Early reduction of WT1 transcripts during induction chemotherapy predicts for longer disease free and overall survival in acute myeloid leukemia

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ABSTRACT

We investigated the prognostic significance of early peripheral blast clearance as assessed by WT1 transcript reduction during the first days of standard induction therapy in 57 adult patients with acute myeloid leukemia (AML). Quantification of WT1 transcript by real-time quantitative PCR in peripheral blood on days 1 and 5 of treatment was performed. WT1 ratio was defined as the ratio of copy number measured on day 1 and on day 5. The median WT1 ratio was greater in patients attaining CR as compared to non-responders (11.68 *vs*. 2.14, respectively; P=0.0006). Furthermore, DFS and OS were significantly longer in patients displaying a WT1 ratio greater than 5.82 (i.e. the median value of whole cohort) than in patients with WT1 ratio of 5.82 or under (P=0.024 and P<0.001, respectively). These data suggest that early decrease of WT1 copy number in peripheral blood predicts for better outcome and should be considered in the management of AML patients.

Key words: acute myeloid leukemia, WT1, peripheral blast clearance, prognosis.

Citation: Gianfaldoni G, Mannelli F, Ponziani V, Longo G, Bencini S, Bosi A, and Vannucchi AM. Early reduction of WT1 transcripts during induction chemotherapy predicts for longer disease free and overall survival in acute myeloid leukemia. Haematologica. 2010;95:833-836. doi: 10.3324/haematol.2009.011908

Introduction

The prognosis of patients with acute myeloid leukemia (AML) is predicted by conventional cytogenetics and gene (*FLT3*[1], *NPM1*[2], *CEBPA*[3], *MLL*[4]) mutational status, which stratifies patients in groups with different probabilities of achieving and maintaining complete remission (CR), providing the basis for risk-oriented treatment. However, this information is not usually available when induction chemotherapy is decided; therefore, risk-oriented treatment concerns post-induction therapy, which is also conditioned by the results of bone marrow (BM) analysis and detection of minimal residual disease (MRD). Quantification of MRD after the induction cycle,[5,6] as well as during the course of post-remission therapy,[7-9] represents a powerful predictor of disease-free (DFS) and overall survival (OS). The *Wilms' tumor gene 1* (*WT1*), which is over-expressed in more than 90% of AML, is a useful marker for monitoring MRD. The quantitative assessment of WT1 levels in BM after induction and consolidation therapy demonstrated high prognostic value irrespectively of cytogenetic or molecular abnormalities.[10-13] Quantification of WT1 transcript level in peripheral blood (PB) leukocytes at recovery from chemotherapy reliably discriminated among patients at different risks of relapse.[14]

The aim of our study was to determine whether assessment of changes in WT1 transcript levels in peripheral blood in the first days during induction therapy would provide information on the chemosensitivity of leukemic blasts and predict for clinically relevant end points.

Design and Methods

Patients

Eligibility criteria for entering the study were a diagnosis of non-M3 AML based on morphological, cytochemical and immunophenotypical criteria and the presence of morphologically identifiable blast cells in PB. Only patients aged 65 and under were included in the study. The study was approved by the local institutional review board, after written informed consent in accordance with the Declaration of Helsinki.

Cytogenetic analysis was performed in BM cells at diagnosis according to the International System for Human Cytogenetic Nomenclature.[15] Cytogenetic risk was assessed according to South West Oncology Group criteria.[16] Presence of FLT-3 internal tandem duplications (ITD) and NPM1 mutations were investigated as described.[17,18] The definition of CR used established criteria.[19] DFS

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Manuscript received on June 4, 2009. Revised version arrived on October 30, 2009. Manuscript accepted on November 2, 2009.
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was measured from the date of CR achieved with the first cycle to relapse or last follow-up. Overall survival (OS) was calculated from diagnosis to the last observation or death; patients receiving allogeneic stem cell transplant (SCT) were censored at the date of transplant.

All patients were treated with a "3+7" induction course: cytarabine 100 mg/m^2 over three hours intravenous infusion every 12 hours on days 1-7; idarubicin 12 mg/m^2 30 min intravenous infused on days 1-3. High-dose cytarabine (3 g/m^2 bid days 1, 3, 5) was used as first consolidation cycle in patients attaining CR.

After the first induction course, patients with persistent disease (i.e. > 5% BM blasts at hematopoietic recovery) received a salvage regimen.

In an intention-to-treat approach, patients with high-risk cytogenetic, FLT3-ITD or adverse clinical features (secondary AML, CR after second course, hyperleukocytosis) were assigned to undergo allogeneic SCT from matched related or unrelated donor, while patients with low-risk cytogenetics were offered autologous PBSCT. Patients with intermediate risk cytogenetics in the absence of FLT3-ITD and adverse clinical features were allocated to allogeneic or autologous SCT according to HLA-identical related donor availability. PBSCs for autologous SCT were mobilized after a DIA course (cytarabine 500 mg/m^2 bid on days 1-6; daunorubicin 50 mg/m^2 on days 4-6). Patients who failed mobilization received two additional courses with high-dose cytarabine.

WT1 quantitative determination

Quantification of WT1 expression levels was performed in PB leukocytes collected on day 1 (immediately before starting therapy) and on day 5 (the fifth day after start of treatment, immediately before cytarabine infusion). This time was chosen on the basis of the kinetics of PB blast disappearance during induction therapy determined with immunophenotyping.[20,21] Mononuclear cells were separated on a Ficoll-Hypaque density gradient (Lymphoprep, Celbio, Italy). Total RNA was extracted using Trizol following the manufacturer's instructions (Invitrogen Ltd, Paisley,U.K., http://www.invitrogen.com) and stored at –80°C until use. RNA was retrotranscribed to cDNA;[22] WT1 copy number was measured using WT1 ProfileQuant Kit from Ipsogen (Marseille, France) in triplicate, and expressed as WT1 copy number per 10,000 ABL copies.[23]

Statistical analysis

Data were analyzed using the SPSS software (StatSoft, Inc., Tulsa, OK, USA). Group comparisons were performed by the Mann-Whitney non-parametric U test or Kruskal-Wallis test with Dunn's multiple comparison post-test. Survival analyses were calculated by Kaplan Meyer survival curves and the log rank test. Univariate and multivariate analyses on caterigorized data were performed using Cox's proportional hazards model.

Results and Discussion

Between May 2004 and March 2007, 57 of 60 consecutive AML patients entering the study completed induction chemotherapy and were evaluable for response; patients' characteristics are shown in Table 1. The median follow-up of the whole cohort of patients was 14 months (1-44).

CR was achieved after the first cycle in 35 patients; of the 22 patients not achieving CR (NCR), 7 patients obtained partial remission while 15 patients were refractory.

Of the 35 patients achieving CR, 18 patients underwent autologous PBSCT, 3 patients received allogeneic SCT and 14 patients received consolidation chemotherapy.

The median number of WT1 transcripts measured on day 1 (pre-treatment value) were 16,218 (61.8-156,314). The number of day 1 WT1 copies was not predictive of CR after the first cycle; patients who eventually achieved CR had a median copy number of 11,500 (range 61.80-156,315) compared to 17,338 (range 784.33-63,241) in those who did not attain CR (P=0.93). Considering the 35 patients achieving CR after the "3+7" cycle, there was no significant difference in the day 1 WT1 copy number between patients persisting in CR (8,044, range 61.80-87,100) or relapsing (22,416, range 180.72-156,315) (P=0.45). These data indicated that the burden of WT1 copies at baseline did not influence disease outcome.

The median number of day 5 WT1 copies were 2,213 (1.70-56,559); day 5 WT1 copies resulted significantly lower in patients who obtained a CR after the first cycle (924.7, range 1.70-20,965) compared to those who did not (7,087, range 9.8-45,656) (P=0.003). All patients but 6 showed reduction of WT1 copy number from day 1 to day 5; of note, 5 of them were primary refractory. For each patient, we calculated the "WT1 ratio" as the ratio of copy

Table 1. Characteristics of patients and treatment outcome.

Patients, n.	57
Median age, years (range)	48 (18-65)
Gender, n.	
Male	29
Female	28
FAB subtypes, n.	
M0	4
M1	10
M2	17
M4	17
M5	7
M6-M7	2
Cytogenetic risk group, n.	
Low	7
Intermediate	32
High	15
Lack of growth	2
Not available	1
Prior myelodysplastic syndrome, n.	4
WBC count, /μL	
Median (range)	13400 (1240-286000)
Peripheral blasts, %	
Median (range)	66 (7-100)
FLT3 status	
FLT3-ITD	14
FLT3-PM	3
FLT3-wt	37
Not available	3
NPM1 status	
NPM1 mutated	16
NPM1 wt	37
Not available	4
Treatment outcome, n. (%)	
CR	35 (61%)
NCR	22 (39%)

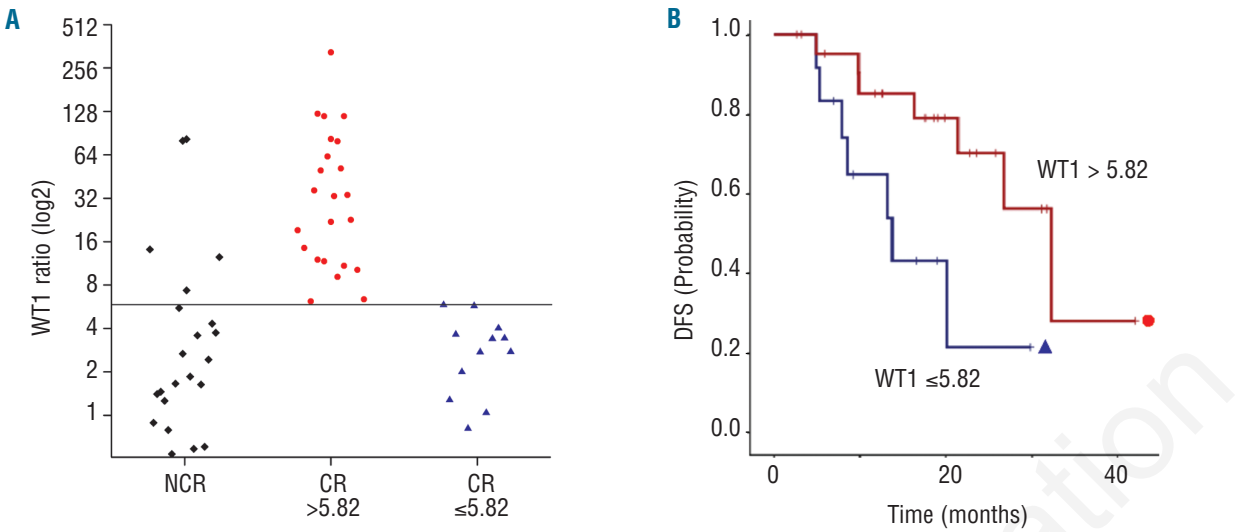

Figure 1. Disease outcome according to WT1 ratio. (A) The distribution of WT1 ratios in responders and non-responders to "3+7" induction course. (B) Among the 3l5 patients achieving CR after the first cycle, there was a significant difference in disease free survival (DFS) for patients with a WT1 ratio above 5.82 (n=23) (•) compared to patients with a WT1 ratio below or equal to 5.82 (n=12) (▲) (*P*=0.024).

number measured on day 1 and on day 5; the median WT1 ratio for the whole patient population was 5.82 (0.54-327.71).

The median WT1 ratio was significantly greater in patients attaining CR (11.68 *vs.* 2.14; *P*=0.0006). Of 28 patients with a WT1 ratio greater than 5.82 (i.e, the median value of the whole population) 23 (82.1%) achieved CR compared to 12 of 29 patients (41.4%) with a ratio of 5.82 or under (*P*=0.002). These data suggested that WT1 ratio was predictive of CR attainment (Figure 1A). Furthermore, we found that among the 35 patients attaining CR after the "3+7" course, the DFS was significantly longer in those displaying a WT1 ratio greater than 5.82 (median 32.3 months) compared to those displaying a ratio of 5.82 or under (median 13.8 months; *P*=0.024) (Figure 1B). Likewise, a WT1 ratio greater than 5.82 predicted for a significantly longer OS in all patients included in the study (median OS not reached) compared to patients with a ratio of 5.82 or under (median OS 13.1 months; *P*<0.001) (Figure 2).

In a multivariate analysis including, age, white blood cell (WBC) count, karyotype and FLT3/NPM1 status, DFS was predicted by both WT1 ratio (*P*=0.01) and WBC count (*P*=0.041), while WT1 ratio was the only variable predicting likelihood of achieving CR and for longer OS (*P*=0.009 and *P*=0.007, respectively). Furthermore, we performed a multivariate analysis within patients attaining CR after the first induction cycle (n=35) including autologous transplant in order to assess a possible impact of post-remission therapy on outcome. Autologous transplantation did not demonstrate any impact on DFS and OS.

Overall, these data suggest that measurement of WT1 ratio after standard chemotherapy represents an important predictor of disease outcome. Specifically, the kinetics of the reduction in WT1 copies correctly predicted the bone marrow response evaluated by standard morphological criteria in the majority of cases. On the other hand, we noted that a subgroup of patients was wrongly predicted as being refractory on the basis of low WT1 ratios; however, it is noteworthy that these patients showed a shorter DFS suggesting poor quality of CR (Figure 1A).

The extent to which WT1 copies were reduced, expressed as WT1 ratio, rather than the absolute count of WT1 copies at day 5, provides an estimate of peripheral

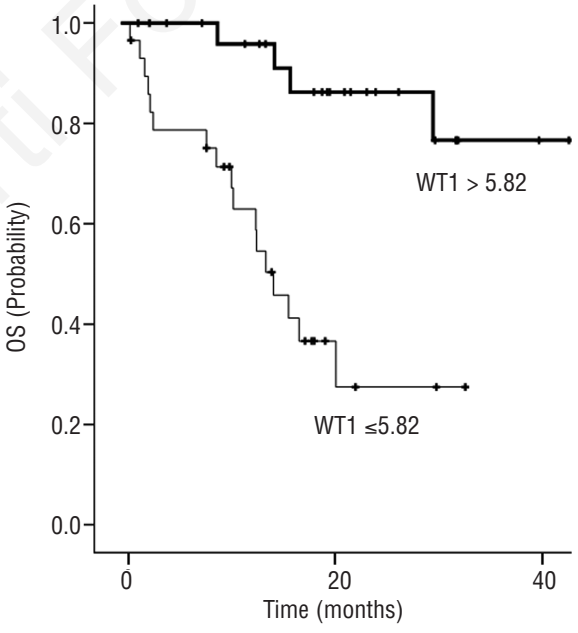

Figure 2. Impact of WT1 ratio on Overall Survival. Within the whole cohort (n=57), there was a significant difference in OS for patients with a WT1 ratio above 5.82 (n=29) compared to patients with a WT1 ratio below or equal to 5.82 (n=28) (*P*<0.001).

blast clearance in each individual patient irrespectively of the baseline leukemic burden.

We used the median value of WT1 ratio measured in this patient population as a cut-off; as such, it should not be intended as an absolute value, and can also depend upon the type of treatment. The fact that measurement of WT1 ratio can easily be performed using PB samples anticipates the relevance of these findings for the management of AML patients. In fact, an early prediction of outcome might provide a means of customizing therapeutic strategy from the first days of induction therapy. Patients with a low reduction in WT1 have a high probability of being refractory or experiencing early relapse, and could be immediately switched to intensification of induction. On the other hand, patients with satisfactory WT1 reduction could continue standard induction protocol, as this correlates with a high rate of CR and longer DFS, avoiding the unnecessary toxicity of an intensified induction.

Authorship and Disclosures

GG, FM, AMV conceived and designed the study, and contributed to manuscript writing. VP and SB contributed to molecular analysis. GL and AB contributed to the analysis and interpretation of data. All Authors approved the final version of the manuscript. The Authors declare they have no potential conflict of interest.

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