# Prognostic Value of *FLT3*-Internal Tandem Duplication Residual Disease in Acute Myeloid Leukemia

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**PURPOSE** The applicability of *FLT3*-internal tandem duplications (*FLT3*-ITD) for assessing measurable residual disease (MRD) in acute myeloid leukemia (AML) in complete remission (CR) has been hampered by patient-specific duplications and potential instability of *FLT3*-ITD during relapse. Here, we comprehensively investigated the impact of next-generation sequencing (NGS)–based *FLT3*-ITD MRD detection on treatment outcome in a cohort of patients with newly diagnosed AML in relation to established prognostic factors at diagnosis and other MRD measurements, ie, mutant *NPM1* and multiparameter flow cytometry.

**METHODS** In 161 patients with de novo *FLT3*-ITD AML, NGS was performed at diagnosis and in CR after intensive remission induction treatment. *FLT3*-ITD MRD status was correlated with the cumulative incidence of relapse and overall survival (OS).

**RESULTS** NGS-based *FLT3*-ITD MRD was present in 47 of 161 (29%) patients with AML. Presence of *FLT3*-ITD MRD was associated with increased risk of relapse (4-year cumulative incidence of relapse, 75% *FLT3*-ITD MRD *v* 33% no *FLT3*-ITD MRD; *P* < .001) and inferior OS (4-year OS, 31% *FLT3*-ITD MRD *v* 57% no *FLT3*-ITD MRD; *P* < .001). In multivariate analysis, detection of *FLT3*-ITD MRD in CR confers independent prognostic significance for relapse (hazard ratio, 3.55; *P* < .001) and OS (hazard ratio 2.51; *P* = .002). Strikingly, *FLT3*-ITD MRD exceeds the prognostic value of most generally accepted clinical and molecular prognostic factors, including the *FLT3*-ITD allelic ratio at diagnosis and MRD assessment by NGS-based mutant *NPM1* detection or multiparameter flow cytometry.

**CONCLUSION** NGS-based detection of *FLT3*-ITD MRD in CR identifies patients with AML with profound risk of relapse and death that outcompetes the significance of most established prognostic factors at diagnosis and during therapy, and furnishes support for *FLT3*-ITD as a clinically relevant biomarker for dynamic disease risk assessment in AML.

## INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous disease that arises from the sequential acquisition of specific driver mutations in the leukemic stem cell.[1] Internal tandem duplications (ITD) in the *FMS-like tyrosine kinase 3* (*FLT3*) receptor gene are among the most common genetic molecular abnormalities in patients with AML.[2,3] In *FLT3*-ITD AML, the FLT3 kinase is constitutively activated resulting in uncontrolled proliferation of leukemic blasts.[4] *FLT3*-ITDs are generally considered late-event mutations in leukemogenesis and are frequently preceded by the appearance of mutations in *DNMT3A* and *NPM1*.[2,3] *FLT3*-ITD has been suggested to characterize an aggressive leukemic phenotype with early relapse and inferior treatment outcome.[3-5] Several features present at diagnosis have been postulated to modify the prognostic effect of *FLT3*-ITD, including the

presence of concurrent mutant *NPM1* and the *FLT3*-ITD clone size, ie, allelic ratio.[5]

Increasing evidence indicates that treatment outcome prediction can be improved by assessing the kinetics and depth of response during therapy by detection of measurable residual disease (MRD).[6] Currently, MRD detection in *FLT3*-ITD AML is carried out by a combination of multiparameter flow cytometry (MFC), mutant *NPM1* real-time quantitative polymerase chain reaction (RQ-PCR), and next-generation sequencing (NGS).[7] In the past, *FLT3*-ITD MRD detection by RQ-PCR and NGS was hampered by the variety of patient-specific *FLT3*-ITD, ie, sequence, position, and length. Advances in sequencing technology now enable accurate molecular detection of *FLT3*-ITD MRD.[8-10]

Clonal evolution studies revealed that late-event mutations in activated signaling genes, such as those in

## CONTEXT

### Key Objective

*FLT3*-internal tandem duplication (ITD) measurable residual disease (MRD) detection in acute myeloid leukemia (AML) has been hampered by the variety in patient-specific duplications. Current guidelines do not recommend *FLT3*-ITD MRD monitoring because of potential instability at relapse. We comprehensively investigated the impact of *FLT3*-ITD MRD detection on treatment outcome in AML.

### Knowledge Generated

To our knowledge, for the first time, we show that next-generation sequencing–based *FLT3*-ITD MRD detection identifies patients with AML with profound relapse risk and death that outweighs currently used prognostic factors. *FLT3*-ITD MRD better identifies patients with AML for relapse compared with multiparameter flow cytometry or next-generation sequencing–based mutant *NPM1* MRD alone.

### Relevance *(C.F. Craddock)*

Although these results require validation in independent data sets, they present a compelling rationale to incorporate *FLT3*-ITD MRD monitoring in AML to guide treatment strategies.*

*Relevance section written by *JCO* Associate Editor Charles F. Craddock, MD.

*FLT3*, are often subclonal and can be unstable during relapse in up to 25% of patients with AML, which may disqualify these markers for MRD detection.[11-16] Systematic studies evaluating the applicability of *FLT3*-ITD MRD detection as a prognostic biomarker in AML are therefore lacking. Here, we present a comprehensive study investigating the impact of NGS-based *FLT3*-ITD MRD detection on treatment outcome in a cohort of patients with newly diagnosed AML, enrolled in multicenter prospective phase III HOVON-SAKK clinical trials, in relation to various other established baseline and MRD prognostic markers.

## METHODS

### Patients and Samples

In total, 161 treatment-naive patients with de novo *FLT3*-ITD AML out of 2,274 patients with AML were included (Fig 1). Patients were enrolled in Dutch-Belgian Cooperative Trial Group for Hematology-Oncology (HOVON) or the Swiss Group for Clinical Cancer Research (SAKK) clinical trials HO42A AML, HO102 AML, and HO132 AML (Fig 1). Trial protocols were approved by the ethics committees at each participating site and performed in accordance with the Declaration of Helsinki and after obtaining patient written informed consent. All patients had achieved complete remission (CR; < 5% blast cells in the bone marrow) after two cycles of induction chemotherapy. Treatments protocols and inclusion criteria have been described previously.[17-19] Of note, in a subset of patients with AML included in the HOVON 132 trial, the residual disease status by MFC and/or mutant *NPM1* RQ-PCR assay was available to the clinical investigator before consolidation therapy to enable subsequent treatment choice.[19] Patient samples were taken at diagnosis and during CR after two cycles of standard induction chemotherapy. Details about

patient and sample selection are provided in the Data Supplement (online only).

### *FLT3*-ITD and Mutant *NPM1* Detection by Targeted NGS

The *FLT3*-ITD status at AML diagnosis was assessed with both capillary fragment length analysis and the NGS TruSight Myeloid Sequencing Panel. Patients were excluded when the presence of *FLT3*-ITD at diagnosis could not be confirmed by NGS or when the *FLT3*-ITD was located in exon 15 (Fig 1). High and low allelic *FLT3*-ITD allelic ratios were determined by capillary fragment length analysis and defined as $\geq 0.5$ or $< 0.5$, respectively.[5,20] *NPM1* mutations at AML diagnosis were determined by NGS. In CR, *FLT3*-ITD and mutant *NPM1* MRD detection was performed with a single-amplicon NGS library panel, covering exon 14 of *FLT3* or exon 12 of *NPM1*, for targeted deep sequencing analysis. The limit of detection of the *FLT3*-ITD MRD assay in CR ranges between variant allele frequencies of 0.01% and 0.001% (Data Supplement). In case of insufficient read coverage in CR (< 300,000 reads), the patient samples were excluded (Fig 1). All patients with *FLT3*-ITD AML were considered for MRD analysis, irrespective of *FLT3*-ITD ratio and/or number of *FLT3*-ITDs detectable by NGS at diagnosis. The NGS libraries were paired-end sequenced ($2 \times 221$-bp) on Illumina NGS platforms according to manufacturer's recommendation (Illumina, San Diego, CA). We used our in-house data analysis pipeline for variant calling as previously described.[21] Details on the experimental procedures, limit of detection, and bioinformatic analyses are available in the Data Supplement.

### Multiparameter Flow Cytometry

Detection of MFC MRD was performed as recommended.[7] The residual disease percentage was defined as the number
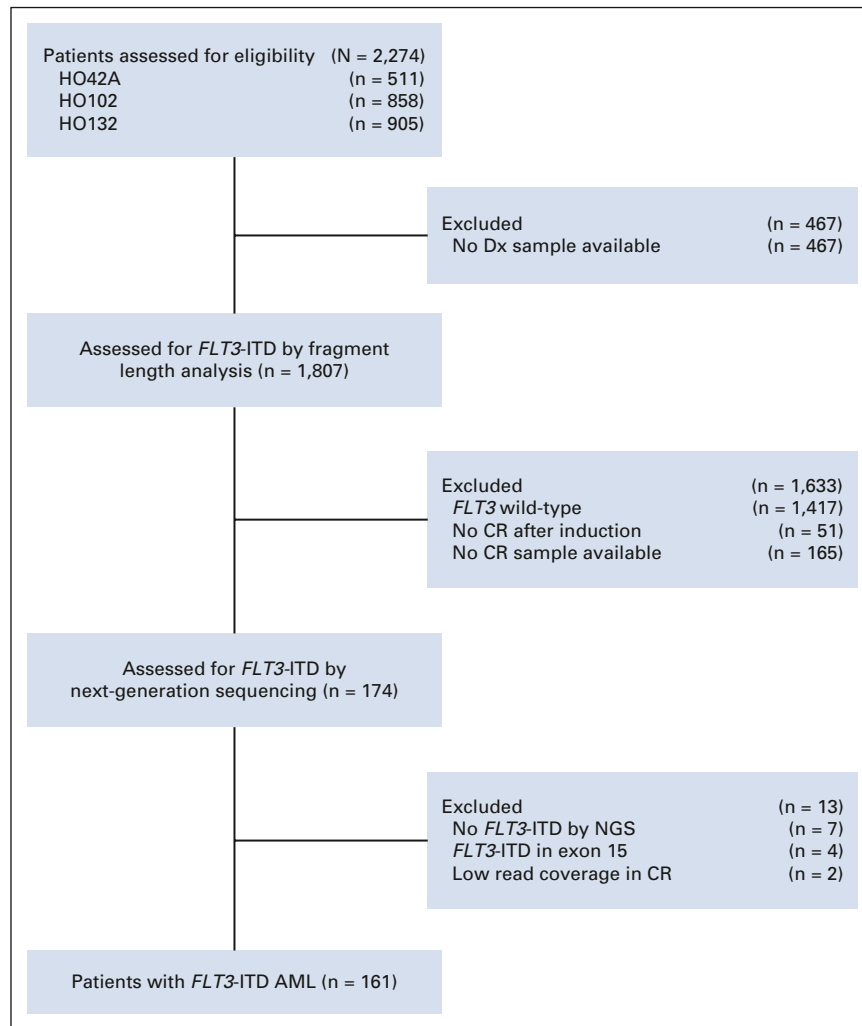
**FIG 1.** Flow diagram of the *FLT3*-ITD MRD study. AML, acute myeloid leukemia; CR, complete remission; Dx, diagnosis; ITD, internal tandem duplication; MRD, measurable residual disease; NGS, next-generation sequencing.

of leukemia-associated immunophenotype cells within the total white blood cell compartment. The threshold between residual disease and no residual disease on the basis of flow cytometry was established at 0.1%. MFC MRD was carried out in 138 out of 161 patients with *FLT3*-ITD AML.

### Statistical Analysis

The primary end point of the study was the cumulative incidence of relapse (CIR). Relapse and survival time were calculated from the date when CR bone marrow samples were taken until the date of the event of interest or censoring set at the date last known alive. Competing-risk analysis was performed for relapse with adjustment for nonrelapse mortality according to the method of Gray[22] and the Fine and Gray[23] model. The secondary end point was overall survival (OS), defined by death from any cause. Survival curves were estimated using the Kaplan-Meier method and differences in survival were assessed with the log-rank test. Multivariable

modeling was performed with the Cox proportional hazards model. The proportional hazards assumption was tested by including the interaction with time-varying coefficients. Differences in patient or molecular characteristics were tested using the Fisher's exact test for categorical variables and Mann-Whitney U test for continuous variables. All statistical tests were two-sided, and *P* values < .05 were considered statistically significant. Statistical analyses were executed with Stata Statistics and Data Science software, Release 17.0 (Stata, College Station, TX).

### RESULTS

### *FLT3*-ITD Detection at Diagnosis, Relapse, and in Complete Remission

NGS-based *FLT3*-ITD detection was carried out in a cohort of 161 patients with AML, with a median survival time of 29.6 months (Table 1 and Fig 1). At diagnosis, 74 out of 161

**TABLE 1.** Patient Characteristics of *FLT3*-ITD Acute Myeloid Leukemia by Detection of *FLT3*-ITD MRD

| Variable | No *FLT3*-ITD MRD (n = 114) | *FLT3*-ITD MRD (n = 47) | Total (n = 161) | P |
|---|---|---|---|---|
| Age, years | | | | .247 |
| Median | 51 | 53 | 51 | |
| Range | 23-66 | 19-65 | 19-66 | |
| Sex, No. (%) | | | | .863 |
| Male | 59 (52) | 23 (49) | 82 (51) | |
| Female | 55 (48) | 24 (51) | 79 (49) | |
| WBC at diagnosis, No. (%) | | | | .069 |
| $\leq 100 \times 10^9$/L | 103 (90) | 37 (79) | 140 (87) | |
| $> 100 \times 10^9$/L | 11 (10) | 10 (21) | 21 (13) | |
| ELN 2017 risk classification, No. (%) | | | | < .001 |
| Favorable | 42 (37) | 4 (9) | 46 (28) | |
| Intermediate | 51 (45) | 18 (38) | 69 (44) | |
| Adverse | 21 (18) | 25 (53) | 46 (28) | |
| Last treatment before first CR, No. (%) | | | | < .001 |
| Cycle I | 101 (89) | 26 (55) | 127 (79) | |
| Cycle II | 13 (11) | 21 (45) | 34 (21) | |
| Consolidation therapy, No. (%) | | | | .075 |
| None | 14 (12) | 9 (19) | 23 (14) | |
| Chemotherapy | 14 (12) | 4 (9) | 18 (11) | |
| Autologous HSCT | 24 (21) | 3 (6) | 27 (17) | |
| Allogeneic HSCT | 62 (55) | 31 (66) | 93 (58) | |
| Cytogenetics, No. (%)[a] | | | | .040 |
| Normal karyotype | 90 (81) | 30 (65) | 120 (76) | |
| Aberrant karyotype | 21 (19) | 16 (35) | 37 (24) | |
| Mutations at diagnosis, No. (%) | | | | |
| *FLT3* | | | | .121 |
| ITD, low ratio | 57 (50) | 17 (36) | 74 (46) | |
| ITD, high ratio | 57 (50) | 30 (64) | 87 (54) | |
| *NPM1* | | | | < .001 |
| Wild-type | 36 (32) | 34 (72) | 70 (43) | |
| Mutant | 78 (68) | 13 (28) | 91 (57) | |
| *DNMT3A* | | | | 1.000 |
| Wild-type | 61 (54) | 25 (53) | 86 (53) | |
| Mutant | 53 (46) | 22 (47) | 75 (47) | |

Abbreviations: CR, complete remission; HSCT, hematopoietic stem cell transplantation; ITD, internal tandem duplication; MRD, measurable residual disease.
[a]Cytogenetics failed in four patients.

(46%) patients had *FLT3*-ITD low allelic ratios and 87 (54%) patients had high allelic ratios. Patients with *FLT3*-ITD AML carried a variety of concurrent gene mutations (Table 1 and Data Supplement). The most frequent coexisting mutations were found in *NPM1* (57%) and *DNMT3A* (47%). At relapse, identical *FLT3*-ITDs were observed in 88% of patients with AML (n = 25). Two patients with AML had gained mutations in different genes at relapse that had been wild-type at diagnosis (Data Supplement).

*FLT3*-ITD MRD was detected in 47 out of 161 (29%) patients with AML, with a median variant allele frequency of 0.008% (range, 0.00031%-3.10%; Table 1 and Data Supplement). All *FLT3*-ITDs found in CR were identical (ie, sequence, position, and length) compared with diagnosis and multiple *FLT3*-ITD MRD clones were detected in five patients with AML. *FLT3*-ITD MRD was significantly associated with aberrant cytogenetics at diagnosis (35% *FLT3*-ITD MRD *v* 19% no *FLT3*-ITD MRD; *P* = .040) and patients with *NPM1*

wild-type AML (72% *FLT3*-ITD MRD *v* 32% no *FLT3*-ITD MRD; *P* < .001; Table 1). Interestingly, *FLT3*-ITD MRD was observed in 22% of patients with triple-mutant AML (*DNMT3A*/*NPM1*/*FLT3*-ITD), whereas none of the patients with double–mutant AML (*NPM1*/*FLT3*-ITD) showed *FLT3*-ITD MRD. Although significantly higher *FLT3*-ITD ratios were observed in patients with *FLT3*-ITD MRD AML (*P* = .035), the association of *FLT3*-ITD MRD with the *FLT3*-ITD allelic ratio status at diagnosis was not significant (*P* = .121). Detection of *FLT3*-ITD MRD was significantly more frequent in patients with AML who needed two cycles rather than one induction cycle to attain CR (45% *FLT3*-ITD MRD *v* 11% no *FLT3*-ITD MRD; *P* < .001; Table 1).

### *FLT3*-ITD Residual Disease and Outcome

We next assessed whether *FLT3*-ITD MRD was prognostic for relapse and OS. *FLT3*-ITD MRD was associated with an increased risk of relapse (4-year CIR, 75% *FLT3*-ITD MRD

*v* 33% no *FLT3*-ITD MRD; hazard ratio [HR], 3.70; 95% CI, 2.31 to 5.94; *P* < .001) and reduced OS (4-year OS, 31% *FLT3*-ITD MRD *v* 57% no *FLT3*-ITD MRD; HR, 2.47; 95% CI, 1.59 to 3.84; *P* < .001; Figs 2A and 2B). To increase the sensitivity of the *FLT3*-ITD MRD assay, we compared 100 ng and 500 ng DNA input in a selected subset of patients with AML for whom sufficient DNA was available (n = 122). Although the number of patients with *FLT3*-ITD MRD AML increased (n = 13) at very low levels (< 0.01%), the association with relapse did not improve (Data Supplement). The *FLT3*-ITD MRD clone size, as indicated by the variant allele frequency in remission, was directly correlated with the risk of relapse (Data Supplement). The number of persisting *FLT3*-ITD MRD clones did not associate with relapse (Data Supplement). Within the mutant *NPM1 FLT3*-ITD AML subset, the prognostic value for relapse (4-year CIR, 77% *FLT3*-ITD MRD *v* 33% no *FLT3*-ITD MRD; HR, 4.87; 95% CI, 1.92 to 12.3; *P* < .001) and
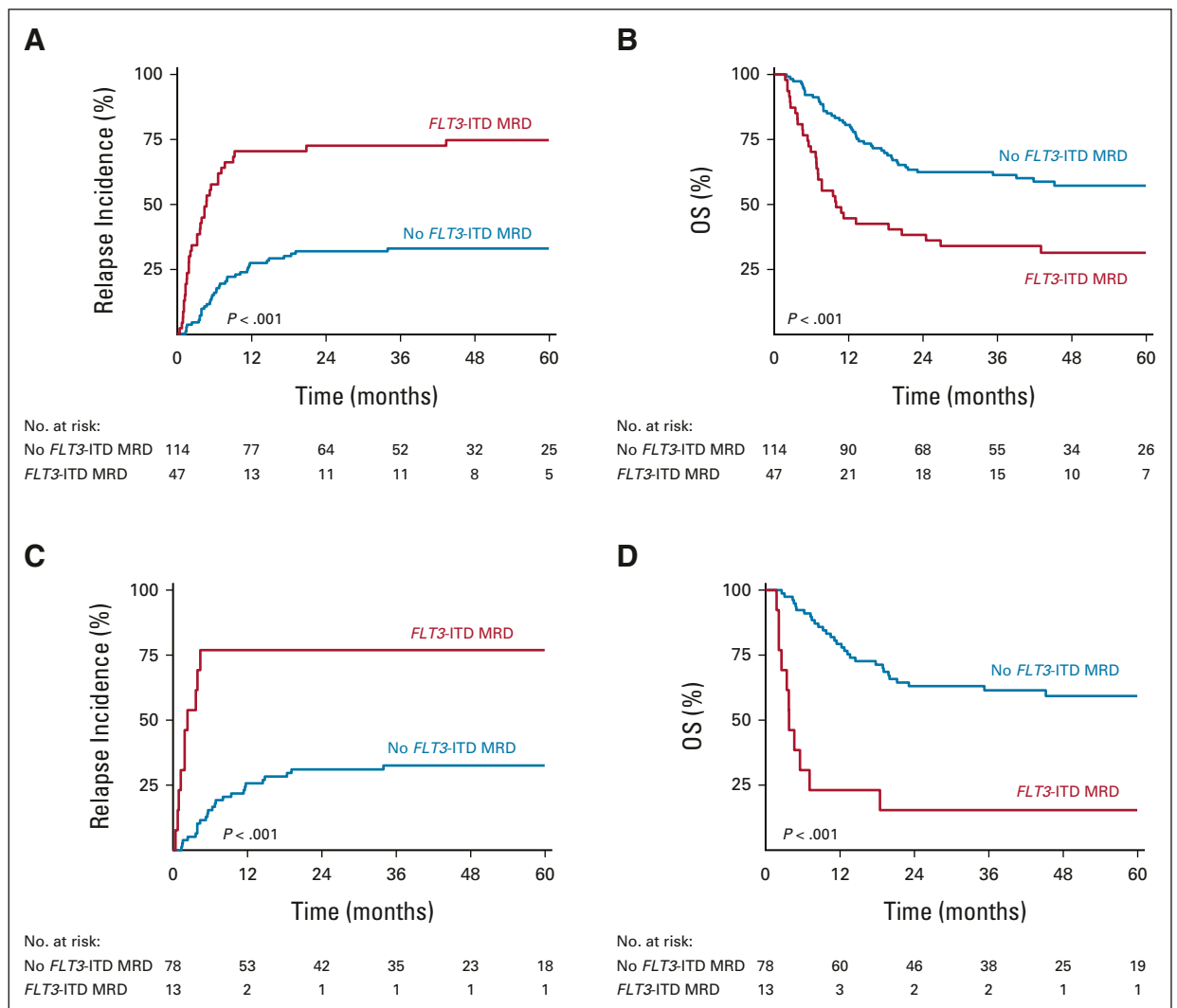


**FIG 2.** Survival outcome of *FLT3*-ITD MRD. (A) Relapse incidence and (B) OS of *FLT3*-ITD AML according to *FLT3*-ITD MRD in complete remission (n = 161). (C) Relapse incidence and (D) OS according to *FLT3*-ITD MRD in patients with mutant *NPM1 FLT3*-ITD AML (n = 91). AML, acute myeloid leukemia; ITD, internal tandem duplication; MRD, measurable residual disease; OS, overall survival.

survival (4-year OS, 15% *FLT3*-ITD MRD *v* 59% no *FLT3*-ITD MRD; HR, 5.36; 95% CI, 2.65 to 10.8; *P* < .001) was preserved (Figs 2C and 2D).

To assess whether the detection of *FLT3*-ITD MRD serves as an independent prognostic factor, we performed univariate analysis and multivariate analysis. In univariate analysis, significantly increased risk of relapse and reduced OS were observed among patients with a high white blood cell count at diagnosis (> 100 × 10⁹/L), late CR (ie, achieved after two cycles of induction chemotherapy), and patients with a high *FLT3*-ITD allelic ratio at diagnosis (Data Supplement) and within the ELN intermediate and adverse risk classification (Data Supplement). In multivariable modeling, *FLT3*-ITD MRD confers profound independent prognostic significance with respect to the relapse rate (HR, 3.55; 95% CI, 1.92 to 6.56; *P* < .001) and OS (HR, 2.51; 95% CI, 1.42 to 4.43; *P* = .002; Table 2). Besides *FLT3*-ITD MRD, only a high white blood cell count and late CR appeared to be independently associated with relapse and OS. Remarkably, the *NPM1* mutation status and the *FLT3*-ITD allelic ratio at diagnosis lost their prognostic value for relapse and survival when *FLT3*-ITD MRD was taken into account (Table 2). In sensitivity analysis, no significant clinically relevant or treatment-related interactions were observed. The prognostic value of *FLT3*-ITD MRD was unaffected in a correction for variation in sampling time (Data Supplement).

### *FLT3*-ITD Residual Disease and Allogeneic Transplantation

Regarding the poor risk features of *FLT3*-ITD AML, many patients will undergo allogeneic transplantation. Therefore, we explored the prognostic value of *FLT3*-ITD MRD in the 93 patients who underwent allogeneic transplantation. In total, 30 patients with AML received myeloablative conditioning (MAC) and 63 patients received reduced-intensity conditioning (RIC). Although the overall risk of relapse is reduced in the transplanted *FLT3*-ITD AML patients, we demonstrate increased relapse incidence and inferior outcome of patients with *FLT3*-ITD MRD AML (Fig 3). The prognostic value of *FLT3*-ITD MRD is comparable in both MAC and RIC groups (*P* = .858). However, the risk of relapse is lower in patients with *FLT3*-ITD MRD AML who had received MAC conditioning. No significant differences for nonrelapse mortality between the RIC and MAC

groups were observed, resulting in improved OS for patients with AML with residual disease after MAC conditioning (Fig 3). In a time-dependent correction for allogeneic hematopoietic stem-cell transplant, the prognostic significance of *FLT3*-ITD MRD is maintained (Data Supplement).

### Residual Disease Detection by Mutant *NPM1* and MFC

MFC MRD and mutant *NPM1* MRD are recommended methods for relapse prediction in patients with AML in CR.[7] We compared the assessment of *FLT3*-ITD MRD with available NGS-based mutant *NPM1* and MFC residual disease measurements in 91 and 138 patients with AML, respectively.

Interestingly, *FLT3*-ITD MRD significantly associated with high relapse risk and adverse OS irrespective of the mutant *NPM1* MRD or MFC MRD status (Fig 4). Increased risk of relapse and inferior survival was observed in patients with AML without *FLT3*-ITD MRD but with persistent mutant *NPM1* MRD; however, the association was not significant (*P* = .081 and *P* = .236). By contrast, MFC MRD without *FLT3*-ITD MRD appeared to confer limited prognostic value for relapse and survival (Figs 4C and 4D).

### DISCUSSION

Current risk classification and management of *FLT3*-ITD AML relies on the assessment of gene mutations at diagnosis and dynamic residual disease response measurements during therapy.[5] However, the prognostic significance of *FLT3*-ITD has been subject of ongoing debate as it may depend on allelic burden and the presence of other gene mutations, in particular mutant *NPM1*.[5] Here, we performed a comprehensive study in *FLT3*-ITD AML investigating the impact of persistence of *FLT3*-ITD in CR after induction chemotherapy and treatment outcome. Our results reveal *FLT3*-ITD MRD as a strong independent prognostic factor that identifies patients with AML with profound risk of relapse and death. Furthermore, *FLT3*-ITD MRD outcompetes the impact of other currently established prognostic factors in *FLT3*-ITD AML, including the *NPM1* mutation status and *FLT3*-ITD allelic ratio at diagnosis and residual disease measurements in CR by NGS-based mutant *NPM1* detection or MFC.

Mutations in activated signaling genes, such as *FLT3*-ITD, generally represent late events in AML development and

**TABLE 2.** Multivariate Analysis of Prognostic Factors for Relapse and OS

| | Relapse Incidence | | OS | |
|---|---|---|---|---|
| **Variable** | **HR (95% CI)** | ***P*** | **HR (95% CI)** | ***P*** |
| *FLT3*-ITD MRD (detection *v* no detection) | 3.55 (1.92 to 6.56) | < .001 | 2.51 (1.42 to 4.43) | .002 |
| Age (per 10 years) | 1.05 (0.86 to 1.29) | .621 | 1.20 (0.97 to 1.49) | .087 |
| WBC at diagnosis (> 100 × 10⁹/L *v* ≤ 100 × 10⁹/L) | 2.96 (1.73 to 5.07) | < .001 | 1.89 (1.05 to 3.43) | .035 |
| *NPM1* mutation at diagnosis (mutant *v* wild-type) | 1.21 (0.68 to 2.16) | .522 | 1.30 (0.75 to 2.23) | .348 |
| *FLT3*-ITD ratio at diagnosis (high *v* low) | 1.76 (1.00 to 3.09) | .050 | 1.60 (0.96 to 2.67) | .070 |
| No. of cycles to attain CR (two cycles *v* one cycle) | 1.84 (1.10 to 3.09) | .020 | 1.73 (0.98 to 3.06) | .058 |

Abbreviations: CR, complete remission; HR, hazard ratio; ITD, internal tandem duplication; MRD, measurable residual disease; OS, overall survival.
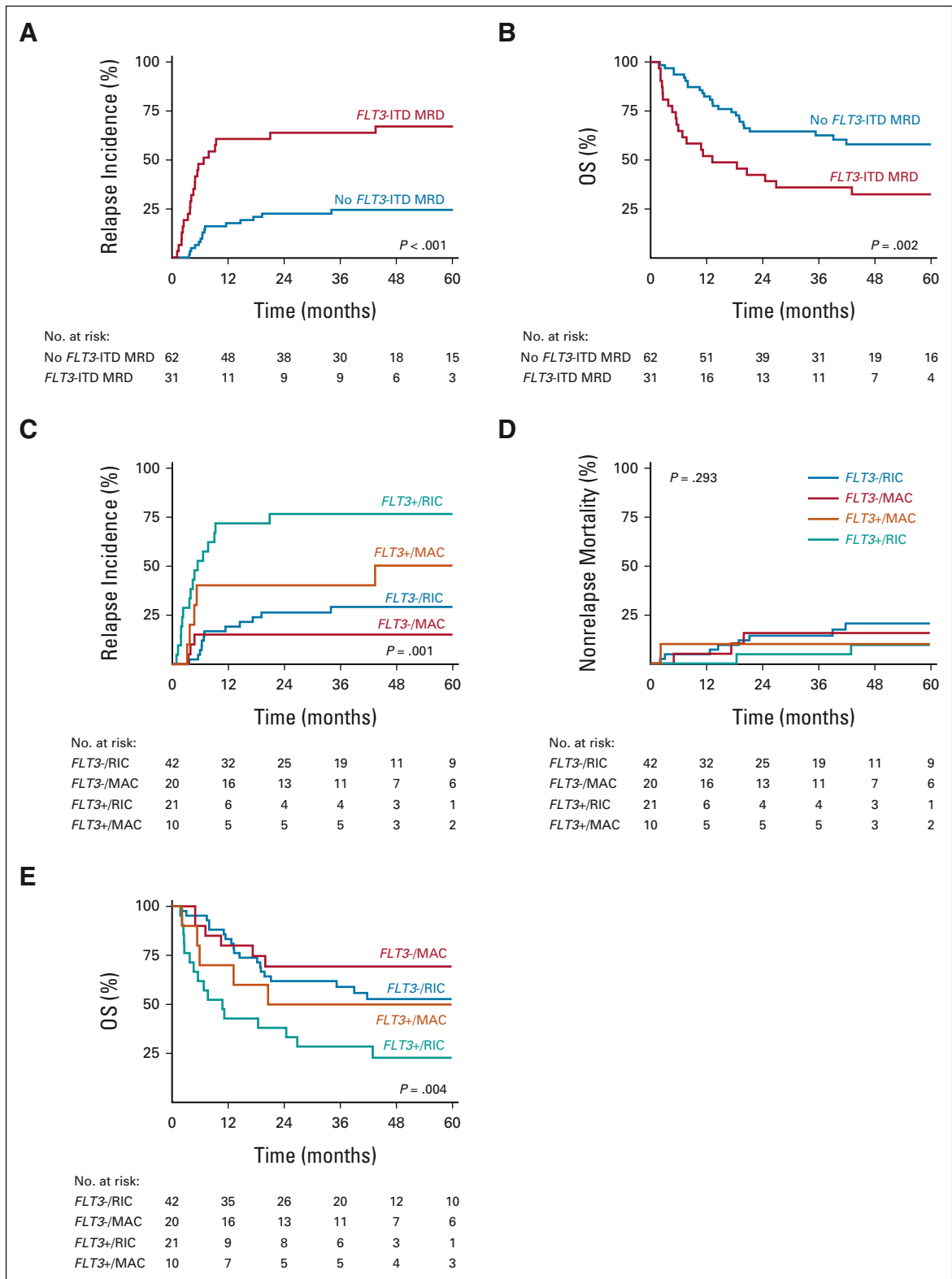
**FIG 3.** Survival outcome of *FLT3*-ITD MRD and allogeneic transplantation. (A) Relapse incidence and (B) OS of *FLT3*-ITD MRD in patients with AML who received allogeneic transplantation (n = 93). (C) Relapse incidence, (D) nonrelapse mortality, and (E) OS of *FLT3*-ITD MRD in patients with AML stratified by RIC or MAC regimens. AML, acute myeloid leukemia; ITD, internal tandem duplication; MAC, myeloablative conditioning; MRD, measurable residual disease; OS, overall survival; RIC, reduced-intensity conditioning.
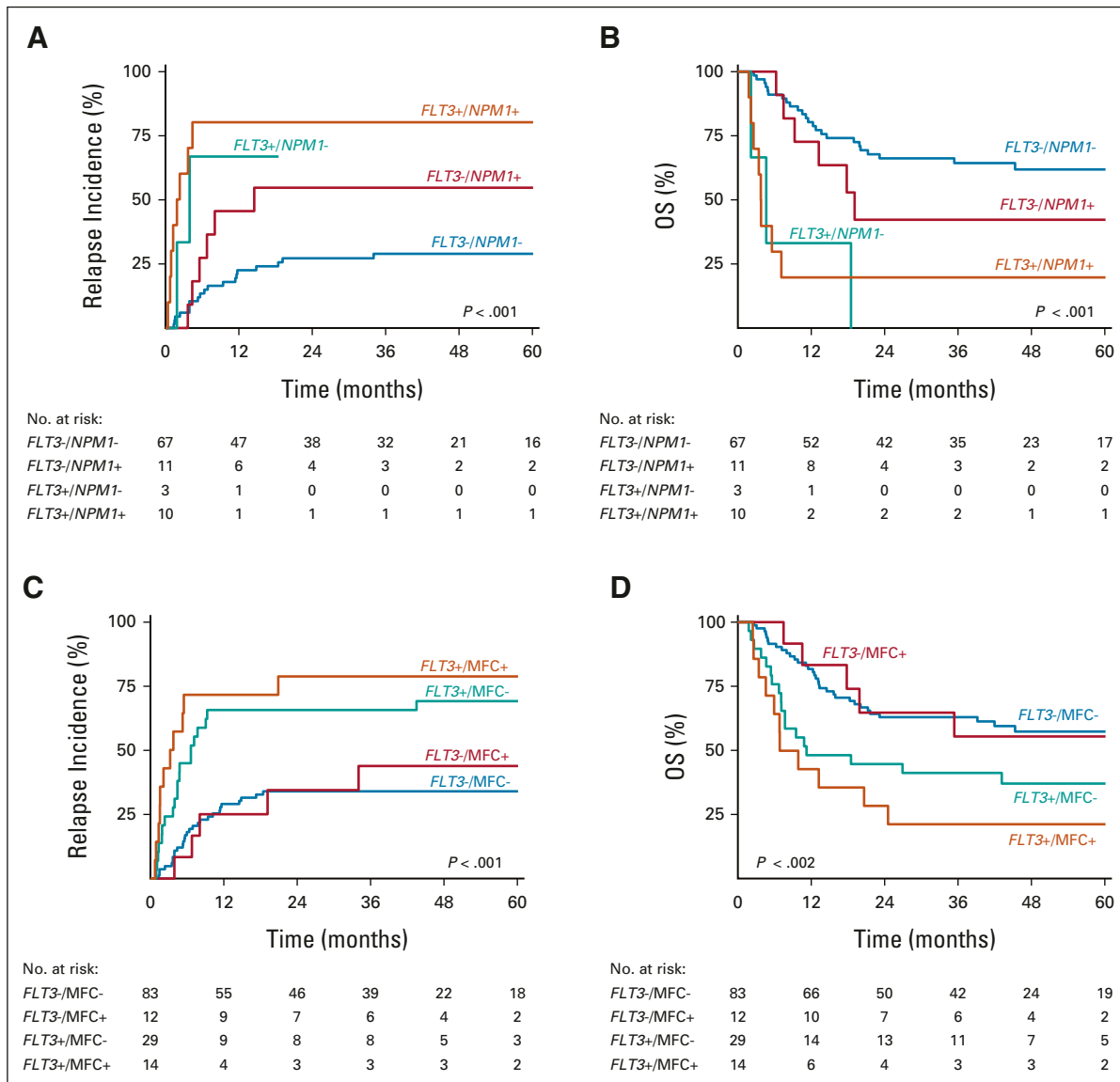
**FIG 4.** Survival outcome of *FLT3*-ITD MRD and NGS-based mutant *NPM1* and MFC MRD. (A) Relapse incidence and (B) OS of *FLT3*-ITD MRD and mutant *NPM1* MRD combined (n = 91). (C and D) Relapse incidence and OS of both *FLT3*-ITD MRD and MFC MRD (n = 138). ITD, internal tandem duplication; MFC, multiparameter flow cytometry; MRD, measurable residual disease; NGS, next-generation sequencing; OS, overall survival.

can be lost or gained at relapse.[11-16] Therefore, residual disease detection of these late mutations have previously been considered to be of limited prognostic value, in contrast to some of the more stable leukemic driver mutations, such as mutant *NPM1*.[7,16] The results of the current study, however, indicate stability of *FLT3*-ITD during relapse in the majority of patients with AML and substantiate residual *FLT3*-ITD in CR as a clinically useful indicator for relapse. We showed that leukemic clones with residual *FLT3*-ITDs that were present at baseline carry high impact on the risk of relapse, whereas AML clones with persistent mutant *NPM1* without *FLT3*-ITD had limited prognostic value for relapse. This implies that, within *FLT3*-ITD AML

with concurrent mutant *NPM1*, residual disease characterized by *FLT3*-ITD better identifies patients with AML for relapse than mutant *NPM1* MRD detection alone. Future studies exploring the comparison of DNA- and RNA-based mutant *NPM1* and *FLT3*-ITD MRD testing as well as using peripheral blood may further improve MRD monitoring of these genetically defined AML subtypes.

*FLT3*-ITD MRD failed to identify all cases of AML relapse. Although NGS-based sequencing was carried out at substantial depth, *FLT3*-ITDs at ultralow levels (< 0.001%) may have been missed. The *FLT3*-ITD assay may even further improve prediction of relapse by the ability to sequence at even higher depth or by increased DNA sample input, as well

as the capability to sequence larger *FLT3*-ITDs and those in exon 15 of the *FLT3* gene. Of note, we studied *FLT3*-ITD MRD at a single time point. With respect to the correlation of *FLT3*-ITD MRD clone size with relapse, it will be of interest to examine how longitudinal monitoring may support relapse prediction or guide different treatment strategies and allogeneic conditioning regimens for *FLT3*-ITD AML.

We have studied *FLT3*-ITD MRD in patients treated with intensive chemotherapy only. It will be interesting to investigate the prognostic value of *FLT3*-ITD MRD in the context of treatments that include the use of FLT3 inhibitors, since these patients have been shown to acquire novel *FLT3*-ITD

mutations or mutations in other activated signaling genes during relapse.[24-26] From this perspective, it will be important to expand NGS-based MRD detection to additional relevant late-event mutations in activated signaling genes and investigate the efficiency of novel and more specific FLT3 inhibitors on the eradication of residual *FLT3*-ITD cells and the prevention of impending relapse in AML.

In conclusion, NGS-based detection of *FLT3*-ITD MRD in CR identifies patients with AML with profound relapse risk and death, and outweighs the prognostic factors that are currently used in AML risk stratification. Therefore, we propose to incorporate *FLT3*-ITD MRD in AML treatment protocols.

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## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

**Prognostic Value of *FLT3*-Internal Tandem Duplication Residual Disease in Acute Myeloid Leukemia**

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