The Clinical Features and Prognosis of NPM1/FLT3-ITD/DNMT3A Triple-mutated Acute Myeloid Leukaemia

Xingchun Luo (✉ luoxch95@163.com)  
Lanzhou Medical College: Lanzhou University  https://orcid.org/0000-0002-4576-9011

Bei Liu  
Lanzhou University First Affiliated Hospital  https://orcid.org/0000-0003-4331-2138

Haiping Liang  
Lanzhou Medical College: Lanzhou University

Long Zhao  
Lanzhou University First Affiliated Hospital

Yuancheng Guo  
Lanzhou Medical College: Lanzhou University

Yue Feng  
Lanzhou Medical College: Lanzhou University

Yu Zhu  
Lanzhou Medical College: Lanzhou University

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Abstract

**Purpose:** Previous studies have shown that patients with NPM1+/FLT3-ITD+ acute myeloid leukaemia (AML) have a poor prognosis, especially those with a high FLT3-ITD allelic ratio. However, no studies have confirmed a clear prognostic impact of DNMT3A on NPM1+/FLT3-ITD+ AML patients.

**Methods:** Our study included a total of 165 patients with newly diagnosed non-acute promyelocytic leukaemia (non-APL) AML at The First Hospital of Lanzhou University between January 2018 and June 2021. Further bioinformatics analysis was performed using the Gene Expression Omnibus (GEO) database.

**Results:** We retrospectively studied 165 patients newly diagnosed non-APL AML and identified 11 (6.7%) patients with NPM1/FLT3-ITD/DNMT3A triple mutations. The patients with triple-mutated AML had advanced age, higher white blood cell (WBC) counts, de novo AML, normal karyotypes, and poor survival, and all were in the M4/M5 French-American-British (FAB) category. Notably, half of the patients with triple-mutated AML had mature monocyte characteristics that were difficult to distinguish from chronic myelomonocytic leukaemia (CMML). We validated the prognosis of patients with triple-mutated AML by further bioinformatics analysis and found that the GNG4 gene, one of the hub genes, was related to triple-mutated AML patients’ survival.

**Conclusion:** Our data demonstrate that DNMT3A gene mutation has adverse prognostic significance in NPM1+FLT3-ITD+comutation AML patients.

1. Introduction

AML is a heterogeneous malignancy, and the median overall survival (OS) is less than 1 year (Burd et al., 2020). In recent years, gene mutations have played an increasingly important role in AML disease diagnosis, prognostic risk stratification and treatment guidelines, with advances in next-generation sequencing technology (Tyner et al., 2018).

More than 90% of patients with AML carry gene mutations, and the higher-frequency mutations are in the NPM1, FLT3-ITD and DNMT3A genes, with incidence rates of approximately 20%. The majority of gene mutations do not occur alone, and coexisting gene mutation numbers range from 2 to 4. The higher the number of comutations, the worse the survival. When the number was more than 7, the 1-year OS rate was less than 30% (Papaemmanuil et al., 2016). For the same gene mutation, different allelic ratios (ARs) and allelic frequencies (AFs) might affect clinical outcomes (S. S. Patel et al., 2018). For example, for FLT3-ITD mutations, a high AR (≥0.5) indicates a worse prognosis (Eisfeld et al., 2020). It has also been shown that the high AF in FLT3-ITD, NPM1, DNMT3A gene mutations and the survival of patients with de novo AML were significantly shortened (Sasaki et al., 2020; Shafik et al., 2021). It is a widely held view that for the same gene mutation and coexisting different mutations, the prognosis is different. An example of this view is NPM1 mutation; the prognosis is better without FLT3-ITD mutation and worse with FLT3-ITD mutation (Herold et al., 2020). Some studies have shown that NPM1/FLT3-ITD/DNMT3A triple mutations are the three most common co-mutations, with a frequency of approximately 7% (Papaemmanuil et al., 2016; Ley et al., 2013). Patients with NPM1/FLT3-ITD/DNMT3A triple-mutated AML often have a worse prognosis (Loghavi et al., 2014).

Here, we report the landscape of gene mutations in 165 patients with AML and find different clinical characteristics of AML with different comutation patterns, especially NPM1/FLT3-ITD/DNMT3A triple-mutated
AML. Further bioinformatics analysis was performed using the GEO database to verify whether patients with triple-mutated AML have a poor prognosis and to explore the possible related mechanisms.

2. Material And Methods

2.1 Patients

Our study included a total of 165 patients with newly diagnosed non-APL AML (de novo or secondary) at The First Hospital of Lanzhou University between January 2018 and June 2021. All patients were diagnosed in combination with peripheral blood counts, bone marrow blasts, bone marrow cell morphology, cytochemical staining, immune cell phenotype by flow cytometry (FCM), karyotype cytogenetics, and molecular biology assessments and met the World Health Organization (WHO) diagnostic criteria. Clinical and survival data were obtained from the electronic medical records and telephone follow-ups. Our study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the First Hospital of Lanzhou University, and all patients or their families signed a written informed consent related to bone marrow aspiration or biopsy and chemotherapy.

2.2 Genetic analyses

Bone marrow cells were cultured for 24 h-48 h, and the karyotypes of 20 metaphase cells were analysed by the R-band technique. Gene mutations were identified by next-generation sequencing and polymerase chain reaction. Eleven genes recurrently mutated in AML patients, including NPM1, FLT3-ITD, DNMT3A, CEBPA double, ASXL1, RUNX1, TET2, IDH1, IDH2, c-KIT, and TP53.

2.3 Statistical analyses

OS was defined from first diagnosis until death or the follow-up cut-off date. Complete remission (CR) was defined as bone marrow blasts <5% without blasts with Auer bodies or the persistence of extramedullary infiltrative leukaemia. Partial remission (PR) was defined as bone marrow blasts less than 20% after treatment (and decreasing by more than 50% compared to pretreatment). Nonremission (NR) was defined as marrow blasts remained at more than 20% after treatment.

General clinical characteristics data, such as peripheral blood counts, bone marrow blasts and age, are expressed as the mean values, and the frequency of gene mutations, proportions of FAB categories and remission rate are expressed as percentages. Statistical analyses were performed using the statistical package SPSS 25. The landscape of gene mutations was presented as waterfall plots drawn using R packages (Version 4.0.3). The swimmer plots of the triple-mutated AML patients’ process of diagnosis and treatment were generated using Microsoft Office Excel software.

2.4 Bioinformatics analysis

We selected a larger sample size dataset in the GEO database: GSE146173, which contained clinical and survival data, especially gene mutations. We downloaded RNA sequencing data from 246 AML samples and found that
27 of them had NPM1+/FLT3-ITD+ co-mutations. Data processing and presentation were performed using R packages (Version 4.0.3).

3. Results

3.1 Patient characteristics

We retrospectively analysed the clinical and survival data of 165 patients with newly diagnosed AML and found that 138 patients (83.6%) had de novo AML and 27 patients (16.4%) had secondary AML and were mostly secondary to myelodysplastic syndrome and CMML.

The patients’ baseline characteristics are listed in Table 1. The demographic characteristics of the 165 patients with AML were as follows: 86 (52.1%) were male, 79 (47.9%) were female, and the average age was 52 years (range, 3 to 84). In the first peripheral blood count of patients in the hospital, the average haemoglobin was 75.8 g/L with a minimum of 27 g/L, the average WBC count was 36.4×10^9/L with a maximum of 392.5×10^9/L, and the average platelet count was 44.3×10^9/L with a minimum of 1×10^9/L. Bone marrow blasts ranged from 20%-98.0%, and the average percentage was 60.2%. The FAB category had high percentages of patients in M5, M4, M2, and only 1 patient each in M0, M1, and M6. The karyotype cytogenetics profile in 107 (64.9%) patients with AML suggested an intermediate prognosis, of which 71 patients had cytogenetically normal AML. Combining karyotype cytogenetics and gene mutations with the latest National Comprehensive Cancer Network (NCCN) guideline risk stratification by genetics, we identified 53 favourable patients (32.2%), 56 intermediate patients (33.9%), and 56 poor patients (33.9%), noting that patients whose karyotype was unknown with no gene mutation were categorized into the intermediate risk stratification group.

Patients received different therapies. Twenty-three (13.9%) of these 165 patients were discharged from the hospital with only supportive therapy because of the patients’ poor performance status, older age, and disadvantaged family economic conditions. The remaining 142 patients received 86 (52.1%) conventional chemotherapy alone, 11 (6.7%) hypomethylating agents (HMAs) alone, 26 (15.8%) conventional chemo- and HMAs, 16 (9.7%) venetoclax and HMAs, and 3 (1.8%) other therapies. After the first cycle of treatment (n=142), 72 (50.7%) patients achieved CR, 44.4% of who were minimal residual disease (MRD)-negative, 16 (11.3%) patients achieved PR, and 26 (18.3%) patients were evaluated for NR.

3.2 Mutation landscape

The gene mutation landscape of 165 patients with newly diagnosed AML is presented in Figure 1. Mutations in NPM1, FLT3-ITD and DNMT3A were the most frequent gene mutations. NPM1 mutation was observed in 37 (22%) patients, FLT3-ITD mutation was observed in 31 (19%) patients, and DNMT3A mutation was observed in 31 (19%) patients. Other gene mutations were identified in TET2 (19%), IDH2 (16%), ASXL1 (16%), CEBPA double (15%), RUNX1 (13%), IDH1 (9%), c-KIT (8%), TP53 (8%), CEBPA other (7%), and FLT3 other (7%). We explored the FLT3 AR value in 31 FLT3-ITD mutated AML cases and found 9/31 (29%) had high AR (≥0.5), 14/31 (45%) had low AR (<0.5), and AR was unknown in 8/31 (26%). DNMT3A-R882 mutation was detected in 55% of DNMT3A-mutated AML cases (17/31), most of which were identified as R882H (14/17 82%), followed by R882C (3/17 18%). The NPM1-W288 hotspot mutation region was observed in 89% (33/37) of NPM1-mutated AML cases. The number of coexisting gene mutations in each patient ranged from 0 to 4. The coexisting pattern gene mutations
of NPM1, FLT3-ITD and DNMT3A in 165 patients with AML are outlined in Table 2. The NPM1+/FLT3-ITD+ frequency was 11.5%, the NPM1+/DNMT3A+ frequency was 12%, and the FLT3-ITD+/DNMT3A+ frequency was 7%. The NPM1/FLT3-ITD/DNMT3A triple mutation frequency was 6.7%, which was unexpectedly high.

### 3.3 NPM1/FLT3-ITD/DNMT3A triple-mutated AML

Baseline characteristics, gene mutations and karyotypes of the 11 NPM1/FLT3-ITD/DNMT3A triple-mutated AML patients are summarized in Table 3. Of these patients, 72.7%(8/11) were female, and 27.3%(3/11) were male, and the average age was 56 years. All 11 triple-mutated AML were M4 18% (2/11) and M5 82% (9/11) FAB categories. The average WBC count was 82×10⁹/L, and 55% (6/11) of AML cases were over 50×10⁹/L. The clinical data of triple-mutated AML patient number 5 are shown in Figure 2. The characteristics of the 11 triple-mutated AML patients' treatment history and efficacy assessment are summarized in Figure 3. The follow-up cut-off date ended August 20, 2021. Only one patient, number 11, received supportive care. NPM1/FLT3-ITD/DNMT3A triple mutations showed a negative impact on the survival of AML patients, and the median OS was merely 4 months. Only the survival time of patient number 5 was over 6 months.

### 3.4. Bioinformatics analysis

To clarify the prognostic impact of DNMT3A on NPM1+/FLT3-ITD+ AML patients, we divided the 27 patients with NPM1+/FLT3-ITD+ AML into two groups by DNMT3A wild-type or mutated. The comparison of survival analysis between the two groups is shown in Figure 4A. Patients with NPM1/FLT3-ITD/DNMT3A triple-mutated AML had inferior OS. Then, the differentially expressed genes (DEGs) between the patients with NPM1+/FLT3-ITD+/DNMT3A+ AML and the patients with NPM1+/FLT3-ITD+/DNMT3A- AML were visualized in a heatmap (Figure 4D). A total of 480 DEGs were identified, including 196 upregulated genes and 284 downregulated genes. The results of the biofunctional analysis, which included Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, are shown in Figure 4B and Figure 4C. We found that the DEGs played important roles in 1) biological processes (BP): positive regulation of cell adhesion, cell–cell junction organization, granulocyte migration and granulocyte chemotaxis; 2) cell composition (CC): extrinsic component of membrane, collagen-containing extracellular matrix, and extrinsic component of plasma membrane; and 3) molecular function (MF): receptor ligand activity, signalling receptor activator activity, cytokine activity, and cytokine receptor binding. The main signalling pathways involved were cytokine–cytokine receptor interaction, osteoclast differentiation, and human T–cell leukaemia virus 1 infection. The protein–protein interaction (PPI) network was obtained from the STRING databases using Cytoscape software (Version 3.8.0) (Figure 4E). The numbers of adjacent nodes are shown in Figure 4F. We found that GNG4, one of the hub genes, had 13 adjacent nodes, but there was no statistically significant prognostic correlation between GNG4 gene expression and survival in NPM1+/FLT3-ITD+/DNMT3A+ AML patients. A possible explanation for this might be the limited sample size, which needs further research.

### 4. Discussion

In our study, we identified NPM1/FLT3-ITD/DNMT3A triple-mutated AML in 11 of 165 newly diagnosed AML patients. The frequency of NPM1/FLT3-ITD/DNMT3A triple mutations was 6.7%, a similar proportion of previous studies (Bezerra et al., 2020). Our study suggested that patients with triple-mutated AML had specific clinical
characteristics, including advanced age, high WBC counts, predominantly M4/M5 FAB category, de novo AML and normal karyotype, which were consistent with current research (Bezerra et al., 2020; Huang et al., 2019; Loghavi et al., 2014).

Interestingly, we found that the bone marrow morphology of five patients with NPM1/FLT3-ITD/DNMT3A triple-mutated AML (patients 5, 8, 9, 10, and 11) had similarity to CMML. The morphology of these patients’ bone marrow cells revealed that the monocyte system was abnormally proliferating, including mature monocytes and primitive and naive monocytes. The percentage of abnormal cell populations was between 65% and 85%, and a majority of them were mature monocytes, with less than 20% primitive and naive mononuclear cells. Therefore, the diagnosis of CMML was based on bone marrow morphology (Arber et al., 2016). However, the five patients’ immune cell phenotypes as determined by FCM supported the diagnosis of AML (subtype M5). Taking patient number 5 as an example, the immune cell phenotype suggested that abnormal cells accounted for approximately 80% of bone marrow nucleated cells, which mainly expressed HLA-DR, CD4, CD11b, CD13, CD14, CD15, CD33, CD38, CD56, CD58, and CD64 CD123, of which approximately 24.5% were CD64bri+CD14+ mature monocytes and approximately 54.5% were CD64bri+CD14-naive monocytes. The immune cell phenotypes of patients 8, 9, 10, and 11 were similar to that of patient number 5. Based on the above findings, we propose that some cases of NPM1/FLT3-ITD/DNMT3A triple-mutated AML exhibited specific monocyte features, which required the identification of CMML at the time of diagnosis. There is other study that differs from our view. Gu J et al. reported a patient with comutation in DNMT3A and FLT3-ITD genes, who had characteristics of monocytes and 8% blast percentage in bone marrow. However, the immune cell phenotype of this case showed immature abnormal monocytes. The researchers considered the diagnosis of this patient was CMML, because of the limited value of immune phenotyping analysis in CMML diagnosis (Gu et al., 2017).

Studies have shown that common gene mutations in CMML patients include ASXL1, SRSF2, TET2, NRAS, RUNX1, and JAK2. However NPM1, FLT3-ITD, DNMT3A gene mutations are rare in CMML (<10%), especially FLT3-ITD (B. J. Patel et al., 2017; Martinez-Verbo et al., 2021; Itzykson et al., 2013), in comparison of 20-30% in AML (Tyner et al., 2018). NPM1+CMML patients have aggressive clinical features, rapid progression to AML, and poor prognosis. A study suggested that NPM1+CMML patients may represent an early phase of patients with AML who have dysplastic features and mature monocytosis (Peng et al., 2016). These studies also pointed out that the NPM1 mutation was proposed as a secondary event in the conversion of CMML to AML (Courville et al., 2013; Lin and Falini, 2015). Some researchers have suggested that wild-type or mutated NPM1 may be a diagnostic basis for AML, irrespective of bone marrow blast percentage (Forghieri et al., 2020). The study by Xu J et al. showed DNMT3A (Arg882) drives CMML through changing gene expression and DNA methylation in hematopoietic cells (Xu et al., 2014). It has also been confirmed that DNMT3A is associated with the progression of CMML to AML (Jankowska et al., 2011), inferior overall and leukaemia-free survival (Patnaik et al., 2017; Kar et al., 2013). Given the rarity of mutation in FLT3-ITD gene in CMML, the prognostic value is unclear (Daver et al., 2013). Up to now, only one CMML patient with NPM1/FLT3/DNMT3A triple mutations has been reported, but it was FLT3-TKD mutation not FLT3-ITD (Falini et al., 2021). The findings from the studies listed above to some extent support the diagnosis of patients 5, 8, 9, 10, and 11 were AML not CMML.

All patients with NPM1/FLT3-ITD/DNMT3A triple-mutated AML had rapid disease progression, were prone to recurrence, and had a poor prognosis. Previous studies have shown that patients with NPM1+/FLT3-ITD+ AML have a poor prognosis, especially those with a high FLT3-ITD AR. However, no studies have confirmed a clear prognostic impact of DNMT3A on NPM1+/FLT3-ITD+ AML patients. According to research findings, the median
OS and disease-free survival (DFS) of patients with AML with NPM1/FLT3-ITD/DNMT3A triple mutations were shorter than those with FLT3-ITD mutated AML (Loghavi et al., 2014). Further analysis revealed that there appeared to be no significant difference in the CR rate between the triple and non-triple mutation groups, but the relapse rate was significantly higher in the triple mutation group, which may explain the poorer prognosis of patients with triple-mutated AML (Bezerra et al., 2020). Comparing treatment regimens, allogeneic haematopoietic stem cell transplantation (allo-HSCT) was found to improve OS and DFS in both groups, but in triple-mutated AML, the advantage of allo-HSCT was not significant, with a 1-year OS rate that was still <30% (Huang et al., 2019). It has been shown that NPM1, FLT3-ITD, and DNMT3A mutations together promote anthracycline resistance during AML treatment via impaired nucleosome remodelling (Guryanova et al., 2016). It has also been found that patients with NPM1/FLT3-ITD/DNMT3A triple-mutated AML had a higher frequency of leukaemic stem cells, a specific GPR56highCD34low immune cell phenotype, and synergistic upregulation of hepatic leukaemia factors (HLFs), and HLF is a key regulator of haematopoietic stem cells (Garg et al., 2019).

To clarify the reason for the poor prognosis of patients with NPM1/FLT3-ITD/DNMT3A triple-mutated AML and to further explore the relevant mechanisms, we used the GEO dataset GSE146173 to search for specific genes with poor prognosis. We identified the GNG4 gene, which plays an important role in triple-mutated AML patient prognosis. The full name of GNG4 is guanine nucleotide-binding protein γ subunit 4, and it is a member of the G-protein γ family. Some studies have found that the expression of GNG4 is associated with gallbladder cancer (Zhu et al., 2021), gastric cancer (Tanaka et al., 2021) and glioblastoma (Pal et al., 2016). However, it is not clear whether GNG4 is a potential oncogenic factor or a tumour suppressor. At present, there are no studies related to GNG4 in haematologic tumours. The prognostic impact of GNG4 on AML requires larger-sample studies as well as further experimental studies.

In conclusion, our study confirms that the high frequency of NPM1/FLT3-ITD/DNMT3A triple mutations (approximately 6.7%) and some triple-mutated cases of AML with mature monocyte characteristics are difficult to distinguish from CMML. Patients with triple-mutated AML have advanced age, higher WBC counts, de novo AML, and normal karyotype, and all are in the M4/M5 FAB category. Most importantly, they have shorter OS and a high relapse rate. By bioinformatics analysis, we found that poor prognosis may be associated with GNG4 gene expression, and further research might explore the related mechanisms between GNG4 and AML prognostic significance. However, the main weakness of this study is the paucity of the sample size of triple-mutated AML patients. We suggest further studies with large case series.

Declarations

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Conflict of interest:

The authors declare that they have no competing interests.

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**Tables**

Table 1

Patient characteristics
| Characteristic                      | Value            |
|-----------------------------------|------------------|
| Age, years                        |                  |
| Median                            | 52               |
| Range                             | 3-84             |
| Sex, n (%)                        |                  |
| Male                              | 86              | 52.1%          |
| Female                            | 79              | 47.9%          |
| Hemoglobin, g/L                   |                  |
| Median                            | 75.8            |
| Range                             | 27-153          |
| Platelet count, $\times 10^9$/L   |                  |
| Median                            | 44.3            |
| Range                             | 1-283           |
| WBC count, $\times 10^9$/L        |                  |
| Median                            | 36.4            |
| Range                             | 0.22-392.5      |
| Bone marrow blasts, %             |                  |
| Median                            | 60.2            |
| Range                             | 20-98.0         |
| Origin of disease, n (%)          |                  |
| de novo AML                       | 138 (83.6)      |
| s-AML                             | 27 (16.4)       |
| FAB category, n (%)               |                  |
| M0                                | 1 (0.6)         |
| M1                                | 1 (0.6)         |
| M2                                | 49 (29.7)       |
| M4                                | 51 (30.9)       |
| M5                                | 60 (36.4)       |
| M6                                | 1 (0.6)         |
| Undetermined                      | 2 (1.2)         |
| Karyotype, n (%)                  |                  |
| Risk stratification, n (%) | Favorable | Intermediate | Poor | Unknown |
|---------------------------|-----------|--------------|------|---------|
|                           | 53(32.2%) | 56(33.9%)    | 56(33.9%) | 23(13.9%) |

| Patients receiving different therapy, n (%) | Conventional chemotherapy alone | HMAs alone | Conventional chemo & HMAs | Venetoclax & HMAs | Other | No chemotherapy |
|--------------------------------------------|---------------------------------|------------|--------------------------|------------------|-------|-----------------|
|                                           | 86(52.1%)                        | 11(6.7%)   | 26(15.8%)                | 16(9.7%)         | 3(1.8%) | 23(13.9%)       |

| Response to chemotherapy (n=142) , n (%) | 1 cycle CR | 1 cycle PR | 1 cycle NR | Other |
|----------------------------------------|------------|------------|------------|-------|
|                                        | 72(50.7%)  | 16(11.3%)  | 26(18.3%)  | 28(19.7%) |

| MRD (1 cycle CR)(n=72) , n (%) | negative | positive | unknown |
|--------------------------------|----------|---------|---------|
|                                | 32(44.4%)| 12(16.7%)| 28(38.9%)|

**Abbreviations:** WBC white blood cell, s-AML secondary AML, FAB French-American British, HMAs hypomethylating agents, CR complete remission, PR partial remission, NR non-remission, MRD minimal residual disease

Table 2

Co-existing pattern of gene mutations of NPM1, FLT3-ITD and DNMT3A
| Group                | n (%)       |
|---------------------|-------------|
| NPM1+               | 37/165 (22%)|
| FLT3-ITD+           | 31/165 (19%)|
| DNMT3A+             | 31/165 (19%)|
| NPM1+/FLT3-ITD+     | 19/165 (11.5%)|
| NPM1+/DNMT3A+       | 20/165 (12%)|
| FLT3-ITD+/DNMT3A+   | 12/165 (7%)|
| NPM1+/FLT3-ITD+/DNMT3A+ | 11/165 (6.7%) |

**Table 3**

The NPM1/FLT3-ITD/DNMT3A triple-mutated AML patient characteristics

| Case No./Sex/Age | FAB  | WBC   | HB  | PLT  | BM  | Karyotype     | gene mutation                                   |
|------------------|------|-------|-----|------|-----|---------------|-------------------------------------------------|
| 1/Female/60      | M5   | 76.03 | 57  | 47   | 82  | 46,XX         | NPM1(W288),DNMT3A(R882H),FLT3-ITD(High),JAK2    |
| 2/Male/59        | M4   | 37.14 | 55  | 22   | 75.5| 46,XY         | NPM1(W288),DNMT3A(R882C),FLT3-ITD(Low),FLT3-TKD |
| 3/Female/39      | M5   | 86.12 | 55  | 120  | 74.5| 46,XX         | NPM1(W288),DNMT3A(R882H),FLT3-ITD(High)         |
| 4/Female/35      | M5   | 6.34  | 70  | 35   | 62.5| 46,XX         | NPM1(W288),DNMT3A(R882H),FLT3-ITD(High)         |
| 5/Female/49      | M5   | 36.03 | 114 | 122  | 54.5| Unknown       | NPM1(W288),DNMT3A(R882H),FLT3-ITD(Low),NRAS     |
| 6/Female/70      | M4   | 129.11| 94  | 23   | 80  | 46,X,-X,+mar  | NPM1(W288),DNMT3A(non-R882),FLT3-ITD(High),TET2 |
| 7/Male/45        | M5   | 254.86| 88  | 103  | 98  | 46,XY         | NPM1(W288),DNMT3A(non-R882),FLT3-ITD(High),CEBPA|
| 8/Female/72      | M5   | 40.14 | 121 | 55   | 73  | 46,XX         | NPM1(W288),DNMT3A(R882H),FLT3-ITD(High),ATM,TET2|
| 9/Female/73      | M5   | 67.45 | 84  | 96   | 65.5| 46,XX         | NPM1(W288),DNMT3A(R882H),FLT3-ITD(Low),LYST,TET2|
| 10/Male/41       | M5   | 34.98 | 71  | 93   | 60  | 46,XY         | NPM1(W288),DNMT3A(R882H),FLT3-ITD(Low),TET2     |
| 11/Female/77     | M5   | 134.5 | 84  | 44   | 80  | Unknown       | NPM1(W288),DNMT3A(R882H),FLT3-ITD(UNKNOWN)      |

**Abbreviations:** FAB French-American British, WBC white blood cell count \times 10^9/L, HB hemoglobin g/L, PLT platelet count \times 10^9/L, BM bone marrow blasts %, High: AR \geq 0.5, Low: \lt 0.5
Figures

Figure 1

Diagram of gene mutations landscape of 165 newly diagnosed non-APL AML patients. The age, gender, FAB category, risk stratification, gene mutation frequency and number, gene mutation status, co-existing gene mutation and co-mutation numbers in AML patients at the initial diagnosis are illustrated. Abbreviations: CEBPA double: biallelic CEBPA mutation; CEBPA other: no biallelic CEBPA mutations; FLT3 other: no ITD FLT3 mutations.
Figure 2

The clinical data of patient number 5 a Bone marrow morphology. b Immune cell phenotype by flow cytometry. c Extra medullary skin infiltration leukemia of lower limbs. d Lower limbs skin biopsy pathology suggested AML (sub-type M5).
Figure 3

Swimmer plots of patients with NPM1/FLT3-ITD/DNMT3A triple-mutations Abbreviations: HMAs: hypomethylating agents, CR: complete remission, PR: partial remission, NR: non-remission, ambiguous: bone marrow morphology is hypoplastic.
Figure 4

Bioinformatics analysis of GEO data: GSE146173 dataset a Kaplan-Meier survival curves for the patients from different groups. b Results of the GO analyses including barplot, bubble, and circus. c Results of the KEGG analyses including barplot, bubble, and circus. d The heat map of the differentially expressed genes (DEGs) between the NPM1+FLT3-ITD+DNMT3A+AML (red) patients and the NPM1+FLT3-ITD+DNMT3A-(blue) patients. e Protein-protein interaction of DEGs using STRING. f Number of adjacent nodes of the DEGs.