Ann Hematol (2012) 91:1855–1860
DOI 10.1007/s00277-012-1546-7

ORIGINAL ARTICLE

Analysis of mutational status, SNP rs16754, and expression levels of Wilms tumor 1 (*WT1*) gene in acute promyelocytic leukemia

Girish Chander Gaur · Safaa M. Ramadan · Laura Cicconi · Nélida I. Noguera · Irene Luna · Esperanza Such · Serena Lavorgna · Jonny Di Giandomenico · Miguel A. Sanz · Francesco Lo-Coco

Received: 12 June 2012 / Accepted: 28 July 2012 / Published online: 16 August 2012

Abstract Overexpression, polymorphisms, and mutations of the *WT1* gene have been reported in several human tumors including acute myeloid leukemia (AML) and variably correlated with prognosis. Acute promyelocytic leukemia (APL) represents the AML subset disclosing higher *WT1* expression levels; however, no *WT1* studies specifically focused on APL have been conducted. We screened for the presence of mutations, SNP rs16754, and expression levels of *WT1* gene in 103 adult patients with newly diagnosed APL. Fms-like tyrosine kinase (*FLT3*) mutations were analyzed as well. *WT1* mutations were identified in four (4 %) patients. At least one copy of the minor SNP rs16754 allele ($WT1^{AG}$ or $WT1^{GG}$) was detected in 30 (29 %) patients. Six patients (6 %) were homozygous for the minor allele ($WT1^{GG}$) and this genotype was associated with higher *WT1* mRNA copies (p=0.018). *FLT3* mutations were found in 37 % of patients and correlated with high *WT1* mRNA expression (p=0.004). Patients heterozygous or homozygous for the minor allele and patients homozygous for major ($WT1^{AA}$) allele did not differ in terms of presenting features. In adult APL, *WT1* gene mutational and polymorphic profile shows similarities with pediatric AML rather than with adult AML.

Keywords Acute promyelocytic leukemia · Wilms tumor 1 · SNP rs16754 · *FLT3* · *WT1* copy number

Introduction

The Wilms tumor 1 (*WT1*) gene encodes a zinc-finger transcription factor which has emerged as an important regulator of normal and malignant hematopoiesis. *WT1* is primarily expressed in the cells of developing genitourinary, central nervous, and hematopoietic systems [1, 2]. *WT1* was originally described as a tumor suppressor gene based on its structural and functional loss in patients with WAGR syndrome characterized by Wilms tumor, aniridia, genitourinary malformations, and mental retardation [3, 4]. However, accumulating evidence also points to an oncogenic role for this transcription factor. *WT1* is highly expressed in a variety of tumors [5–7] including acute myeloid leukemia (AML) [8, 9]. In addition, its expression is inversely associated with the degree of cell differentiation [10, 11]. Consequently, *WT1* is currently considered as a potential target for immunotherapy in both leukemias and solid tumors.

Over the last decade, several studies have documented in AML increased expression levels of *WT1* transcript [12–14], mutations in the coding regions of *WT1* exons 7 and 9

G. C. Gaur · S. M. Ramadan · L. Cicconi · S. Lavorgna · J. Di Giandomenico · F. Lo-Coco (✉)
Department of Biopathology, University of Rome "Tor Vergata", Via Montpellier 1, 00133 Rome, Italy
e-mail: francesco.lo.coco@uniroma2.it

G. C. Gaur · L. Cicconi · N. I. Noguera · S. Lavorgna · J. Di Giandomenico · F. Lo-Coco
Laboratorio di Neuro-Oncoematologia, Fondazione Santa Lucia, 00143 Rome, Italy

S. M. Ramadan
Department of Medical Oncology, National Cancer Institute—Cairo University, Cairo, Egypt

I. Luna · E. Such · M. A. Sanz
Department of Hematology, Hospital Universitario La Fe, 46009 Valencia, Spain

[15–17], and more recently specific allelic variation (SNP rs16754) [18–20]. Among distinct French-American-British (FAB) AML subsets, AML-M5 is associated with a significantly lower *WT1* expression [21] whereas AML-M3 is characterized by the highest *WT1* expression levels [13]. Interestingly, a progressive rise in *WT1* mRNA expression levels has been described in chronic myelogenous leukemia (CML) [8] and myelodysplastic syndromes (MDS) [22] during disease progression. Yet, conflicting data are currently available on the prognostic impact of these alterations in AML. Two recent studies observed a correlation between high *WT1* mRNA expression and improved outcome in acute myeloid leukemia [17, 23].

On the other hand, mutations in the coding region of the *WT1* gene are detected in approximately 10 % of adults with cytogenetic normal AML [CN-AML; 10, 15, 24]. While two studies showed an association between *WT1* mutation and poor overall survival (OS), disease free survival (DFS) [17, 24] and higher risk of relapse [16, 17], two more recent studies [10, 19] reported lack of *WT1* mutational status impact on prognosis. Almost all *WT1* mutations in AML cluster in exons 7 and 9. The N-terminal region of exon 7 harbors two mutational hotspot regions located between nucleotide 1285 and 1360 [17] where more than 90 % of the exon 7 mutations are clustered.

The mutational hotspot located in exon 7 also harbors the *WT1* SNP rs16754. This is a synonymous SNP consisting of a substitution of the nucleotide adenine with guanine at nucleotide position 1293 and is present in a homozygous ($WT1^{AA}$ or $WT1^{GG}$) or heterozygous state ($WT1^{AG}$).

Four main studies investigated the prognostic impact of SNP rs16754 in adult and pediatric AML. Damm et al. [18] identified the combined homozygous and the heterozygous *WT1* SNP rs16754 as an independent favorable prognostic marker in adult CN-AML. Subsequently, the Cancer and Leukemia Group B (CALGB) [25] reported that the homozygous form $WT1^{GG}$ is significantly associated with better DFS and OS. In pediatric AML, Ho et al. [20] observed a better outcome in favorable-risk AML harboring SNP rs16754. On the other hand, Hollink et al. [19] could not identify a prognostic relevance of SNP rs16754 in pediatric AML.

Fms-like tyrosine kinase (*FLT3*) is a type III receptor tyrosine kinase with important roles in the survival and proliferation of hematopoietic stem cell. Mutations in *FLT3* gene occur in 45 % of APL patients [26]. Both internal tandem duplication (ITD) of the juxtamembrane domain and point mutation D835 of the kinase domain (TKD) constitutively activate *FLT3*. *FLT3*-ITD mutation is specifically found in AML and is associated with high levels of *WT1* transcripts and inferior outcome in both pediatric and adult AML [27, 28].

To date, no studies have investigated in detail *WT1* gene variations in APL. Therefore, in addition to *WT1* expression level analysis, we carried out a comprehensive study of *WT1* mutations and polymorphisms in 103 newly diagnosed APL patients. Finally, we explored the association between mutations, polymorphisms, and *WT1* expression level.

Design and methods

Patient samples

Bone marrow samples from newly diagnosed adult APL patients were collected at diagnosis. Of the 103 patients included in the study, 77 patient samples were collected at Policlinico Tor Vergata, Rome and 26 patient samples were collected from La Fé Hospital, Valencia, Spain. Patients were selected as consecutive cases for which both RNA and DNA were available. APL diagnosis was confirmed in all cases at the genetic level by RT-PCR detection of the *PML-RARA* fusion gene. In all patients included in the study, infiltration of bone marrow by leukemic blasts was >70 %. Written informed consent for the study was obtained from all patients and the study was approved by the IRBs of both participating institutions.

Mutation analysis and SNP rs16754

Genomic DNA was extracted from 103 diagnostic bone marrow samples using Nucleospin blood kit (Macherey-Nagel GmbH & Co., Germany). Polymerase chain amplification of *WT1* exons 7 and 9 was performed on genomic DNA by using two primer pairs. The primers used for exon 7 were *WT1* ex7 forward: 5′-CTCCAGTGCTCACTCTCCCTC-3′ and *WT1* ex7 reverse: 5′- CCTTAGCAGTGTGAGAGCCTG-3′ while exon 9 primers were those reported by Gaidzik et al. [15]. SNP rs16754 and *WT1* mutations were confirmed by direct sequencing of the amplified and purified PCR product. Direct sequencing was carried out by using the Big Dye Terminator V3.1 cycle sequencing reaction (Applied Biosystems, Foster City, CA) and the reaction was analyzed on an ABI 3130 Genetic analyzer (Applied Biosystems, USA). Abnormal sequencing results were confirmed by at least two repeated sequencing analysis.

Molecular analysis of *FLT3* ITD and TKD

Molecular analysis of *FLT3* internal tandem duplication (ITD) in the juxtamembrane and D835 point mutation in the tyrosine kinase domain (TKD) were performed in available samples from 94 patients as described in Noguera et al. [26].

RNA extraction and cDNA synthesis

Total RNA was extracted from leukemic blasts using Trizol (Life technologies, Invitrogen, USA). Reverse transcription was performed on 1 μg total RNA as per standard protocol (Applied Biosystems, Foster City, CA). *PML-RARA* isoforms were amplified as reported by RT-PCR based method [29].

Expression analysis of *WT1* mRNA

Real-time quantitative polymerase chain reaction (RQ-PCR) assay was performed using patient cDNAs (available for 97 cases) on a 7900 real-time PCR system (Applied Biosystems) using the *WT1* ProfileQuant Kit as described in the manufacturer's instructions (Ipsogen, Marseille, France). For analysis of samples and controls, those with detectable *WT1* copy numbers were expressed per 10^4 *ABL* copies according to EAC criteria [30].

Statistical methods

Chi square test was used to compare the categorical variables between the groups. However, Fisher's exact test was used wherever chi square test was not appropriate. For *WT1* expression analysis, the APL samples were divided into different groups accordingly. To compare continuous variables according to different groups, nonparametric Mann–Whitney *U* test (comparing two groups) and Kruskal–Wallis test (comparing >2 groups) were used. Results with *p* values less than 0.05 were considered statistically significant. All statistical analysis was conducted using SPSS 16.

Results

WT1 copy number and patient presenting features

The distribution of *WT1* copy number was similar among patients classified as low/intermediate (n=81) or high risk (n=17) according to Sanz criteria [31]. The median expression of *WT1* copy number in relation to the breakpoint cluster region in the *PML* gene was also comparable among patients with BCR1 (n=48), BCR2 (n=6) and BCR3 (n=41; data not shown).

Mutational screening of *WT1*

WT1 mutations were identified in 4 (4 %) out of the 103 newly diagnosed APL patients (Table 1). All four mutations were heterozygous. The three mutations located in exon 7 were frameshift mutations and resulted in a premature stop codon at the protein level. The fourth mutation located in exon 9 was a missense mutation which resulted in replacement of a histidine residue at codon 397 by an arginine.

Table 1 Types of *WT1* mutation identified in four APL patients

UPN No.	Mutation	Exon	Protein effect
12	1581C>A	9	Missense mutation (H397R)
16	1317-1324 del 7 bp	7	Frameshift, premature stop
23	1295-1296 ins T	7	Frameshift, premature stop
79	1314-1319 ins 5 bp	7	Frameshift, premature stop

Mutations are reported according to Accession No. NM_024426

Analysis of SNP rs16754

The minor allele of the synonymous SNP rs16754 ($WT1^{AG/GG}$) was found in 30 (29 %) of the analyzed patients of whom 6 (20 %) were homozygous ($WT1^{GG}$). All the four patients who had *WT1* mutations were homozygous for major allele ($WT1^{AA}$). There was no significant difference in median age, WBC and platelet counts at presentation or *PML/RARA* fusion isoform between patients with and without SNP rs16754. Finally, analysis of SNP rs16754 according to ethnicity showed that all five analyzable patients of Asian descent included in the study were homozygous or heterozygous for the SNP rs16754 ($WT1^{AG/GG}$). Therefore, at variance with the remaining 99 patients of Caucasian origin, there was a prevalence of the G allele in the population of Asian ethnicity (data not shown).

FLT3 mutational status

Out of 94 cases evaluated for *FLT3*, 35 patients (37 %) had mutations. Of these, 25 patients carried an ITD, 9 cases a TKD, and one patient had concomitant *FLT3* ITD and TKD mutations.

Table 2 Expression of *WT1* in relation to SNP rs16754 and *WT1* mutations

Expression of *WT1* (n=97)	*WT1* median expression[a] (range)
Overall (97)	29590 copies (239.51–265694.88)
SNP rs16754	
AA (70)	27920 copies (1119.89–265694.88)
AG (22)	19119 copies (239.52–141458.14)
AG+GG (27)	31490 copies (239.51–163659.56)
GG (5)	78880 copies (37475.64–163659.56)*
WT1 mutation	
WT1 wild type (93)	31487 copies (239.51–265694.86)
Mutations (4)	12750 copies (3389.84–59285.41)

*p=0.018

[a] Number of copies *WT1*/10^4 ABL

Table 3 Expression of *WT1* in relation to *FLT3* mutation

FLT3 mutation	*WT1* copy number[a] median(range)
FLT3 wild type	16760 copies (239.51–265694.86)
FLT3 (ITD or TKD)	59290 copies* (2331.55–221827.86)
FLT3 (ITD)	69850 copies (2331.55–221827.86)
FLT3 (TKD)	57910 copies** (14190.51–163659.56)

*p=0.004; **p=0.019

[a] Number of copies *WT1*/10^4 ABL

Correlation between *WT1* mutations, *FLT3* mutations, SNP rs16754, and *WT1* expression levels

FLT3 ITD and TKD mutations were detected at comparable frequencies in patients with and without *WT1* SNP rs16754. *WT1* median copy number was significantly higher in patients with ($WT1^{GG}$) than in patients heterozygous for the minor allele ($WT1^{AG}$) or homozygous for major allele ($WT1^{AA}$). However, this difference disappeared combining patients homozygous and heterozygous for minor allele and comparing them with patients homozygous for major allele. We also observed a relatively lower median *WT1* copy number in the group of patients with *WT1* mutations compared to patients with wild-type *WT1* but the difference was not statistically significant (Table 2). Finally, we observed a significantly lower median copy number of *WT1* transcript in patients with wild type *FLT3* as compared to those harboring *FLT3* mutations (p=0.004, Table 3).

As concerning correlation with outcome, we were unable to carry out the analysis on the whole series due to heterogeneity of treatment context. However, a subanalysis of the relapse cohort revealed that out of seven patients undergoing disease recurrence, five carried *WT1* genetic variations (two in the form of mutations and three heterozygous for the rs16754 SNP) while four had *FLT3* mutations (three *FLT*3-ITD and one *FLT3*-TKD).

Discussion

To the best of our knowledge, the present study is the first comprehensive analysis of *WT1* gene variations in a large series of newly diagnosed APL patients. Three of the four *WT1* mutations were frameshift mutations located in the exon 7 "hotspot" region already described in AML [17] which result in a premature stop codon. As previously reported, mutations affecting *WT1* gene coding region may result in a protein incapable of proper binding to DNA in turn leading to transcriptional abnormalities [15]. All patients with mutations were homozygous for the major allele of SNP rs16754 consistent with data reported by Ho et al in pediatric AML [20].

At variance with previous reports on AML [32], of the four patients with *WT1* mutations in our study, two harbored mutation in *FLT3*-TKD region and none had *FLT3*-ITD mutation. Two of the four patients with *WT1* mutations relapsed shortly after remission including one of those who had *FLT3*-TKD mutation. This patient was considered as high risk according to Sanz risk score. For AML patients, it has been also reported that *WT1* expression is significantly higher in cases harboring mutations in this gene [20, 33, 34]. However in our cohort, patients harboring *WT1* mutations had a relatively lower *WT1* copy numbers (median, 12750 copies). We recognize however the limitation of these comparisons, which are based on only four cases mutated for *WT1*.

As to the *WT1* SNP rs16754, the frequency of homozygosity for the minor allele in our APL series appears higher than that reported in two studies conducted in adult AML (5.8 % vs. 1.6 % and 2.1 %; Damm et al. [18] and Hollink et al. [19]) but in line with results in pediatric AML [20]. Recently, synonymous SNP rs16754 has been correlated with higher expression levels of *WT1* in pediatric AML patients [20]. In keeping with these observations, we found a higher number of *WT1* transcripts in patients with the homozygous minor allele ($WT1^{GG}$) compared to other allelic variants.

In our cohort, both patients with *FLT3*-ITD or *FLT3*-TKD mutations had a high median *WT1* copy number. Two recent studies on AML patients have shown that high *WT1* expression was specifically correlated with presence of *FLT3*-ITD mutation [27, 35]. However, in our series, we observed a significant correlation between *WT1* expression and *FLT3*-TKD mutation.

We could not find any significant association between *WT1* status and patient presenting features. As concerning clinical outcome, the analysis was hampered by several factors including the very small number of relapses and treatment heterogeneity. In particular, distinct therapeutic options were given for post-induction in the three different regimens adopted by the Gruppo Italiano Malattie Ematologiche dell'Adulto and Programa para el Estudio y la Terapéutica en Hemopatía Maligna groups in the considered period. However, the available data hereby presented on relapsed patients may suggest that *WT1* genetic variations could have an impact on prognosis. Larger studies in homogeneously treated patients are needed to better clarify the prognostic role of *WT1* gene status in APL.

Acknowledgments Prof. F. Lo-Coco acknowledges the study support from the Associazione Italiana contro le Leucemie (AIL) and Associazione Italiana per la Ricerca sul Cancro (AIRC).

Disclosures The authors declare that there are no competing financial interests in relation to the work described in this paper.

References

1. Scharnhorst V, van der Eb AJ, Jochemsen AG (2001) WT1 proteins: functions in growth and differentiation. Gene 273:141–161
2. Yang L, Han Y, Suarez Saiz F, Minden MD (2007) A tumor suppressor and oncogene: the WT1 story. Leukemia 21:868–876
3. Riccardi VM, Sujansky E, Smith AC, Francke U (1978) Chromosomal imbalance in the Aniridia-Wilms' tumor association: 11p interstitial deletion. Pediatrics 61:604–610
4. Haber DA, Buckler AJ, Glaser T, Call KM, Pelletier J, Sohn RL, Douglass EC, Housman DE (1990) An internal deletion within an 11p13 zinc finger gene contributes to the development of Wilms' tumor. Cell 61:1257–1269
5. Oji Y, Miyoshi S, Maeda H, Hayashi S, Tamaki H, Nakatsuka S, Yao M, Takahashi E, Nakano Y, Hirabayashi H, Shintani Y, Oka Y, Tsuboi A, Hosen N, Asada M, Fujioka T, Murakami M, Kanato K, Motomura M, Kim EH, Kawakami M, Ikegame K, Ogawa H, Aozasa K, Kawase I, Sugiyama H (2002) Overexpression of the Wilms' tumor gene WT1 in *de novo* lung cancers. Int J Cancer 100:297–303
6. Koesters R, Linnebacher M, Coy JF, Germann A, Schwitalle Y, Findeisen P, von Knebel DM (2004) WT1 is a tumor-associated antigen in colon cancer that can be recognized by in vitro stimulated cytotoxic T cells. Int J Cancer 109:385–392
7. Thorner P, Squire J, Plavsic N, Jong R, Greenberg M, Zielenska M (1999) Expression of WT1 in pediatric small cell tumors: report of two cases with a possible mesothelial origin. Pediatr Dev Pathol 2:33–41
8. Inoue K, Sugiyama H, Ogawa H, Nakagawa M, Yamagami T, Miwa H, Kita K, Hiraoka A, Masaoka T, Nasu K, Kyo T, Dohy H, Nakauchi H, Ishidate T, Akiyama T, Kishimoto T (1994) WT1 as a new prognostic factor and a new marker for the detection of minimal residual disease in acute leukemia. Blood 84:3071–3079
9. Inoue K, Ogawa H, Sonoda Y, Kimura T, Sakabe H, Oka Y, Miyake S, Tamaki H, Oji Y, Yamagami T, Tatekawa T, Soma T, Kishimoto T, Sugiyama H (1997) Aberrant overexpression of the Wilms tumor gene (WT1) in human leukemia. Blood 89:1405–1412
10. Virappane P, Gale R, Hills R, Kakkas I, Summers K, Stevens J, Allen C, Green C, Quentmeier H, Drexler H, Burnett A, Linch D, Bonnet D, Lister TA, Fitzgibbon J (2008) Mutation of the Wilms' tumor 1 gene is a poor prognostic factor associated with chemotherapy resistance in normal karyotype acute myeloid leukemia: the United Kingdom Medical Research Council Adult Leukaemia Working Party. J Clin Oncol 26:5429–5435
11. Noronha SA, Farrar JE, Alonzo TA, Gerbing RB, Lacayo NJ, Dahl GV, Ravindranath Y, Arceci RJ, Loeb DM (2009) WT1 expression at diagnosis does not predict survival in pediatric AML: a report from the Children's Oncology Group. Pediatr Blood Cancer 53:1136–1139
12. Miwa H, Beran M, Saunders GF (1992) Expression of the Wilms' tumor gene (WT1) in human leukemias. Leukemia 6:405–409
13. Cilloni D, Gottardi E, De Micheli D, Serra A, Volpe G, Messa F, Rege-Cambrin G, Guerrasio A, Divona M, Lo Coco F, Saglio G (2002) 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 16:2115–2121
14. Cilloni D, Renneville A, Hermitte F, Hills RK, Daly S, Jovanovic JV, Gottardi E, Fava M, Schnittger S, Weiss T, Izzo B, Nomdedeu J, van der Heijden A, van der Reijden BA, Jansen JH, van der Velden VH, Ommen H, Preudhomme C, Saglio G, Grimwade D (2009) Real-time quantitative polymerase chain reaction detection of minimal residual disease by standardized WT1 assay to enhance risk stratification in acute myeloid leukemia: a European LeukemiaNet study. J Clin Oncol 27:5195–5201
15. Gaidzik VI, Schlenk RF, Moschny S, Becker A, Bullinger L, Corbacioglu A, Krauter J, Schlegelberger B, Ganser A, Döhner H, Döhner K, German-Austrian AML Study Group (2009) Prognostic impact of WT1 mutations in cytogenetically normal acute myeloid leukemia: a study of the German-Austrian AML Study Group. Blood 113:4505–4511
16. Hou HA, Huang TC, Lin LI, Liu CY, Chen CY, Chou WC, Tang JL, Tseng MH, Huang CF, Chiang YC, Lee FY, Liu MC, Yao M, Huang SY, Ko BS, Hsu SC, Wu SJ, Tsay W, Chen YC, Tien HF (2010) WT1 mutation in 470 adult patients with acute myeloid leukemia: stability during disease evolution and implication of its incorporation into a survival scoring system. Blood 115:5222–5231
17. Ho PA, Zeng R, Alonzo TA, Gerbing RB, Miller KL, Pollard JA, Stirewalt DL, Heerema NA, Raimondi SC, Hirsch B, Franklin JL, Lange B, Meshinchi S (2010) Prevalence and prognostic implications of WT1 mutations in pediatric acute myeloid leukemia (AML): a report from the Children's Oncology Group. Blood 116:702–710
18. Damm F, Heuser M, Morgan M, Yun H, Grosshennig A, Göhring G, Schlegelberger B, Döhner K, Ottmann O, Lübbert M, Heit W, Kanz L, Schlimok G, Raghavachar A, Fiedler W, Kirchner H, Döhner H, Heil G, Ganser A, Krauter J (2010) Single nucleotide polymorphism in the mutational hotspot of WT1 predicts a favorable outcome in patients with cytogenetically normal acute myeloid leukemia. J Clin Oncol 28:578–585
19. Hollink IH, van den Heuvel-Eibrink MM, Zimmermann M, Balgobind BV, Arentsen-Peters ST, Alders M, Willasch A, Kaspers GJ, Trka J, Baruchel A, Creutzig U, Pieters R, Reinhardt D, Zwaan CM (2010) No prognostic impact of the WT1 gene single nucleotide polymorphism rs16754 in pediatric acute myeloid leukemia. J Clin Oncol 28:e523–e526
20. Ho PA, Kuhn J, Gerbing RB, Pollard JA, Zeng R, Miller KL, Heerema NA, Raimondi SC, Hirsch BA, Franklin JL, Lange B, Gamis AS, Alonzo T, Meshinchi S (2011) WT1 synonymous single nucleotide polymorphism rs16754 correlates with higher mRNA expression and predicts significantly improved outcome in favorable-risk pediatric acute myeloid leukemia: a report from the children's oncology group. J Clin Oncol 29:704–711
21. Siehl JM, Reinwald M, Heufelder K, Menssen HD, Keilholz U, Thiel E (2004) Expression of Wilms' tumor gene 1 at different stages of acute myeloid leukemia and analysis of its major splice variants. Ann Hematol 83:745–750
22. Tamaki H, Ogawa H, Ohyashiki K, Ohyashiki JH, Iwama H, Inoue K, Soma T, Oka Y, Tatekawa T, Oji Y, Tsuboi A, Kim EH, Kawakami M, Fuchigami K, Tomonaga M, Toyama K, Aozasa K, Kishimoto T, Sugiyama H (1999) The Wilms' tumor gene WT1 is a good marker for diagnosis of disease progression of myelodysplastic syndromes. Leukemia 13:393–399
23. Miglino M, Colombo N, Pica G, Grasso R, Clavio M, Bergamaschi M, Ballerini F, Ghiso A, Ghiggi C, Mitscheunig L, Beltrami G, Cagnetta A, Vignolo L, Lucchetti MV, Aquino S, Pierri I, Sessarego M, Carella AM, Gobbi M (2011) WT1 overexpression at diagnosis may predict favorable outcome in patients with *de novo* non-M3 acute myeloid leukemia. Leuk Lymphoma 52:1961–1969
24. Paschka P, Marcucci G, Ruppert AS, Whitman SP, Mrózek K, Maharry K, Langer C, Baldus CD, Zhao W, Powell BL, Baer MR, Carroll AJ, Caligiuri MA, Kolitz JE, Larson RA, Bloomfield CD (2008) Wilms' tumor 1 gene mutations independently predict poor outcome in adults with cytogenetically normal acute myeloid leukemia: a cancer and leukemia group B study. J Clin Oncol 26:4595–4602
25. Becker H, Maharry K, Radmacher MD, Mrózek K, Metzeler KH, Whitman SP, Schwind S, Kohlschmidt J, Wu YZ, Powell BL, Carter TH, Kolitz JE, Wetzler M, Carroll AJ, Baer MR, Moore JO, Caligiuri MA, Larson RA, Marcucci G, Bloomfield CD (2011) Clinical outcome and gene- and microRNA-expression profiling according to the Wilms tumor 1 (WT1) single nucleotide polymorphism rs16754 in adult *de novo* cytogenetically normal acute

myeloid leukemia: a Cancer and Leukemia Group B study. Haematologica 96:1488–1495
26. Noguera NI, Breccia M, Divona M, Diverio D, Costa V, De Santis S, Avvisati G, Pinazzi MB, Petti MC, Mandelli F, Lo Coco F (2002) Alterations of the FLT3 gene in acute promyelocytic leukemia: association with diagnostic characteristics and analysis of clinical outcome in patients treated with the Italian AIDA protocol. Leukemia 16:2185–2189
27. Staffas A, Kanduri M, Hovland R, Rosenquist R, Ommen HB, Abrahamsson J, Forestier E, Jahnukainen K, Jónsson ÓG, Zeller B, Palle J, Lönnerholm G, Hasle H, Palmqvist L, Ehrencrona H, Nordic Society of Pediatric Hematology and Oncology (NOPHO) (2011) Presence of FLT3-ITD and high BAALC expression are independent prognostic markers in childhood acute myeloid leukemia. Blood 118:5905–5913
28. Brunet S, Labopin M, Esteve J, Cornelissen J, Socié G, Iori AP, Verdonck LF, Volin L, Gratwohl A, Sierra J, Mohty M, Rocha V (2012) Impact of FLT3 internal tandem duplication on the outcome of related and unrelated hematopoietic transplantation for adult acute myeloid leukemia in first remission: a retrospective analysis. J Clin Oncol 30:735–741
29. van Dongen JJ, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, Gottardi E, Rambaldi A, Dotti G, Griesinger F, Parreira A, Gameiro P, Diáz MG, Malec M, Langerak AW, San Miguel JF, Biondi A (1999) 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 13:1901–1928
30. Gabert J, Beillard E, van der Velden VH, Bi W, Grimwade D, Pallisgaard N, Barbany G, Cazzaniga G, Cayuela JM, Cavé H, Pane F, Aerts JL, De Micheli D, Thirion X, Pradel V, González M, Viehmann S, Malec M, Saglio G, van Dongen JJ (2003) 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 17:2318–2357
31. Sanz MA, Lo Coco F, Martín G, Avvisati G, Rayón C, Barbui T, Díaz-Mediavilla J, Fioritoni G, González JD, Liso V, Esteve J, Ferrara F, Bolufer P, Bernasconi C, Gonzalez M, Rodeghiero F, Colomer D, Petti MC, Ribera JM, Mandelli F (2000) Definition of relapse risk and role of nonanthracycline drugs for consolidation in patients with acute promyelocytic leukemia: a joint study of the PETHEMA and GIMEMA cooperative groups. Blood 96: 1247–1253
32. Summers K, Stevens J, Kakkas I, Smith M, Smith LL, Macdougall F, Cavenagh J, Bonnet D, Young BD, Lister TA, Fitzgibbon J (2007) Wilms' tumour 1 mutations are associated with FLT3-ITD and failure of standard induction chemotherapy in patients with normal karyotype AML. Leukemia 21:550–551
33. Willasch AM, Gruhn B, Coliva T, Kalinova M, Schneider G, Kreyenberg H, Steinbach D, Weber G, Hollink IH, Zwaan CM, Biondi A, van der Velden VH, Reinhardt D, Cazzaniga G, Bader P, Trka J, European Study Group on WT1 Expression in Childhood AML (2009) Standardization of WT1 mRNA quantitation for minimal residual disease monitoring in childhood AML and implications of WT1 gene mutations: a European multicenter study. Leukemia 23:1472–1479
34. Heesch S, Goekbuget N, Stroux A, Tanchez JO, Schlee C, Burmeister T, Schwartz S, Blau O, Keilholz U, Busse A, Hoelzer D, Thiel E, Hofmann WK, Baldus CD (2010) Prognostic implications of mutations and expression of the Wilms tumor 1 (WT1) gene in adult acute T-lymphoblastic leukemia. Haematologica 95:942–949
35. Spassov BV, Stoimenov AS, Balatzenko GN, Genova ML, Peichev DB, Konstantinov SM (2011) Wilms' tumor protein and FLT3-internal tandem duplication expression in patients with *de novo* acute myeloid leukemia. Hematology 16:37–42