Low WT1 transcript levels at diagnosis predicted poor outcomes of acute myeloid leukemia patients with \( t(8;21) \) who received chemotherapy or allogeneic hematopoietic stem cell transplantation

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Abstract

**Background:** Acute myeloid leukemia (AML) with \( t(8;21) \) is a heterogeneous disease. Identifying AML patients with \( t(8;21) \) who have a poor prognosis despite achieving remission is important for determining the best subsequent therapy. This study aimed to evaluate the impact of Wilm tumor gene-1 (WT1) transcript levels and cellular homolog of the viral oncogene \( \nu-KIT \) receptor tyrosine kinase (\( \nu\text{-KIT} \)) mutations at diagnosis, and RUNX1-RUNX1T1 transcript levels after the second consolidation chemotherapy cycle on outcomes.

**Methods:** Eighty-eight AML patients with \( t(8;21) \) who received chemotherapy only or allogeneic hematopoietic stem cell transplantation (allo-HSCT) were included. Patients who achieved remission, received two or more cycles of consolidation chemotherapy, and had a positive measureable residual disease (MRD) test result (defined as <3-log reduction in RUNX1-RUNX1T1 transcript levels compared to baseline) after 2–8 cycles of consolidation chemotherapy were recommended to receive allo-HSCT. Patients who had a negative MRD test result were recommended to receive further chemotherapy up to only 8 cycles. WT1 transcript levels and \( \nu\text{-KIT} \) mutations at diagnosis, and RUNX1-RUNX1T1 transcript levels after the second consolidation chemotherapy cycle were tested.

**Results:** Patients who had a \( \nu\text{-KIT} \) mutation had significantly lower WT1 transcript levels than patients who did not have a \( \nu\text{-KIT} \) mutation (6.7% ± 10.6% vs. 19.5% ± 19.9%, \( P < 0.001 \)). Low WT1 transcript levels (≤5.0%) but not \( \nu\text{-KIT} \) mutation at diagnosis, a positive MRD test result after the second cycle of consolidation chemotherapy, and receiving only chemotherapy were independently associated with high cumulative incidence of relapse in all patients (hazard ratio [HR] = 3.53, 2.30, and 11.49; 95% confidence interval [CI] 1.64–7.62, 1.82–7.56, and 4.43–29.82; \( P = 0.002, 0.034, \) and \( <0.001, \) respectively); these conditions were also independently associated with low leukemia-free survival (HR = 3.71, 2.33, and 5.85; 95% CI 1.82–7.56, 1.17–4.64, and 2.75–12.44; \( P = 0.002, 0.016, \) and \( <0.001, \) respectively) and overall survival (HR = 3.50, 2.32, and 4.34; 95% CI 1.56–7.82, 1.09–4.97, and 1.98–9.53; \( P = 0.002, 0.030, \) and \( <0.001, \) respectively) in all patients.

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Conclusions: Testing for WT1 transcript levels at diagnosis in patients with AML and t(8;21) may predict outcomes in those who achieve remission. A randomized study is warranted to determine whether allo-HSCT can improve prognosis in these patients.

Keywords: Acute myeloid leukemia, RUNX1-RUNX1T1 transcript level, WT1 transcript level, C-KIT mutation, Allogeneic hematopoietic stem cell transplantation

Background
Patients who have acute myeloid leukemia (AML) with t(8;21) have a better prognosis than those with other cytogenetic subtypes [1, 2]. Nevertheless, 30%–40% of patients with t(8;21) relapse, and many die of advanced leukemia [3–5]. Consequently, identifying AML patients with t(8;21) who have a poor prognosis despite achieving remission is important for determining the best subsequent therapy.

Several variables are associated with outcomes in AML patients with t(8;21) [3, 5–14]. Some variables can be ascertained at diagnosis, some after therapy, and others either at diagnosis or after therapy. Many of these variables are confounded. For example, the association between cellular homolog of the viral oncogene v-KIT receptor tyrosine kinase (C-KIT) mutation at diagnosis and poorer outcomes after chemotherapy is masked by the predictive value of Runt-related transcription factor 1-RUNX1 translocation partner 1 (RUNX1-RUNX1T1) transcript levels after achieving remission [12, 13]. This is not surprising since response to therapy is usually a better predictor of outcomes than a measurement at diagnosis.

Wilm tumor gene-1 (WT1) is a transcription factor overexpressed in diverse neoplasms, including AML. We and others reported that more than 70% of AML patients with overexpressed WT1 at diagnosis [15–18]. Studies in mice indicated that, in some settings, WT1 overexpression was required for the development of leukemia [19]. Whether WT1 transcript levels at diagnosis predict outcomes of AML patients is controversial [20–26]. No study has examined AML patients with t(8;21) who achieved remission.

In our study, we examined 88 AML patients with t(8;21) who enrolled in the multicenter AML05 trial (registered at http://www.chictr.org as #ChiCTR-OCH-12002406); after achieving remission, these patients received chemotherapy only or allogeneic hematopoietic stem cell transplantation (allo-HSCT) [13]. We evaluated the association between WT1 transcript levels at diagnosis and therapy outcomes, after adjusting for other potential prognostic variables, including C-KIT mutations at diagnosis and RUNX1-RUNX1T1 transcript levels after the second consolidation chemotherapy cycle.

Methods

Patient selection
Between January 2007 and December 2012, 124 consecutive AML patients with t(8;21) (median age 37 years; age range 14–60 years) from three centers (Peking University People’s Hospital, Beijing No. 6 Hospital, and Beijing Rehabilitation Hospital) were enrolled in the AML05 trial. After one or two cycles of induction chemotherapy, 108 patients achieved complete remission; of these, 101 patients had high-quality RNA samples extracted from bone marrow mononuclear cells at diagnosis.

As we previously reported [13], induction chemotherapy was composed of 1–2 cycles of induction with an anthracycline (either daunorubicin 45 mg/m² or idarubicin 8–10 mg/m² for 3 days) in combination with cytarabine 100 mg/m² for 7 days. The first and second cycles of consolidation chemotherapy included intermediate-dose cytarabine (IDAC 1–2 g/m² every 12 h for 3 days) with or without an anthracycline (daunorubicin 45 mg/m² or mitoxantrone 8 mg/m² for 3 days). Post-consolidation chemotherapy was performed as reported previously [13], which included IDAC for 2 cycles, then followed by daunorubicin/idarubicin in combination with cytarabine, homoharringtonine with cytarabine, mitoxantrone with cytarabine, or aclamycin with cytarabine. Among the 101 patients, 1 received no further therapy, 42 received 1–6 cycles of post-consolidation chemotherapy, and 13 received 1–4 cycles of post-consolidation chemotherapy followed by autologous-hematopoietic stem cell transplantation (auto-HSCT); the remaining 45 received no post-consolidation chemotherapy (n = 7) or 1–6 courses of post-consolidation chemotherapy (n = 38), followed by allo-HSCT from a human leukocyte antigen (HLA)-identical sibling (n = 22), a HLA haplotype-matched relative (n = 19), or a HLA-matched unrelated donor (n = 4). Therapy recommendation was based on the results of measurable residual disease (MRD) testing [27] after 2–8 cycles of consolidation chemotherapy. The real treatment selection was based on both physician’s recommendation and patient’s preference.

RNA extraction, real-time quantitative polymerase chain reaction (RQ-PCR) testing, and C-KIT mutation testing were performed at Peking University Institute of Hematology. The cutoff date for follow-up was October 31, 2014. We did not consider WT1 transcript levels at
diagnosis when deciding what post-consolidation therapy was received by patients who achieved remission and received consolidation chemotherapy.

Ethics, consent, and permissions
Our study was approved by the Ethics Committee of Peking University People’s Hospital. In accordance with the Declaration of Helsinki, all patients offered signed informed consent to participate in the study.

RNA extraction and complementary DNA (cDNA) synthesis
Trizol Reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA. A High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) was used to synthesize cDNA.

Detection of RUNX1-RUNX1T1 and WT1 transcripts
As described previously, TaqMan-based RQ-PCR technology was used to detect RUNX1-RUNX1T1 and WT1 transcript levels [18, 28–30]. Primer and probe sequences for Abelson (ABL) and RUNX1-RUNX1T1 from the report of the Europe against cancer program [31, 32] were used. Primer and probe sequences used for WT1 detection were performed as those published previously [30, 33]. Transcript levels were calculated as percent of RUNX1-RUNX1T1 or WT1 transcript copies/ABL copies. We previously identified an upper limit of 0.5% of WT1 transcript level detection in normal bone marrow samples [18].

In a prior study, the median baseline level of RUNX1-RUNX1T1 transcripts was 388% (range 138%–848%) [34]. We defined a positive MRD test result as a less than 3-log reduction in RUNX1-RUNX1T1 transcript level compared to baseline (>0.4%) after the second cycle of consolidation chemotherapy. Although a positive or negative MRD test result after the second cycle of consolidation chemotherapy was used in the univariate and multivariate analyses, therapy recommendations were based on results of MRD testing at each time point.

Detection of C-KIT mutations in exons 17 and 8
cDNA was used for PCR to detect C-KIT mutations in exons 17 and 8 [10]. The PCR products were analyzed by bidirectional sequencing on an ABI 3730 sequencer (Applied Biosystems). Sensitivity of mutation detection was 10%–20%.

Statistical analyses and definitions
Fisher’s exact test was used to compare the differences in variable frequencies between cohorts. Martingale residual plot and receiver operating characteristic (ROC) curves based on overall mortality were used to determine the potentially optimal WT1 cutoff levels. Survival functions were estimated using the Kaplan–Meier method and compared by the log-rank test within the same treatment group. The starting point for comparing outcomes between the cohorts was the point when complete remission was declared. Left-truncated analyses were used to eliminate the potential bias caused by the relapse or death which made the patient unable to receive an allo-HSCT. At each study time point in this model, the risk set in the non-transplant cohort consisted of all patients who were still in the study, whereas the risk set in the transplant cohort included only patients whose waiting time to undergo allo-HSCT was shorter than the current study period and who were still in the study.

Univariate probabilities of overall survival (OS) and leukemia-free survival (LFS) were calculated using a left-truncated version of the Kaplan–Meier estimator with 95% confidence intervals (CIs). To accommodate competing risks, cumulative incidence of relapse (CIR) and treatment-related mortality (death in complete remission) were calculated using a left-truncated version of the cumulative incidence function (CIF). To adjust for the differences in baseline characteristics, left-truncated versions of the Cox proportional hazards regression models were used to evaluate the relative risk of patients who received chemotherapy only versus those who received allo-HSCT. The proportionality assumption was tested by adding a time-dependent covariate. A backward stepwise model selection approach was used to identify all significant risk factors. Furthermore, we analyzed the association of variables with clinical outcomes in the chemotherapy-only and allo-HSCT cohorts. Results of the multivariate analysis were confirmed by fitting a Cox model with a time-dependent treatment assignment in which all patients were considered non-transplant patients and were switched to the transplant cohort when the current study time point passed each individual's transplant time. LFS was measured from the date that complete remission was detected. Events for LFS included relapse or death after achieving complete remission. Patients or their relatives were queried at the date of the last follow-up or censored on the date the patients were last known to be alive. P values less than or equal to 0.05 were considered statistically significant. SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) and SPSS version 13.0 (IBM Corporation, Armonk, NY, USA) software packages and GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA) were used for data analyses. Standard definitions of complete remission and relapse were used as reported previously [35]. Relapses included those occurred at the bone marrow and/or extra-medullary sites.
Table 1 Unadjusted outcomes at 3 years of acute myeloid leukemia (AML) patients with t(8;21) who achieved complete remission after 1–2 induction chemotherapy cycles

| Group            | CIR rate (95% CI) | LFS rate (95% CI) | OS rate (95% CI) |
|------------------|-------------------|-------------------|-----------------|
| Chemotherapy only| 71% (63%–80%)     | 26% (15%–43%)     | 41% (25%–58%)   |
| Auto-HSCT        | 8% (0%–71%)       | 85% (51%–96%)     | 92% (57%–99%)   |
| Allo-HSCT        | 16% (10%–22%)     | 72% (56%–83%)     | 77% (61%–87%)   |
| All cohorts      | 37% (25%–50%)     | 56% (45%–65%)     | 65% (54%–74%)   |

CIR: cumulative incidence of relapse; LFS: leukemia-free survival; OS: overall survival; allo-HSCT: allogeneic hematopoietic stem cell transplantation; auto-HSCT: autologous-hematopoietic stem cell transplantation

Results

Patients and outcomes

Among the 101 patients studied, median follow-up time for those who survived was 38 months (range 4–93 months). Twenty-six chemotherapy-only patients, 1 auto-HSCT patient, and 6 allo-HSCT patients experienced a relapse. Twenty-two chemotherapy-only patients, 1 auto-HSCT patient, and 6 allo-HSCT patients experienced a relapse after two cycles of consolidation chemotherapy. The Martingale residuals plot indicated that the potential breakpoint for WT1 transcript levels at diagnosis was significantly associated with WT1 transcript levels and a C-KIT mutation (mean ± standard deviation: 6.7 ± 10.6% vs. 19.5 ± 19.9%, P < 0.001; Fig. 1).

Determining the best WT1 transcript breakpoint

The Martinale residuals plot indicated that the potential breakpoint for WT1 transcript levels was 5.0%–10.0%. We identified 5.0% (≤5.0% vs. >5.0%) as the most appropriate WT1 breakpoint using the maximized partial likelihood method. In this study, 5.0% was the largest likelihood among 5.0%, 8.0%, and 10.0%. The same WT1 level breakpoint was identified through ROC curve analyses (data not shown). Based on these data, 40 patients with WT1 transcript levels ≤5.0% at diagnosis were characterized as having low WT1 transcript levels, and 48 patients were characterized as having high WT1 transcript levels.

Relationship between WT1 transcript level and C-KIT mutation at diagnosis

Among 88 patients, 82 (93.2%) had WT1 overexpression (transcript level >0.5%) at diagnosis, including 48 (54.5%) with WT1 overexpression >1-log above the upper limit of normal bone marrow cells (transcript level >5.0%). Thirty patients had C-KIT mutations detected at diagnosis. Patients who had a C-KIT mutation had significantly lower WT1 transcript levels than those who did not have a C-KIT mutation (mean ± standard deviation: 6.7% ± 10.6% vs. 19.5% ± 19.9%, P < 0.001; Fig. 1).

Forty patients had high WT1 transcript levels and no C-KIT mutation, 22 patients had low WT1 transcript levels and a C-KIT mutation, 18 patients had low WT1 transcript levels and no C-KIT mutation, and 8 patients had high WT1 transcript levels and a C-KIT mutation. Having low WT1 transcript levels at diagnosis was significantly associated with C-KIT mutation (22 of 40 vs. 8 of 48; P < 0.001).

Associations of WT1 transcript level and C-KIT mutation at diagnosis with outcomes

Of the 43 patients who received chemotherapy only, 17 who had low WT1 transcript levels at diagnosis had significantly higher 3-year CIR and lower LFS and OS rates than the 26 patients who had high WT1 transcript levels at diagnosis (CIR 100% vs. 44% [95% CI 32%–56%], P < 0.001; LFS 0% vs. 51% [95% CI 26%–71%], P < 0.001;

Table 2 Variables at diagnosis of AML patients with t(8;21) who achieved complete remission and received chemotherapy only and allo-HSCT

| Variable                        | All (88) | Chemotherapy only (43) | Allo-HSCT (45) | P value |
|---------------------------------|----------|------------------------|----------------|---------|
| Total                            | 88       | 43                     | 45             | 0.36    |
| Age (years)                     | 36 (14–60)| 42 (14–60)              | 36 (14–54)     | 0.36    |
| Males                           | 47 (53%) | 19 (44%)               | 28 (62%)       | 0.13    |
| WBC (×10⁹/L)                    | 8.3 (1.3–112)| 8.1 (1.3–112)         | 8.6 (1.2–83)   | 0.41    |
| Blast cells percentage in the bone marrow (%) | 46% (18%–87%) | 46% (23%–87%) | 48% (18%–83%) | 0.82 |
| Platelet count (×10⁹/L)         | 29 (4–187) | 30 (5–187)              | 28 (4–160)     | 0.38    |
| Other cytogenetic abnormality than t(8;21) | 54 (64%) | 24 (59%)               | 30 (70%)       | 0.36    |
| RUNX1-RUNX1T1 transcript level  | 466% (97%–2545%) | 532% (186%–2545%)      | 422% (97%–933%)| 0.31    |
| C-KIT mutation                  | 30 (34%) | 15 (35%)               | 15 (33%)       | 1.00    |

RUNX1-RUNX1T1: Runt-related transcription factor 1–RUNX1 translocation partner 1, C-KIT: cellular homolog of the viral oncogene v-KIT receptor tyrosine kinase

* These values are presented as number of patients followed by percentage in parentheses; other values are presented as median followed by a range in parentheses.
OS 15% [95% CI 3%–36%] vs. 66% [95% CI 41%–82%], \( P = 0.001 \); Fig. 2). Similarly, the 15 patients who had a \( C\)-\( KIT \) mutation at diagnosis had significantly higher 3-year CIR and lower LFS and OS rates than patients without a \( C\)-\( KIT \) mutation (CIR 100% vs. 52% [95% CI 30%–70%], \( P = 0.001 \); LFS 0% vs. 43% [95% CI 22%–63%], \( P = 0.002 \); OS 19% [95% CI 3%–44%] vs. 57% [95% CI 34%–74%], \( P = 0.018 \)). The 16 patients who had a positive MRD test after the second cycle of consolidation chemotherapy had significantly higher 3-year CIR and lower LFS and OS rates than the 27 patients who had a negative MRD test (CIR 89% [95% CI 79%–95%] vs. 59% [95% CI 39%–75%], \( P < 0.001 \); LFS 11% [95% CI 1%–35%] vs. 41% [95% CI 19%–61%], \( P < 0.001 \); OS 25% [95% CI 7%–49%] vs. 58% [95% CI 34%–76%], \( P = 0.001 \)).

Next, we grouped patients undergoing chemotherapy only according to \( WT1 \) transcript level and \( C\)-\( KIT \) mutation at diagnosis. The 6 patients who had low \( WT1 \) transcript levels and no \( C\)-\( KIT \) mutation had similar 3-year CIR, LFS, and OS rates as the 15 patients who had a \( C\)-\( KIT \) mutation (11 with low and 4 with high \( WT1 \) transcript levels at diagnosis; CIR 100% vs. 100%, \( P = 0.491 \); LFS 0% vs. 0%, \( P = 0.491 \); OS 16% [95% CI 1%–32%] vs. 19% [95% CI 7%–30%], \( P = 0.640 \); Fig. 2); these patients were merged in further analyses. The 21 patients who had

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**Fig. 1** Patients with a cellular homolog of the viral oncogene v-KIT receptor tyrosine kinase (\( C\)-\( KIT \)) mutation had significantly lower Wilm tumor gene-1 (\( WT1 \)) transcript levels at diagnosis than patients who did not have a \( C\)-\( KIT \) mutation. Line represents median \( WT1 \) transcript levels.

**Fig. 2** Both \( WT1 \) transcript level and \( C\)-\( KIT \) mutation at diagnosis were associated with outcomes in patients who received chemotherapy only. Patients were grouped by \( WT1 \) transcript level and/or \( C\)-\( KIT \) mutation at diagnosis. a, d, g cumulative incidence of relapse (CIR) rate (%); b, e, h leukemia-free survival (LFS) rate (%); c, f, i overall survival (OS) rate (%). Each row has the same labels that are shown on the right of the row.
low WT1 transcript levels or C-KIT mutation at diagnosis had a significantly higher 3-year CIR and worse LFS and OS than the 22 patients with high WT1 transcript levels and no C-KIT mutation (CIR 100% vs. 36% [95% CI 9%–65%], P < 0.001; LFS 0% vs. 57% [95% CI 30%–78%], P < 0.001; OS 16% [95% CI 3%–37%] vs. 71% [95% CI 43%–87%], P = 0.001; Fig. 2). Based on these data, we classified patients who had low WT1 transcript levels or a C-KIT mutation at diagnosis as being high-risk and those with high WT1 transcript levels and no C-KIT mutation at diagnosis as being low-risk. Using WT1 transcript levels at diagnosis allowed us to reclassify six patients as high-risk compared to using C-KIT mutation state data only to calculate risk.

**Allo-HSCT outcomes**

Forty-five patients received allo-HSCT. Patients with low WT1 transcript levels at diagnosis (n = 23) had 3-year CIR, LFS, and OS rates similar to patients with high WT1 transcript levels at diagnosis (n = 22; CIR 13% [95% CI 3%–30%] vs. 15% [95% CI 4%–34%], LFS 64% [95% CI 40%–80%] vs. 80% [95% CI 55%–92%], OS 66% [95% CI 42%–82%] vs. 86% [95% CI 63%–95%]; all P > 0.05). Likewise, patients with a C-KIT mutation at diagnosis (n = 15) had 3-year CIR, LFS, and OS rates similar to patients without a C-KIT mutation at diagnosis (n = 30; CIR 14% [95% CI 2%–35%] vs. 14% [95% CI 5%–29%]; LFS 79% [95% CI 49%–93%] vs. 69% [95% CI 48%–82%]; OS 78% [95% CI 47%–92%] vs. 75% [95% CI 55%–87%]; all P > 0.05). Patients who had a positive MRD test (n = 19) had similar 3-year CIR, LFS, and OS rates as those who had a negative MRD test (n = 26; CIR 11% [95% CI 2%–28%] vs. 16% [95% CI 5%–33%]; LFS 68% [95% CI 43%–84%] vs. 76% [95% CI 53%–88%]; OS 74% [95% CI 48%–88%] vs. 79% [95% CI 57%–91%]; all P > 0.05).

**Univariate and multivariate analyses in the combined population**

WT1 transcript levels at diagnosis, C-KIT mutation state at diagnosis, MRD test results after the second cycle of consolidation chemotherapy, and subsequent therapy received (chemotherapy only vs. allo-HSCT) were entered into multivariate analysis for CIR, LFS, and OS. All variables except C-KIT mutation state at diagnosis were independently associated with these outcomes (Table 3).

**Comparison of outcomes between allo-HSCT and chemotherapy within different risk groups**

Of the 40 patients with low WT1 transcript levels at diagnosis, the 17 who received chemotherapy only had significantly lower LFS and OS rates than the 23 who received allo-HSCT (LFS: HR = 6.70 [95% CI 2.63–17.05], P < 0.001; OS: HR = 4.71 [95% CI 1.83–12.07], P = 0.001). Of the 48 patients with high WT1 transcript levels at diagnosis, the 26 who received chemotherapy only had similar LFS and OS rates as the 22 who received allo-HSCT (LFS: HR = 3.44 [95% CI 0.96–12.41], P = 0.059; OS: HR = 2.66 [95% CI 0.63–11.16], P = 0.183).
Discussion
Leukemia is one of the leading causes of cancer death [36]. AML is a type of heterogeneous leukemia. In the current study, we found that WT1 transcript level at diagnosis was significantly associated with CIR, LFS, and OS in patients with AML and t(8; 21) who achieved remission with conventional therapy. Although others have studied the prognostic effect of WT1 at diagnosis in patients with AML, the results are controversial; some studies reported that high WT1 transcript levels were associated with a good outcome [20–22], and others reported the contrary [23–26]. These studies included diverse populations and were not restricted to patients who achieved remission.

We also found an association between low WT1 transcript levels and C‑KIT mutation at diagnosis. Others have reported similar results, but they examined fewer patients [37]. Martingale residual plot and ROC curve analyses indicated that a WT1 transcript level of ≤5.0 or >5.0% distinguished patients with different CIR, LFS, and OS probabilities.

Multivariate analyses showed that WT1 transcript levels at diagnosis and results of MRD testing after the second cycle of consolidation chemotherapy, but not C‑KIT mutation at diagnosis, independently associated with CIR, LFS, and OS in patients who received chemotherapy only and in patients who received allo-HSCT. C‑KIT mutation is a widely recognized adverse prognostic factor in AML patients with t(8; 21) [8, 10, 38–42]. Although WT1 levels at diagnosis associated with C‑KIT mutation, we also noted discordant results. We found that patients with either low WT1 transcript levels or a C‑KIT mutation at diagnosis had poor prognosis despite achieving remission. However, WT1 levels at diagnosis were a better predictor of outcome than C‑KIT mutation, and combining them enabled us to reclassify six patients as being at high-risk.

Our previous study suggested that allo-HSCT could improve the prognosis of high-risk patients with t(8;21) after 2–8 cycles of consolidation chemotherapy [13]. Multivariate analyses showed an independent association between therapy type and CIR, LFS, and OS.

Outcomes of patients with low WT1 transcript levels at diagnosis, whom we defined as high-risk, were better for those who received allo-HSCT than for those who received chemotherapy only. However, because patients with high WT1 transcript levels at diagnosis were not randomized to receive either allo-HSCT or chemotherapy only, we cannot be certain that receiving allo-HSCT resulted in better outcomes.

Why low WT1 transcript levels at diagnosis are associated with poor prognosis in patients with AML is unclear. WT1 functions are complex, including activation and repression of transcription and oncogenic and tumor-suppressor properties [43–46].

Our study has several important limitations. First, it was not randomized. Second, a substantial number of patients with a positive MRD test result declined their therapy assignment, including some patients who relapsed before allo-HSCT could be done. Third, MRD testing continued to be done at diverse time points following the analysis completed after the second consolidation cycle. To interrogate associations of variables we analyzed with outcomes, we used the results of the test that was conducted after the second consolidation cycle in univariate and multivariate analyses; however, therapy recommendations were sometimes made based on test results from later time points. Fourth, the small sample size of subgroups resulted in relatively low statistical power. These considerations could have biased our results, and a randomized trial is warranted to test the validity of our conclusions.

Conclusions
Low WT1 transcript levels at diagnosis are associated with poor outcomes of AML patients with t(8;21) who achieve remission. Patients who received allo-HSCT instead of chemotherapy only had better outcomes. For patients with AML and t(8;21) with low WT1 transcript levels at diagnosis, a randomized trial of allo-HSCT versus chemotherapy only after consolidation chemotherapy is needed to determine if allo-HSCT improves outcomes.

Abbreviations
C‑KIT mu (−): without C‑KIT mutation; C‑KIT mu (+): with C‑KIT mutation.

Authors’ contributions
XJH designed the research; YZQ, YW, HHZ, RPG, MJZ, and XJH analyzed the data and wrote the report. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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