This is the peer reviewd version of the followng article:

Concomitant monitoring of WT1 and FLT3-ITD expression in FLT3-ITD acute myeloid leukemia patients: which should we trust as a minimal residual disease marker? / De Marchi, F., Candoni, A., Zannier, M.E., Haley, L., Lau, B.W.Y., Fanin, R.. - In: AMERICAN JOURNAL OF HEMATOLOGY. - ISSN 0361-8609. - 92:5(2017), pp. E72-E74. [10.1002/ajh.24686]

*Terms of use:*

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

15/11/2026 21:17

(Article begins on next page)

**Title: Concomitant monitoring of *WT1* and *FLT3-ITD* expression in *FLT3-ITD* Acute Myeloid Leukemia patients: which should we trust as a minimal residual disease marker?**

**Authors:**

**De Marchi Federico[1,3], Candoni Anna[1], Zannier Maria Elena[1], Lau Wing Yin Bonnie[2], Haley Lisa[3], Fanin Renato[1].**

[1]Division of Hematology and BMT, Department of Experimental and Clinical Medical Sciences, Azienda Sanitaria Universitaria Integrata di Udine, Udine, Italy

[2]Department of Pediatric Hematology/Oncology, Johns Hopkins University School of Medicine, Johns Hopkins Hospital, Baltimore, MD 21287, USA.

[3]Department of Pathology, Johns Hopkins University School of Medicine, Johns Hopkins Hospital, Baltimore, MD 21287, USA.

**Address for correspondence:** Federico De Marchi MD, Department of Pathology, Johns Hopkins University School of Medicine, Johns Hopkins Hospital, Baltimore, MD 21287, USA. E-mail: federicodm86@gmail.com

**Correspondence**

To the Editor:

*FLT3-ITD* mutations (FMS-like tyrosine kinase 3) are the most common molecular alterations observed in Acute Myeloid Leukemia (AML). The presence of these mutations has an unfavorable prognostic significance and is the target of specific *FLT3-ITD* inhibitor drugs, currently in advanced stage clinical trials **[1,2]**. In recent years minimal residual disease (MRD) detection in AML has taken a very important prognostic role, becoming essential for the choice of the most appropriate consolidation strategy **[1-4]**. A recent study by Gaballa et al has shown how *FLT3-ITD*

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1002/ajh.24686

This article is protected by copyright. All rights reserved.

Accepted Article

molecular status at the time of transplant is a key predictor of disease relapse and survival in patients with *FLT3-ITD* AML **[3]**. Similarly, a study by our group has demonstrated the high predictive value of MRD assessment by the Wilms' tumor (*WT1*) gene expression at the time of stem cell transplant in this subset of patients **[4]**. However, to date, there are no studies comparing the effectiveness of *FLT3-ITD* and *WT1* detection on MRD monitoring in *FLT3-ITD* AML.

The reliability of the *FLT3-ITD* mutation as a MRD marker has not yet been clarified and the use of polymerase chain reaction (PCR) techniques for its detection do not seem to guarantee high sensitivity ("PCR bias") **[1,2]**. Therefore, in order to verify the usefulness of serial *FLT3-ITD* tests along the course of the disease, we compared its expression level with the quantitative analysis of *WT1,* a known pan leukemic marker available for MRD assessment in AML **[5]**.

We analyzed 24 consecutive *FLT3-ITD* positive AML patients (diagnosed at the University of Udine-Hematology Department), all with *WT1* overexpression at diagnosis. The bone marrow samples were collected at three specific time-points for each AML case: at diagnosis, post-induction, and finally either post consolidation therapy (in cases in cytological remission), post salvage therapy (in primary refractory cases) or at cytological relapse (in cases of relapse after a previous remission). Of note, none of these patients received therapy with *FLT3-ITD* inhibitors. The expression level of *FLT3-ITD* mutation was determined by PCR (Applied Biosystem, Foster City, CA), followed by capillary gel electrophoresis to determine allelic burden **[6]**. Concomitantly, at the same time points, we performed quantitative PCR for *WT1* gene expression, in accordance with the LeukemiaNet guidelines and the detection of *WT1* copies > 250/104 copies of the control gene Abelson (*Abl*) was defined as WT1 overexpression (Ipsogen *WT1* ProfileQuant Kit, Qiagen, EU) **[5]**.



This article is protected by copyright. All rights reserved.

Accepted Article

At diagnosis, the median bone marrow blast cells (BC) count was 90% (range 20-100%), with a 37% median *FLT3-ITD* allelic burden (range 3% -83%) and 9970 copies of WT1 (range 481-36423). As reported in **TABLE 1**, patients were divided into three groups according to the cytologic remission status of their AML after induction therapy: 1) patients with disease refractory (RES) to induction chemotherapy (AML-RES, 8 patients, median bone marrow BC after chemotherapy of 30%, range 15%-80%); 2) patients in cytologic Complete Remission (CR) post Induction therapy (AML-CR, 10 patients, BC<5%); 3) patients in relapse (REL) post CR (AML-REL, 6 patients, median bone marrow BC at relapse of 35%, range 20% -100%). We found that in those patients who achieved and maintained a cCR after induction (10 AML-CR, group 1), both *WT1* and *FLT3-ITD* became negative in a concordant manner: 8/10 cases (80%) were double negative after induction and 2/10 (20%) were double positive (concordance 100%); after consolidation 10/10 cases (100 %) were double negative. In cases with resistant AML (8 AML-RES, group 2) or at relapse (6 AML-REL, group 3) post chemotherapy, *WT1* was overexpressed in the totality of cases (14/14, 100%), while *FLT3-ITD* was positive only in 9/14 cases (64%), being negative in 5/14 (36%) cases, even in the presence of cytologically evident bone marrow disease. We have therefore observed a discrepancy between *WT1* and *FLT3-ITD* expression levels in relapsed (AML-REL) and disease-resistant (AML-RES) patients, with WT1 showing greater stability as a marker of MRD and a stronger concordance with cytology.

These data, within the limitations of a small series, suggests that *WT1* could be a reliable MRD marker and highlight the instability of *FLT3-ITD*, with possible switch from *FLT3-ITD* positivity at diagnosis to *FLT3-ITD* negativity at the time of cytological relapse or during post-chemotherapy resistance. This probably reflects the fact that the AML is constituted by leukemic subclones heterogeneous for *FLT3* expression and that a FLT3-ITD negative subclone may be responsible for the recurrence or resistance of AML **[1,2]**.



This article is protected by copyright. All rights reserved.

Conversely, overexpression of the pan leukemic marker *WT1* seems to be more stable along the course of the disease and more homogeneous in the different leukemic subclones, making this an appropriate and reliable marker for MRD detection; furthermore, overexpression of *WT1* appears to have a high degree of concordance with cytology (100% in this series).

In summary, these data suggest that *FLT3-ITD* is not, by itself, an optimal MRD marker and that the current available technique for its detection has lower sensitivity compared to the one in use for *WT1*. More sensitive molecular methods, such as new PCR based tests or next-generation sequencing (NGS) assays, could increase *FLT3-ITD* sensitivity and its use as MRD marker [**2,7**]. However, even with the current technique, 2 patients in our series were negative for *WT1* and positive for *FLT3-ITD* after induction and later relapsed. It might therefore be useful, until more efficient methods are routinely available, to test both *WT1* and *FLT3-ITD* markers at diagnosis and throughout the AML course to better evaluate the depth of remission and to guide MRD driven therapy in *FLT3-ITD* positive AML.

## References

1. Kayser S, Levis M. FLT3 tyrosine kinase inhibitors in acute myeloid leukemia: clinical implications and limitations. Leuk Lymphoma 2014;55:234-55.

2. Lewis M. FLT3 as a marker of minimal residual disease: Time to re-think ? Am J Hematol 2017; Epub ahead of print

3. Gaballa S, Saliba R, Oran B, et al. Relapse Risk and Survival in Patients with FLT3 Acute Myeloid Leukemia Undergoing Stem Cell Transplantation. Am J Hematol 2017; Epub ahead of print



This article is protected by copyright. All rights reserved.

Accepted Article

Accepted Article

4. Candoni A, De Marchi F, Zanini F, et al. Predictive value of pre transplant molecular minimal residual disease assessment by WT1 gene expression in FLT3 Acute myeloid leukemia. Exp Hematol J 2017; Epub ahead of print

5. Cilloni D, Renneville A, Hermitte F, et al. 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 2009;27:5195-201.

6. Noguera NI, Ammatuna E, Zangrilli D, et al. Simultaneous detection of NPM1 and FLT3-ITD mutations by capillary electrophoresis in acute myeloid leukemia. Leukemia 2005;19:1479-82.

7. Grunwald MR, Tseng LH, Lin MT, et al. Improved FLT3 internal tandem duplication PCR assay predicts outcome after allogeneic transplant for acute myeloid leukemia. Biol Blood Marrow Transplant 2014;**20**:1989-95.



This article is protected by copyright. All rights reserved.

**TABLE 1. Comparative analysis of FLT3 and WT1 according to AML remission status after induction chemotherapy.**

| GROUP 1 AML-cCR (10 CASES) | Diagnosis | Post Induction (in cCR) | Post Consolidation |
|---|---|---|---|
| **FLT3 POSITIVE** (allelic burden-%) | 10/10<br>48,5 (29-76) | 2/10 (20%)*<br>(3; 6) | 0/10 |
| **WT1 POSITIVE** (number of copies/10$^4$ Abl) | 10/10<br>11488 (4164-26393) | 2/10 (20%)*<br>(1154; 438) | 0/10 |
| **GROUP 2 AML-RES (8 CASES)** | **Diagnosis** | **Post Induction (RES)** | **Post Salvage CHT (RES)** |
| **FLT3 POSITIVE** (allelic burden-%) | 8/8<br>38 (3-83) | **6/8 (75%)**<br>30,5 (3,4-89) | **6/7° (85%)**<br>44,5 (16-98) |
| **WT1 POSITIVE** (number of copies/10$^4$ Abl) | 8/8<br>10733 (481-36423) | 8/8 (100%)<br>5266 (752-17047) | 7/7° (100%)<br>6081 (757-28371) |
| **GROUP 3 AML-REL (6 CASES)** | **Diagnosis** | **Post Induction (in cCR)** | **Relapse** |
| **FLT3 POSITIVE** (allelic burden-%) | 6/6<br>34 (4,7-39) | 2/6 (33%)**<br>(5; 10,5) | **3/6 (50%)**<br>(43; 37; 48,4) |
| **WT1 POSITIVE** (number of copies/10$^4$ Abl) | 6/6<br>7497 (1328-13719) | 2/6 (33%)**<br>(1451; 447) | 6/6 (100%)<br>11613 (651-21589) |

Accepted Article



This article is protected by copyright. All rights reserved.

Accepted Article

cCR, Cytologic Complete Remission; RES, Refractory; REL, relapse; CHT, chemotherapy; Abl, Abelson gene.

° 1 AML-RES patient died after CHT.

* MRD positive=**2/2** (with both FLT3-ITD positive and WT1 positive).

** MRD positive =**4/6** (2/6 only FLT3-ITD positive and 2/6 only WT1 positive); MRD negative=2/6 (with both FLT3-ITD negative and WT1 negative).



This article is protected by copyright. All rights reserved.