The effect of haploidentical hematopoietic stem cell transplantation on comutations based on next-generation sequencing in adult acute myeloid leukemia patients with the FLT3-ITD mutation

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Article

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Abstract

The objective of this study was to investigate which comutations based on next-generation sequencing (NGS) at diagnosis affect the clinical prognosis of de novo AML patients with FLT3-ITD mutations and the effect of haploidentical hematopoietic stem cell transplantation (haplo-HSCT) on the comutations. We analyzed 95 de novo AML patients with *FLT3*-ITD mutations from January 2018 to August 2021 based on the NGS 99-gene platform. Forty-one other types of molecular mutations were detected. The most common cooccurring mutations were NPM1 (n = 43, 45.3%) and DNMT3A (n = 21, 22.1%). NPM1 mutation status did not affect the clinical outcomes. AML patients with FLT3-ITD and DNMT3A comutations had significantly worse 3-year DFS (25.2% and 62.6%, P = 0.003) and OS rates (57.3% vs. 73.1%, P = 0.047) than those without DNMT3A mutations and the survival was significantly more favorable after haplo-HSCT than chemotherapy (3-year DFS,77.1% vs. 15.4%, P = 0.009; 3-year OS, 82.8% vs. 46%, *P* = 0.001, respectively). By multivariate analysis, DNMT3A mutation was a risk factor for DFS and OS, while haplo-HSCT was a protective factor. DNMT3A mutation might be a poor prognostic factor in adult AML patients with FLT3-ITD mutations and haplo-HSCT could overcome the poor prognostic of DNMT3A comutation.

Introduction

FMS-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) mutations are found in 25–30% of AML patients, predominantly in those with cytogenetically normal disease[1–4]. Numerous studies have confirmed that FLT3-ITD-mutated AML is strongly related to a shorter remission duration and poorer survival outcomes[5–7]. Allogeneic hematopoietic stem cell transplantation (allo-HSCT), especially haploidentical hematopoietic stem cell transplantation (haplo-HSCT) for those lacking matched donors, improves the survival of patients with FLT3-ITD mutations[8–10]. In the 2022 European LeukemiaNet (ELN), all AML cases with FLT3-ITD mutations are now categorized as intermediate risk irrespective of the FLT3-ITD allelic ratio (AR) or concurrent presence of NPM1 mutation, which is one of the most important changes made to the previous risk classification that included the 2017 ELN and 2022 National Comprehensive Cancer Network (NCCN)[11,12]. This suggests that widely accepted prognostic factors predicting the clinical outcome of newly diagnosed FLT3-ITD-positive AML are still lacking. Hence, the molecular characteristics of FLT3-ITD need to be studied, and the assessment of additional potential prognostic markers is urgent.

Next-generation sequencing (NGS) is widely used in the clinic to help diagnose AML and stratify prognosis and has the advantages of high throughput, high sensitivity, and high stability. Additionally, FLT3-ITD comutations might have important prognostic significance and can even lead to changes in treatment decisions. The most common FLT3-ITD comutation is the nucleophosmin member 1 gene mutation (NPM1 mut), which is no longer a factor in FLT3-ITD-mutated AML per the 2022 ELN but still helps to stratify the prognosis in the 2022 NCCN[11,12]. Additionally, whether other NGS comutation genes can add layers to FLT3-ITD is unclear. Previous studies reported that FLT3-ITD-positive patients with

DNMT3A may suffer from poor outcomes[13–15]. Whether the poor outcomes of FLT3-ITD comutations can be overcome by haplo-HSCT has not been reported.

Although there are many studies on FLT3-ITD-mutated AML patients, prior studies lack either specific data regarding the effect of haplo-HSCT on the NGS comutations or comparisons between haplo-HSCT and chemotherapy for AML patients with *FLT3*-ITD mutations[13, 16, 17]. Hence, in the present study, we focused specifically on the detailed molecular profiles of *FLT3* mutations based on NGS and the effect of haplo-HSCT on AML patients with *FLT3*-ITD mutations and their comutations.

Methods

Patients

This study analyzed 95 de novo AML patients (other than acute promyelocytic leukemia) with *FLT3*-ITD mutations who were diagnosed based on the NGS 99-gene platform and treated at the Peking University Institute of Hematology from January 2018 to August 2021. The clinical outcomes of patients who were subsequently treated with haplo-HSCT (n = 40) or chemotherapy (n = 55) were compared. AML was diagnosed according to the 2016 revision World Health Organization classification of myeloid neoplasms and acute leukemia[18]. Patients who died before induction were excluded. The last follow-up date was 30 April 2022. Informed consent was obtained from all patients. This study was performed in accordance with the Declaration of Helsinki and was approved by the ethics committee of Peking University People's Hospital.

Detection of FLT3-ITD mutations

The FLT3-ITD assay was performed using fluorescent polymerase chain reaction (PCR) with primers and amplification conditions as described previously[19]. The FLT3-ITD AR was calculated as the ratio of the area under the curve of mutant to wild-type alleles (FLT3-ITDmut/FLT3wt). NGS was used to detect comutations. In addition, three main mutants of the NPM1 gene, A, B and D, were detected by real-time fluorescence quantitative PCR[20].

Treatment

Induction therapy

According to the Chinese guidelines for the diagnosis and treatment of adult AML (not acute promyelocytic leukemia) (2021)[21], the induction regimens of patients fit for intensive therapy were as follows: IA, HAA, and CAG. One of these regimens could be chosen after discussion between the doctors and patients, with the patient's family financial situation and tolerance to the drugs also considered. The use of sorafenib during induction therapy was allowed but not mandatory because sorafenib was not recommended for *FLT3*-ITD AML in the Chinese guidelines[21]. The induction regimens of patients unfit for intensive therapy were venetoclax and azacitidine (VEN + AZA) with or without sorafenib as follows: oral

venetoclax 100 mg d1, 200 mg d2, 400 mg on days 3–28. If a strong CYP3A inhibitor was required after dose escalation, venetoclax was decreased from 400 mg to 100 mg; azacitidine 75 mg/m^2 was administered IV or subcutaneously on days 1 to 7; and sorafenib 400 mg Bid was or was not administered on days 1–28.

Consolidation therapy

For the ELN favorable-risk group, consolidation chemotherapy involved a total of 6–7 cycles, including four courses of intermediate-dose cytarabine (IDAC, 2 g/m2/q12 h for three days) and 2–3 courses of '3 + 7' regimens (three days of anthracycline and seven days of cytarabine). For the ELN intermediate-risk and adverse-risk groups, patients received 2–3 cycles of consolidation chemotherapy of intermediate-dose cytarabine or '3 + 7' regimens, and allo-HSCT was then performed.

Haplo-HSCT

Patients received a myeloablative conditioning regimen (MAC) without in vitro T-cell depletion. The conditioning therapy for haploidentical donors (HIDs) was modified to BUCY2 plus ATG (thymoglobulin)[22, 23]. All patients received G-CSF-mobilized, fresh, and unmanipulated G-CSF-primed bone marrow cells plus G-CSF-primed peripheral blood stem cells. Additionally, all patients received cyclosporine, mycophenolate mofetil, and short-term methotrexate for GVHD prophylaxis[22, 23]. Sorafenib maintenance (400 mg orally twice daily) was recommended after discussion between the doctors and patients, with the patient's family financial situation and tolerance to drugs also considered when hematopoietic recovery occurred within 60 days post-transplantation. Sorafenib was administered at 31–60 days posttransplantation and continued until at least day 180 and up to day 365. The dose modifications and use time of sorafenib were based on adverse events and tolerance. An alternative FLT3 inhibitor could also be started posttransplantation as maintenance after allo-HSCT.

NGS

NGS was performed in all 95 patients using a panel of 99 genes, which covered all the mutation hotspots of acute leukemia, myelodysplastic syndrome and myeloproliferative neoplasms. Bone marrow samples from 101 patients were collected. GDNA was extracted with the QIAsymphony SP nucleic acid extraction and purification analyzer (product of Kaijie company in Germany). An Illumina standard library was constructed. Ninety-nine genes related to myeloid tumors (Supplementary Table 1) were captured using the target sequence of a hematological malignancy customized probe (product of twist Bioscience, USA), and pe150 was sequenced on a Nova SEQ (Illumina, USA). The analysis included the determination of point mutation (SNV), insertion and deletion (indel), ITD and partial tandem duplication (PTD). The algorithm independently developed by Guangzhou Jinyu company is used to detect the variation in SNV, INDEL, ITD and PTD. The variation detection results were annotated with software such as ANNOVAR. To ensure the accuracy of variation, the original variation detection results were filtered: the average effective depth of each sample capture target area was ≥ 2000×. The read ratio mass and base mass values supporting the mutant were higher than 30 and had both positive and negative chain support.

Definition of clinical end points

Composite complete remission comprised complete remission, complete remission with incomplete platelet recovery, and complete remission with incomplete hematological recovery. Complete remission was defined as bone marrow blasts less than 5%, the absence of circulating blasts and blasts with Auer rods, the absence of extramedullary disease, an absolute neutrophil count of 1·0 × 10 /L or higher and a platelet count of 100 × 10 /L. Complete remission with incomplete platelet recovery met all criteria for complete remission except that for platelets (platelets < 100 × 10 /L), and complete remission with incomplete hematological recovery met all criteria for complete remission except that for residual neutropenia (absolute neutrophil count < 1·0 × 10 /L), thrombocytopenia (platelets < 100 × 10 /L) or both. Relapse was defined as more than 5% blasts in the BM, reappearance of blasts in the peripheral blood, or extramedullary leukemia in patients with previously documented CR. Overall survival (OS) was defined as the time from diagnosis to death or last follow-up. Disease-free survival (DFS) was defined as the period from the date CR to relapse or death, and patients who did not achieve CR were excluded.

Statistical analysis

Continuous variables are expressed as the mean ± standard deviation (SD), and categorical variables are expressed as percentages. The independent sample t test, chi-square test/Fisher's exact test and Mann–Whitney test were used for comparisons between groups. Competing risk analysis was used to calculate the cumulative rates of relapse, and Gray's test was used to test for the differences between groups. The DFS and OS rates were estimated using the Kaplan–Meier method and compared by the log-rank test. Cox regression was used for the analysis of prognosis. Factors for univariate analysis included age, white blood cell count at diagnosis, cytogenetic risk, comutations detected in more than three patients, sorafenib use, venetoclax use, and haplo-HSCT. Multivariate analysis was performed for risk factors with a $P < 0.1$ by univariate analysis. P values < 0.05 were considered statistically significant. Hazard ratios (HRs) were calculated with their 95% confidence intervals (CIs). The Statistical Package for the Social Sciences (SPSS), version 13.0 (SPSS, Inc., Chicago, IL, USA) was used for the data analysis.

Results

Clinical features

The baseline characteristics of the patients are listed in Table I. The median (range) follow-up for all survivors was 17.1 (7.3–58.8) months. The median (range) age of the patients was 44 (16–74) years, and 23.2% (22/95) were aged ≥ 55 years. The median (range) FLT3-ITD AR was 0.38 (0.01–2.9). According to the cytogenetic risk group, there were 10 patients (10.5%) with favorable-risk karyotypes, 77 (81.2%) with intermediate-risk karyotypes and 8 (8.4%) with high-risk cytogenetics. The most common induction therapy was IA (n = 49, 48.5%). The CR/CRi rate after two cycles of induction therapy was 84.2% (80/95). No difference in the CR rate was found between the different induction groups (P = 0.289). Sorafenib was added in 47.4% (45/95) of patients during the first induction treatment, and gilteritinib was

added in 6 patients with refractory/relapse disease. A total of 40 (42.1%) patients received haplo-HSCT, and 29 patients received sorafenib as maintenance treatment after haplo-HSCT, which accounted for 72.5% haplo-HSCTs.

Correlation of FLT3-ITD mutation with other molecular mutations

The details of NGS mutations are presented in Fig. 1. Forty-one types of other molecular mutations were detected in 86 (90.5%) patients, and 9 (9.5%) had no additional molecular mutations. The median (range) number of comutations was 2 (0–6). The most common cooccurring mutations were in the NPM1 (n = 43, 45.3%), DNMT3A (n = 21, 22.1%), NRAS (n = 13, 13.7%), IDH2 (n = 8, 8.4%), TET2 (n = 7, 7.4%), RUNX1 (n = 7, 7.4%), WT1 (n = 7, 7.4%), and RAD21 (n = 7, 7.4%) genes. Other familial mutations, such as biallelic mutations in the CEBPA (n = 4, 4.2%), IDH1 (n = 4, 4.2%), TP53 (n = 3, 3.2%), CSF3R (n = 3, 3.2%), ASXL1 (n = 2, 2.1%) and GATA2 (n = 2, 2.1%) genes, were also found.

Impact of haplo-HSCT on FLT-ITD

FLT3-ITD–positive AML patients who underwent allo-HSCT had significantly more favorable outcomes than those who received chemotherapy. The estimated DFS and OS were 78.6% vs. 48% (P = 0.02, Fig. 2A) and 82% vs. 61.1% (P = 0.04, Fig. 2B), respectively. To further analyze the effect of CR1 status on allo-HSCT, we compared the effect of the CR1 outcome on allo-HSCT with that of the non-CR1 outcome. FLT3-ITD–positive AML patients who underwent allo-HSCT with CR1 had significantly more favorable outcomes than those who received chemotherapy and undergo allo-HSCT with non-CR1. The estimated DFS and OS were 63.6% vs. 50.6% (P = 0.016, Fig. 2C) and 77.4% vs. 63.4% (P = 0.004, Fig. 2D), respectively. The DFS and OS were comparable between the *FLT3* AR low (< 0.5) group (n = 60) and the *FLT3* AR high (≥ 0.5) group (n = 35) according to a *FLT3* AR below or above the 2017ELN and 2022 NCCN guideline cutoff value of 0.5. The estimated DFS and OS were 59.7% vs. 48.9% (P = 0.439, Fig. 2E) and 77.7% vs. 72.4% (P = 0.485, Fig. 2F), respectively.

Impact of NPM1 mutation

The OS rates were 76.9% and 75% in the NPM1 mutation group and NPM1 wild-type group (P = 0.782, Fig. 3A), respectively, and the DFS rates were 45.9% and 82.6% in the NPM1 mutation group and NPM1 wild-type group (P = 0.014, Fig. 3B), respectively. In the NPM1 + FLT3-ITD + AML group, patients who underwent allo-HSCT had significantly more favorable outcomes than those who did not receive allo-HSCT. The estimated DFS and OS were 77.9% vs. 66.8% (P = 0.03, Fig. 3C) and 80% vs. 47.8% (P = 0.004, Fig. 3D), respectively. In the NPM1-FLT3-ITD + AML group, patients who underwent allo-HSCT had significantly more favorable outcomes than those who received chemotherapy. The estimated DFS and OS were **69.5%** vs. **57.1%** (P = 0.034, Fig. 3E) and 78.9% vs. 57.8**%** (P = 0.017, Fig. 3F), respectively.

Impact of other cooccurring mutations

The results showed that FLT3-ITD mutation cooccurring with DNMT3A mutation (n = 23) had a significantly worse DFS (24.2% and 56.5%, P = 0.006, Fig. 4A) and OS rate (57.3% vs. 73.1%, P = 0.047,

Fig. 4B) at 3 years than lack of the DNMT3A mutation (n = 78). Patients with FLT3-ITD mutation cooccurring with DNMT3A mutation who underwent haplo-HSCT (n = 9) had significantly more favorable outcomes than those who did not receive allo-HSCT (n = 14). The estimated DFS and OS were 77.1% vs. 15.4% (P = 0.007, Fig. 4C) and 66.7% vs. 46% (*P* = 0.05, Fig. 4D), respectively. Other common cooccurring mutations, such as NRAS, TET2, IDH2, WT1, and RUNX1, did not influence the outcomes of FLT3-ITD-mutated AML.

Prognostic factors

The multivariate analysis results are shown in Table 2. Age ≥ 55 years, DNMT3A mutation and haplo-HSCT were finally entered into the DFS and OS multivariate analysis. Cooccurring DNMT3A mutation had a significantly worse 3-year DFS (HR = 2.987, 95% CI = 1.329–6.7111, P = 0.008) and 3-year OS than patients without DNMT3A mutations (HR = 1.14, 95% CI = 0.468–2.776, P = 0.044), respectively. Patients with cooccurring DNMT3A mutation had a significantly better 3-year DFS (HR = 0.429, 95% CI = 0.184–1.004, P = 0.05) and 3-year OS(HR = 0.109, 95% CI = 0.026–0.465, P = 0.003) after haplo-HSCT, respectively. Thus, cooccurring DNMT3A mutation had a significantly inferior survival outcome, and haplo-HSCT might overcome the inferior outcome of cooccurring DNMT3A mutation in FLT3-ITD-mutated AML.

Discussion

To our knowledge, this is the first study to analyze the effect of haplo-HSCT on cooccurring mutations at diagnosis based on NGS with a detailed subgroup analysis and to compare haplo-HSCT and chemotherapy for adult AML patients with FLT3-ITD mutations. Our analysis demonstrated that the most common cooccurring mutations were NPM1 and DNMT3A. NPM1 mutation status did not affect the clinical outcomes of adult AML patients with FLT3-ITD mutation. DNMT3A mutation might be a poor prognostic factor in adult AML patients with *FLT3*-ITD mutations, and haplo-HSCT might overcome the adverse influence of DNMT3A mutation.

Genetic mutations cooccurring with *FLT3*-ITD mutations have been evaluated in recent years as pretreatment parameters[13]. This study analyzed gene expression in 95 de novo AML patients diagnosed by NGS and showed that 9 (9.5%) had no additional molecular mutations. The most common cooccurring mutations were found successively, and it was discovered that most of the comutations clustered in methylation-related genes, chromatin-modifying genes and transcription factor genes. *NPM1* and *DNMT3A* were more frequently identified in this study, while mutations in *TP53*, CSF3R, ASXL1 and GATA2 were rare. The distribution was similar to other reported genomic and epigenetic landscapes of adult de novo AML patients[13, 24]. Genes involved in DNA methylation (such as TET2 and DNMT3A) were frequently mutated in *FLT3*-ITD-mutated AML, but the relapse/event-free survival of patients with TET2 comutations was not significantly different from that of patients with wild-type AML.

In this study, the outcomes of *FLT3*-ITD patients, irrespective of *NPM1* mutation status and FLT3-ITD AR, who underwent haplo-HSCT were significantly more favorable than those of patients who received only

chemotherapy. NPM1 mutation status and FLT3-ITD AR did not affect the clinical outcomes of adult AML patients with FLT3-ITD mutation, which is one of the most important changes in 2022 ELN and different from 2022NCCN and 2017ELN[11]. The reason for this relates to methodological issues with standardizing the assay to measure the FLT3-ITD AR, the modifying impact of FLT3 inhibitor-based therapy on FLT3-ITD without NPM1 mutation, and the increasing role of MRD in treatment decisions[25]. It should be noted that the OS and DFS after haplo-HSCT in the present study, which were up to approximately 80%, are further improved compared with previous studies. The high rate (72.5%) of maintenance treatment of sorafenib after transplantation might further contribute to the improvement of survival after transplantation, which was confirmed in previous studies[26]. In addition, we further found that DNMT3A comutations in FLT3-ITD mutation AML conferred a worse outcome, which is in accordance with previous studies[14, 15]. Although Tang et al reported that FLT3-ITD with DNMT3A mutation is a poor prognostic factor in Chinese patients with AML after allo-HSCT, the effect of haplo-HSCT on FLT3-ITD with DNMT3A mutation was not analyzed separately[15]. In this study, AML patients with FLT3-ITD mutations and cooccurring DNMT3A mutations achieved a superior 3-year DFS and OS after haplo-HSCT. These results suggested that adult AML patients with FLT3-ITD mutations and DNMT3A mutations might benefit from haplo-HSCT.

This study had several limitations. First, it was a single-center, retrospective study. Second, it must be stated that the patients were not randomized to receive chemotherapy or haplo-HSCT. Third, the shortest follow-up duration was 7.3 months for surviving patients, which included 12 patients with a follow-up duration of less than 10 months (which was the median time of relapse). Finally, there was a significant difference in age between the two groups: the transplant cohort was younger than the nontransplant cohort.

In conclusion, this study first analyzed the effect of haplo-HSCT on other cooccurring mutations at diagnosis based on NGS. Our analysis demonstrated that the most common cooccurring mutations are NPM1 and DNMT3A. DNMT3A mutation might be a poor prognostic factor in adult AML patients with *FLT3*-ITD mutations. Haplo-HSCT might overcome the adverse influence of DNMT3A mutation. Further well-designed, prospective, randomized trials are needed to confirm this conclusion.

Declarations

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Puissant A, Fenouille N, Alexe G, Pikman Y, Bassil CF, Mehta S *et al.* SYK is a critical regulator of FLT3 in acute myeloid leukemia. *Cancer Cell* 2014; **25**(2): 226–242. e-pub ahead of print 2014/02/15; doi: 10.1016/j.ccr.2014.01.022
2. Yanada M, Matsuo K, Suzuki T, Kiyoi H, Naoe T. Prognostic significance of FLT3 internal tandem duplication and tyrosine kinase domain mutations for acute myeloid leukemia: a meta-analysis. Leukemia 2005; **19**(8): 1345–1349. e-pub ahead of print 2005/06/17; doi: 10.1038/sj.leu.2403838
3. Frohling S, Schlenk RF, Breitruck J, Benner A, Kreitmeier S, Tobis K *et al.* Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. *Blood* 2002; **100**(13): 4372–4380. e-pub ahead of print 2002/10/24; doi: 10.1182/blood-2002-05-1440
4. Marcucci G, Haferlach T, Dohner H. Molecular genetics of adult acute myeloid leukemia: prognostic and therapeutic implications. J Clin Oncol 2011; **29**(5): 475–486. e-pub ahead of print 2011/01/12; doi: 10.1200/JCO.2010.30.2554
5. Tao S, Wang C, Chen Y, Deng Y, Song L, Shi Y *et al.* Prognosis and outcome of patients with acute myeloid leukemia based on FLT3-ITD mutation with or without additional abnormal cytogenetics. Oncol Lett 2019; **18**(6): 6766–6774. e-pub ahead of print 2019/12/07; doi: 10.3892/ol.2019.11051
6. Daver N, Schlenk RF, Russell NH, Levis MJ. Targeting FLT3 mutations in AML: review of current knowledge and evidence. Leukemia 2019; **33**(2): 299–312. e-pub ahead of print 2019/01/18; doi: 10.1038/s41375-018-0357-9
7. Qiu QC, Wang C, Bao XB, Yang J, Shen HJ, Ding ZX *et al.* The impact of FLT3 mutations on treatment response and survival in Chinese de novo AML patients. Hematology 2018; **23**(3): 131–138. e-pub ahead of print 2017/09/07; doi: 10.1080/10245332.2017.1372248
8. Brunet S, Labopin M, Esteve J, Cornelissen J, Socie G, Iori AP *et al.* Impact of FLT3 internal tandem duplication on the outcome of related and unrelated hematopoietic transplantation for adult acute myeloid leukemia in first remission: a retrospective analysis. J Clin Oncol 2012; **30**(7): 735–741. e-pub ahead of print 2012/02/01; doi: 10.1200/JCO.2011.36.9868
9. Schmid C, Labopin M, Socie G, Daguindau E, Volin L, Huynh A *et al.* Outcome of patients with distinct molecular genotypes and cytogenetically normal AML after allogeneic transplantation. Blood 2015; **126**(17): 2062–2069. e-pub ahead of print 2015/09/10; doi: 10.1182/blood-2015-06-651562
10. Zhang YY, Mo XD, Zhang XH, Xu LP, Wang Y, Yan CH *et al.* FLT3 internal tandem duplication does not impact prognosis after haploidentical allogeneic hematopoietic stem cell transplantation in AML patients. *Bone Marrow Transplant* 2019; **54**(9): 1462–1470. e-pub ahead of print 2019/02/03; doi: 10.1038/s41409-019-0456-x

11. Dohner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H *et al.* Diagnosis and Management of AML in Adults: 2022 ELN Recommendations from an International Expert Panel. *Blood* 2022. e-pub ahead of print 2022/07/08; doi: 10.1182/blood.2022016867
12. Dohner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Buchner T *et al.* Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* 2017; **129**(4): 424–447. e-pub ahead of print 2016/11/30; doi: 10.1182/blood-2016-08-733196
13. Zhang Y, Zhao BD, Wang CC, Wang YG, Wang HF, Wang JH *et al.* A novel prognostic scoring model for newly diagnosed FLT3-ITD-positive acute myeloid leukemia. Am J Cancer Res 2020; **10**(12): 4527–4537. e-pub ahead of print 2021/01/09;
14. Ma J, Dunlap J, Paliga A, Traer E, Press R, Shen L *et al.* DNMT3A co-mutation is required for FLT3-ITD as an adverse prognostic indicator in intermediate-risk cytogenetic group AML. Leuk Lymphoma 2018; **59**(8): 1938–1948. e-pub ahead of print 2017/11/23; doi: 10.1080/10428194.2017.1397659
15. Tang S, Shen H, Mao X, Dai H, Zhu X, Xue S *et al.* FLT3-ITD with DNMT3A R882 double mutation is a poor prognostic factor in Chinese patients with acute myeloid leukemia after chemotherapy or allogeneic hematopoietic stem cell transplantation. *Int J Hematol* 2017; **106**(4): 552–561. e-pub ahead of print 2017/06/16; doi: 10.1007/s12185-017-2256-7
16. Liu SB, Qiu QC, Bao XB, Ma X, Li HZ, Liu YJ *et al.* Pattern and prognostic value of FLT3-ITD mutations in Chinese de novo adult acute myeloid leukemia. *Cancer Sci* 2018; **109**(12): 3981–3992. e-pub ahead of print 2018/10/16; doi: 10.1111/cas.13835
17. Sakaguchi M, Yamaguchi H, Najima Y, Usuki K, Ueki T, Oh I *et al.* Prognostic impact of low allelic ratio FLT3-ITD and NPM1 mutation in acute myeloid leukemia. *Blood Adv* 2018; **2**(20): 2744–2754. e-pub ahead of print 2018/10/21; doi: 10.1182/bloodadvances.2018020305
18. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM *et al.* The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016; **127**(20): 2391–2405. e-pub ahead of print 2016/04/14; doi: 10.1182/blood-2016-03-643544
19. Meshinchi S, Woods WG, Stirewalt DL, Sweetser DA, Buckley JD, Tjoa TK *et al.* Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukemia. *Blood* 2001; **97**(1): 89–94. e-pub ahead of print 2001/01/03; doi: 10.1182/blood.v97.1.89
20. Ruan GR, Li JL, Qin YZ, Li LD, Xie M, Chang Y *et al.* Nucleophosmin mutations in Chinese adults with acute myelogenous leukemia. *Ann Hematol* 2009; **88**(2): 159–166. e-pub ahead of print 2008/08/30; doi: 10.1007/s00277-008-0591-8
21. Leukemia, Lymphoma Group CSoHCMA. [Chinese guidelines for the diagnosis and treatment of adult acute myeloid leukemia (not APL) (2021)]. *Zhonghua Xue Ye Xue Za Zhi* 2021; **42**(8): 617–623. e-pub ahead of print 2021/09/23; doi: 10.3760/cma.j.issn.0253-2727.2021.08.001
22. Tang F, Mo X, Zhang X, Wang Y, Yan C, Chen Y *et al.* Influence of the degree of donor bone marrow hyperplasia on patient clinical outcomes after allogeneic hematopoietic stem cell transplantation. *Sci China Life Sci* 2020; **63**(1): 138–147. e-pub ahead of print 2019/08/23; doi: 10.1007/s11427-018-9509-y

23. Tang F, Xu Y, Chen H, Xu L, Zhang X, Wang Y *et al.* Comparison of the clinical outcomes of hematologic malignancies after myeloablative haploidentical transplantation with G-CSF/ATG and posttransplant cyclophosphamide: results from the Chinese Bone Marrow Transplantation Registry Group (CBMTRG). Sci China Life Sci 2020; **63**(4): 571–581. e-pub ahead of print 2019/08/20; doi: 10.1007/s11427-019-9594-7
24. Medinger M, Passweg JR. Acute myeloid leukaemia genomics. Br J Haematol 2017; **179**(4): 530–542. e-pub ahead of print 2017/06/28; doi: 10.1111/bjh.14823
25. Dohner K, Thiede C, Jahn N, Panina E, Gambietz A, Larson RA *et al.* Impact of NPM1/FLT3-ITD genotypes defined by the 2017 European LeukemiaNet in patients with acute myeloid leukemia. Blood 2020; **135**(5): 371–380. e-pub ahead of print 2019/12/12; doi: 10.1182/blood.2019002697
26. Xuan L, Wang Y, Huang F, Fan Z, Xu Y, Sun J *et al.* Sorafenib maintenance in patients with FLT3-ITD acute myeloid leukaemia undergoing allogeneic haematopoietic stem-cell transplantation: an open-label, multicentre, randomised phase 3 trial. *Lancet Oncol* 2020; **21**(9): 1201–1212. e-pub ahead of print 2020/08/14; doi: 10.1016/S1470-2045(20)30455-1

Tables

Table 1
The baseline characteristics of the AML patients with FLT3-ITD mutation

Variables	Number (%) /Median (range)	Chemotherapy n = 55	Haplo-HSCT n = 40	P
Sex				0.77
Male	47	26	21	
Female	48	29	19	
Age, years, median (range)	44 (16–74)	54 (26–74)	45 (16–62)	0.022
FLT3-ITD Mutation/wt	0.38(0.01–2.9)	0.34(0.01–2.3)	0.55(0.01–2.9)	0.85
Marrow blast (range, %)	65(16–96)	60(16–91)	66(22–96)	0.78
Median WBC count, $\times 10^9$/L (range)	27(1-443)	26(1-319)	29(3-443)	0.91
Median HB level, g/L (range)	82(36–154)	84(43–154)	79(36–145)	0.62
Median PLT count, $\times 10^9$/L (range)	62(6-404)	65(9-404)	61(6-377)	0.72
Cytogenetic risk group				0.001
Favorable risk	10	7	3	
Intermediate risk	77	46	31	
High risk	8	2	6	
Induction therapy				0.45
IA	46	25	21	
HAA	20	11	9	
CAG	17	9	8	
VEN + AZA	12	10	2	
Follow-up time of survivor, median (range), months	17.1(7.3–58.8)	16.9(7.3–58.8)	19.2(11.2–51.4)	0.27

Haplo-HSCT haploidentical hematopoietic stem cell transplantation, *AML* acute myeloid leukemia,

IA idarubicin and cytarabine, *HAA* homoharringtonine aclacinomycin and cytarabine, *CAG* aclacinomycin cytarabine and granulocyte colony-stimulating factor,

Table 2
Multivariate analysis of risk factors associated with haplo-HSCT outcomes

Risk factor	Multivariate analysis		
	HR	95% CI	P value
DFS			
Age ≥ 55 years	1.440	0.621–3.338	0.396
NPM1 mutation	1.674	0.633–4.432	0.299
DNMT3A gene mutation	2.987	1.329–6.711	0.008
Haplo-HSCT	0.429	0.184–1.004	0.05
OS			
Age ≥ 55 years	1.870	0.361–2.095	0.756
DNMT3A gene mutation	1.140	0.468–2.776	0.044
Haplo-HSCT	0.109	0.026–0.465	0.003
Haplo-HSCT haploidentical hematopoietic stem cell transplantation			

Figures

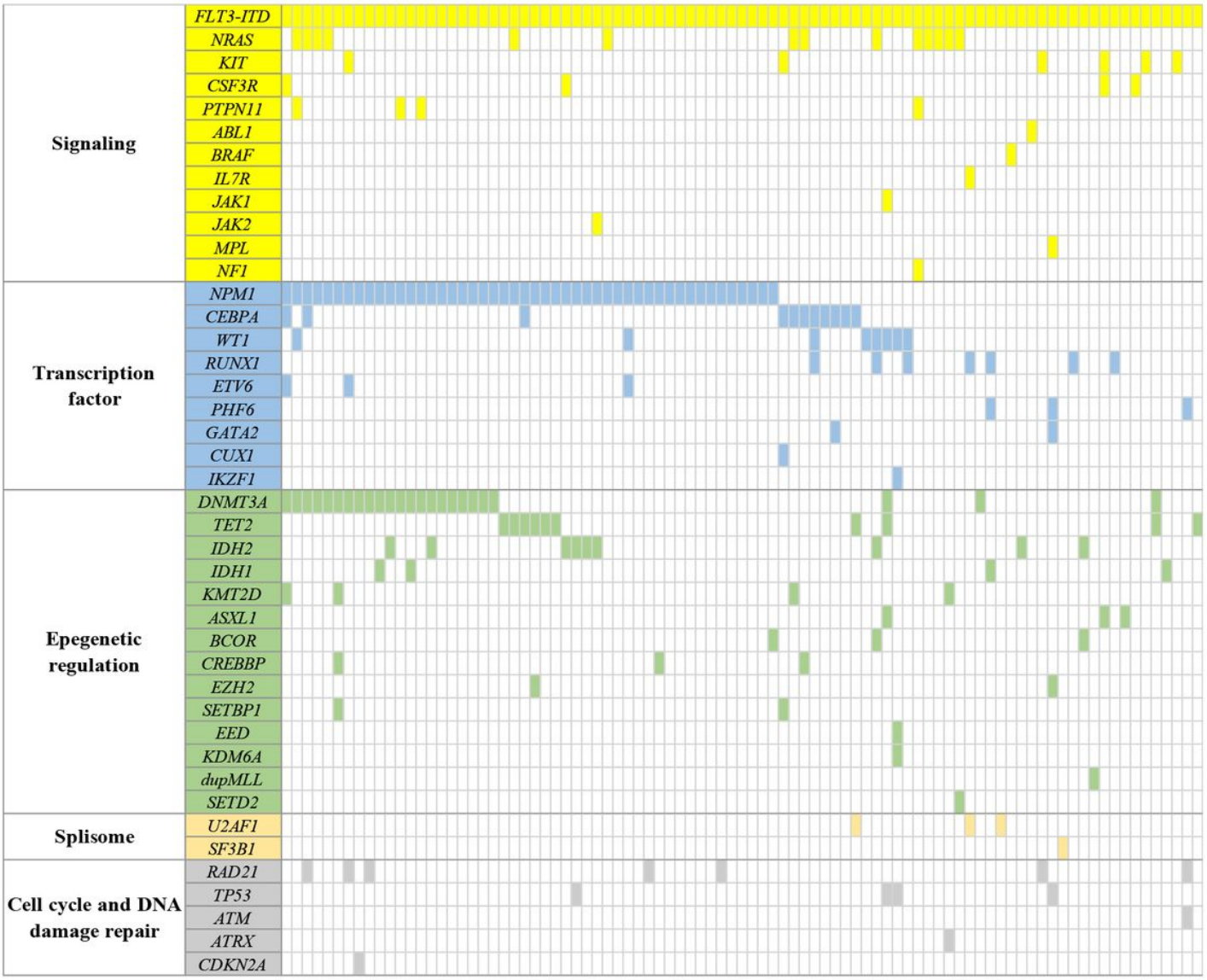

Figure 1

Mutational landscape in 95 patients with FLT3-ITD-positive AML.

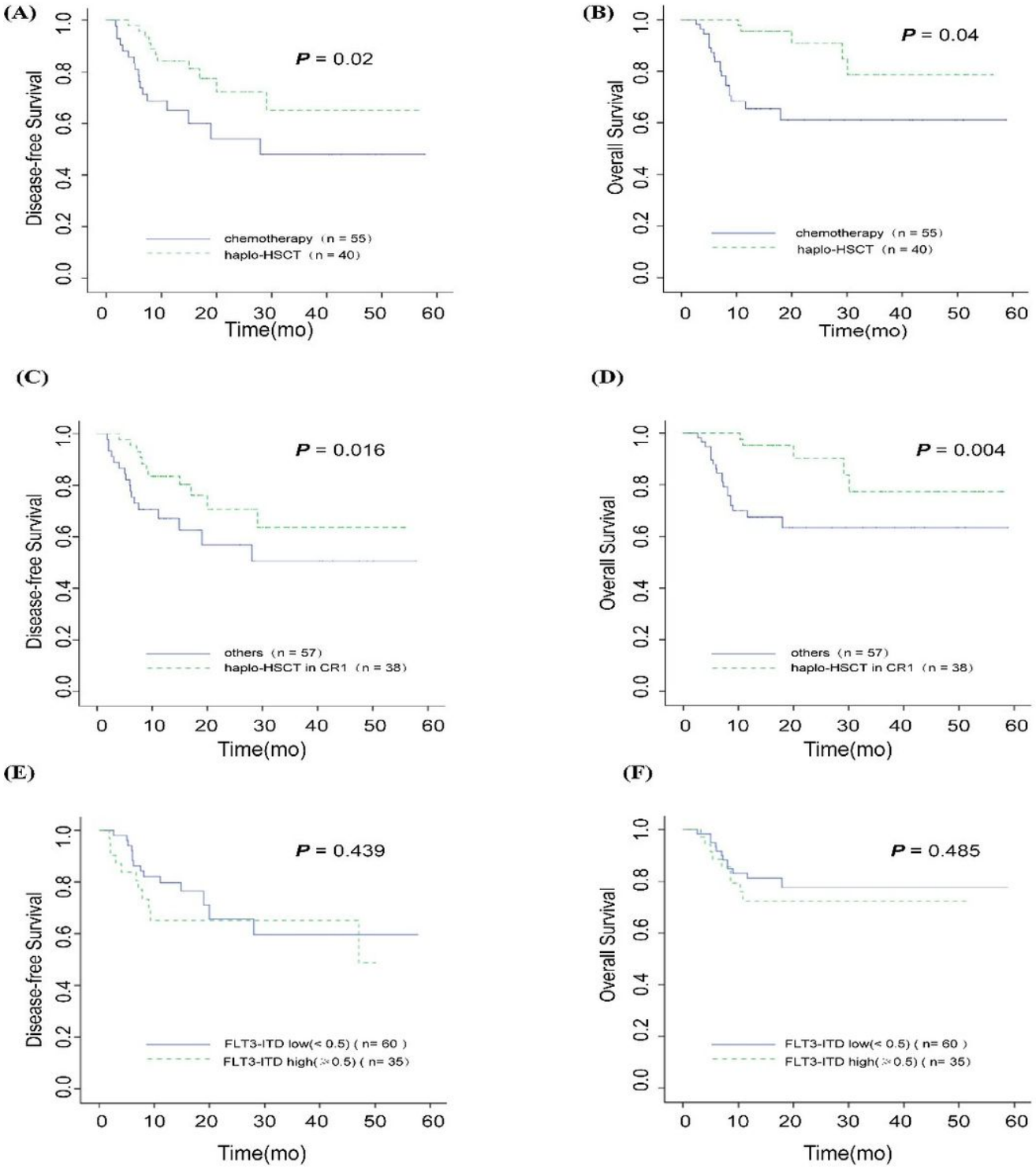

Figure 2

Impact of halpo-HSCT and AR (the cut off value is 0.5) on FLT-ITD FLT3-ITD-positive AML. (A) DFS between haplo-HSCT and chemotherapy; (B) OS between haplo-HSCT and chemotherapy; (C) DFS between haplo-HSCT with CR1 and chemotherapy; (D) OS between haplo-HSCT with CR1 and chemotherapy; (E) DFS between FLT3-ITD AR low (<0.5) and FLT3 AR high (≥0.5); (F) OS between FLT3-ITD AR low (<0.5) and FLT3 AR high (≥0.5).

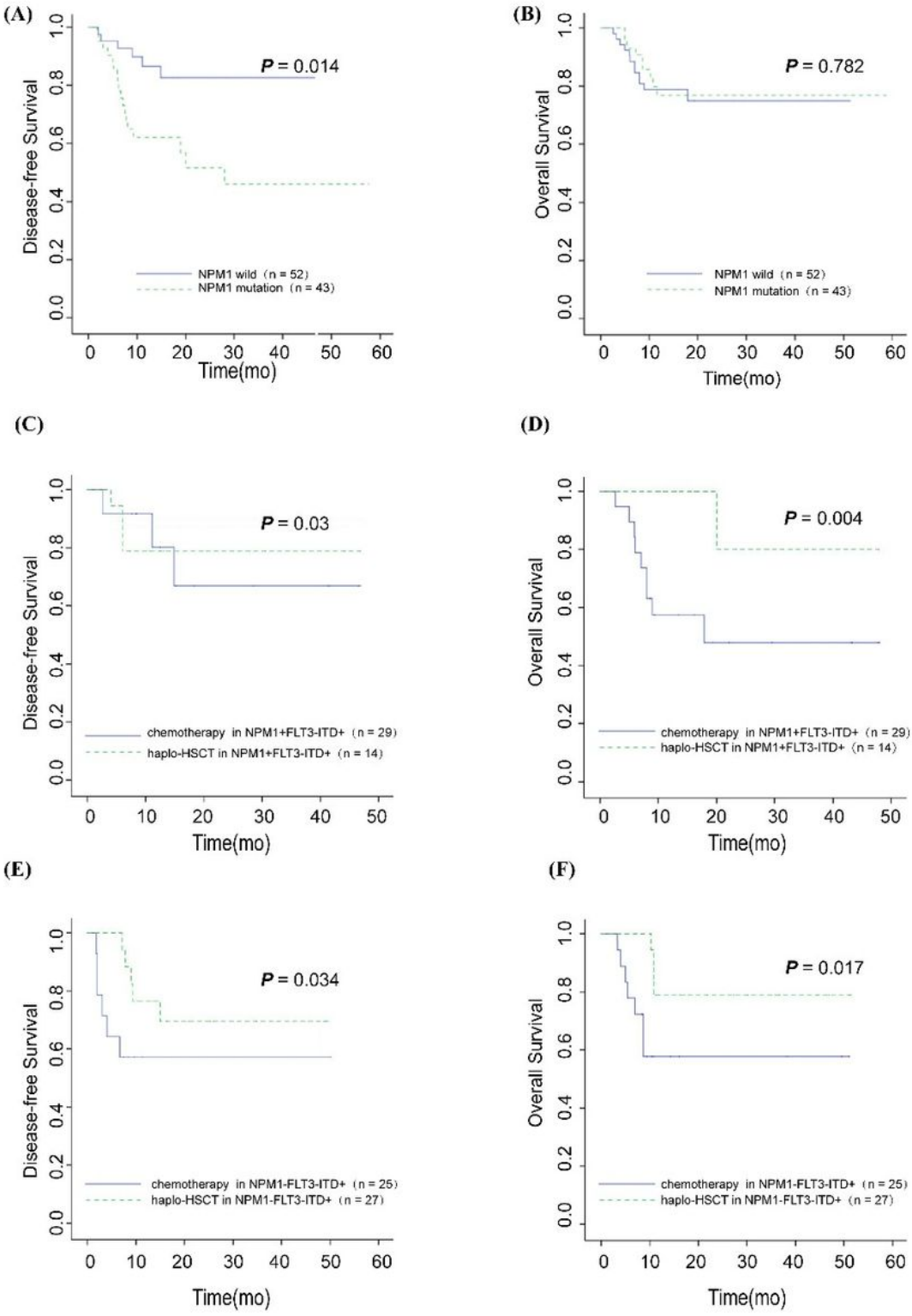

Figure 3

Impact of NPM1 mutation on FLT3-ITD-positive AML. (A) OS between NPM1 mutation and NPM1 wild; (B) DFS between NPM1 mutation and NPM1 wild; (C) DFS in NPM1+FLT3-ITD+ between haplo-HSCT and chemotherapy; (D) OS in NPM1+FLT3-ITD+ between haplo-HSCT and chemotherapy; (E) DFS in NPM1-FLT3-ITD+ between haplo-HSCT and chemotherapy; (F) OS in NPM1-FLT3-ITD+ between haplo-HSCT and chemotherapy.

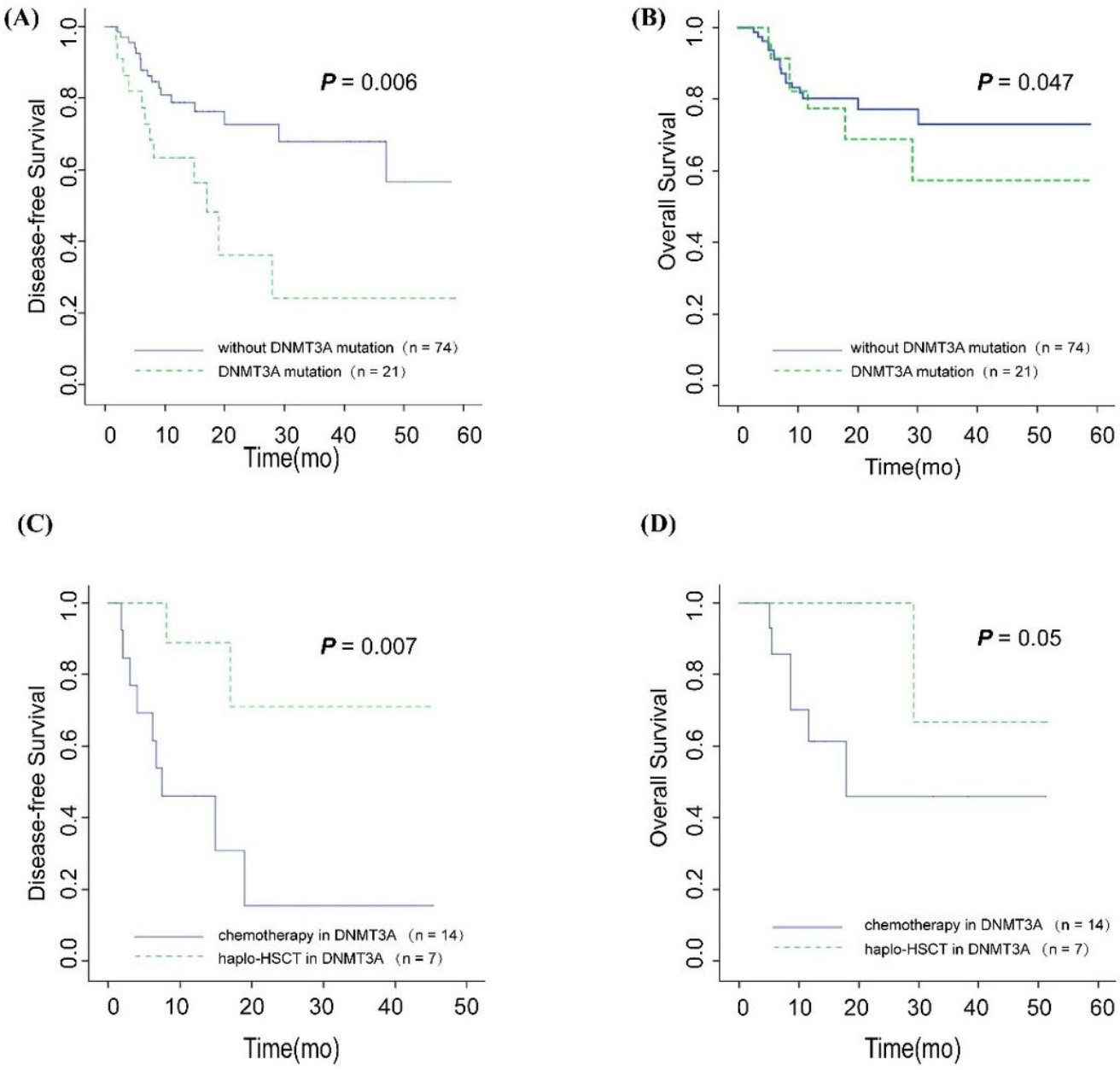

Figure 4

Impact of DNMT3A mutation on FLT3-ITD-positive AML. (A) DFS between DNMT3A comutation and without DNMT3A mutation; (B) OS between DNMT3A comutation and without DNMT3A mutation; (C) DFS in FLT3-ITD+ DNMT3A+ between haplo-HSCT and chemotherapy; (D) OS in FLT3-ITD+ DNMT3A+ between haplo-HSCT and chemotherapy.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.docx