European Journal of Haematology ISSN 0902-4441

ORIGINAL ARTICLE

Quantitative assessment of *WT1* gene expression after allogeneic stem cell transplantation is a useful tool for monitoring minimal residual disease in acute myeloid leukemia

Anna Candoni[1], Mario Tiribelli[1], Eleonora Toffoletti[1], Daniela Cilloni[2], Alexia Chiarvesio[1], Angela Michelutti[1], Erica Simeone[1], Corrado Pipan[3], Giuseppe Saglio[2], Renato Fanin[1]

[1]Division of Hematology and Bone Marrow Transplantation, Department of Medical and Morphologic Research, University of Udine, Udine; [2]Department of Clinical and Biological Sciences, Division of Internal Medicine and Hematology, University of Turin, Turin; [3]Institute of Hygiene and Epidemiology, DPMSC, University of Udine, Udine, Italy

Abstract

Introduction: WT1 overexpression is described in several oncological diseases including acute myeloid leukemia (AML). Quantification of *WT1* in bone marrow samples may be useful as a marker of minimal residual disease (MRD) and may predict the relapse of AML after allogeneic hematopoietic stem cell transplant (HSCT). *Methods and results:* The quantitative expression of *WT1* was measured in 38 AML patients (16 males and 22 females) at diagnosis, at the time of transplant and after the allogeneic HSCT (at precise time points). All cases showed high *WT1* expression levels at diagnosis with a mean of 4189 (SD 3325) and a median of 3495 (range 454–13923) copies *WT1*/10^4 *Abl.* At transplant, 25 patients (66%) were in complete cytologic remission (CcR) and 13 (34%) had refractory or relapsed AML. Bone marrow samples from patients transplanted in CcR showed significantly lower *WT1* expression levels during HSCT compared with the samples from patients with a relapsed or refractory AML ($P = 0.004$). After HSCT, a rapid decline in *WT1* expression levels was observed in all patients who attained or maintained a condition of CcR. Six of 38 patients (13%) relapsed after HSCT and all of them had an increase in *WT1* expression at/or before relapse. Five of these six patients died of leukemia and one was successfully reinduced with donor lymphocyte infusion (DLI) + chemotherapy with a rapid reduction of *WT1* levels. Besides, we found a complete concordance between *WT1* expression levels and other disease markers (when available). *Conclusions:* In our experience, there was a complete concordance between *WT1* expression levels (measured by quantitative RT-PCR at precise time points) and status of AML before and after allogeneic HSCT. *WT1* may be useful as a non-specific leukemia marker for monitoring MRD and as a predictor of AML clinical relapse. Based on these results, cases with increase of *WT1* levels after HSCT and without graft vs. host disease may be candidate to discontinuation of immunosuppression and/or DLI therapy.

Key words WT1; MRD; acute leukemia; allogeneic hematopoietic stem cell transplant

Correspondence Anna Candoni, MD, Division of Hematology and Bone Marrow Transplantation Unit, University of Udine, Udine, Italy. Tel: 0432 559662; Fax: 0432 559661; e-mail: candoni.anna@aoud.sanita.fvg.it

Accepted for publication 10 September 2008 doi:10.1111/j.1600-0609.2008.01158.x

Evaluation of therapeutic efficacy in cancer patients requires a precise evaluation of minimal residual disease (MRD) after treatment. In hematological malignancies, and particularly in acute and chronic leukemia, definition of remission often requires not only a morphological analysis, but also lack of disease evidence at sub-microscopic level. This is highly important after a potentially curative approach as allogeneic hematopoietic stem cell transplant (HSCT), where early detection of relapse may lead to the modulation of

immunosuppressive therapy or the use of donor lymphocyte infusion (DLI).

In acute myeloid leukemia (AML), various approaches have been used to define MRD: immunophenotyping, cytogenetic and molecular analysis. Unfortunately, the methods with higher sensibility (i.e. molecular tests) may be hampered by the lack of a specific target of study. A majority of AML cases, in fact, do not have a specific cytogenetic and/or molecular alteration that can be used as a sensible marker of MRD after morphological remission. Wilms tumor gene (*WT1*) has been cloned in 1990 and identified as a onco-suppressor gene encoding a transcription factor that plays a fundamental role in the development of Wilms tumor (1, 2). Various papers have reported an over-expression of *WT1* in various hematological malignancies, including AML where *WT1* is highly expressed at diagnosis in about 80% of cases. Moreover, it is now evident that *WT1* values correlate with disease status, thus being a good candidate for the evaluation of MRD, especially in the relevant percentage of patients who lack a specific molecular marker of their disease (2–13). The aim of this study was to explore whether the *WT1* quantitative expression evaluation may be a useful marker of MRD in AML cases after allogeneic HSCT. We therefore evaluated, sequentially and using a quantitative RT-PCR technique, *WT1* expression in 38 consecutive AML patients who overexpressed *WT1* at diagnosis and who underwent allogeneic transplant procedure at our center.

Methods

Quantitative assessment of *WT1*

The quantitative assessment of the *WT1* transcript amount was performed by real-time quantitative polymerase chain reaction (RQ-PCR) in 42 AML patients at diagnosis (of which 38/42 overexpressed *WT1*). In the 38 positive cases, *WT1* was evaluated before and after transplantation procedure, monthly, for the first 6 months and then every 2 months. A total of 730 samples were collected and analyzed with a mean of 19 samples per patient.

Mononuclear cells were separated from bone marrow (BM) samples on a Ficoll–Hystopaque 1077 (Sigma Aldrich Company, Saint Louis, MO, USA) density gradient. Total RNA was extracted using RNeasy mini kit, following the manufacturer's instructions (Qiagen, Hilden, Germany). The RT (reverse transcription) step was adapted from the BIOMED 1 protocol (14). Starting from 1 μg of total RNA, random hexamers were used at a concentration of 25 μM and to the reaction mixture 100 U of the reverse transcriptase was added (Applera, Milan, Italy) to obtain a significant enhancement of the assay sensitivity (11). RQ-PCR reactions and fluorescence measurements were performed on the ABI PRISM 7300 Sequence Detection System (Applied Byosystems, Foster City, CA, USA).

For quantitative assessment of *WT1*, we used *WT1* ProfileQuant Kit (Nanogen, San Diego, CA, USA) following the previously reported method by Cilloni *et al.* (11). Primers and probe for *WT1* were: forward primer (located on exon 7): 5′-CAGGCTGCAATAAGAGATATTTTAAGCT-3′; reverse primer (located on exon 8): 5′-GAAGTCACACTGGTATGGTTTCTCA-3′; TaqMan probe (located on exon 7): 5′-CTTACAGATGCACAGCAGGAAGCACACTG-3′. The fluorescent probe was labelled with 6-carboxy-fluorescein phosphoramide as reporter dye at the 5′-end and with the quencher dye carboxy-tetramethyl-rhodamine at the 3′-terminus.

The calibration curve, for quantitative assessment of *WT1*, was constituted by serial dilutions ranging from 10^6 to 10^1 molecules of a linearized plasmid obtained by cloning the target *WT1* sequence (Ipsogen, Marseille, France). The sensitivity of the RQ-PCR assay for *WT1* could constantly detect 10 plasmid molecules. The sensitivity score calculated according to Gaber *et al.* in the follow up samples was −3.6 (15). In accordance with the indications of the European SANCO concerted action and manufacturer's instructions of ProfileQuant Kit, the *WT1* transcript values obtained were normalized with respect to the number of *ABL* transcripts and expressed as gene copy number every 10^4 copies of *ABL* ($WT1/ABL \times 10^4$). The cut-off of normal value for BM samples was 70 copies $WT1/10^4$ copies ABL, as previously established (11, 15). All experiments were carried out in duplicate with appropriate negative controls. The results showing a discrepancy >1 Ct between the two wells were excluded and repeated. The samples containing less of 1000 copies of ABL were evaluated as degraded and inadequate for analysis.

Patients

Our study included 38 AML patients (16 males and 22 females) who underwent allogeneic HSCT at our Division of Bone Marrow Transplantation between December 2005 and December 2007. All of them overexpressed WT1 at diagnosis of AML (more than 70 copies $WT1/10^4$ copies ABL on BM samples). Results were analyzed as of May 31, 2008.

Patient characteristics at diagnosis of AML are listed in Table 1A. Median age at diagnosis was 45 yr (range: 18–69), most prevalent FAB subtypes were M0-M1 and M4-M5. Seventeen patients (45%) displayed an abnormal karyotype at diagnosis, but only seven of 38 (18%) had a specific molecular marker that could allow for MRD monitoring (three cases with AML1/ETO, two cases with MLL rearrangements, one case with

Table 1 (A) Patient characteristics at diagnosis of AML; (B) Outcome after allo-HSCT

(A)	
No. of cases	38 (16M/22F)
Age (yrs)	
Mean	46 ± 15
Median	45 (18–69)
FAB	
M0-M1	17/38 (45%)
M2	5/38 (13%)
M3	1/38 (3%)
M4-M5	15/38 (39%)
Karyotype	
Abnormal	17/38 (45%)
Normal	21/38 (55%)
Molecular biology	
Specific marker	7/38 (18%)
(B)	
Follow-up post-HSCT (months)	
Mean	17 ± 12
Median	14 (3–50)
Status of patients after HSCT	
Alive	30/38 (79%)
Death	8/38 (21%)
Status of AML after HSCT	
CR	32/38 (84%)
Relapse	6/38 (16%)
Causes of death after HSCT	
AML	5/8
TRM	3/8 (2 GVHD, 1 MI)

MI, myocardial infarction; TRM, transplant related mortality; GVHD, graft vs. host disease.

DEK-CAN, one case with PML/RARα). At the time of transplant, 25 patients (66%) were in first complete remission (CR), while 13/38 (34%) had a partial remission (PR) or resistant disease (RES); 89% of the patients (31/38) were considered at high risk of relapse according to disease status at HSCT (PR or RES) and clinical or biological characteristics at diagnosis (unfavorable cytogenetic asset, high WBC count, AML secondary to myelodysplastic syndrome, overexpression of PGP multidrug-resistance proteins). Twenty-two patients (58%) received grafts from a sibling donors, while 16 (42%) were transplanted from matched unrelated donors (MUD). Myeloablative conditioning regimen was used in 21 patients (12/21 sibling and 9/21 MUD) and reduced-intensity conditioning (RIC) regimen in 17 patients (10/17 sibling and 7/17 MUD).

Definition of response to therapy before and after transplantation was expressed according to the published National Cancer Institute criteria, revised by the International Working Group (16). CR was defined as absence of any tumor and $<5\%$ BM blasts with polymorph nuclear cells $>1\times10^9$/L, platelets $>100\times10^9$/L and independence of transfusions. Partial remission was defined as 5–15% blasts in BM of adequate cellularity with evidence of trilineage regeneration. Patients who did not meet the criteria for CR or PR were categorized as resistant (RES).

Statistical analysis

Categorical variables were compared by chi-squared test; P values less than 0.05 were considered statistically significant. Data were analyzed using the NCSS60 software package (NCSS Company, Kaysville, UT, USA) and Microsoft Office Excel software (Microsoft Corporation, Redmont, WA, USA).

Results

All the 38 AML patients included in our study expressed high levels of *WT1* at diagnosis, with a mean value of 4189 ± 3325 *WT1* copies/10^4 ABL and a median value of 3495 (range: 454–13923) *WT1* copies/10^4 *ABL*. At the time of HSCT, the 25 patients in CR displayed a median *WT1* copy number of 64 (range: 10–265), significantly lower than the 13 patients with active disease (median value 2662; range 271–6413) ($P = 0.004$). In fact, while in CR patients, mean *WT1* values decreased from diagnosis (4435) to transplant (64), in RES/PR cases, the *WT1* copies at transplant (median 2662) were substantially identical to those at disease onset (median 2640) (Fig. 1A and B). Moreover, all the 13 cases with active leukemia at HSCT maintained high *WT1* values, confirming the strict concordance between expression of this molecular marker and leukemia burden (Fig. 2).

Of the 25 patients transplanted while in CR, those who maintained CR after HSCT displayed *WT1* copy values persistently low during all the follow-up period, as shown in Fig. 3. In the nine patients who received transplant with active disease attaining a sustained CR post-HSCT, *WT1* levels rapidly decreased to normal range in the first 2 months after transplant procedure and remained low through the entire study period (Fig. 4).

Among the patients with sustained CR after HSCT, there was a complete concordance between low levels of *WT1*, full donor chimerism (100%), BM blasts (less than 5%) and, in the few patients with a molecular marker (three cases with AML1/ETO, two cases with MLL rearrangements, one case with DEK-CAN), negativity of disease-specific marker.

In the six patients who relapsed after HSCT, *WT1* was always overexpressed at the time of cytological and hematological relapse. None of the relapsed patient had low *WT1* copy number at disease recurrence, another evidence of concordance between this molecular marker and disease status. As shown in Fig. 5, four of six patients who relapsed after HSCT had active leukemia at the time

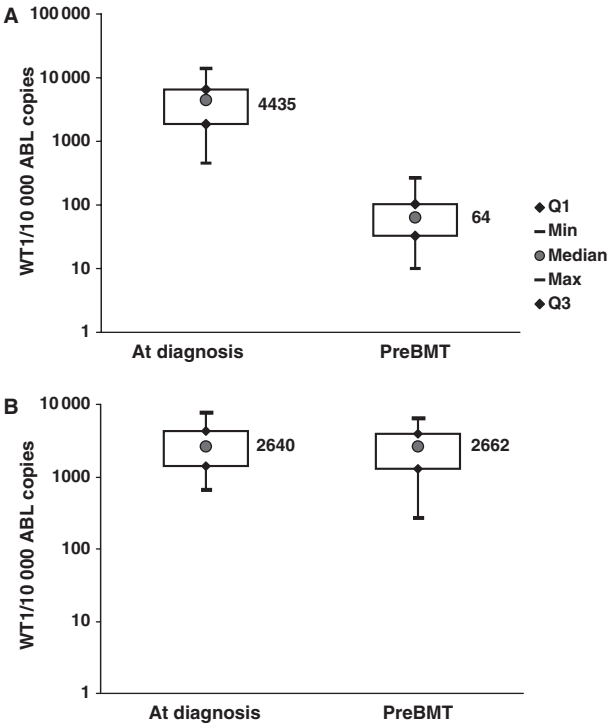


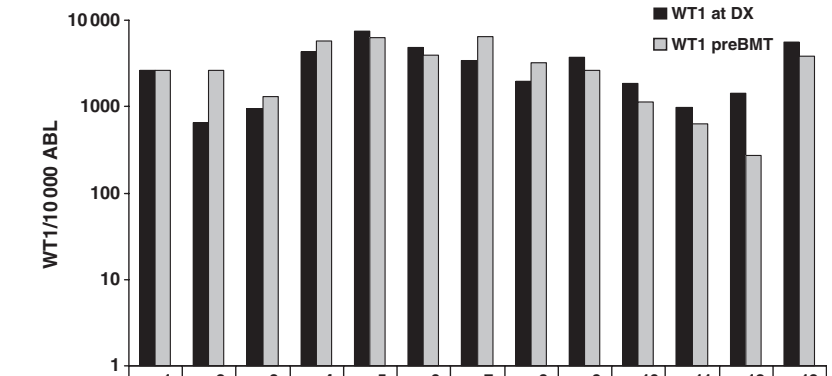
Figure 1 Comparison between *WT1* transcript levels at diagnosis of acute myeloid leukemia and before hematopoietic stem cell transplant (HSCT) in patients who performed HSCT in complete remission (A) and in those who performed HSCT with active disease (partial remission or refractory) (B).

of transplant. Three of these cases (Pt 1, 2, 3) had AML recurrence in the first 6 months after HSCT, with a relatively low and steady increase in *WT1* expression, while the fourth patient (Pt 4) relapsed lately, but again with a slow kinetics of *WT1* increase. In contrast, two patients (Pt 5 and Pt 6) transplanted while in CR, relapsed more lately (14 and 16 months after HSCT, respectively) and displayed a faster growth of *WT1* expression levels. So, in these cases, *WT1* increase was concomitant with cytological detection of AML relapse, and molecular analysis did not allow anticipating disease recurrence. Of six relapsed cases, only one (Pt 4) had a specific molecular abnormality (*PML/RARα)*, whose reappearance of which was concomitant with *WT1* overexpression after HSCT. The other five patients lacked a marker amenable to detection by cytogenetic and/or PCR.

Outcome after HSCT are listed in Table 1B. With a median follow-up time of 14 months (range: 3–50), 30 patients are alive (79%) and eight patients are dead, five for AML recurrence and three for transplant-related mortality (two GVHD, one myocardial infarction) while in CR. Five of the six patients who relapsed after HSCT, five had died, while one achieved a second CR with salvage chemotherapy and DLI. In this patient, attainment of CR was consensual to a decrease in *WT1* copy level below 70 copies/10^4 ABL. We did not find any significant difference in *WT1* expression and kinetics, between patients transplanted with conventional or RIC conditioning regimens, or according to stem cell donor type (sibling or MUD).

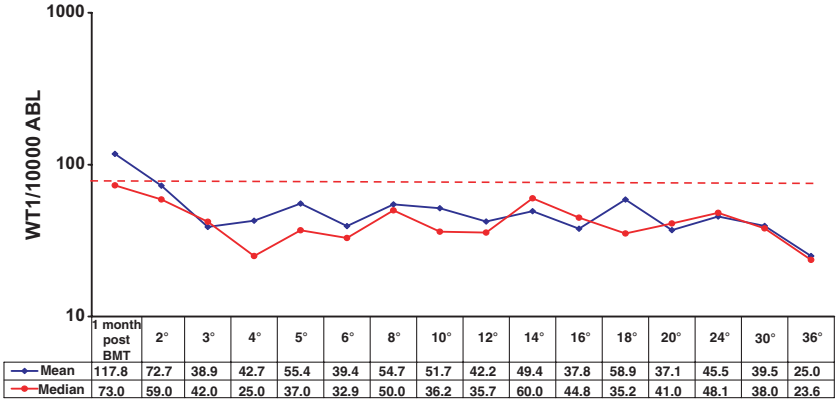
Discussion

Accurate assessment and monitoring of MRD after therapy are of crucial importance in leukemic patients, as it allows determining the efficacy of treatment and establishing early diagnosis of impending relapse (17–20). Unfortunately, less than 40% of AML cases display a specific molecular marker for MRD evaluation. The usefulness of *WT1* as a 'panleukemic marker', based on the highly prevalence of *WT1* overexpression in AML patients at diagnosis (more than 80% of cases), has been proposed by various papers (2, 3, 5, 7, 10, 11, 13, 19, 21). However, there is still no consensus on the real efficacy of *WT1* as a marker of MRD in AML after chemotherapy or allogeneic stem cell transplant. Discrepancies may be due to the different methods employed to determine *WT1* expression, as some studies used a quantitative



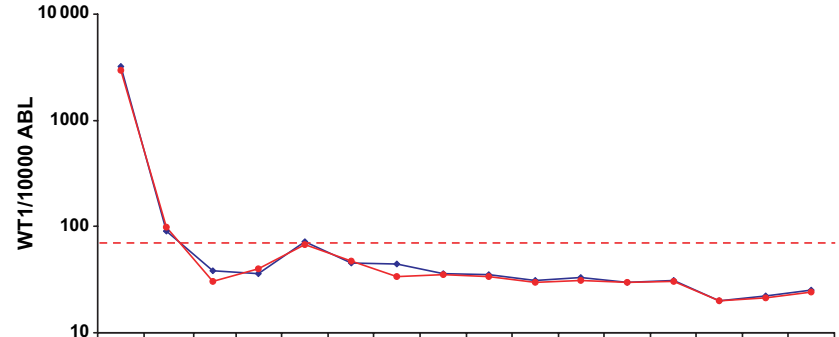
	pz 1	pz 2	pz 3	pz 4	pz 5	pz 6	pz 7	pz 8	pz 9	pz 10	pz 11	pz12	pz13
WT1 at DX	2640	658	941	4272	7560	4771	3368	1980	3679	1834	967	1414	5526
WT1 preBMT	2662	2638	1291	5692	6272	3931	6413	3237	2607	1117	638	271	3838

Figure 2 Concordance between overexpression of *WT1* at diagnosis and before allo-HSCT in 13 patients who performed transplantation procedure with partial remission or refractory acute myeloid leukemia.



	1 month post BMT	2°	3°	4°	5°	6°	8°	10°	12°	14°	16°	18°	20°	24°	30°	36°
Mean	117.8	72.7	38.9	42.7	55.4	39.4	54.7	51.7	42.2	49.4	37.8	58.9	37.1	45.5	39.5	25.0
Median	73.0	59.0	42.0	25.0	37.0	32.9	50.0	36.2	35.7	60.0	44.8	35.2	41.0	48.1	38.0	23.6

Figure 3 Dynamic longitudinal evaluation of quantitative *WT1* expression at precise time points during follow-up after hematopoietic stem cell transplant (HSCT) in acute myeloid leukemia patients transplanted in complete remission (CR) who maintained CR after HSCT. The broken line represents the upper normal limit (70 copies *WT1*/10^4 ABL).



	WT1 Pre BMT	1°month	2°	3°	4°	5°	6°	8°	10°	12°	13°	14°	16°	18°	20°	24°
Mean	3250.0	90.1	38.1	36.2	71.7	45.8	44.6	35.7	35.0	31.0	33.0	30.0	31.0	20.0	22.3	25.0
Median	2949.5	99.0	30.5	40.0	67.0	47.5	34.2	34.9	34.0	30.0	31.0	29.7	30.6	20.0	21.5	24.0

Figure 4 Dynamic longitudinal quantitative evaluation of *WT1* expression at precise time points during follow-up after hematopoietic stem cell transplant (HSCT) in acute myeloid leukemia patients transplanted without complete remission (CR) who reached and maintained CR after HSCT. The broken line represents the upper normal limit (70 copies *WT1*/10^4 ABL).

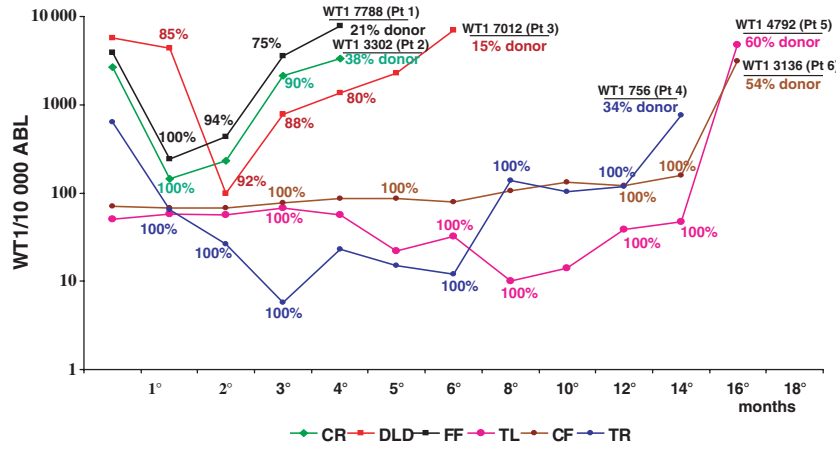


Figure 5 Dynamic longitudinal quantitative evaluation of WT1 expression at precise time points after allo-HSCT in six acute myeloid leukemia patients who relapsed after HSCT. The corresponding percentages (%) of donor chimerism have been reported.

or semi-quantitative methodologies, but other authors reported *WT1* expression on a solely qualitative basis with low specificity and sensitivity of the assay (7, 8, 12, 13, 22–26). While the former studies constantly found a correlation between *WT1* increase and leukemic relapse, the latter failed to link *WT1* expression with disease course (6, 7, 12, 19, 22, 23). In particular, the work by Elmaagacli *et al.* suggests that detection of *WT1* transcripts does not predict leukemic relapse after allogeneic HSCT and that, therefore, *WT1* could not be considered a suitable marker for MRD detection in this setting (22). However, these negative conclusions should be taken cautiously if we consider that the authors evaluated *WT1* expression only by a qualitative PCR, whose results can be altered by expression of *WT1* gene at low levels in normal hematopoietic stem cells. Moreover, the patient

population of this study included both AML and acute lymphoblastic leukemia (ALL) cases. Patients were evaluated at different but no precise time points after transplant. Only a portion of this study population expressed *WT1* before HSCT, and no data about *WT1* expression at diagnosis are reported (22). In contrast, the paper by Ogawa and co-workers, who used a quantitative RT-PCR methods, found a strict correlation between MRD kinetics, as expressed by *WT1* levels after allogeneic HSCT and probability of leukemic relapse in 72 patients with AML, ALL or advanced phase chronic myeloid leukemia (25). These data were confirmed by Cilloni *et al.* in a smaller set of patients with acute leukemia, tested for *WT1* expression by real-time quantitative PCR before and after allogeneic HSCT (27).

Consistent with these two reports, we found an exact concordance between *WT1* gene expression and disease status after allogeneic HSCT. In our study, we evaluated a highly homogenous group of patients, as all had a diagnosis of AML and overexpressed *WT1* at disease onset. To validate the usefulness of *WT1* as predictor of relapse, we monitored our patients closely and at precise time points, as BM samples were tested for quantitative *WT1* expression before allogeneic HSCT and every month after transplant for the first 6 months and then bimonthly for the first 2 yr, or until relapse. To minimize the variability linked to sampling differences, we used exclusively BM as a source for RNA. In the published experiences in AML after chemotherapy, both BM and peripheral blood (PB) samples have been used for *WT1* analysis. A recent paper by Ommen *et al.* seems to suggest that relapse has been detected earlier using monthly BM than PB samples providing longer window of opportunity before relapse (13). However, according to predictive model presented in this paper, also PB samples may be useful for AML relapse prediction, but only when shorter sampling intervals are possible (at least bimonthly PB samples collection) (13).

Our results revealed a concordance between normal levels of *WT1* gene expression and remission status in the patients in CR before transplant and in those who attained and/or maintained a CR after HSCT. In all cases, *WT1* levels remained within the range of normality throughout the follow-up period, and were consistent with morphological BM analysis, full donor chimerism and molecular specific markers (when available). Conversely, the patients with a post-transplant increase in *WT1* gene levels from normal to high, inevitably experienced disease recurrence. In no case, at the time of morphological relapse, *WT1* levels were low, confirming that *WT1* overexpression is a marker of AML activity and that increase in quantitative PCR results can predict relapse. Nonetheless, we have found a variability in the kinetics of *WT1* increase in the patients who relapsed after allogeneic HSCT, as some of them (Pt 1,2,3,4) displayed a low and steady rise of *WT1* copies, while others (Pt 5 and 6) had a rapid growth of gene expression levels, so that molecular and morphological diagnosis of AML relapse were concomitant. So, the patients in CR after transplant but with a progressive and slow increase in *WT1* levels, and without sign of GVHD, could be candidates to a modulation of immune suppression, either by suspension of drug therapy or, in selected cases, with pre-emptive DLI (24, 25, 27–30). In fact, immunotherapy is clearly more effective for patients who have a low tumor burden (i.e. at molecular relapse) compared with those with cytological AML recurrence (25, 28–30); thus, monitoring of MRD by quantitative assessment of *WT1* level may favorably impact on prognosis of relapsed patients after allo-HSCT. Unfortunately, in few patients, *WT1* increase after HSCT was very rapid and the detection of molecular and morphological relapse was concomitant, so that no pre-emptive immunotherapy was possible to avoid the overt disease reappearance.

The data presented in this paper, along with those reported by Ogawa *et al.,* show that an accurate quantitative assessment of the *WT1* transcript expression allows the monitoring of MRD after allogeneic HSCT in the majority of AML patients (24, 25). *WT1* confirms to be a good candidate as a 'panleukemic' marker, as it is expressed in about 80% of AML cases at diagnosis and its levels of expression correlate with disease status. Early detection of post-transplant relapse by sequential molecular analysis could permit, at least in those patients with a slow kinetic of *WT1* expression increase, a timely therapeutic approach with modulation of immune suppression and/or preventive use of DLI. Our study confirms the importance of quantitative assessment of WT1 as a marker of MRD after HSCT.

Further studies on larger number of allo-transplanted patients are warranted, to investigate the significance and causes of the individual kinetics of molecular relapse and to explore the concordance between chimerism and WT1 overexpression in those patients who relapsed after HSCT. Also we need to explore better the sensitivity for *WT1* analysis of PB samples to MRD monitoring after allogeneic HSCT (12, 13).

Acknowledgements

This work has been supported by a grant from AIL (Associazione Italiana contro le Leucemie), Udine section.

References

1. Call KM, Glaser T, Ito CY, *et al.* Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. *Cell* 1990;**60**:509–20.

2. Yang L, Han Y, Saurez F, *et al*. A tumor suppressor and oncogene: The *WT1* story. *Leukemia* 2007;**3**:1–9.
3. Miyagi T, Ahuja H, Kudota T, *et al*. Expression of the candidate Wilms' tumor gene, *WT1*, in human leukemia cells. *Leukemia* 1993;**7**:970–7.
4. Brieger J, Weidmann E, Fenchel K, *et al*. The expression of the Wilms' tumor gene in acute myelocytic leukemias as a possible marker for leukemic blast cells. *Leukemia* 1994;**8**:2138–43.
5. Menssen HD, Renkl H-J, Rodeck U, *et al*. Presence of Wilm's tumor gene (*wt1*) transcripts and the *WT1* nuclear protein in the majority of human acute leukemia. *Leukemia* 1995;**9**:1060–9.
6. Inoue K, Ogawa H, Sonoda Y, *et al*. Aberrant overexpression of the Wilms tumor gene (*WT1*) in human leukemia. *Blood* 1997;**89**:1405–12.
7. Inoue K, Ogawa H, Yamagami T, *et al*. Long-term follow-up of minimal residual disease in leukemia patients by monitoring *WT1* (Wilms tumor gene) expression levels. *Blood* 1996;**88**:2267–78.
8. Inoue K, Sugiyama H, Ogawa H, *et al*. *WT1* as a new prognostic factor and a new marker for the detection of minimal residual disease in acute leukemia. *Blood*. 1994;**84**:3071–9.
9. Bergmann L, Miething C, Mourer U, *et al*. High levels of Wilms' tumour gene (*WT1*), mRNA in acute myeloid leukemias are associated with a worse long term outcome. *Blood* 1997;**90**:1217–25.
10. Niegemann E, Wehner S, Kornhuber B, *et al*. *WT1* gene expression in childhood leukemias. *Acta Haematol* 1999;**102**:72–6.
11. Cilloni D, Gottardi A, De Micheli D, *et al*. Quantitative assessment of *WT1* expression by real time quantitative PCR may be a useful tool for monitoring minimal residual disease in acute leukemia patients. *Leukemia* 2002;**16**:2115–21.
12. Cilloni D, Messa F, Arruga F, *et al*. Early prediction of treatment outcome in acute myeloid leukemia by measurement of WT1 transcript levels in peripheral blood samples collected after chemotherapy. *Haematologica* 2008;**93**:921–4.
13. Ommen HB, Nyvold GC, Breaendstrup K, Andersen BL, Ommen IB, Hasle H, Hokland P, Ostegaard M. Relapse prediction in acute myeloid leukaemia patients in complete remission using *WT1* as a molecular marker: developement of a mathematical model to predict time from molecular to clinical relapse and define optimal sampling intervals. *Br J Haematol*, 2008;**141**:782–91.
14. van Dongen JJ, Macintyre EA, Gabert JA, *et al*. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. *Leukemia* 1999;**13**: 1901–28.
15. Gabert J, Beillard E, van der Velden VH, *et al*. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia-a Europe Against Cancer program. *Leukemia*. 2003;**17**:2318–57.
16. Cheson DB, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH. Revised recommendations of the international Working Group for Diagnosis, Standardization of response Criteria, Treatment Outcomes, and reporting Standards for therapeutic Trials in acute myeloid leukemia. *J Clin Oncol* 2003;**21**:4642–9.
17. Reichle A, Rothe G, Krause S, *et al*. Transplant characteristics: minimal residual disease and impaired megakaryocytic colony growth as sensitive parameters for predicting relapse in acute myeloid leukemia. *Leukemia* 1999;**13**:1227–34.
18. Chung NG, Ausch V, Radich JP. The detection and significance of minimal residual disease in acute and chronic leukaemia. *Tissue Antigens* 2006;**68**:371–85.
19. Lapillonne H, Renneville A, Auvrignon A, *et al*. High *WT1* expression after induction therapy predicts high risk of relapse and death in pediatric acute myeloid leukaemia. *J Clin Oncol* 2006;**24**:1507–15.
20. Porter D, Roth M, McGarigle C, *et al*. The graft-versus-leukemia (GVL) effect of donor mononuclear cell (MNC) infusions for patients with relapsed acute leukemia and myelodysplasia (MDS) after allogeneic bone marrow transplantation (BMT). *Blood* 1994;**84**:338a.
21. Cilloni D, Saglio G. WT1 as a universal marker for minimal residual disease detection and quantification in Myeloid Leukemias and in Myelodysplastic Sindrome. *Acta Haematol* 2004;**112**:79–84.
22. Elmaagacli AH, Beelen DW, Trenschel R, *et al*. The detection of wt-1 transcripts is not associated with an increased leukemic relapse rate in patients with acute leukemia after allogeneic bone marrow transplantation or peripheral blood stem cell transplantation. *Bone Marrow Transplant* 2000;**25**:91–6.
23. Mizuta S, Ito Y, Kohno A, *et al*. Accurate quantitation of residual tumor burden at bone marrow harvest predicts timing of subsequent relapse in patients with common ALL treated by autologous bone marrow transplantation. Nagoya BMT Group. *Bone Marrow Transplant* 1999;**24**:777–84.
24. Ogawa H, Tamaki H, Ikegame K, *et al*. The usefulness of monitoring *WT1* gene transcripts for the prediction and management of relapse following allogeneic stem cell transplantation in acute type leukemia. *Blood* 2003;**101**:1698–704.
25. Ogawa H, Tsuboi A, Oji Y, *et al*. Successful donor leukocyte transfusion at molecular relapse for a patient with acute myeloid leukemia who was treated with allogeneic bone marrow transplantation: importance of monitoring of minimal residual disease by *WT1* assay. *Bone Marrow Transplant* 1998;**21**:525–7.

26. Weisser M, Kern W, Rauhut S, *et al*. Prognostic impact of RT-PCR-based quantification of *WT1* gene expression during MRD monitoring of acute myeloid leukaemia. *Leukemia* 2005;**19**:1416–23.
27. Cilloni D, Gottardi E, Fava M, Messa F, Carturan S, Busca A, Guerrasio A, Saglio G. Usefulness of quantitative assessment of the *WT1* gene transcript as a marker of minimal residual disease detection. *Blood* 2003;**102**:773–4.
28. Loren AW, Porter DL. Donor leukocyte infusions for the treatment of relapsed acute leukaemia after allogeneic stem cell transplantation. *Bone Marrow Transplant* 2008;**41**:483–93.
29. Kolb H-J, Schattenberg A, Goldman JM, *et al*. Graft-versus leukemia effect of donor lymphocyte transfusions in marrow grafted patients. *Blood* 1995;**86**:2041–50.
30. Dominetto A, Pozzi S, Miglino M, *et al*. Donor lymphocyte infusions for the treatment of minimal residual disease in acute leukemia. *Blood* 2007;**109**:5063–4.