



A Prospective Evaluation of WT1 Gene Expression in Primary Acute Myeloid Leukemia: Bench to Bedside

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Abstract

CONTEXT: Acute myeloid leukaemia (AML) is a heterogenous disease with marked variation in response to therapy. New prognostic markers such as gene mutations including NPM1, FLT3, and WT1 have been identified.

OBJECTIVE: Evaluation of WT1 gene expression, mutations including AML-ETO, CBFB-MYH11, NPM1, FLT3-ITD and PML-RARA as biomarkers for therapy response in primary AML.

DESIGN: Prospective Clinical Research

SETTING: Study done at a tertiary cancer care center

PATIENTS OR OTHER PARTICIPANTS: A total of 114 cases of diagnosed AML, having blast percentage of $\geq 20\%$ blasts in peripheral blood or bone marrow on Day-0 and treated with induction therapy for 28 days following which morphological remission occurred (blast percentage $\leq 5\%$) were enrolled.

MATERIALS & METHODS: RNA was extracted from blood/bone marrow samples at diagnosis (Day-0) and on completion of induction therapy (Day-28). Haematological workup and flowcytometric immunophenotypes were done. WT1 expression was done using real-time qPCR, and was normalized against endogenous control genes: HBG2, GAPDH, $\beta 2$ microglobulin. Detection of mutations including AML-ETO, CBFB-MYH11, NPM1, FLT3-ITD and PML-RARA was done in 47 cases. Cytogenetics was done in 40 cases and FISH for PML-RARA in 9 cases.

RESULTS: WT1 expression was seen to be reduced at Day-28 in 96/114(84.2%) cases; whereas in 18/114(15.8%) cases, WT1 expression was seen to have increased at Day-28 as compared to Day-0 levels. Blast count percentage in these 18 cases varied from 28% to 80%. AML-ETO mutation was detected in 14/47(29.7%) cases, CBFB-MYH11 in 3/47(29.8%) cases, NPM1 mutations in 5/47(10.6%) cases and FLT3-ITD in 8/47(17%) cases. PML-RARA mutation was detected in 9 cases of APML [confirmed by cytogenetics and FISH in 6/9 cases].

CONCLUSIONS: WT1 gene expression shows significant reduction in more than 80% of cases showing morphological remission at the end of induction therapy. WT1 expression along with blast count percentage can act as good biomarker for assessment of remission. Molecular analysis in AML cases has diagnostic as well as prognostic significance. More prospective studies with larger sample size are needed to further validate these results.

Background

- AML is a heterogeneous disease with marked variation in response to therapy.
- New prognostic markers, such as gene mutations, including NPM1, FLT3, and WT1 are being identified.
- Wilm's tumor 1 gene (WT1) is located at chromosome 11p13, and it encodes transcriptional regulatory protein for genes involved in cellular growth and metabolism.
- Recurrent somatic mutations in WT1 appear to occur in approximately 6–15% of *de novo* AML.
- Therefore, WT1 gene mutation and its related significance in AML needs to be studied to emphasize its potential role as a marker of disease progression.

AIM: Evaluation of WT1 gene expression and mutations, including AML-ETO, CBFB-MYH11, NPM1, FLT3-ITD, and PML-RARA, as biomarkers for therapy response in primary AML.

Objective

Design: Prospective clinical research

Setting: Study done at a tertiary cancer care center

Patients: A total of 114 cases of diagnosed AML, having $\geq 20\%$ blasts in peripheral blood or bone marrow on day 0, and treated with induction therapy for 28 days, following which morphological remission occurred (blast %age $\leq 5\%$), were enrolled.

Materials & Methods

- RNA was extracted from blood/bone marrow samples at diagnosis (day 0) and on completion of induction therapy (day 28).
- Hematological workup and flowcytometric immunophenotypes were done.
- WT1 expression was done using real-time qPCR and was normalized against endogenous control genes: HBG2, GAPDH, and $\beta 2$ -microglobulin.
- Detection of mutations, including AML-ETO, CBFB-MYH11, NPM1, FLT3-ITD, and PML-RARA, was done in 47 cases.
- Cytogenetics was done in 40 cases, and FISH for PML-RARA in 9 cases.

Results

- WT1 expression: was seen to be reduced at day 28 in 96/114 (84.2%) cases, whereas in 18/114 (15.8%) cases, WT1 expression was seen to have increased at day 28 compared to day 0 levels.
- Blast count %age in these 18 cases: 28% to 80%.
- AML-ETO mutation: in 14/47 (29.7%) cases; CBFB-MYH11 in 3/47 (29.8%) cases; NPM1 mutations in 5/47 (10.6%) cases; FLT3-ITD in 8/47 (17%) cases.
- PML-RARA mutation: in 9 cases of APML (confirmed by cytogenetics and FISH in 6/9 cases).

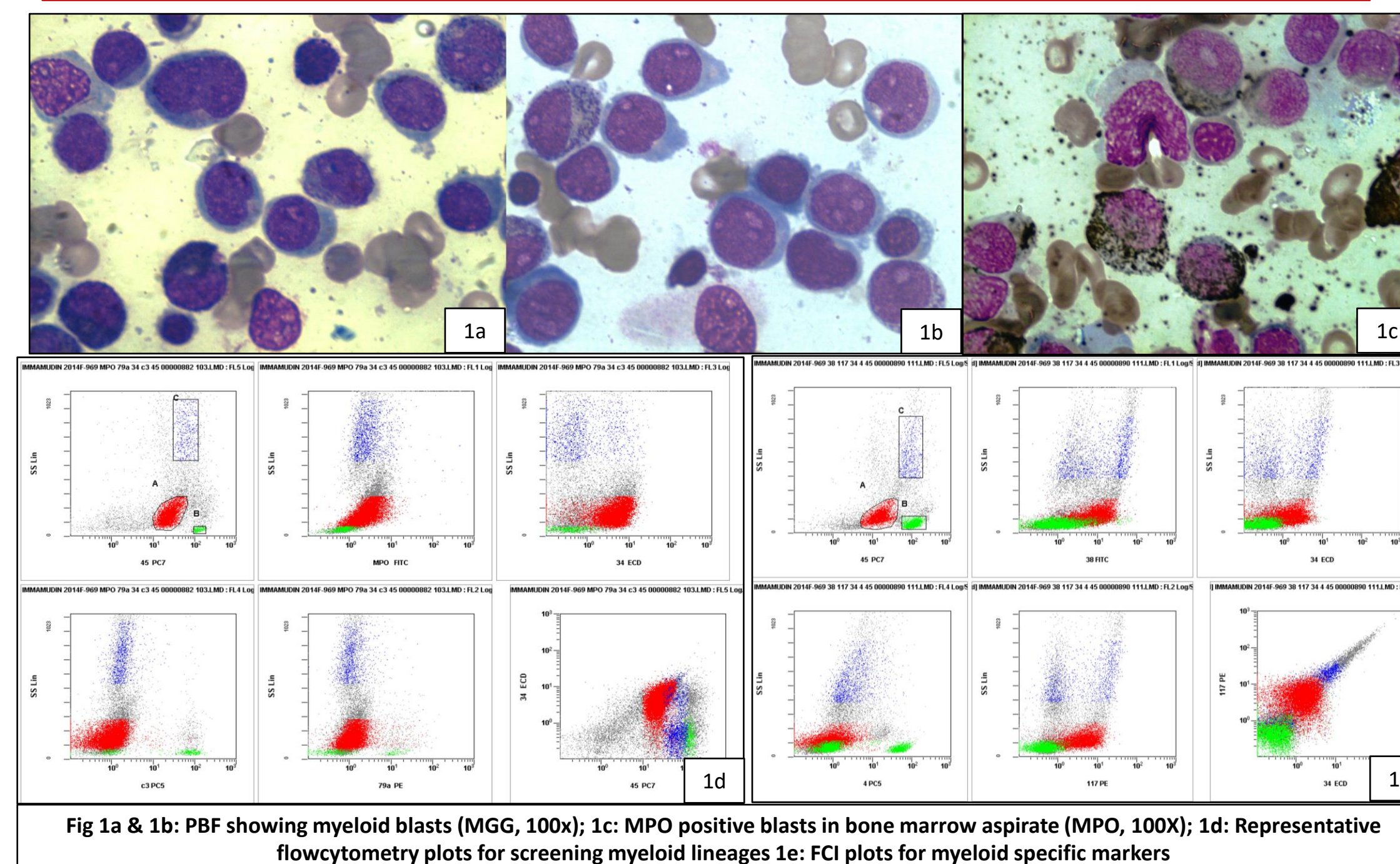


Fig 1a & 1b: PBF showing myeloid blasts (MGG, 100x); 1c: MPO positive blasts in bone marrow aspirate (MPO, 100X); 1d: Representative flowcytometry plots for screening myeloid lineages 1e: FCI plots for myeloid specific markers

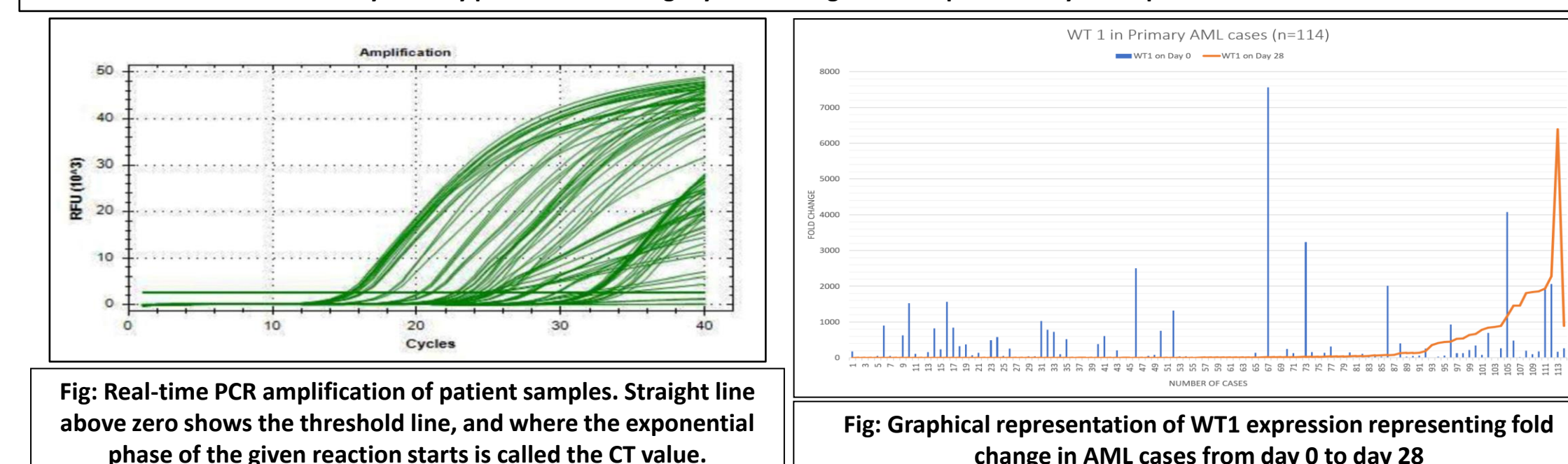


Fig: Real-time PCR amplification of patient samples. Straight line above zero shows the threshold line, and where the exponential phase of the given reaction starts is called the CT value.

Fig: Graphical representation of WT1 expression representing fold change in AML cases from day 0 to day 28

Discussion

- WT1 has a role in the epigenetic modifications in AML.
- WT1 mutations are associated with younger age, FLT3-ITD mutations & CEBPA mutations. Also anticorrelated with TET2 and IDH1/2 mutations.
- Increased levels of WT1 in AML are associated with resistance to therapy, a higher incidence of relapse, and poor overall survival.

Summary

- WT1 gene expression shows significant reduction in $>80\%$ of cases showing morphological remission at the end of induction therapy.
- WT1 expression along with blast count percentage can act as a good biomarker for assessment of remission.
- Molecular analysis in AML cases has diagnostic as well as prognostic significance.
- More prospective studies with larger sample sizes are needed to further validate these results.

References

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