buccisano F, Maurillo L, Spagnoli A, et al. Cytogenetic and molecular diagnostic

characterization combined to postconsolidation minimal residual disease assessment by flow cytometry improves risk stratification in adult acute myeloid leukemia. *Blood.* 2010;116(13): 2295-2303.

14. Buccisano F, Maurillo L, Del Principe MI, et al. Prognostic and therapeutic implications of minimal residual disease detection in acute myeloid leukemia. *Blood.* 2012;119(2):332-341.

15. Alonzo TA, Ho PA, Gerbing RB, et al. Conventional cytogenetics, molecular profiling, and flow cytometric response data allow the creation of a two-tiered risk-group system for risk-based therapy allocation in childhood AML: a report from the Children's Oncology Group [abstract]. *Blood.* 2010;116:761a.

16. Campana D, Pui C-H. Detection of minimal residual disease in acute leukemia: methodologic advances and clinical significance. *Blood.* 1995; 85(6):1416-1434.

17. Campana D. Determination of minimal residual disease in leukaemia patients. *Br J Haematol.* 2003;121(6):823-838.

18. Rubnitz JE, Inaba H, Dahl G, et al. Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial. *Lancet Oncol.* 2010;11(6): 543-552.

19. Schnittger S, Weisser M, Schoch C, Hiddemann W, Haferlach T, Kern W. New score predicting for prognosis in PML-RARA+, AML1-ETO+, or CBFBMYH11+ acute myeloid leukemia based on quantification of fusion transcripts. *Blood.* 2003; 102(8):2746-2755.

20. Krauter J, Gorlich K, Ottmann O, et al. Prognostic value of minimal residual disease quantification by real-time reverse transcriptase polymerase chain reaction in patients with core binding factor leukemias. *J Clin Oncol.* 2003;21(23):4413-4422.

21. Leroy H, de Botton S, Grardel-Duflos N, et al. Prognostic value of real-time quantitative PCR (RQ-PCR) in AML with t(8;21). *Leukemia.* 2005; 19(3):367-372.

22. Perea G, Lasa A, Aventín A, et al; Grupo Cooperativo para el Estudio y Tratamiento de las Leucemias Agudas y Miel. Prognostic value of

minimal residual disease (MRD) in acute myeloid leukemia (AML) with favorable cytogenetics [t(8;21) and inv(16)]. [t(8;21) and inv(16)] *Leukemia.* 2006;20(1):87-94.

23. Weisser M, Haferlach C, Hiddemann W, Schnittger S. The quality of molecular response to chemotherapy is predictive for the outcome of AML1-ETO-positive AML and is independent of pretreatment risk factors. *Leukemia.* 2007;21(6): 1177-1182.

24. Lane S, Saal R, Mollee P, et al. A >or=1 log rise in RQ-PCR transcript levels defines molecular relapse in core binding factor acute myeloid leukemia and predicts subsequent morphologic relapse. *Leuk Lymphoma.* 2008;49(3):517-523.

25. Qin YZ, Li JL, Zhu HH, et al. [Detection of common fusion transcript levels in untreated leukemia patients by real-time quantitative RT-PCR technique]. *Zhonghua Xue Ye Xue Za Zhi.* 2007;28(7):433-437.

26. Döhner H, Estey EH, Amadori S, et al; European LeukemiaNet. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood.* 2010;115(3):453-474.

27. Lu DP, Dong LJ, Wu T, et al. Conditioning including antithymocyte globulin followed by unmanipulated HLA-mismatched/ haploidentical blood and marrow transplantation can achieve comparable outcomes with HLA-identical sibling transplantation. *Blood.* 2006;107(8): 3065-3073.

28. Anderson JR, Cain KC, Gelber RD. Analysis of survival by tumor response. *J Clin Oncol.* 1983; 1(11):710-719.

29. Liu Yin JA, O'Brien MA, Hills RK, et al. Minimal residual disease monitoring by RT-qPCR in core-binding factor AML allows risk-stratification and predicts relapse: results of the UK MRC AML-15 trial. *Blood.* 2012;120(14):2826-2835.

30. Jourdan E, Boissel N, Chevret S, et al. Prospective evaluation of gene mutations and minimal residual disease in patients with core binding factor acute myeloid leukemia. *Blood.* 2013;121(12):2213-2223.

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