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Wilms' Tumor Gene 1 Transcript Levels in Leukapheresis of Peripheral Blood Hematopoietic Cells Predict Relapse Risk in Patients Autografted for Acute Myeloid Leukemia

Carlo Messina [1], Anna Candoni [2], Matteo G. Carrabba [1], Cristina Tresoldi [3], Elisa Sala [1], Michela Tassara [3], Alessandra Crippa [3], Jacopo Peccatori [1], Andrea Assanelli [1], Salvatore Gattillo [3], Laura Bellio [3], Renato Fanin [2], Fabio Ciceri [1,*], Massimo Bernardi [1]

[1] *Hematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, Milan, Italy*
[2] *Division of Hematology and Bone Marrow Transplantation Unit, University Hospital of Udine, Udine, Italy*
[3] *Immuno-hematology and Transfusion Medicine, San Raffaele Scientific Institute, Milan, Italy*

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A B S T R A C T

Autologous hematopoietic stem cell transplantation (ASCT) is a curative option alternative to allogeneic transplantation for patients with acute myeloid leukemia (AML). Relapse after ASCT can be due to contamination with leukemic blasts of autologous peripheral blood stem cells (PBSCs) collected by leukapheresis (LK). Identification and quantification of a minimal residual disease (MRD) marker in PBSCs could be relevant in determining the relapse risk after ASCT. High levels of the *WT1* gene transcript in bone marrow of AML patients after treatment completion predict disease relapse. We evaluated *WT1* transcript levels in autologous PBSC from LK used for ASCT in 30 consecutive AML patients in complete remission (CR) and established a correlation with clinical outcome. At diagnosis, all patients had *WT1* overexpression. All patients were in morphological and genetic CR at the time of PBSC collection and before ASCT. Real-time quantitative PCR of *WT1* was performed in samples of each LK, using TaqMan technology on RNA from mononucleated cells. The median *WT1* transcript level in the PBSC graft (WT1-LK) of patients who relapsed was significantly higher than of those who did not relapse after transplantation ($P < .0001$). We defined a cut-off level of 80 WT1-LK copies/ABL 10e4 copies to discriminate between positive and negative PBSC grafts. The cut-off level was strongly associated with disease recurrence, DFS and OS. Our study represents the largest series of patients evaluating *WT1* as a marker of MRD in PBSC LK products using a completely standardized real-time WT1-reverse transcriptase-PCR based assay. These data, if confirmed by prospective study, will help to determine an individual patient's adapted postremission allocation strategy.

INTRODUCTION

Conventional treatment of acute myeloid leukemia (AML) consists of remission induction and postremission or consolidation therapy. Induction results in complete remission (CR) in 70% to 80% of younger and 50% to 60% of elderly patients. Consolidation therapy after attaining remission is necessary to prevent relapse. More than 50% of patients, however, experience disease recurrence during or after treatments.

Remission induction with a combination of cytarabine and anthracycline is the current standard for patients eligible for a curative approach. Postremission treatments include chemotherapy with high-dose cytarabine, allogeneic hematopoietic stem cell transplantation, or autologous hematopoietic stem cell transplantation (ASCT) [1]. The choice of the best postremission strategy is usually based on a risk-adapted approach together with evaluation of patient comorbidities and donor availability.

Allogeneic hematopoietic stem cell transplantation after an initial CR (CR1) is the best option for AML with intermediate or high genetic risk and/or other adverse prognostic factors (eg, hyperleucocytosis, therapy related). ASCT is considered for consolidation in low and intermediate cytogenetic groups and for high-risk patients with no allogeneic transplant option available [2] because it offers an advantage compared with chemotherapy [3], showing a lower relapse incidence in newly diagnosed AML patients in CR1 [4].

The persistence of minimal residual disease (MRD) in the bone marrow (BM) and/or the contamination with leukemic

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* Correspondence and reprint requests: Fabio Ciceri, MD, Hematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, IRCCS, Milano, via Olgettina 60, 20132, Italy.
E-mail address: ciceri.fabio@hsr.it (F. Ciceri).

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blasts of autologous BM harvest or peripheral blood stem cells (PBSCs) collected by leukapheresis (LK) may limit the potential benefit of ASCT in AML. Identification and quantification of a reliable marker of MRD in the BM and LK of autologous PBSCs could be relevant in determining the relapse risk after ASCT. Multiparameter flow cytometry, PCR, and fluorescence in situ hybridization are useful tools for detection of MRD in AML patients. PCR-based quantification of MRD has a higher sensitivity, but its applicability is restricted to subgroups of AML with leukemia-specific molecular targets (eg, AML1-ETO, CBFb-MYH11, NPM1). These cases comprise less than 40% of all AML cases. The Wilms' tumor gene 1 is overexpressed in more than 90% of AML patients at diagnosis. Several studies have shown that quantification of Wilms' tumor gene 1 transcripts WT1 in the BM and PB of patients in CR after induction and consolidation therapy demonstrated high prognostic value, irrespectively of cytogenetic or molecular abnormalities, reliably discriminating among patients at different risk of relapse [5]. Moreover, elevated levels of *WT1* in BM harvest have been associated with a higher relapse risk in patients autografted for AML [6]. Siehl et al. [7] showed that PBSCs from AML patients have significantly higher *WT1* levels as compared with those obtained from solid cancer patients, indicating a possible contamination with leukemic blasts.

In our study, we measured by European LeukemiaNet (ELN) standardized assay [8] *WT1* transcript levels in PBSCs from LK (WT1-LK) used for ASCT in 30 consecutive AML patients in CR1. We then established a correlation with clinical outcome.

METHODS

Patient Characteristics

Samples from LK products and from BM, at diagnosis and subsequent controls; clinical data; and outcomes were available from 30 consecutive patients with a diagnosis of AML who received an ASCT when in CR1 between August 2005 and February 2013. Eighteen patients were treated at the Hematology and Bone Marrow Transplantation Unit of the San Raffaele Scientific Institute and 12 patients at the Division of Hematology and Bone Marrow Transplantation of the University of Udine.

According to the ELN genetic prognostic classification of AML, 4 patients (13%) were in the favorable group, 23 (77%) in intermediate-I, 0 in intermediate-II, and 3 (10%) in the adverse group [1]. At diagnosis, all patients had *WT1* overexpression.

All patients were in CR1 before PBSC collection, after 1 or 2 induction cycles, and at least 1 consolidation cycle containing high-dose cytarabine. Median patient age was 61 years (range, 28 to 76), and other patient and disease characteristics are summarized in Table 1. Data were analyzed on March 31, 2013.

Treatment and WT1 Monitoring

Induction chemotherapy consisted of the combination of fludarabine (30 mg/m^2 on days 1 to 5), cytarabine (2 g/m^2 days 1 to 5), and idarubicin (10 mg/m^2 on days 1 to 3) (19 patients) or of daunorubicin (60 mg/m^2 on days 1 to 3) and continuous infusion cytarabine (100 mg/m^2 on days 1 to 7) (34 patients) or of idarubicin or daunorubicin associated with continuous infusion of cytarabine and etoposide (100 mg/m^2 on days 1 to 7) (idarubicin regimen, 3 patients; daunorubicin regimen, 1 patient). Three patients received 2 induction cycles.

After induction, all patients received 1 or 2 courses of consolidation treatments containing high-dose cytarabine. PBSC LK was performed after the last course. ASCT with myeloablative conditioning was administered as final consolidation. The following conditioning regimens were used: busulfan 16 mg/kg + cyclophosphamide 120 mg/kg for 13 patients and treosulfan 30 g/m^2 + fludarabine 150 mg/m^2 + cytarabine 10 g/m^2 for 17 patients. PBSC graft was 4.94 × 10^6/kg CD34$^+$ cells (range, 1.90 to 9.10 × 10^6/kg). Treatments were administered according to the policy of each center.

All patients were in morphological and cytogenetic CR1 at the time of PBSC collection and before ASCT. Definition of response to therapy was expressed according to the published National Cancer Institute criteria, revised by the International Working Group [9]. After ASCT, no further treatments were administered to patients in continuous CR, up to last contact.

WT1 cut-off values for pathological samples were defined as higher than 250 copies *WT1*/10^4 ABL copies in BM, according to Cilloni et al. [8]. *WT1* levels were assessed at diagnosis, in the BM before LK, in the LK product, and before ASCT. After ASCT, *WT1* levels were assessed in BM monthly during the first 3 months; at months 6, 9, and 12, and then annually or if clinically relevant.

PBSC Collection and Cryopreservation

PBSCs were collected using the COBE Spectra Apheresis System (Terumo BCT, Inc., Lakewood, CO) by continuous collection with manual control of hematocrit or automatic spill-over protocol. PBSCs were cryopreserved and stored according to Jacie Standards, 2nd to 5th editions.

SAMPLE PREPARATION AND QUANTIFICATION OF *WT1* TRANSCRIPTS

The quantitative assessment of *WT1* transcripts was performed using real-time quantitative PCR (RQ-PCR) on mRNA of mononuclear cells from LK samples, which had been frozen immediately after collection and thawed immediately before PCR analysis. Mononuclear cells were obtained by Ficoll Hypaque density gradient centrifugation and stored at 80°C. Total RNA was then extracted by phenol-chloroform assay (TRIzol, Life Technologies, Grand Island, NY) and retrotranscribed by the BIOMED 1 adapted protocol (standardized RT-PCR protocol according to report of the BIOMED 1 [Biomedecine and Health Programme] concerned action and modified according to EAC protocol [Europe Against Cancer]). Starting from 1 μg of total RNA, random hexamers were used at a concentration of 50 μM, and 100 U of the reverse transcriptase were added to the reaction mixture to obtain significant enhancement of the assay sensitivity. Finally, RQ-PCR assays were performed on cDNA to amplify and simultaneously quantify *WT1* transcripts [10,11].

WT1 transcripts were quantified using the TaqMan technology, through the WT1 ProfileQuant kit ELN (QIAGEN Sciences, Germantown, MD), whose components have been validated together in the context of a collaborative study led by a group of experts from the ELN consortium. This kit includes plasmids specific for *WT1* and ABL respectively, and specific primers and FAM-TAMRA probe mixes for *WT1* and ABL. *WT1* primers and probes were localized on exons 1 and 2. The WT1 ProfileQuant (ELN) assay uses validated standard curves plasmid-based (3 plasmid standard dilutions for the ABL control gene and 5 standard dilutions for the *WT1* gene) to calibrate and normalize the RQ-PCR results. By generating standard curves based on the known concentration of plasmid dilutions of both genes, it precisely measures the copy number of the ABL control and *WT1* transcript in human cell samples. The *WT1* transcript values obtained were normalized with respect to the number of ABL transcripts and expressed as *WT1* copy number every 10e4 copies of ABL (WT1/ABL 10e4).

Statistical Analysis

Clinical characteristics and response rates of the therapeutic subgroups were compared using the chi-square test or Fisher exact test for categorical variables, according to the sample size. To assess whether the population mean ranks differ, the Wilcoxon signed-rank test was used (Mann-Whitney U test). Survival curves were constructed according to the method of Kaplan-Meier [12] and compared using the log rank test. Differences were considered significant when $P \leq .05$ (2-tailed). All survival analyses were performed using the SPSS, version 14 (IBM Corporation, Armonk, NY).

Table 1
Patient Characteristics

Patient No.	Age (yr)	FAB	Cytogenetic or Molecular Abnormality	ELN Classification Genetic Risk Group	Number of Induction Cycles to Obtain CR	WT1 BM before ASCT	WT1 BM after ASCT	Relapse Post-ASCT	DFS (d)	WT1 Levels in LK Infused
1	71	M2	No	Intermediate-I	1	55	23	No	2737	7
2	62	M1	FLT3 TKD	Intermediate-I	1	8	8	No	2515	7
3	64	M4	No	Intermediate-I	1	NA	NA	No	2404	36
4	69	M5	No	Intermediate-I	1	NA	NA	No	2385	17
5	41	M2	No	Intermediate-I	1	NA	NA	No	2377	8
6	61	M5	No	Intermediate-I	1	25	19	No	2189	5
7	47	M5	No	Intermediate-I	1	69	48	No	2006	61
8	76	M1	No	Intermediate-I	1	NA	1156	Yes	1809	82
9	52	M1	No	Intermediate-I	1	210	47	No	1805	4
10	57	M5	FLT3 ITD	Intermediate-I	1	90	75	No	1784	11
11	24	M1	No	Intermediate-I	1	55	100	No	930	10
12	28	M0	No	Intermediate-I	1	115	91	No	929	20
13	60	M2	t(8:21)	Favorable	1	44	10	No	876	15
14	68	M5	No	Intermediate-I	1	32	14	No	747	17
15	72	M0	No	Intermediate-I	2	7	12	Yes	626	75
16	68	undiff.	No	Intermediate-I	1	NA	NA	Yes	534	90
17	58	M5	CEBPAα mut	Favorable	1	267	45	No	420	24
18	60	M0	No	Intermediate-I	2	NA	NA	Yes	368	780
19	44	M5	No	Intermediate-I	1	90	212	Yes	364	50
20	68	M4	No	Intermediate-I	1	45	185	Yes	351	194
21	57	M1	No	Intermediate-I	1	75	164	Yes	313	17
22	69	M2	No	Intermediate-I	1	200	165	Yes	226	272
23	65	sAML	No	Intermediate-I	1	78	3	Yes	168	1
24	52	M2	inv(16)	Favorable	1	12	0	Yes	141	23
25	72	M4	CEBPAα mut	Favorable	1	86	506	Yes	116	61
26	75	sAML	No	Intermediate-I	1	324	82	Yes	93	840
27	77	M4	FLT3 ITD + NPM1mut	Intermediate-I	1	23	15	No	93	7
28	67	M4	No	Intermediate-I	1	3	34	Yes	69	81
29	71	M0	No	Intermediate-I	1	17	9	No	53	110
30	53	M4	Complex cariotype	Adverse	1	1060	1619	Yes	52	421

FAB indicates, French-American-British; WT1, Wilms' tumor gene 1; M0-5, AML subtypes according to FAB classification; FLT3, fms-like tyrosine kinase 3; TKD, *FLT3* tyrosine kinase domain; NA, not available; ITD, internal tandem duplication; undiff, undifferentiated; CEBPAα, CCAAT/enhancer-binding protein alpha; sAML, secondary AML.

Overall survival (OS) was calculated from the date of ASCT to death or to the last date of follow-up, whereas disease-free survival (DFS) was calculated from the day of transplant to the first relapse date, progression or death, or to the last date of follow-up. Survival rates were reported as 1-year OS ± standard error. Impact on survival of WT1-LK levels was evaluated by comparing the survival curves by means of the log-rank test. Adjustment for multiple group comparisons was performed according to Bonferroni method (significance level = .05/number of tests). Comparison of the therapeutic variables was performed retrospectively. The independent prognostic value of variables was analyzed using the Cox proportional hazard model. Backward stepwise regression was performed to identify the most powerful predictors of survival. All probability values were 2-sided. Analyses were carried out using the Statistica 4.0 statistical package for Windows (Statsoft Inc, 1993, Tulsa, OK). Values for $P < .05$ were considered statistically significant.

RESULTS

We evaluated data from 30 patients with AML in CR1 who received an ASCT between August 2005 and February 2013. No patient died from transplant-related causes; after a median follow-up of 812 days (range, 52 to 2737) from ASCT, 14 patients (47%) relapsed and 16 were alive in CR. Median days of DFS and OS were, respectively, 580 (range, 52 to 2737) and 812 (range, 53 to 2737). Four patients received an allogeneic hematopoietic stem cell transplantation after relapse, 3 died for toxicities related to the transplant, and 1 patient is alive in second remission.

Median *WT1* transcript level in the PBSC graft of all 30 patients was 23 (range, 0 to 839). As a "negative" control group, we evaluated the *WT1* levels in the PBSCs collected from 15 patients with different hematological diseases without marrow involvement at time of LK and with no *WT1* overexpression as molecular marker of active disease (14 multiple myeloma, 2 Hodgkin lymphoma and 6 non-Hodgkin lymphoma): median value was 16.6 (range, 1.5 to 71.8). The difference between the 2 groups was not statistically significant ($P = .1$), although the range was broader in AML patients: we postulated that most of our AML patients had "negative" values. Full data are reported in Table 2. The median *WT1* transcript level in the PBSC graft of patients who relapsed was significantly higher than levels of those who did not relapse after transplantation: 82 (range, 4 to 839) and 13 (range, 4 to 109) ($P < .0001$), respectively (Figure 1).

Cilloni et al. [8] previously published median *WT1* levels in PBSCs of mobilized healthy donors ranging from 0 to 39 (median, 6.1). In our control series the highest value of *WT1* in LK of nonmyeloid tumor–mobilized patients was 71.8 and the value discriminating the 95th percentile was 68.9. We defined a cut-off level of 80 (ie, 1 decade above the 95th percentile) to increase the specificity of our analysis. In fact, using this value to define positive and negative PBSC grafts, 21 patients (group A) received negative and 9 patients (group B) positive PBSCs, which strongly correlated with disease recurrence ($P = .01$, Fisher test). The defined cut-off level had a positive predictive value for relapse of 88.8% and negative predictive value for relapse of 71.4%, with a sensitivity of 57.1% and a specificity of 93.7%.

Table 2
WT1 Quantitative Assessment in LK Control Samples

	Type of Sample	Number of Samples	*WT1* Copy Number/ 104 ABL Copies	
			Median	Range
Non-Hodgkin lymphoma	LK	14	11.85	1.55-71.89
Hodgkin lymphoma	LK	2	27.16	11.54-42.78
Multiple myeloma	LK	6	30.46	1.55-51.82
Total	LK	22	16.66	1.55-71.89

The cumulative incidence of relapse (RI) was 30% in group A and 87% in group B ($P = .0001$). The observed 6- and 12-month RI was, respectively, 14% and 24% for group A and 35% and 61% for group B (Figure 2).

One-year DFS and OS rates were, respectively, 75% (95% confidence interval [CI], 58% to 97%) and 90% (95% CI, 77% to 100%) for group A and 25% (95% CI 8% to 84%) and 25% (95% CI 8% to 84%) for group B. Median DFS was not reached for group A, whereas it was 351 days for group B ($P = .0001$) (Figure 3). Median OS was significantly better for group A than for group B: not reached and 351 days, respectively ($P = .001$) (Figure 4).

WT1 transcript levels were evaluated in the BM of 25 and 24 patients before PBSC collection by LK and before ASCT, respectively. Median *WT1* transcript levels in the BM before LK and ASCT were 56 (range, 6 to 1048) and 62 (range, 3 to 1060), respectively. Five patients had BM *WT1* levels > 250 before LK (copies: 283, 583, 646, 950, and 1048); 2 of these patients were LK negative and 1 did not relapse. Three patients had BM *WT1* levels > 250 before ASCT (copies: 267, 324, and 1060); 1 of these patients received a PBSC product that was LK negative and is still in remission.

Table 1 also shows *WT1* levels in BM after ASCT in 25 evaluated patients. In 5 patients *WT1* levels in the PBSC graft and in the first BM evaluated after ASCT (mostly around day +30) did not correlate: in 4 patients who were BM negative WT1-LK was positive and 3 of them relapsed, and only 1 patient who was WT1-LK negative became BM positive and relapsed. These data suggest a possible stronger correlation with outcome of WT1-LK than day +30 BM-WT1 levels, but numbers are still too small to draw any conclusion.

As previously reported by the European Group for Blood and Marrow Transplantation (EBMT) survey [13], the dose of $CD34^+$ cells infused could influence the relapse rate after ASCT. In particular, infusion of $>7.16 \times 10^6$/kg $CD34^+$ cells correlated with increased relapse incidence and lower leukemia-free survival (LFS). We analyzed the correlation of the dose of $CD34^+$ cells infused in our patients with outcome and WT1-LK levels. In our series, 6 patients received a $CD34^+$ cell dose $\geq 7 \times 10^6$/kg and 5 patients relapsed. All received a WT1-LK product positive, and the patient who did not relapse received a WT1-LK product negative.

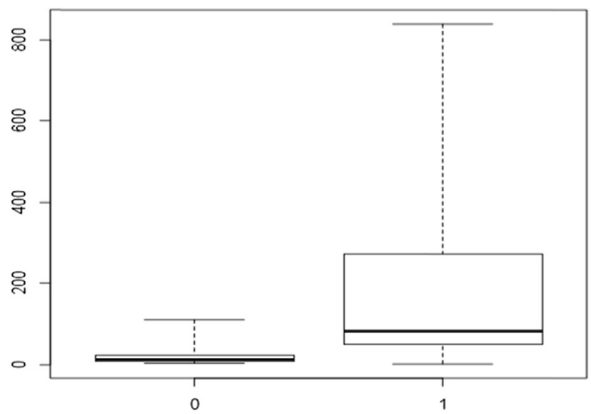

Figure 1. Median LK-WT1 copy number (copies/ABL 10e4) of AML relapsed patients (right column, n = 14) and in those in continuous CR (left column, n = 16): 82 (range, 4 to 839) versus 13 (range, 4 to 109), respectively ($P < .001$).

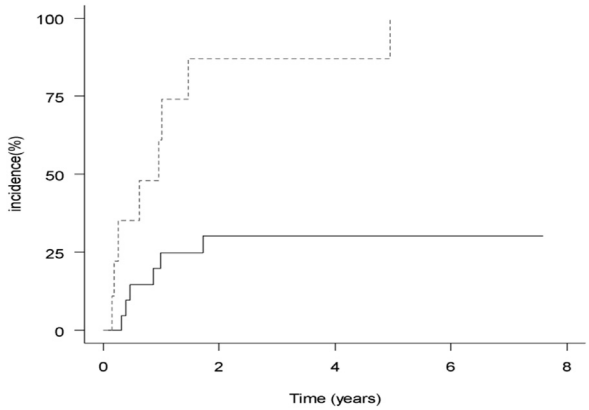

Figure 2. Cumulative incidence of relapse of 30 patients receiving autologous transplantation with apheresis containing ≥ 80 *WT1* copies/ABL 10e4 (dotted line, n = 9) or <80 *WT1* copies/ABL 10e4 (continuous line, n = 21), $P = .0001$.

DISCUSSION

Postremission ASCT can extend survival in a selected subgroup of AML patients [14]. Several studies reported that ASCT using mobilized PBSCs offers a faster hematopoietic recovery and is associated with limited morbidity and treatment-related mortality than after BM harvest infusion [15-17]. However, a retrospective cohort analysis from the EBMT of 2165 AML patients in CR1 reported a higher incidence of relapse after ASCT with PBSCs than with BM stem cells [18]. The authors postulated that recruitment of leukemic cells during mobilization of PBSC with granulocyte

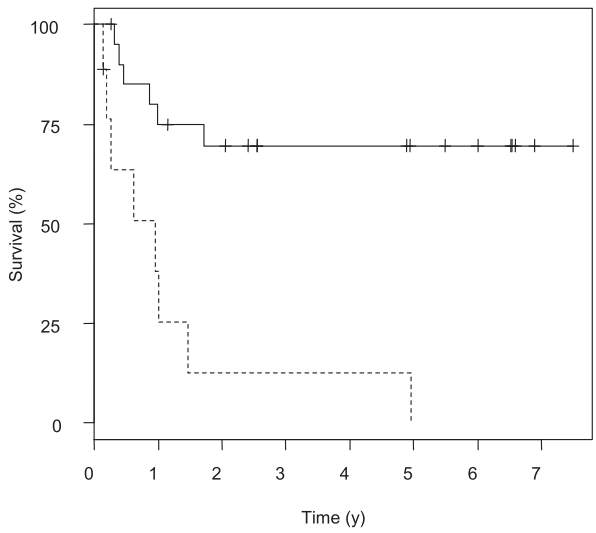

Figure 3. DFS of 30 patients receiving autologous transplantation with apheresis containing ≥80 *WT1* copies/ABL 10e4 (dotted line, n = 9) or <80 *WT1* copies/ABL 10e4 (continuous line, n = 21), $P = .0002$.

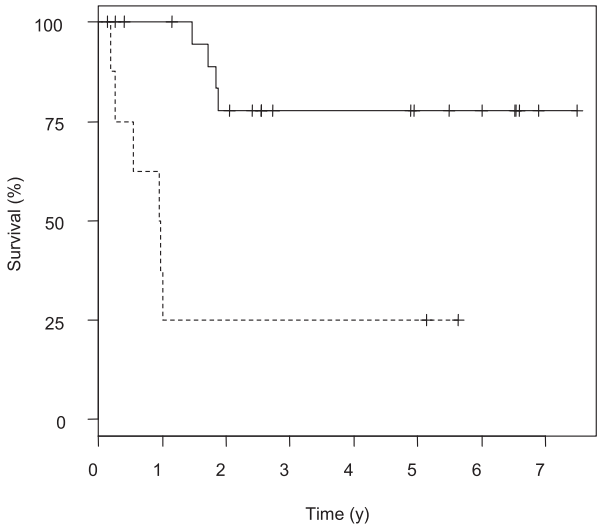

Figure 4. OS of 30 patients receiving autologous transplantation with apheresis containing ≥80 *WT1* copies/ABL 10e4 (dotted line, n = 9) or <80 *WT1* copies/ABL 10e4 (continuous line, n = 21), $P \leq .0001$.

colony-stimulating factor and consequent graft contamination may have accounted for the higher incidence of relapse after transplantation. This hypothesis finds support from the following observations: (1) a direct correlation between RI, LFS, and $CD34^+$ PBSCs infused reported by a retrospective EBMT study [13] and (2) a 32% reduction in RI and 29% increase in 4-year LFS by "in vitro" purging with mafosfamide and a 25% increase in 3-year LFS by "in vitro" purging with 4-hydroperoxycyclophosphamide, respectively, reported by the EBMT [19,20] and by the Center for International Blood and Marrow Transplant Research [21,22].

In this context and in the perspective of optimization of ASCT clinical indication, a quantitative assessment of a reliable marker of MRD in LK of autologous PBSCs could be a useful tool in determining the relapse risk after ASCT. *WT1* is a tumor-suppressor gene coding for a zinc-finger transcription factor located on chromosome 11p13, involved in the pathogenesis of the Wilms' tumor [23]. In normal PB and BM, *WT1* expression is low and often undetectable even by qualitative reverse transcriptase PCR [24], whereas it is overexpressed in nearly 90% of AML cases [25-27]. Our data show that real-time quantification of *WT1* transcripts in PBSC collections of patients with multiple myeloma, non-Hodgkin lymphoma, and Hodgkin lymphoma were low (16.6; range, 1.5 to 71.8). Based on these results and considering that, once prospectively validated, *WT1* levels on LK could be potentially used to tailor treatment of most patients with positive LK toward more aggressive approaches, we choose to value specificity over sensitivity of the exam. Thus, we defined 80, which was 1 decade above the value of the 95th percentile of our controls, as the cut-off value.

In our study, all patients with WT1-LK levels above 80 relapsed, and the difference in relapse incidence and DFS was significant when compared with patients with WT1-LK levels lower than 80. Of note, 4 of 9 patients with higher WT1-LK levels and who relapsed had normal BM *WT1* levels at the time of LK. This may suggest that *WT1* in BM is less predictive than *WT1* in LK after mobilization. This difference could be explained by the recruitment of leukemic cells from all body sites under granulocyte colony-stimulating factor stimulation and supports *WT1* in LK as a useful marker of what is really infused. Our results are also in accordance with those from a study that quantified MRD in BM harvests used for autografting in AML. According to those data, higher *WT1* levels detected by PCR were associated with a worse relapse-free survival [6].

In a similar attempt to establish individual prognostic tools, Milone et al. [28] analyzed 96 AML patients in CR1 for peak $CD34^+$ cell levels in PB during PBSC mobilization and harvest, irrespective of postremission treatment received. The peak of $CD34^+$ cells in PB was an independent predictor for DFS in multivariate analysis. Studies in AML using flow cytometry have demonstrated that the levels of cells expressing an aberrant phenotype in BM harvests correlated with disease recurrence [29].

Regarding the relationship between the number of $CD34^+$/kg infused and *WT1* levels in LK and RI post-ASCT in our series, only 1 in 5 patients who received a megadose of CD34 did not relapse. This patient was the only who received *WT1* negative LK. If confirmed in larger trials, these data would support the role of MRD evaluation in PBSC product irrespective to CD34 dose infused.

Our study represents the largest series of patients evaluating *WT1* as a marker of MRD in PBSC LK products. However, we would like to remark on 2 points of our study. First, the number of patients is still insufficient to provide a strong recommendation about postconsolidation strategy. The low number of patients in our study is due to both the competing option of allogeneic transplantation, which at the 2 centers is offered to patients up to age 70 years, and the rate of successful autologous LK, which was around 50% to 60%. Second, we could not explain with certainty if patients with low WT1-LK did better because of less tumor contamination of the LK products or because they were the better responders to chemotherapy. We observed only 3 patients with *WT1* BM positive before ASCT and 2 of them, both with WT1-LK product positive, relapsed after ASCT. Only 4 patients had *WT1* BM negative before ASCT and WT1-LK products positive, and 3 of them relapsed. Patients received similar treatments, according to internal policies at the 2 centers. Although we cannot formally exclude that the outcome of patients was influenced by the differences among the chemotherapic regimens, in our opinion this point was not relevant for the purposes of our study because all regimens administered can be considered equivalent options for AML treatment.

In summary, our results validate the use of RQ-PCR of *WT1* transcripts for MRD quantification in PBSC products of AML patients and suggest that higher MRD levels reflect a higher grade of contamination by leukemic cells. We conclude that a completely standardized real-time WT1-reverse transcriptase-PCR–based assay could address patients with negative PBSC LK products to ASCT with a projected long-term survival without AML relapse, whereas patients with contaminated PBSCs (≥80 WT1-LK copies) could better benefit of further in vivo purging with chemotherapy before a second PBSC collection. Alternatively, in a context in which an alternative donor (eg, matched unrelated donor, haploidentical donor, cord blood) is available for most patients and reduced-intensity conditioning can be offered also to healthy patients older than 65 years, we suggest that these patients should be considered for allogeneic stem cell transplantation. We believe our results represent the basis for a prospective study incorporating operationally a *WT1* MRD analysis of the PBSC graft as an individual patient's adapted postremission allocation strategy.

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