


**QUANTITATIVE ASSESSMENT OF WT1 GENE EXPRESSION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IS A USEFUL TOOL FOR MONITORING MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA.**

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**INTRODUCTION.** WT1 overexpression is described in several oncological diseases including acute myeloid leukemias (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 HSCT. **METHODS and RESULTS.** The quantitative expression of WT1 was measured in 38 AML pts (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 pts (66%) were in complete cytologic remission (CcR) and 13 (34%) had refractory or relapsed AML. Bone marrow samples from pts transplanted in CcR showed at HSCT significantly lower WT1 expression levels compared to the samples from pts with a relapsed or refractory AML (P=0.004). After HSCT a rapid decline of WT1 expression levels was observed in all pts that attained or maintained a condition of CcR. Six out 38 pts (13%) relapsed after HSCT and all of them had an increase in WT1 expression at/or before relapse. Five of these six pts died with leukemia and one was successfully reinduced with 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 (NSLM) 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 GVHD may be candidate to discontinuation of immunosuppression and/or DLI therapy.

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**[The significance of quantification of MDR1 and WT1 gene expression in acute myeloid leukemia]**

[Article in Chinese]

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**OBJECTIVE:** To study the quantification of MDR1 and WT1 gene expression in patients with de novo acute myeloid leukemia (AML) and to explore the role of these two genes in clinical drug resistance and their correlation with risk stratification. **METHODS:** A real time quantitative reverse transcriptase polymerase chain reaction method was established for

detecting MDR1 and WT1 gene expression levels in 63 de novo AML patients. RESULTS: The expression of WT1 and MDR1 was significantly higher in de novo AML patients than in normal controls ( $P < 0.001$ ). WT1 levels were significantly correlated with corresponding levels of MDR1 gene in de novo AML patients ( $P = 0.004$ ). Expression levels of WT1 and MDR1 gene were not associated with FAB subtype and risk stratification ( $P > 0.05$ ). AML patients with FLT3-ITD mutations had a significantly higher WT1 expression level as compared to those without ( $P < 0.05$ ), on the contrary MDR1 expression was not associated with FLT3-ITD mutations ( $P > 0.05$ ). Patients with co-expression of high levels of WT1 and MDR1 had a significantly lower complete remission rate after induction therapy than those with low levels ( $P < 0.05$ ). CONCLUSION: There is a positive correlation between MDR1 gene expression and WT1 gene expression in AML. Quantification of the two gene expression together is more effective for judgement of prognosis in AML.

#### **Development of minimal residual disease-directed therapy in acute myeloid leukemia.**

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The last three decades have seen major advances in understanding the genetic basis of acute myeloid leukemia (AML). Comprehensive molecular and cytogenetic analysis can distinguish biologically and prognostically distinct disease subsets that demand differing treatment approaches. Definition of these pretreatment characteristics coupled with morphological response to induction chemotherapy provides the framework for current risk-stratification schemes, aimed at identifying subgroups most (and least) likely to benefit from allogeneic transplant. However, since such parameters lack the precision to distinguish the individual patient likely to be cured with conventional therapy from those destined to relapse, there has been considerable interest in development of multiparameter flow cytometry, identifying leukemia-associated aberrant phenotypes, and real-time quantitative polymerase chain reaction (RQ-PCR) detecting leukemia-specific targets (eg, fusion gene transcripts, NPM1 mutation) or genes overexpressed in AML (eg, WT1), to provide a more precise measure of disease response. Minimal residual disease (MRD) monitoring has been shown to be a powerful independent prognostic factor and is now routinely used to guide therapy in patients with the acute promyelocytic leukemia (APL) subtype. We consider the challenges involved in extending this concept, to develop a more tailored personalized medicine approach to improve the management and outcome of other forms of AML.

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treatment approaches. Definition of these pretreatment characteristics coupled with morphological response to induction chemotherapy provides the framework for current risk-stratification schemes, aimed at identifying subgroups most (and least) likely to benefit from allogeneic transplant. However, since such parameters lack the precision to distinguish the individual patient likely to be cured with conventional therapy from those destined to relapse, there has been considerable interest in development of multiparameter flow cytometry, identifying leukemia-associated aberrant phenotypes, and real-time quantitative polymerase chain reaction (RQ-PCR) detecting leukemia-specific targets (eg, fusion gene transcripts, NPM1 mutation) or genes overexpressed in AML (eg, WT1), to provide a more precise measure of disease response. Minimal residual disease (MRD) monitoring has been shown to be a powerful independent prognostic factor and is now routinely used to guide therapy in patients with the acute promyelocytic leukemia (APL) subtype. We consider the challenges involved in extending this concept, to develop a more tailored personalized medicine approach to improve the management and outcome of other forms of AML.

**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.**

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**PURPOSE:** To determine the clinical relevance of Wilms' tumor 1 (WT1) gene mutations in acute myeloid leukemia (AML) with normal karyotype (NK). **PATIENTS AND METHODS:** Exons 7 and 9 of WT1 were screened in samples from 470 young adult NK AMLs using a combination of direct sequencing and high-resolution capillary electrophoresis. **RESULTS:** Overall, 51 mutations were detected in 47 cases (10%): 46 frameshift mutations with insertion/deletion of one to 28 base pairs in exon 7 (n = 45) or exon 9 (n = 1), with a median mutant level of 45% (range, 8% to 86%), and five substitutions in exon 9: D396N (n = 3), H397Y (n = 1) and H397Q (n = 1). Patients with WT1 mutations had an inferior response to induction chemotherapy compared with wild-type cases (complete remission rate, 79% v 90%, odds ratio [OR] = 3.02; 95% CI, 1.17 to 7.82; P = .02), a higher rate of resistant disease (15% v 4%; OR = 9.33; 95% CI, 2.38 to 36.6; P = .001), an increased cumulative incidence of relapse (67% v 43%, hazard ratio [HR] = 3.02; 95% CI, 1.69 to 5.38; P = .0008), with a reduction in both relapse-free survival (22% v 44%; HR = 2.16; 95% CI, 1.32 to 3.55; P = .005) and overall survival (26% v 47%; HR = 1.91; 95% CI, 1.23 to 2.95; P = .007) at 5 years. In multivariate analysis, which included FLT3 internal tandem duplication and NPM1 mutation status, the presence of a WT1 mutation remained an independent adverse prognostic factor. **CONCLUSION:** WT1 mutations are a negative prognostic indicator in NK AML and may be suitable for the development of targeted therapy.

**Minimal residual disease detection in acute myeloid leukemia by mutant nucleophosmin (NPM1): comparison with WT1 gene expression.**

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**BACKGROUND:** Molecular analysis of minimal residual disease is only applicable in acute myeloblastic leukemia (AML) patients with genetic markers (20-30%). This study analyzes the feasibility of the real-time quantitative polymerase chain reaction (RQ-PCR) assay to detect mutant nucleophosmin (NPM1) during follow-up in AML patients. Moreover, we compare the NPM1 results with those of WT1 expression to MRD assessment. **METHODS:** The study includes 97 samples from 24 AML patients with type A NPM1 mutation at diagnosis. MRD was evaluated simultaneously by RQ-PCR assay to detect NPM1-mutated and WT1 expression. **RESULTS:** The expression levels of WT1 and NPM1 in 93 paired samples showed a strong positive correlation ( $r=0.81$ ;  $p<0.0001$ ). However, the kinetics of disappearance were different, WT1 decreased rapidly after induction but maintained these residual levels after treatment in patients in complete remission, whereas NPM1 experienced a mild reduction after induction but was undetectable in long survivor patients. **CONCLUSIONS:** This study shows the feasibility of the RQ-PCR assay to monitor MRD in AML patients carrying NPM1 mutations and its advantage over RQ-PCR assay for WT1. Owing to NPM1-mutated is specific of leukemic cells and shows higher levels at presentation.