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ORIGINAL ARTICLE

The prognostic significance of Wilms' tumor gene 1 (WT1) expression at diagnosis in adults with Ph-negative B cell precursor acute lymphoblastic leukemia

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Abstract

The prognostic significance of Wilms' tumor gene 1 (WT1) expression at diagnosis in adults with B cell precursor acute lymphoblastic leukemia (BCP-ALL) remains poorly understood. A total of 257 adults with Ph-negative BCP-ALL who were consecutively diagnosed and received at least 1 course of induction therapy at our institute were retrospectively analyzed. The WT1 expression patterns were significantly different among the molecularly and cytogenetically defined groups (E2A-PBX1, TEL-AML1, and MLL rearrangements; high hyperdiploidy and B-other). By considering the WT1 expression pattern and the relapse status, 2 cutoff values, 1.8% and 7.2%, were arbitrarily selected to place patients into WT1-low, WT1-inter, and WT1-high groups. In the B-other patients who achieved complete remission (CR), WT1-low and WT1-high patients had similar 3-year relapse-free survival (RFS), disease-free survival (DFS), and overall survival (OS) rates, which were all significantly lower than those of WT1-inter patients. The combined WT1-low/high expression group ($n = 132$) had significantly lower 3-year RFS, DFS, and OS rates compared with the WT1-inter group ($n = 63$) of B-other patients (RFS and DFS all $P < 0.0001$; OS $P = 0.0018$ and 0.0008). WT1 low/high expression as well as treating with chemotherapy only was independent poor prognostic factors for RFS, DFS, and OS in the B-other patients who achieved CR. Therefore, the molecularly and cytogenetically defined adult Ph-negative BCP-ALL groups have characteristic WT1 expression patterns, and WT1 low/high expression at diagnosis predicts poor outcome in B-other patients.

Keywords B cell precursor acute lymphoblastic leukemia · Ph-negative · WT1 transcript levels · At diagnosis · Prognosis

Introduction

Treatment outcomes for adults with B cell precursor acute lymphoblastic leukemia (BCP-ALL) remain suboptimal, with only a 30–40% long-term survival rate [1, 2]. Accurate predictors for prognosis and individualized management have been important for the successful treatment of BCP-ALL. In addition to traditional clinical factors, cytogenetic and molecular abnormalities such as high hyperdiploid karyotypes, BCR-ABL, E2A-PBX1, TEL-AML1, and MLL rearrangements, which have been confirmed to be highly predictive of outcome, more genetic factors need to be identified in order to improve risk stratification and treatment outcome, especially in other BCP-ALL (B-other) patients.

Abnormal gene expression might be prognostic in acute leukemia. The Wilms' tumor 1 (WT1) gene, located on chromosome 11p13, encodes a transcription factor. Normal hematopoietic stem/progenitor cells have low WT1 expression, and acute leukemia patients commonly overexpress WT1. Many studies have explored the role of the WT1 transcript level as an index for minimal residual disease (MRD) monitoring in acute leukemia [3, 4]. However, the prognostic significance of WT1 expression at diagnosis is still under debate. Compared with adult acute myeloid leukemia (AML) [5–7], there are

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limited data on the prognostic significance of the diagnostic WT1 expression in adult ALL, and the results are conflicting. As examples, Chiusa et al. found that high WT1 expression was an independent predictor of poor disease-free survival (DFS) and overall survival (OS) [8], whereas Busse et al. found that high WT1 expression had no prognostic impact on outcome [9]. Therefore, comprehensive and thorough investigations need to be conducted.

In the current study, by retrospectively evaluating 257 adult consecutive Ph-negative BCP-ALL patients who were receiving treatment at our institute over the past decade, we investigated their WT1 expression patterns at diagnosis and the prognostic impacts on their outcomes.

Patients and methods

Patients and treatment

Consecutive adults with Ph-negative BCP-ALL who were < 65 years old and received at least 1 cycle of induction chemotherapy at our hospital from June 2008 to December 2017 were included. The diagnosis was based on bone marrow morphology, immunophenotyping, karyotyping, and molecular testing. The cutoff date for follow-up was June 30, 2018.

Treatment

The chemotherapy procedure consisted of induction, consolidation, and maintenance chemotherapy as reported previously [10]. Briefly, the CODP±L regimen was used during induction; the revised hyper-CVAD regimen or MTX+ Lasparaginase and CAM for 8 cycles were used during consolidation; mercaptopurine and MTX were used for 2 years during maintenance. Intrathecal methotrexate and/or cytosine arabinoside administration was used for central nervous system leukemia (CNSL) prevention or treatment. Consenting patients with appropriate donors received allogeneic-hematopoietic stem cell transplantation (allo-HSCT) during their first complete remission (CR1). Transplantation regimens were reported previously [11].

Detection of WT1 and fusion transcript levels

All patients' bone marrow samples collected at diagnosis were used for TaqMan-based real-time quantitative PCR (RQ-PCR) to measure the WT1 transcript as well as BCR-ABL1, E2A-PBX1, TEL-AML1, and MLL rearrangement (MLL-AF4, MLL-AF9, MLL-AF1p, and MLL-AF1q) fusion transcripts as described in our previous studies [12, 13]. ABL was selected as the control gene [14]. In addition, IKZF1 deletion was tested in 146 patients who were diagnosed after 2014 [15]. The PCR experiments were performed in duplicate, and the WT1 transcript level was calculated as the percentage of WT1 transcript copies/ABL copies. The upper limit of the WT1 transcript levels in normal bone marrow (NBM) samples in our laboratory is 0.60% [12, 13].

Minimal residual disease evaluation

MRD monitoring was based on the identification of cells with aberrant phenotypic features identified by flow cytometry at diagnosis. MRD was evaluated after the end of induction and after each cycle of consolidation. The results are presented as the percentage of cells with an aberrant phenotype / nucleated cell numbers.

Definitions

The B-other group was defined as having no high hyperdiploid karyotype (51–65 chromosomes) and no BCR-ABL1, E2A-PBX1, TEL-AML1, or MLL rearrangement according to previous reports [16, 17]. CR was defined as the presence of trilineage hematopoiesis and less than 5% BM blast cells, neutrophil counts of more than 1×10^9/L, platelet counts of more than 100×10^9/L, the absence of extramedullary disease, and no recurrence for 4 weeks [2]. Relapse-free survival (RFS) and disease-free survival (DFS) were measured from the date when CR was achieved. The events measured were relapse for RFS and death during CR1 or relapse for DFS. The event measured for overall survival (OS) was death (regardless of the cause), and patients were queried at the date of the last follow-up to determine whether they were still alive; patients who were no longer alive were censored from the study on the date they were last known to be alive.

Statistical analysis

Pairwise comparisons of the variables between groups were performed using the Mann–Whitney U test for continuous variables and Fisher's exact test for categorical variables. A receiver operating characteristic (ROC) curve was used to identify the optimal cutoff levels that best discriminated patients in relapse. Survival functions were estimated using the Kaplan–Meier method and were compared using the log-rank test. The variables with $P < 0.20$ by the univariate analysis were entered into a multivariate model using a Cox proportional hazards model to identify the most statistically significant parameters associated with RFS and OS. The level for a statistically significant difference was set at $P < 0.05$ for all univariate tests. The SPSS 16.0 software package (SPSS Inc., Chicago, IL) and GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA) were used for the data analysis.

Results

Patients

A total of 257 patients with Ph-negative BCP-ALL were included in this study. A total of 131 (51.0%) patients were male. The median age at diagnosis was 32 years (range, 16–64 years). The patients' characteristics are shown in Table 1.

A total of 216 (84.0%) patients achieved CR after the first cycle of induction chemotherapy, and a total of 234 (91.1%) eventually achieved CR. Thereafter, 5 patients did not receive consolidation treatment, 91 (35.4%) received chemotherapy alone, and 138 (53.7%) received chemotherapy followed by allo-HSCT (matched sibling donor, $n = 38$; haploidentical related donor, $n = 98$; matched unrelated donor, $n = 2$) in their CR1.

The median follow-up period was 17.0 months (range, 1.5–119.0 months) for the entire cohort and 30.0 months (range, 1.5–119.0 months) for the 157 (61.1%) patients who were still alive at the study's conclusion. Fifty-six patients (61.5%) that received chemotherapy alone and 25 patients (18.1%) that received allo-HSCT subsequently relapsed at a median of 7.2 months (range, 2.7–69.8 months) after achieving CR. The 3-year rates of RFS, DFS, and OS in the patients who achieved CR were 59.6% (95% confidence interval (CI), 52.0–66.4%), 52.0% (95% CI, 44.6–58.9%), and 57.7% (95% CI, 49.9–64.7%), respectively, and the 3-year OS rate in the entire cohort was 53.2% (95% CI, 45.8–59.9%).

Distribution and outcomes by molecularly and cytogenetically defined groups

In the 257 patients with Ph-negative BCP-ALL, 8 (3.1%) had a high hyperdiploidy karyotype; 19 (7.4%) had a MLL rearrangement, including MLL-AF4 ($n = 18$) and MLL-AF1p ($n = 1$); 14 (5.4%) had the E2A-PBX1 rearrangement; 1 (0.4%) had the TEL-AML1 fusion transcript; and the remaining 215 (83.7%) patients belonged to the B-other ALL group.

The median follow-up period was 18.0 months (range, 1.5–119.0 months) and 32.0 months (range, 2.0–119.0 months) in all of the B-other ALL patients and 134 patients in the B-other ALL group who were still living at the end of the study, respectively. A total of 195 patients (90.7%) achieved CR. The 3-year RFS rate was 61.2% (95% CI, 52.6–68.7%), which was significantly higher than that in the E2A-PBX1 and MLL rearrangement groups (E2A-PBX1 31.7% [95% CI, 8.6–58.4%], MLL rearrangement 39.8% [95% CI, 15.9–63.1%], $P = 0.0094$ and 0.017) and tended to be lower than that in those with a high hyperdiploidy karyotype (100%, $P = 0.10$).

WT1 expression patterns by molecularly and cytogenetically defined groups

The median WT1 transcript level at diagnosis of the whole cohort was 3.0% (range, 0.001–331.1%), and 183 patients (71.2%) overexpressed WT1 compared with the upper limit of NBM (0.6%). The median WT1 transcript levels in the

Table 1 Characteristics of patients at diagnosis

Variable	All	WT1 expression			*P* value
		Low	Inter	High	
Total	257	102	67	88	
Age (year, median, range)	32 (16–64)	31 (16–64)	31 (16–61)	31 (16–61)	0.93
Males (%)	131 (51%)	55 (54%)	35 (51%)	41 (47%)	0.58
WBC (× 10E9/L; median; range)	8.1 (0.4–464)	7.2 (0.4–225)	9.3 (1.0–151)	12 (0.3–464)	0.038
Hemoglobin (g/L)	87 (31–165)	112 (42–165)	86 (31–162)	82(41–138)	0.014
Platelet count (× 10E9/L; median; range)	72 (3.0–510)	78 (9–368)	78 (3.0–368)	50 (3.0–510)	0.22
Molecularly and cytogenetically defined group					< 0.0001
High hyperdiploid	8	5	0	3	
E2A-PBX1	14	14	0	0	
TEL-AML1	1	1	0	0	
MLL rearrangement	19	1	0	18	
B-other	215	81	67	67	
Cytogenetic categories* (%) ($n = 202$)					< 0.0001
Standard/intermediate	144 (71%)	51 (64%)	52 (98%)	41 (59%)	
High/very high	58 (29%)	29 (36%)	1 (2%)	28 (41%)	
IKZF1 deletion (%) ($n = 146$)	39 (27%)	11 (19%)	14 (35%)	14 (29%)	0.19

*Based on the ECOG 2993 trial, fusion transcript testing results by RQ-PCR were also considered

MLL rearrangement, E2A-PBX1, TEL-AML1, high hyperdiploidy, and B-other groups were 45.7% (range, 1.3–331.1%), 0.19% (range, 0.01–1.6%), 0.95% (range, 0.95–0.95%), 0.82% (range, 0.02–40.2%), and 3.0% (range, 0.001–169.3%), respectively ($P < 0.0001$). As shown in Fig. 1a, the WT1 transcript level of the B-other group was significantly lower than that of the MLL rearrangement group ($P < 0.0001$), was higher than that of the E2A-PBX1 ($P = 0.0001$) group, and was similar to that of the high hyperdiploidy group ($P = 0.99$). Furthermore, great variation existed in the B-other group, with a coverage of 4.9 logs.

Determining optimal cutoff values of the WT1 transcript levels for grouping

The optimal cutoff values of the WT1 transcript levels for grouping were determined in the B-other ALL group. The ROC curves showed that the WT1 transcript levels at diagnosis could not significantly differentiate 195 patients who achieved CR in relapse (area under curve 0.52, $P = 0.69$). However, there seemed to be 2 distinct WT1 expression patterns among the relapsed patients: one group had low WT1 transcript levels, and the other had high WT1 transcript levels (Fig. 1b). This finding implied that using 2 cutoff values to categorize the patients into 3 groups might be optimal to investigate the prognostic significance of the WT1 transcript levels.

To relate the patients' WT1 transcript levels with their relapse status, we arbitrarily selected 1.8% and 7.2% as the 2 cutoff values, which corresponded to a 3-fold and 12-fold increase compared with the upper limit of NBM (0.6%). Thus, WT1 transcript levels < 1.8%, 1.8–7.2%, and > 7.2% were defined as WT1-low, WT1-inter, and WT1-high expression, respectively; 81 (37.6%), 67 (31.2%), and 67 (31.2%) patients in the B-other group had WT1-low, WT1-inter, and WT1-high expression, respectively.

In the entire cohort, 102 (39.7%), 67 (26.1%), and 88 (34.2%) patients had WT1-low, WT1-inter, and WT1-high expression. The characteristics of the patients in each group are shown in Table 1. WT1-inter expression was significantly related to the B-other group (67/215 vs 0/42, 31.1% vs 0, $P < 0.0001$). In addition, the WBC count, hemoglobin, and cytogenetic categories were significantly different among the 3 groups.

WT1 expression at diagnosis had no impact on CR achievement

In both the B-other ALL and the whole cohort, CR achievement after the first induction therapy was similar among patients with WT1-low, WT1-inter, and WT1-high expression (80.2% vs 91.0% vs 80.6%, $P = 0.14$; 81.4% vs 91.0% vs 81.8%, $P = 0.19$).

Low or high WT1 expression predicted poor outcomes

As shown in Fig. 2a–c, of the 195 B-other ALL patients who achieved CR, those with WT1-low expression had similar 3-year RFS, DFS, and OS rates compared with those with WT1-high expression (RFS 54.0% [95% CI, 40.0–66.1%] vs 49.3% [95% CI, 33.5–63.3%], $P = 0.98$; DFS 45.5% [95% CI, 32.6–57.5%] vs 41.9% [95% CI, 27.9%–55.3%], $P = 0.83$; OS 50.0% [95% CI, 36.0–62.5%] vs 55.0% [95% CI, 39.8–67.9%], $P = 0.90$), and they both had significantly lower 3-year RFS, DFS, and OS rates compared with the WT1-inter group (RFS 85.4% [95% CI, 72.8–92.4%], $P = 0.0002$ and 0.0004; DFS 78.3% [95% CI, 64.8–87.1%], $P = 0.0003$ and 0.0003; OS 80.7% [95% CI, 67.0–89.2%], $P = 0.0030$ and 0.0068). Similar results existed if the patients who underwent allo-HSCT were censored at the time of transplantation (Fig. 2d–f). Patients with WT1-low expression had similar 3-year RFS, DFS, and OS rates compared with those with WT1-high expression (RFS 28.3% [95% CI, 10.5–49.3%] vs 24.5% [95% CI, 6.8–47.9%], $P = 0.49$; DFS 27.5% [95% CI, 10.2–48.1%] vs 20.6% [95% CI, 5.6–41.9%], $P = 0.23$; OS 26.5% [95% CI, 8.8–48.4%] vs 25.7% [95% CI, 8.5–47.3%],

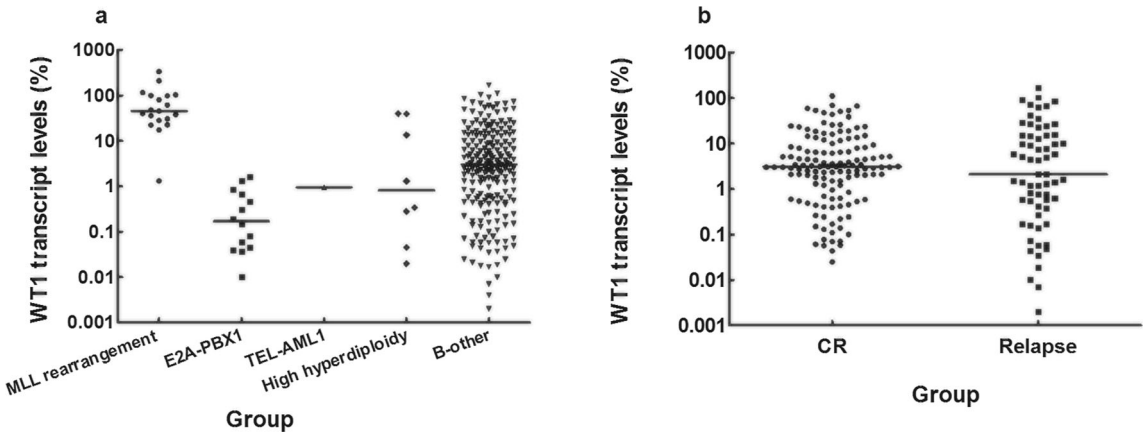

Fig. 1 WT1 expression patterns. **a** Molecularly and cytogenetically defined groups. **b** Relapsed and continuous CR patients in the B-other group

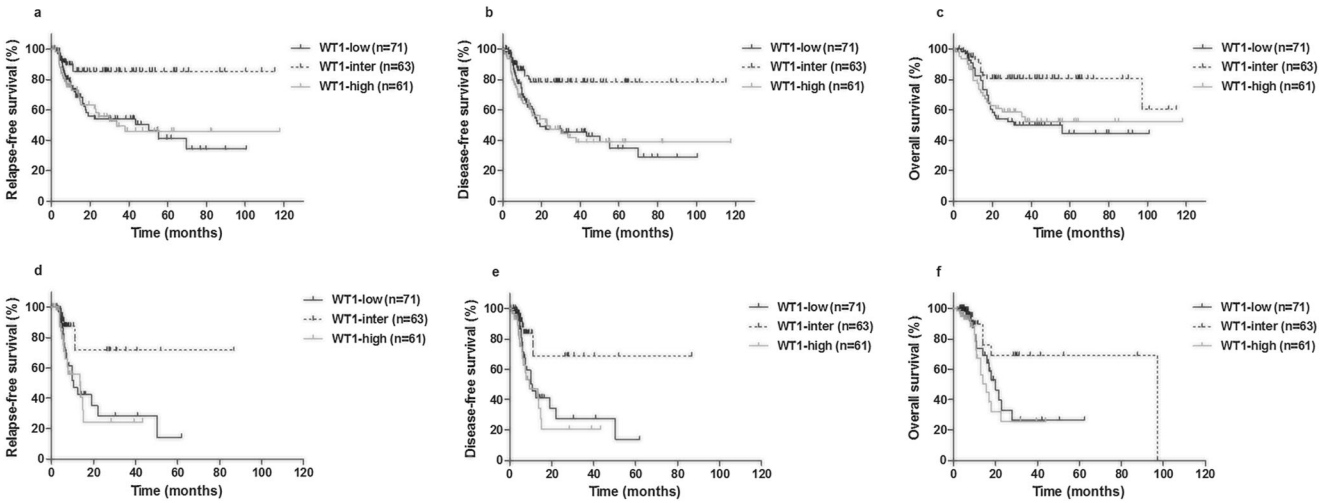

Fig. 2 Outcomes of B-other ALL patients who achieved CR. **a** RFS, no censoring. **b** DFS, no censoring. **c** OS, no censoring. **d** RFS, censoring at the time of allo-HSCT. **e** DFS, censoring at the time of allo-HSCT. **f** OS, censoring at the time of allo-HSCT

$P = 0.30$), and they both had significantly lower 3-year RFS, DFS, and OS rates compared with patients in the WT1-inter group (RFS 71.6% [95% CI, 43.6–87.4%], $P = 0.0073$ and 0.0034; DFS 68.8% [95% CI, 41.8–85.2%], $P = 0.0096$ and 0.0010; OS 69.1% [95% CI, 41.0–85.8%], $P = 0.047$ and 0.0093).

We further performed data validation within our patients. All 195 B-other patients achieving CR were ranked by the strokes of their names and splitted into group 1 (the 1st to 98th patient) and group 2 (the 99th to 195th patient), which had similar pretreatment parameters and the frequencies of treating with chemotherapy only (Table S1). The similar tendencies existed in RFS, DFS, and OS among patients with WT1-low, WT1-inter, and WT1-high expression within both groups although some differences were not significant (Fig. S1 and Table S2).

Due to the similar outcomes, the WT1-low and WT1-high groups were combined into a WT1-low/high group. As shown in Fig. 3a–c, the WT1-low/high group had significantly lower 3-year RFS, DFS, and OS rates compared with the WT1-inter group among the B-other patients who achieved CR (RFS 52.0% [95% CI, 41.0–61.4%] vs 85.4% [95% CI, 72.8–92.4%], $P = 0.0001$; DFS 43.8% [95% CI, 34.3–52.9%] vs 78.3% [95% CI, 64.8–87.1%], $P < 0.0001$; OS 52.7% [95% CI, 42.5–61.9%] vs 80.7% [95% CI, 67.0–89.2%], $P = 0.0021$). WT1 expression stratified the B-other ALL patients and defined 32% (63/195) of them as having low relapse risk.

Furthermore, similar results were observed in the whole cohort (Fig. 3d–f): the WT1-low/high group had significantly lower 3-year RFS, DFS, and OS rates compared with the WT1-inter group (RFS 50.7% [95% CI, 41.6–59.1%] vs 85.4% [95% CI, 72.8–92.4%], $P < 0.0001$; DFS 42.5% [95% CI, 34.0–50.7%] vs 78.3% [95% CI, 64.8–87.1%], $P < 0.0001$; OS 49.3% [95% CI, 40.2–57.8%] vs 80.7% [95% CI, 67.0–89.2%], $P = 0.0008$).

Low or high WT1 expression independently predicted poor outcomes in the B-other ALL patients who achieved CR

Univariate analysis of RFS, DFS, and OS in the B-other ALL patients who achieved CR was performed. As shown in Table 2, in addition to WT1 expression at diagnosis, platelet count and treatment modality were significantly related to RFS; additionally, age and treatment modality were significantly related to DFS and OS (all $P < 0.05$). The multivariate analysis showed that WT1-low/high status and treatment with chemotherapy only both independently predicted lower RFS, DFS, and OS rates in the B-other ALL patients who achieved CR (Table 3).

Discussion

In the current study, we showed that molecularly and cytogenetically defined groups had characteristic WT1 expression patterns at diagnosis, and WT1-low/high expression independently predicted poor outcomes in B-other ALL.

In agreement with previous data [18–22], we found that the outcomes were significantly different among groups in BCP-ALL. However, the outcomes did not correspond to WT1 transcript levels. The WT1 transcript levels in the MLL rearrangement group were uniformly high, whereas those in the E2A-PBX1 group were uniformly low, with no overlap between the two groups, and the WT1 transcript levels were intermediate in the B-other group. Furthermore, although WT1 expression was similar between patients who achieved CR and who relapsed in the B-other group, patients who suffered from relapse seemed to have two separate types of patterns. These phenomena implied that low or high WT1 expression might both be relevant to relapse. Therefore, using 2

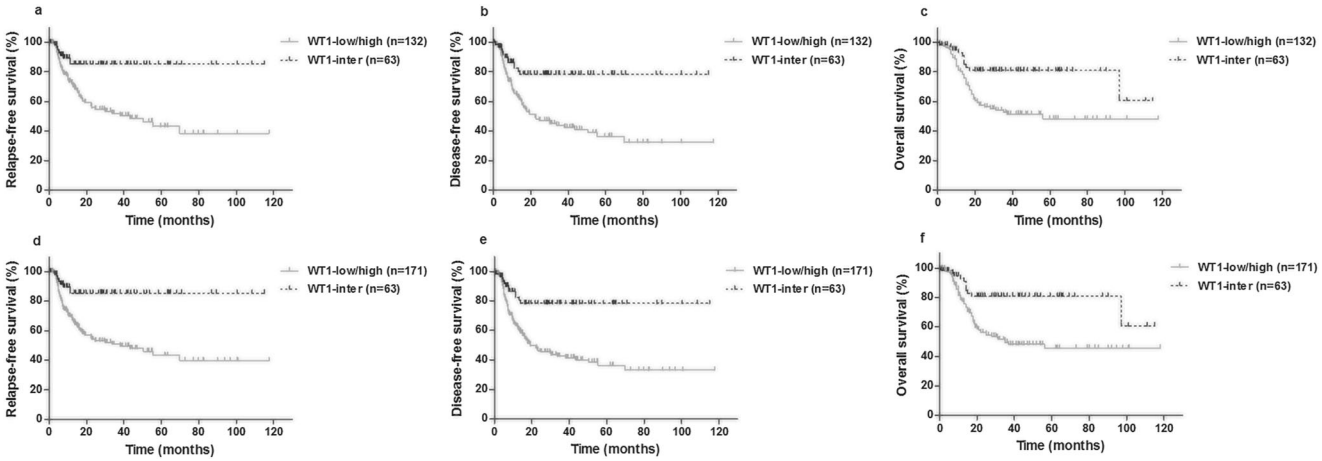

Fig. 3 Outcomes of the B-other and Ph-negative ALL patients grouped by WT1 expression at diagnosis. **a** RFS, B-other ALL. **b** DFS, B-other ALL. **c** OS, B-other ALL. **d** RFS, Ph-negative ALL. **e** DFS, Ph-negative ALL. **f** OS, Ph-negative ALL

cutoff values to categorize patients into 3 groups might be optimal to investigate the prognostic value of WT1 expression. As expected, WT1-low and WT1-high groups had similarly poor outcomes compared with the WT1-inter group. After combination, low/high WT1 expression were shown to be independent poor prognostic factors for RFS, DFS, and OS in the B-other patients who achieved CR in addition to treating with chemotherapy only.

Boublikova et al. evaluated 106 childhood BCP-ALL patients and found that an abnormal increase or decrease in WT1 expression was independently associated with a significantly increased risk of relapse [23], which is in agreement with our data. Similarly, Heesch et al. evaluated 238 adult T-ALL patients and found aberrant high or negative WT1 expression was related to a higher relapse rate and an inferior outcome compared with intermediate-WT1 expression [24]. These results implied a similar mechanism of WT1 expression between adults and children and between BCP and T-ALL. In contrast, Chiusa et al. analyzed 20 adult BCP-ALL patients and showed that high WT1 expression independently predicted poor DFS and OS [8]. Busse et al. evaluated 158 adult BCP-ALL patients and found no independent prognostic role of WT1 expression level for DFS and OS [9]. The discrepancy might be caused by the difference in patient composition and treatment. Furthermore, lack of standardization in methodologies to measure WT1 transcripts might be another factor.

Due to differences in control gene, primers and probe sequences, plasmid standards, and laboratory procedure, interlaboratory variability is common in RQ-PCR testing. The application of laboratory-specific conversion factor (CF) is one way to achieve standardization among laboratories, which is only applicable for BCR-ABL1 transcript in chronic myeloid leukemia (CML) to date [25]. EURO-MRD consortium recently described the first result on standardization of RQ-PCR for the e1a2 BCR-ABL1 transcript in Ph + ALL [26]. Cilloni and Willasch et al. individually reported the

Table 2 *P* values of the univariate analysis of RFS, DFS, and OS in the B-other ALL patients who achieved CR ($n = 195$)

Variable	RFS	DFS	OS
WT1 expression (low/high vs inter)	*0.0001*	*< 0.0001*	*0.0021*
Sex (male vs female)	0.58	0.28	0.47
Age (year, < 45 vs ≥ 45)	*0.062*	*0.0030*	*0.0010*
White blood cell count (×10E9/L) (< 30 vs ≥ 30)	*0.087*	*0.19*	0.44
Hemoglobin (g/L) (≤ 90 vs > 90)	0.91	0.37	*0.16*
Platelets (× 10E9/L)(≤ 60 vs > 60)	*0.010*	*0.067*	*0.11*
Cytogenetic categories* (standard/intermediate vs high/very high) ($n = 145$)	*0.17*	0.26	0.22
CR induction courses (1 vs > 1)	0.67	0.75	0.69
IKZF1 deletion (yes vs no) ($n = 114$)	0.65	*0.16*	0.21
Treatment modality (chemotherapy only vs allo-HSCT)	*< 0.001*	*< 0.001*	*< 0.001*
MRD at the end of the first induction (≤ 0.1% vs > 0.1%)	0.35	0.47	0.95

Italicized numbers $P < 0.20$

*Based on the ECOG 2993 trial, fusion transcript testing results by RQ-PCR were also considered

Table 3 Multivariate analysis of RFS, DFS, and OS in the B-other ALL patients who achieved CR ($n = 195$)

Variable	RFS		DFS		OS	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
WT1 transcript level (low/high vs inter)	3.7 (1.5–8.7)	0.003	3.9 (1.6–9.2)	0.002	2.6 (1.4–5.0)	0.004
Treatment modality (chemotherapy only vs allo-HSCT)	9.5 (4.7–19.1)	< 0.001	4.5 (2.4–8.5)	< 0.001	4.2 (2.5–7.0)	< 0.001

results of standardization for WT1 transcript launched in Europe [4, 27]. In order to make the current results comparable with others, 1.8% and 7.2%, which corresponded to a 3-fold and 12-fold increase compared with the upper limit of NBM, were chosen as cutoff values for patient grouping.

The role of WT1 in the process of leukemogenesis is not well understood. WT1 has been demonstrated to cause both transcriptional activation and transcriptional repression and possesses both oncogenic and tumor suppressor properties [28, 29], which may reflect its inconsistent, or even opposite, prognostic impacts on AML and ALL. We suspected that the poor prognostic impact of low and high WT1 expression might have different molecular mechanisms.

There are several limitations. First, this was a retrospective study, and the treatment regimens were not uniform. Second, some data were incomplete; that is, the cytogenetic and IKZF1 deletion results were unavailable for a certain number of patients. Third, a range of distinct, recurrent abnormalities in B-other ALL has recently been revealed by several genomic studies. However, these analyses were not performed for the patients in the current study.

In conclusion, molecularly and cytogenetically defined adult Ph-negative BCP-ALL groups have distinct WT1 expression patterns at diagnosis. WT1-low or WT1-high expression predicted poor outcome in B-other and Ph-negative BCP-ALL. At present, allo-HSCT is the recommended treatment for BCP-ALL due to the general poor outcome. The current study provides evidence that WT1 expression at diagnosis might be useful for patient stratification. Multicenter prospective studies are warranted. In addition, the standardization of the methodology of quantitative WT1 testing needs to be improved.

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Compliance with ethical standards

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

Ethical approval The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Peking University People's Hospital.

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

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