RESEARCH ARTICLE

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Exploring the Prevalence and Prognostic Impact of Wilms Tumor *1 Exon 7* Mutation Status with Combinations of *FLT3-ITD* and *NPM1* Mutations as Potential Molecular Biomarkers in Acute Myeloid Leukemia Patients with Normal Cytogenetics

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Abstract

Aim: This study explores the prognostic impact of *FLT3-ITD, NPM1*, and *WT1* mutations both independently and in combination in Cytogenetically Normal Acute Myeloid Leukemia (CN-AML) patients as they exhibit varying clinical outcomes. **Methods:** 150 CN-AML patients were selected to assess the prevalence and prognostic significance of *WT1* mutations in combination with FLT3-ITD and NPM1 status using polymerase chain reaction (PCR) followed by Sanger sequencing. **Results:** *WT1 exon 7* mutations were present in 12.6% of patients. Elderly individuals, with a mean age of 49.4 years, were more prone to NPM1 mutations, though the association was not statistically significant (p=0.094). Significant associations were observed between lactate dehydrogenase (LDH) and hemoglobin (Hb) levels with *FLT3-ITD* and *NPM1* mutations (p=0.003 and p=0.04, respectively). The M4 subtype exhibited the highest prevalence of WT1 mutations (p=0.0036). Patients with NPM1 mutations had a higher overall survival rate compared to NPM1 wild-type cases (p=0.057). There was no significant correlation between overall survival and *WT1* and *FLT3-ITD* mutations. Regarding relapse-free survival, NPM1 mutation cases exhibited a higher survival probability compared to NPM1 wild-type cases. Similarly, *WT1* mutated cases had a higher survival probability compared to WT1 wild-type cases, although these differences were not statistically significant. The combined mutation statuses of *NPM1/FLT3-ITD* with *WT1* did not yield significant outcomes. The study suggests that larger cohort studies may reveal more relevant associations, given the relatively small cohort in this study. **Conclusion:** This study found a significant association between patient survival outcomes and *NPM1* mutation status, as well as the combined *FLT3-ITD* and *NPM1* status. Profiling both *NPM1* and *FLT3-ITD* mutations at the time of diagnosis serves as a robust prognostic marker in AML treatment. *WT1* mutation status did not show a significant association with patient outcomes. Larger population studies may provide more relevant insights.

Keywords: *CN AML- FLT3 ITD- NPM1-* Prognosis- *WT1* Mutation

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Introduction

Acute myeloid leukemia (AML) is the most prevalent and ineffably heterogeneous kind of leukemia in adults, accounting for around 80% of all cases [1]. The clonal proliferation of immature "blast cells" in the bone marrow and peripheral circulation leads to inefficient erythropoiesis and bone marrow failure [2]. Recent advances in management advice have increased cure rates for patients under 60 years old to over 40% and for patients over 60 years old to up to 15% [3]. The treatment outcome for the elderly population is still poor despite

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improvements in treatment protocols [4-6].

Cytogenetic screening is the first step in categorizing AML patients so that appropriate treatment options may be made and a baseline prognosis can be determined. Chromosomal aberrations are significant prognostic and recurrence risk factors that are found in 50–60% of AML patients. According to WHO recommendations, those without any discernible chromosomal aberrations were identified as having cytogenetically normal AML (CN AML) and placed in the intermediate-risk group [7]. CN AML has significant patient treatment outcome heterogeneity while being in the intermediate-risk group. Mutations have been discovered in cytogenetically normal (CN) instances, which account for 40–50% of all AML cases [8]. For accurately assessing the risk status of the CN AML cohort, molecular screening is a must. New genetic mutations related to acute myeloid leukemia have recently been found as a result of the advent of large sequencing technologies. *FLT3, NPM1, CEBPA, WT1, DNMT3A, TET2, IDH1, IDH2*, and *MLL* are frequently mutated genes that were found to interfere with the disease outcome of patients [9].

FLT3(FMS FMS-like tyrosine Kinase) is located in chromosome band 13q12 and is a class III receptor tyrosine kinase family member. Although a few studies have been conducted in the Indian community of CN AML patients. *FLT3 ITD* is one of the proven poor prognostic indicators in AML. Leucocytosis and a higher number of blast cells in the bone marrow and peripheral blood of AML patients are both substantially correlated with *FLT3 ITD* mutation. [10, 11]. Although the WHO defines FMS-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD) as a potent molecular genetic marker significantly impacting the clinical prognosis in patients with AML in some specific cytogenetic risk groups [12]. Many large cohorts have shown that FLT3-ITD is a poor predictive factor for both overall survival (OS) and relapse-free survival (RFS) [11, 13, 14].

NPM1(Nucleophosmin 1) is a protein-coding gene located in chromosome 5q35.1. *NPM1* contributes to the European Leukemia Net (ELN) categorization of CN-AML due to its high frequency of mutation and consistent prognostic impact [15]. Exon 12 *NPM1* mutations in the absence of *FLT3-ITD* mutation are associated with favorable prognostic outcomes [16-19].

WT1 (Wilm's Tumor 1) is a transcription regulator located in the chromosomal band 11p13. By promoting the transcription of genes involved in cellular development and metabolism, such as extracellular matrix elements, growth factors, and other transcription regulators, *WT1* encourages the inhibition of cell proliferation [20]. *WT1* mutations exist in 10-13% of AML patients and 20% of cases with phenotypic leukemia [21-24]. According to several studies, *WT1* mutation in patients with CN-AML was an unfavorable and independent risk factor for overall survival (OS) [25-27]. *WT1* mutations were also linked to the failure of conventional induction chemotherapy in the presence of additional mutations, such as *FLT3-ITD* [28].

Materials and Methods

Study design and Patient selection

The current study evaluated 312 cytopathologically verified adult de novo Acute Myeloid Leukemia (AML) patients registered at the Regional Cancer Centre (RCC), Thiruvananthapuram, India, with samples collected from February 2018 to March 2020. In accordance with the Ethical clearance from the Institutional Review Board Iof RCC and Declaration of Helsinki, all patients provided written informed consent for the use of their pre-treatment blood and bone marrow samples (fresh or cryopreserved) and medical records. Patients with acute promyelocytic leukemia (APML), secondary AML, those younger than 18 or older than 75, and individuals with severe comorbidities were excluded from the study.

A total of 150 patients bone marrow sample were identified as cytogenetically normal using conventional G-banding (GTG banding) and molecular cytogenetic techniques (Fluorescence In Situ Hybridization, FISH). These patients, with proper follow-up, were recruited for additional molecular analysis. Overall survival (OS) and relapse-free survival (RFS) were measured for a minimum of twenty-four months. Age, sex, French-American-British (FAB) subtypes, platelet count, bone marrow blast percentage, lactate dehydrogenase (LDH) level, hemoglobin (Hb), and white blood cell (WBC) counts were statistically assessed to determine their correlation with the cohort's *FLT3-ITD, NPM1*, and *WT1 exon 7* mutation profile statuses.

The current study aims to explore the prognostic impact of *WT1* mutation status and to correlate it with *FLT3-ITD* and *NPM1* mutations based on clinical and survival data.

Cytogenetic analysis

G-banded karyotype analysis was performed according to standard protocols. Using Genasis Software (Applied Spectral Imaging, Migdal Ha'Emek, Israel), karyotypes were analyzed according to the International System for Human Cytogenetic Nomenclature (ISCN 2020). According to ELN (European Leukemia Network) guidelines at least 20 metaphase cells from cultured bone marrow (BM) samples were evaluated. AML FISH panels for recurrent cytogenetic abnormalities (AML-ETO, PML-RARA, CBFβ-MYH11) were also performed to confirm that each CN AML patient was devoid of any recurrent cytogenetic abnormalities to ensure the accuracy of the results.

Molecular Profiling

Genomic DNA was extracted from bone marrow samples or peripheral blood samples of AML patients who were confirmed as CN by conventional as well as molecular cytogenetic profiling, using Quick DNA Miniprep Kit by ZYMO RESEARCH according to the manufacturer's protocols. The screening of *NPM1, WT1 Exon 7*, and *FLT3 ITD* mutations was performed by PCR and direct Sanger sequencing. The selection of the exon of this study was based on previous reports. *NPM1*, *FLT3 ITD*, and *WT1* were amplified from genomic DNA by

PCR using the Emerald Amp GT PCR Master Mix (2 X Premix) by Takara. Primers were designed and supplied by Geno Biosciences Pvt Ltd. The total reaction volume of 25µl contained approximately 5µl of 20 ng DNA,1µl of 10 pmol of each primer for *WT1*, *NPM1*, and *FLT3 ITD*, 12.5µl of master mix, and 5.5µl of nuclease-free water. DNA was amplified using the following PCR conditions: Cycling conditions were as follows: *FLT3 ITD*-1 cycle 9 min at 95°C,35 cycles at 94°C 30 sec,1 min at 60°C,2 min at 72°C,10 min at 72°C. *WT1*-1 cycle 5′ at 95°C, 35 cycles 1′ at 95°C, 1′ at 58.5°C, 1′ at 72°C, and 1 cycle 10′ at 72°C.*NPM1*-1 cycle 5 min at 95°C, 35 cycles 30" at 95°C, 1′ at 57°C, 1′ at 72°C, and 1 cycle 10'.The primers used were as follows *NPM1*-F-5'CCTGGACAACATTTATCA3'*NPM1*-Rev-5′-CCA CCC TGG GTG GTT AAAG-3′, Fw-, *FLT3 ITD*-F 5' CAA TTT AGG TAT GAA AGC CAGC, 3'FLT3-ITD-R-5'CTTTCAGCATTTTGACGGCAACC.3'Fw-*WT1*-Ex7-5'GACCTAC GTGAATGT TCACATG-3', Rev-*WT1*-Ex7-5'ACAACACCTGGATCAGAC CT-3'.

Clinical characteristics of the study population

Of the 150 patients, 80(53.3%) were males and 70(46.7%) were females. The FAB subtype M4 is identified with a higher frequency of incidence with 44(29.3%) patients and M0 with the lowest frequency of 1(0.7%). The baseline characteristics of the patients were given in the supplementary data Table 1.

Statistical Analysis

Data entry and baseline analysis were performed using the Microsoft Excel program and the statistical analysis was done with the package of social science software (SPSS) Version 28.0. In the baseline analysis, the student's t-test was done for Age, BMB, and Hb as they are normally distributed, and the Mann-Whitney U test for WBC, platelet, and LDH. Except for Hb, all other variables are nonnormal. Hence for Hb, an independent sample t-test was used and for the rest of the variables Mann-Whitney U test was used. Kaplan–Meier survival analysis was used to find out OS and RFS.

Results

Frequencies of FLT3, NPM1, and WT1 mutations

We identified at least one mutation among *WT1, FLT3-ITD*, or *NPM1* in 61.3% of the total cohort studied. 38 (25.3%) of the 150 pathologically confirmed de novo CN AML patients have *NPM1* mutations and *FLT3 -ITD* mutations were found in 35 cases (23.3%). *WT1 Exon 7* mutations were found in 19 (12.6 %) (Figure 1). Isolated mutation frequencies for *NPM1, FLT3 ITD*, and *WT1Exon 7* were 20 (52.6%), 17 (48.6%), and 6(31.6%) respectively. Eight patients (5.3%) tested positive for both *NPM1* and *WT1*, 13 cases (8.7%) tested positive for both *NPM1* and FLT3-ITD, and 8 (5.3%) cases identified mutated for FLT3-ITD/*WT1*. Three instances tested positive for each of the three genes. The frequencies for various combinations were given in detail in Table 1 and mutations identified in *WT1 Exon 7* were given in Figure 1.

Baseline characteristics According to FLT3-ITD, NPM1 and WT1 mutation status

With p-values of 0.39, 0.094, and 0.465, respectively, none of the results for patients' ages with *FLT3- ITD, NPM1*, and *WT1* mutational status are statistically correlated. The WBC, platelet count, and BMB % did not show any obvious relationships. We were able to determine that the LDH level of *FLT3-ITD* mutant cases is, on average, greater (1281.35 u/L) than that of the non-mutated cases (756.7 u/L) with a p-value of 0.003. Between the mean Hb level of 8.63 g/L in *NPM1*wt patients and 7.9 g/L in *NPM1* mutant patients, there is a significant connection with a p-value of 0.04. Patients with the French-American-British (FAB) M4 subtype of AML had the highest incidence (42.1%) of *WT1* mutation (P = .0036).

The baseline characteristics according to *FLT3-ITD*, *NPM1*, and *WT1* status were shown below in Table 2.

Statistical analysis of Mutation status with patients' clinical/laboratory characteristics

Following the most recent National Comprehensive Cancer Network (NCCN) recommendations, we categorized patients into separate groups based on various combinations of mutation statuses to assess the significance of the independent and combined impact of *WT1* with *FLT3 -ITD, NPM1* mutation status.

OS and RFS according to independent and combined NPM1, FLT3-ITD, and WT1 mutational statuses

According to a 24-month follow-up, the survival probability (SP) for *FLT3 ITD* wild-type patients is 44% with Standard Error (SE) 5, whereas it is 33.8% with SE 9.3 for mutant cases, with a p-value of 0.58 and a 95% Confidence Interval (CI). whereas survival probability for patients with *NPM1wt* is 37 percent with SE 5.1 according to a 24-month analysis, but it is 55.7% with SE 8.7 % for mutant instances (0.057). In *WT1*wt cases, the SP is 41.3 % with SE 4.7%, while it is 47.8 % with SE 12.4% for mutant cases (p-0.744). Kaplan–Meier survival Estimates for OS (24 months) of *FLT3-ITD*, *NPM1*, and *WT1* were

Table 1. Frequency of *FLT3-ITD, NPM1*, and *WT1* Mutations and Their Combinations

NPM1, FLT3-ITD &WT1 status	n =150 patients	Frequency (%)
FLT3-ITD+	35	23.3
NPM1 mut	38	25.3
WT1 mut	19	12.6
FLT3-ITD+/NPM1 wt/*WT1* mut	5	3.3
FLT3-ITD+/NPM1 wt/*WT1* wt	17	11.3
FLT3-ITD-/NPM1 mut/*WT1*mut	5	3.3
FLT3-ITD-/NPM1 mut/*WT1*wt	20	13.3
FLT3-ITD-/NPM1 wt/*WT1*mut	6	4.0
FLT3-ITD-/NPM1 wt/*WT1* wt	84	56.0
FLT3-ITD+/NPM1 +/WT1 +	3	2.0
FLT3-ITD+/NPM1 mut/*WT1* wt	10	6.6

+ or mut indicates the presence of mutation, wt-Wildtype, or without mutation

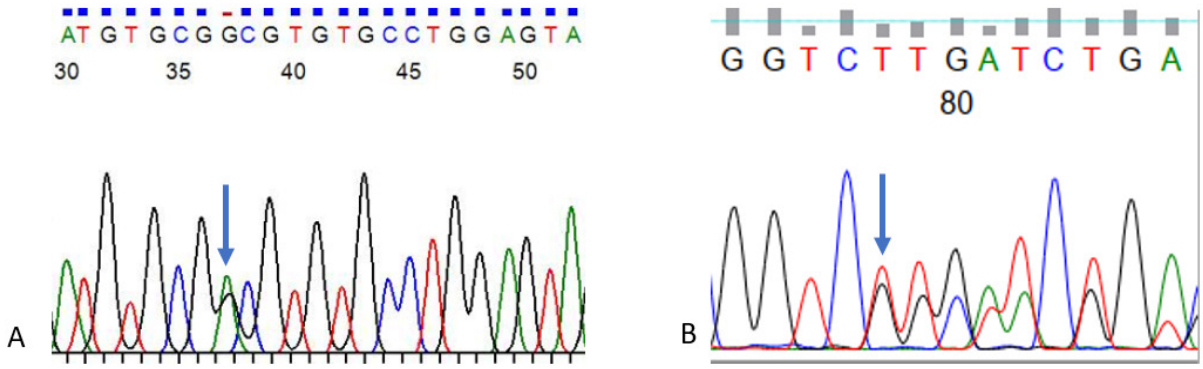

Figure 1. Mutations and Polymorphic Variants Identified in WT1 Exon 7, Polymorphic Variant(A)- c.1122A>G, CGA>CGG, p.Arg374Arg (rs17654),Mutant(B)- c.1148-1157dupTTGTACGGTC

shown in Figure 2.

Overall Survival

For *WT1* wt cases, the SP is 44.4% with a SE of 18.9%, while for WT1 mutant cases, the SP is 66.7% with a SE of 27.2% (p- 0.655) in the *FLT3-ITD+/NPM1* mut status.*WT1* wt exhibits a 2-year SP of 24.4% with SE 11.9 percent in *FLT3-ITD+/NPM1* wt status, while *WT1*mut exhibits SP of 26.7 % with SE 22.6% and a (p- 0.327). Within *FLT3-ITD-/NPM1* wt status, the *WT1*wt presented SP of 38.5% with SE 5.8% while the *WT1*mut had SP of 66.7% with SE 19.2 (p- 0.335). The *WT1*wt cases in the *FLT3-ITD/NPM1* mut category had an SP of 63.3% and a SE of 11.2 percent, while the *WT1* mut cases had an SP of 30 percent and a SE of 23.9% (p- 0.394).

Disease-Free survival

In *WT1*wt instances, the survival probability is 35.6% with SE of 18.6%, however, in *WT1* mutant cases, the survival probability is 100% (perhaps because there aren't many patients in that group) with a p-value of 0.139 in the *FLT3 ITD+/NPM1* mut category. In the *FLT3-ITD+/NPM1* wt category, *WT1* mutant cases present with an SP of 50% with SE 35.4% while the *WT1* wt cases show

Table 2. Baseline Characteristics According to Gene Mutations

Patient's characteristics	*FLT3-ITD-*	*FLT3-ITD+*	p value	*NPM1*wt	*NPM1* mut	p value	*WT1* wt	*WT1* mut	p value
Age									
number of patients	115	35		112	38		131	19	
Mean	45.62	47.91	0.386	45.06	49.37	0.094	46.47	44	0.465
STD Deviation	13.6	13.9		13.85	12.78		13.33	16.09	
BMB (%)									
number of patients	114	34		110	38		129	19	
Mean	63.65	67.44	0.404	63.29	68.08	0.27	64.93	61.74	0.576
STD Deviation	22.9	24.17		23.52	21.986		22.66	26.855	
WBC (X 10^9)									
number of patients	115	35		112	38		131	19	
Mean	42.25	60.96	0.062	42.57	58.53	0.16	44.7	59.76	0.387
STD Deviation	62.02	83.78		61.19	84.41		64.96	86.36	
Platelet(X10^9)									
number of patients	115	35		112	38		131	19	
Mean	67.32	64.62	0.6	67.54	64.21	0.35	69.99	43.96	0.139
STD Deviation	68.16	53.8		71.01	42.95		67.92	30.99	
Hb (g/L)									
number of patients	115	35		112	38		131	19	
Mean	8.41	8.56	0.682	8.63	7.9	0.04*	8.38	8.89	0.267
STD Deviation	1.99	1.58		1.98	1.55		1.95	1.44	
LDH(u/L)									
number of patients	109	34		105	38		105	38	
Mean	756.66	1281.35	0.003**	911.15	799.24	0.93	911.15	799.24	0.24
STD Deviation	833.27	1726.23		1232.79	772.02		1232.79	772.023	

*, significant association

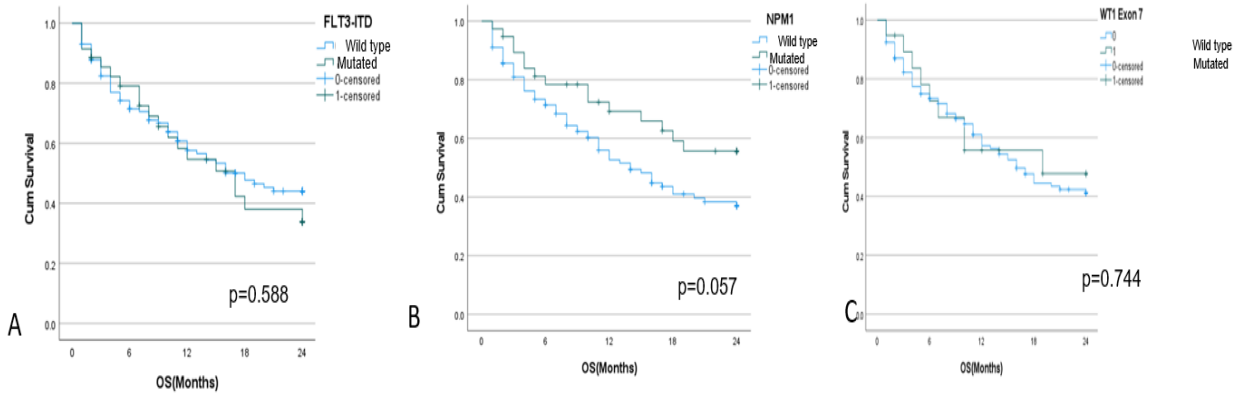

Figure 2. Kaplan–Meier Survival Estimates for OS (24 months) of FLT3-ITD(A), NPM1(B), and WT1(C).

an SP of 22.9% with SE 11.5% having a p-value of 0.67. Under the *FLT3-ITD-/NPM1-* category *WT1* mut instances exhibit SP of 50% with SE 35.4% whereas *WT1* wt showed 26.8% SP with 5.8% SE (p- 0.133). In the *FLT3-ITD-/NPM1+* instances, *WT1* wt cases presented SP of 45.5% having a SE of 11.7% while *WT1* mut cases showed SP of 25% with SE of 21.7% (p-0.780).

Relapse-Free Survival (RFS)

Out of the 150 patients, 35 have *FLT3 ITD* mutations and displayed SP of 35% with SE of 10%; the remaining Wild Type cases were observed with SP of 31.8 with SE of 5.1 % (p- 0.674). The *NPM1* wildtype presented with an SP of 27.3 percent with SE 5.2% (p-0.091), whereas the 38 *NPM1* mut instances exhibited an SP of 45.3 percent with SE 8.9 percent. *WT1* wt instances showed SP of 30.5% with SE 4.75 while the mutated with 49.4% SP and 16.8% SE (p-0. 067). Kaplan–Meier survival Estimates for RFS (24 months) of *FLT3-ITD*, *NPM1*, and *WT1* were shown in Figure 3. Within the *FLT3-ITD+/NPM1*mut category, *WT1* mut cases showed SP of 49.4%with SE of 16.8%, and *WT1*wt cases presented with an SP of 22.9% and 11.5% SE (p-0.675). In the *FLT3-ITD+/NPM1*wt category, the *WT1* mutated cases showed SP of 25% with SE 21.7% while the wildtype cases showed SP of 45.5% with SE 11.7% (p-value 0.780). In the *FLT3-ITD-/NPM1*-category the wild-type cases exhibited SP of 26.8% with SE of 5.8% and the mut cases showed SP of 50% and SE of 35.4% (p-0.133). Within the *FLT3-ITD-/NPM1+* instances, the *WT1* wildtype cases showed SP of 35.6% with SE of 18.6% and mut cases showed SP of 100 % (p- 0.139). p-values for OS and RFS for combined mutation statuses were shown in Table 3.

Subgroup (*WT1* mutation status with Combined mutation status of *ITD* and *NPM1*) analysis of patients' OS, DFS, and RFS were given in the supplementary data (from Supplementary Table 2, Supplementary Figure 1 to Supplementary Table 13, Supplementary Figure 12).

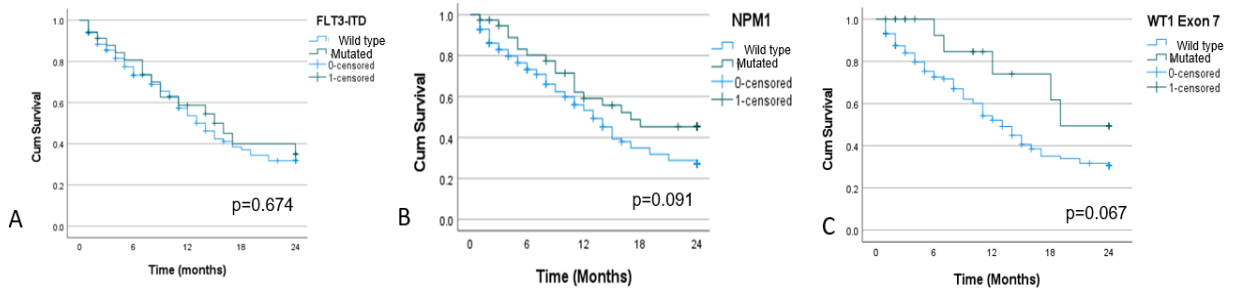

Figure 3. Kaplan–Meier Survival Estimates for RFS (24 months) of FLT3-ITD(A), NPM1(B), and WT1(C).

Table 3. OS, and RFS for Combined Mutation Status of *WT1*

Mutation status	Mean OS	P-value	Mean RFS	P-value
*FLT3-ITD+/NPM1*wt/*WT1* mut	5.8		5.2	
*FLT3-ITD+/NPM1*wt/*WT1* wt	11.6	0.65	10.6	0.78
*FLT3-ITD+/NPM1*mut/*WT1* mut	13.4		12.6	
*FLT3-ITD+/NPM1*mut/*WT1* wt	16.95	0.394	12.9	0.14
*FLT3-ITD+/NPM1*wt /*WT1* mut	15.17		11.8	
*FLT3-ITD+/NPM1*wt /*WT1* wt	12.2	0.335	10.2	0.13
FLT3-ITD+/NPM1 mut /*WT* mut	19.3		19.3	
FLT3-ITD+/NPM1 mut/*WT1* wt	20.2	0.327	12.4	0.67

Treatment response

Response of the patients was categorized into 4 groups, patients with Complete Remission (CR), Relapse, Persistent disease (PD), and induction death (ID-treatment-related mortality). After analyzing the survival, 32(21.3 %) of the patients achieved CR,71(47.3%) relapsed, and 30(20%) patients with persistent disease. Induction death occurred in 5(3.3%) patients.

Discussion

There have been only two studies [26, 27] on *WT1* done in AML patients in the Indian population to date. But no studies were conducted in India on CN AML patients specifically to analyze the prognostic implications of *WT1* mutation status as well as combined mutation status with *NPM1* and *FLT3 ITD*. Recent research [28]. from Pakistan found that the *WT1* gene was linked to the recruitment of three distinct germline mutations in *AML* patients. This study examined the incidence, prognostic significance, and interactions of *FLT3-ITD*, *WT1*, and *NPM1* statuses in adult patients with CN-AML. Our patients had a 23.3 % incidence of *FLT3-ITD*+, which was comparable to the 19.7 to 28 % reported by certain studies [29, 30, 16] but consistent with other research findings (30%, 31 %) [31, 32]. Patients with *ITD* mutation were treated with *FLT3* inhibitors such as first-generation FLT3 inhibitors, such as Midostaurin and Sorafenib, target multiple kinases and have shown efficacy in treating FLT3-mutated AML, though they come with broader side effects and issues of resistance [33]. Second-generation FLT3 inhibitors, including Gilteritinib, Quizartinib, and Crenolanib, are more selective and potent against FLT3 mutations, offering improved tolerability and effectiveness, particularly in relapsed or refractory cases. These newer inhibitors address some limitations of the first-generation drugs, providing essential advancements in personalized AML treatment [34]. Moreover, FLT3 inhibitors are considered as valuable tools in the fight against AML, particularly for patients with *FLT3* mutations.

Contrarily, our study's prevalence of *NPM1*mut (25.3%) was lower than that of the majority of previously published research, which showed a 45–64% incidence of *NPM1*mut [35]. De Jonge et al. [36] reported a frequency of *NPM1* mutation that was more in line with our findings (25%). *WT1* mutations were found in 12.6 % of the patients and which was higher than that of the majority of previously published research 6.7% [24], 8.2 % [28, 18, 23, 20],10 % [30] 10.7 % [16]. The difference in incidences may be due to ethnic variations. More studies on *WT1* mutation in CN AML patients are needed to find whether there is a higher incidence of *WT1* mutation in the Indian population. 13 (37%) of the 35 *FLT3-ITD*+ patients associated with *NPM1* mutations and 8 (22.9%) of the ITD+ cases were co-occurred with *WT1* mutations. Of the 38 *NPM1* mutant cases, 8 (21.1%) also had *WT1* mutations. Only three individuals identified with all three mutations together. Hence statistical analysis results were not given into consideration for this category. Statistical analysis revealed that there is a strong association between Hb levels and the *NPM1* mutation status of patients. The Hb levels were significantly lower among the *NPM1* mutated cases (p-value 0.04). In the *FLT3-ITD*+cases, the LDH levels were identified as relatively higher compared with wild-type cases (p-value -0.003). A higher proportion of WT1-mutated patients had M4 morphology in comparison with *WT1* wild-type patients (42.1%, p- 0.036) [22]. But there are no significant correlations were found among other baseline parameters such as BMB %, WBC count, age, sex, and platelet count with mutation status. Patients with *NPM1* mutation showed a higher *OS* when compared with *NPM1*wt (55.7% Vs 37%) with a p-value of 0.05* [24, 37]. The combined mutation status of *FLT3-ITD, NPM1*, and *WT1* does not deliver any significant association with OS and RFS. We were not able to correlate the independent mutation statuses of *FLT3-ITD, NPM1*, and *WT1* with RFS as well as the prognostic impact of *WT1* mutation status with *NPM1*/ FLT3-ITD combined status. At least one genetic mutation among W*T1, FLT3-ITD* or *NPM1* was discovered in 61.3% of the population investigated, revealing the heterogeneity of CN AML patients.

In conclusion, this study found a significant association between patient survival outcomes and *NPM1* mutation status, as well as the combined *FLT3-ITD* and *NPM1* status. Profiling both *NPM1* and *FLT3-ITD* mutations at the time of diagnosis serves as a robust prognostic marker in AML treatment. *WT1* mutation status did not show a significant association with patient outcomes. Larger population studies may yield more relevant insights.

Author Contribution Statement

All authors contributed equally in this study.

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