haematologica
Journal of the Ferrata Storti Foundation

Long-term outcomes in *FLT3*-mutated acute myeloid leukemia after frontline hypomethylating agent, venetoclax and a FLT3 inhibitor

by Nicholas J. Short, Sanam Loghavi, Musa Yilmaz, Omer Karrar, Kunhwa Kim, Courtney D. DiNardo, Tapan M. Kadia, Manuel Maroun, Gautam Borthakur, Ghayas C. Issa, Joseph Jabbour, Betul Oran, Elisabeth J. Shpall, Uday Popat, Keyur P. Patel, Mark Routbort, Marina Konopleva, Farhad Ravandi, Hagop Kantarjian and Naval Daver

Received: June 24, 2025.
Accepted: September 24, 2025.

Citation: Nicholas J. Short, Sanam Loghavi, Musa Yilmaz, Omer Karrar, Kunhwa Kim, Courtney D. DiNardo, Tapan M. Kadia, Manuel Maroun, Gautam Borthakur, Ghayas C. Issa, Joseph Jabbour, Betul Oran, Elisabeth J. Shpall, Uday Popat, Keyur P. Patel, Mark Routbort, Marina Konopleva, Farhad Ravandi, Hagop Kantarjian and Naval Daver. Long-term outcomes in FLT3-mutated acute myeloid leukemia after frontline hypomethylating agent, venetoclax and a FLT3 inhibitor.
Haematologica. 2025 Oct 2. doi: 10.3324/haematol.2025.288553 [Epub ahead of print]

Title: Long-term outcomes in *FLT3*-mutated acute myeloid leukemia after frontline hypomethylating agent, venetoclax and a FLT3 inhibitor

Running title: Outcomes of triplet regimens in *FLT3*-mutated AML

Authorship and Affiliations:

Nicholas J. Short[1*], Sanam Loghavi[2*], Musa Yilmaz[1], Omer Karrar[1], Kunhwa Kim[1], Courtney D. Dinardo[1], Tapan M. Kadia[1], Manuel Maroun[1], Gautam Borthakur[1], Ghayas C. Issa[1], Joseph Jabbour[1], Betul Oran[3], Elizabeth J. Shpall[3], Uday Popat[3], Keyur P. Patel[2], Mark Routbort[2], Marina Konopleva[4], Farhad Ravandi[1], Hagop Kantarjian[1], Naval Daver[1]

[1]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX
[2]Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX
[3]Dpartment of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX
[4]Department of Oncology, Montefiore Einstein Cancer Center & Albert Einstein College of Medicine, New York, NY

* N.J.S and S.L. contributed equally to this study

Article type: Regular article

Corresponding authors: Nicholas J. Short, MD, Department of Leukemia, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 428, Houston, TX 77030; e-mail: nshort@mdanderson.org

Naval Daver, MD, Department of Leukemia, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 428, Houston, TX 77030; e-mail: ndaver@mdanderson.org

Conflict of interest statement: N.J.S. has received consulting fees from Amgen, Pfizer Inc., GSK, Adaptive Biotechnologies, Autolus, and Sanofi; research funding from Takeda Oncology, Astellas Pharma Inc., Xencor, GSK, NextCure, Ascentage, Novartis, Hemogenyx, and Vironexis; and honoraria from Adaptive Biotechnologies, Novartis, Amgen, Takeda Oncology, Pfizer Inc., Astellas Pharma Inc., and Sanofi. S.L declares research support from Amgen and Astellas and consulting fees from AbbVie, Arima, Blueprint

Medicine, BMS, Caris Diagnostics, Daiichi-Sankyo, Immunogen, Kura Oncology, Recordati, Servier, Stemline, Syndax, Tempus AI. M.K. is a member of the advisory boards of AbbVie, Auxenion, Dark Blue Therapeutics, Legend, MEI Pharma, Menarini/Stemline Therapeutics, received consulting fees from AbbVie, Adaptive, Curis, Intellisphere, Janssen, Menarini/Stemline Therapeutics, Novartis, Sanofi Aventis, Servier, Syndax, Vincerx, and has received research funding by AbbVie, Janssen, Klondike Biopharma. ND has received research funding from Astellas Pharma, AbbVie, Genentech, Daiichi Sankyo, Gilead Sciences, ImmunoGen, Pfizer, Bristol Myers Squibb, Trovagene, Servier, Novimmune, Incyte, Hanmi Pharm, Fate Therapeutics, Amgen, Kite Pharma, Novartis, Astex Pharmaceuticals, KAHR, Sumitomo, Shattuck, Sobi, Arcellx, Nerviano, Avencell, SOBI, Astra-Zeneca, Vincerx, Caribou, and Trillium; has been an advisor for Astellas Pharma, AbbVie, Genentech, Daiichi Sankyo, Novartis, Jazz Pharmaceuticals, Amgen, Servier, Karyopharm Therapeutics, Trovagene, Trillium, Syndax, Gilead Sciences, Pfizer, Bristol Myers Squibb, Kite Pharma, Actinium Pharmaceuticals, Arog Pharmaceuticals, ImmunoGen, SOBI, Arcellx, Caribou, Avencell, Dark Blue, Charm, Merrill life, Vincerx, Astra-Zeneca, and Shattuck labs; has been a data monitoring committee member for Kartos Therapeutics, KEROS, and Jazz Pharmaceuticals. The rest of the authors have no relevant conflicts to disclose.

Authorship contributions: N.J.S. conceptualized the study, collected and analyzed the data, treated patients and wrote the first draft of the manuscript; S.L. collected and analyzed the data and performed pathological analysis and interpretation, M.Y., C.D.D., T.M.K., G.B., G.C.I., B.O., E.J.S., U.P., M.K., F.R., and H.K. treated patients; K.P.P. and M.R. performed pathological analysis and interpretation; O.K., J.J., K.K., and M.M. collected and analyzed the data and performed statistical analyses; N.D. conceptualized the study and treated patients. All authors reviewed and edited the manuscript and approve of the final version.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Funding source: This research is supported in part by the MD Anderson Cancer Center Leukemia SPORE CA100632, and the NIH/NCI Cancer Center Support Grant P30 CA016672.

Key words: acute myeloid leukemia, venetoclax, FLT3, targeted therapy, gilteritinib,

Abstract

Triplet regimens with a hypomethylating agent, venetoclax and a FLT3 inhibitor yield high rates of response in newly diagnosed *FLT3*-mutated AML. However, the long-term outcomes and patterns of relapse with these triplet regimens are not well-established. In this retrospective analysis, 73 patients with newly diagnosed *FLT3*-mutated AML received a frontline FLT3 inhibitor-containing triplet regimen. The composite complete remission (CR) and CR with incomplete hematologic recovery (CRi) rate was 93%. Next-generation sequencing *FLT3*-ITD MRD negativity (sensitivity: 0.005%) was achieved in 60% of patients after cycle 2 and 90% after cycle 4. The estimated 3-year relapse-free survival (RFS) for *FLT3*-ITD-mutated and *FLT3* TKD-mutated AML was 38% and 76%, respectively, and the 3-year overall survival (OS) was 45% and 76%, respectively. Neither age, *NPM1* co-mutation, ELN 2022 risk, nor allogeneic stem cell transplantation in first remission significantly impacted OS. Baseline *RAS* pathway mutations were associated with poor long-term survival (3-year OS 22% versus 63% without RAS pathway mutation). *FLT3* wild type relapses accounted for 65% of relapses, and new RAS pathway mutations were observed in 24% of relapses. Outcomes were poor after relapse (median OS of 6.1 months), particularly for those with persistently detectable *FLT3* mutations. Triplet combinations of an HMA, venetoclax and a FLT3 inhibitor result in durable remission and encouraging long-term OS in older adults with newly diagnosed *FLT3*-mutated AML. However, better strategies to prevent *FLT3* wild type relapses and to overcome RAS pathway-mediated resistance are still needed.

Introduction

In older patients with acute myeloid leukemia (AML) who are unfit for intensive chemotherapy, the standard of care frontline regimen is a hypomethylating agent (HMA) plus venetoclax.[1] While this regimen significantly improves response rates and overall survival (OS) as compared with azacitidine alone, some molecular features predict for lesser benefit with the HMA plus venetoclax regimen. The presence of *FLT3*-ITD, *NRAS*, *KRAS,* and/or *TP53* mutations have been shown to be associated with both primary and secondary resistance to this regimen.[2,3] In a subgroup analysis of the VIALE-A trial, patients with *FLT3*-ITD-mutated AML did not appear to derive significant clinical benefit from the addition of venetoclax to azacitidine, and the median OS in this population was approximately 10 months.[2] These relapses are largely driven by expansion of the *FLT3*-ITD-mutated subclone.[3] While "doublet" therapies evaluating an HMA plus a FLT3 inhibitor have been explored, the durability of remissions with these regimens are modest.[4-7] In a randomized phase III trial, azacitidine plus gilteritinib resulted in higher response rates as compared with azacitidine alone in older adults with newly diagnosed *FLT3*-mutated AML but did not significantly improved OS.[7]

To overcome the observed *FLT3*-mediated resistance to an HMA plus venetoclax, novel "triplet" regimens consisting of an HMA, venetoclax and a FLT3 inhibitor have been developed.[4] In a phase I/II study of azacitidine, venetoclax and gilteritinib in older adults with *FLT3*-mutated AML, the composite complete remission (CR) and CR with incomplete hematologic recovery (CRi) rate was 96%, and the estimated 18-month OS was 72%, which compares favorably to historical expectations with azacitidine plus venetoclax in *FLT3*-mutated AML.[8] In a similar study of frontline decitabine, venetoclax and quizartinib, the CR/CRi rate was 92%, and the median OS was not yet reached.[9] However, despite these encouraging early data, the follow-up is limited, and the long-term efficacy of these regimens is

therefore not well-established. The predictors of long-term outcomes and mechanisms of relapse with these novel FLT3 inhibitor-containing triplet regimens has also not been comprehensively evaluated.

Methods

Study design and participants

We retrospectively evaluated the long-term outcomes and patterns of relapse in adults with newly diagnosed *FLT3*-mutated AML who received a triplet regimen consisting of an HMA, venetoclax and a FLT3 inhibitor. Only patients with *FLT3*-ITD or *FLT3*-tyrosine kinase domain (TKD) mutations (e.g. D835/D836) with variant allelic frequency (VAF) ≥1% were included in this analysis. All patients were treated on prospective clinical trials (NCT03404193, NCT03661307, NCT04140487, NCT05010122 and NCT05520567). The details of the specific treatment regimens have been previously published.[8-12] This study was conducted at a single academic center (The University of Texas MD Anderson Cancer Center [UTMDACC]). This study was approved by the Institutional Review Board of UTMDACC and was conducted in accordance with the Declaration of Helsinki.

Baseline molecular testing

Mutational analysis was prospectively performed at diagnosis and at relapse using an 81-gene next-generation sequencing (NGS) panel, with a sensitivity of 2% VAF **(Supplemental Table 1)**.[13,14] Multiplex polymerase chain reaction (PCR) for *FLT3*-ITD or the *FLT3* kinase domain (D835/D836), which has a sensitivity of 1%, was performed at diagnosis and relapse.

Response and outcomes definitions

Responses were determined according to the European LeukemiaNet (ELN) 2022 guidelines.[15] Multiparameter flow cytometry (MFC) with sensitivity of 0.1-0.01% was performed on bone marrow samples for measurable residual disease (MRD) assessment.[16,17] Error-corrected NGS-based MRD assessment for *FLT3*-ITD was retrospectively performed on bone marrow samples. Molecular barcode-tagged primers were utilized to perform polymerase chain reaction (PCR) amplification for the detection of the *FLT3*-ITD. Bidirectional paired-end NGS of the PCR products was performed on the Illumina MiSeq Sequencer. The genomic reference sequence used is genome GRCh37/hg19. Illumina Experiment Manager 1.19.1, Miseq Control Software 4.1.0.656, Sequence Analysis Viewer 2.4.7, MiSeq Reporter 2.5.1, Invivoscribe dockerized MRD software (Invivoscribe®, San Diego, CA) were utilized in the experimental setup and data analysis. This assay has an analytical sensitivity of 5×10^{-5} mutant alleles per total alleles (VAF 0.005%). The analytical sensitivity of this assay was validated for an ITD length of 30bp. While the maximum ITD length detectable by this assay is 252bp, the detectable size and sensitivity vary depending on the insertion location and sequence of the ITD. NGS MRD negativity was defined as *FLT3*-ITD <0.005%. Relapse-free survival (RFS) was calculated from time of response until relapse or death, censored if alive at last follow-up. OS was calculated from time of treatment initiation until death, censored if alive at last follow-up.

Statistical methods

Patient characteristics were summarized using median (range) for continuous variables and frequencies (percentages) for categorical variables. To compare two groups with continuous variables, the Wilcoxon rank-sum test was performed. The Kaplan-Meier method was used to estimate the probabilities for RFS and OS and differences between groups were evaluated with the log-rank test. All statistical analyses were performed using GraphPad Prism 9.

Results

Baseline characteristics

The baseline characteristics of the study population (N=73) are shown in **Table 1.** The median age was 70 years (range, 18 to 88 years), and 26 patients (36%) were ≥75 years of age. Fifty-eight patients (80%) had only a *FLT3*-ITD mutation, 14 patients (19%) had only a *FLT3*-TKD mutation, and 1 patient (1%) had both *FLT3*-ITD and TKD mutations. The most common FLT3 inhibitors used were gilteritinib (n=49, 67%) and quizartinib (n=18, 25%). Patients with *FLT3*-TKD-mutated AML only received gilteritinib (n=13) or midostaurin (n=1). The median *FLT3* VAF for ITD mutations was 23% (range, 1-80%) and for TKD mutations was 19% (range, 2-57%). The most common co-mutations were *DNMT3A* and *NPM1*, present in 47% patients each. A *RAS* pathway mutation (defined as *KRAS, NRAS, PTPN11, CBL, NF1* and/or *BRAF*) was detected in 19 patients (26%). The rate of *RAS* pathway mutations in *FLT3*-ITD and *FLT3*-TKD-mutated AML was similar (25% [15/59] and 29% [4/14], respectively).

Dose intensity

The median number of cycles received was 3 (range, 36 cycles). The median durations of the hypomethylating agent, venetoclax and the FLT3 inhibitor in cycle 1 was 7 days (range, 2-10 days), 14 days (range, 2-28 days), and 14 days (range, 2-28 days), respectively. In cycle 4, the median durations were 5 days (range, 2-5 days), 7 days (range, 3-21 days), and 21 days (range, 7-28 days). Granulocyte-colony stimulating factor (G-CSF) was given to 58% of responders (42/72) in cycle 1 and to 36% (10/28) in cycle 4.

Response rates

Sixty-nine patients (82%) achieved CR and 8 patients (11%) achieved CRi, for a CR/CRi rate of 93%. (**Supplemental Table 2).** An additional 4 patients (6%) achieved morphological leukemia-free state. There was one early death. Among 59 evaluable responders, 48 (81%) achieved MRD negativity by MFC as best response. MFC MRD negativity was achieved in 48% (20/42) after cycle 1, 63% (19/30) after cycle 2, 70% (14/20) after cycle 3, and 69% (9/13) after cycle 4. Among *FLT3*-ITD-mutated patients, *FLT3* NGS MRD negativity at 0.005% sensitivity was achieved in 6% (1/17) after cycle 1, 60% (11/17) after cycle 2, 82% (9/11) after cycle 3, and 90% (9/10) after cycle 4. Rates of *FLT3*-ITD NGS MRD negativity after each cycle and cumulatively are shown in **Figure 1.**

Disposition

The disposition for the 73 patients is shown in **Supplemental Figure 1.** Among the 72 responders, 30 (42%) underwent allogeneic stem cell transplant (alloSCT) in first remission after a median of 4.5 months from the start of treatment and after a median of 3 cycles of protocol therapy. Among the 30 transplanted patients, 12 subsequently died (6 from alloSCT-related complications and 6 due to relapsed AML) and the remaining are alive and in remission at last follow-up. Seventeen transplanted patients (57%) received a post-alloSCT FLT3 inhibitor. Thirteen patients (18%) relapsed in the absence of alloSCT, 6 (8%) died in remission (3 from infection, 2 from unknown cause, and 1 from aortic dissection), and 23 (32%) are in ongoing remission without alloSCT.

Survival outcomes

The median follow-up was 26 months (range, 1 to 56 months). For the entire cohort, the median RFS and OS were 28.8 months and 38.5 months, respectively, and the estimated 3-year RFS and OS rates were 46% and 52%, respectively **(Supplemental Figure 2).** For patients with a *FLT3*-ITD mutation, the median RFS and OS were 16.7 months and 28.1 months, respectively, and the 3-year RFS and OS rates

was 38% and 45%, respectively **(Figure 2A-B)**. For patients with *FLT3*-TKD mutation only, the median RFS and OS were 36.6 months and 39.3 months, respectively, and the 3-year RFS and OS rates were both 76% **(Figure 2A-B)**.

Predictors of survival

When stratified by age <75 versus ≥75 years, outcomes were similar (3-year OS: 53% and 49%, respectively; P=0.99) **(Supplemental Figure 3).** Age also did not impact outcomes in either the *FLT3*-ITD or *FLT3*-TKD-mutated subgroups (<75 versus ≥75 years; P=0.73 for ITD and P=0.43 for TKD) **(Supplemental Figure 4).** Neither *NPM1* co-mutation status nor ELN 2022 risk stratification impacted OS (P=0.85 for *NPM1* mutated versus wild type; P=0.91 for adverse versus favorable/intermediate risk) **(Supplemental Figure 5).** Patients with *FLT3*-ITD, *NPM1*, and *DNMT3*A "triple" mutations had numerically worse OS than those who were *FLT3*-ITD and *NPM1*-mutated but *DNMT3A* wild type (3-year OS: 36% versus 66%, respectively; P=0.35), although this was not statistically significant (**Supplemental Figure 6).** The strongest predictor for survival outcomes was a baseline RAS pathway mutation. Presence of a baseline RAS pathway mutation was associated with a trend towards worse survival (3-year OS: 22% versus 63% in those with no RAS pathway mutation; P=0.07) **(Figure 3).** *RAS* pathway mutations were associated with poor outcomes in both *FLT3*-ITD and *FLT3*-TKD-mutated AML (3-year OS of 15% and 38%, respectively).

A landmark analysis was performed to evaluate the impact of alloSCT in first remission. The baseline characteristics of the transplanted and non-transplanted groups are shown in **Supplemental Table 3.** As expected, patients who underwent alloSCT in first remission were significantly younger than those who did not undergo alloSCT (median age: 67 years versus 72 years; P=0.001). The relapse rate in patients who underwent alloSCT was 20% versus 28% in those who did not undergo alloSCT (P=0.45). The rates of death in remission for alloSCT versus no alloSCT were 20% and 13%, respectively (P=0.42). Survival

outcomes were similar regardless of alloSCT consolidation (3-year OS: 55% for alloSCT versus 61% for no alloSCT; P=0.49) **(Supplemental Figure 7).** Similarly, no impact of alloSCT was observed in patients <75 years of age (P=0.32), those with *FLT3*-ITD-mutated AML (P=0.71), nor in those with ELN 2022 adverse risk disease (P=0.72) **(Supplemental Figures 8-10).** Among non-transplanted patients with *FLT3*-ITD-mutated AML, those who achieved high-sensitivity *FLT3* NGS MRD negativity by the end of cycle 4 had superior outcomes compared to those who remained MRD-positive (3-year OS: 61% versus 0%, respectively; P=0.02 (**Figure 4A-B).** Among evaluable transplanted patients, 3 of 4 (75%) who were *FLT3* NGS MRD-positive prior to alloSCT subsequently relapsed, compared with 2 of 11 (18%) who were MRD-negative (P=0.04), although no difference in OS was observed. In transplanted patients, the number of cycles received prior to alloSCT (<3 versus ≥3) did not impact post-alloSCT relapse rates (15% versus 24%, respectively; P=0.58).

Relapse characteristics

Overall, 19 patients relapsed (26% of responders), and the median duration of response in the relapsed patients was 9.4 months (range, 2.3 to 26.6 months). One relapse was extramedullary-only (cerebrospinal fluid and skin). Seventeen patients underwent repeat cytogenetic and molecular sequencing at relapse to evaluate for clonal evolution. Using the *FLT3* PCR assay (sensitivity 1%), the *FLT3* mutation was no longer detected at relapse in 11 patients (65% of evaluable relapses), and these patients comprise the "*FLT3* wild type relapse" group for subsequent analyses. The rate of *FLT3* wild type relapse was similar in patients with pretreatment *FLT3*-ITD or *FLT3*-TKD mutations (62% [8/13] and 75% [3/4], respectively; P=0.62). To evaluate for the presence of low-level *FLT3*-mutated subclones in patients with "*FLT3* wild type" relapse as assessed by conventional PCR, the high-sensitivity *FLT3*-ITD NGS MRD assay was retrospectively performed on 7 relapses samples with available bone marrow

material. Five of these relapse samples were undetectable for *FLT3*-ITD with the high-sensitivity NGS MRD assay and 2 had low-level *FLT3*-ITD detected at 0.01% and <0.001% VAF, respectively.

Twelve of the 17 evaluable patients (71%) had new cytogenetic or molecular abnormalities at relapse **(Supplemental Table 4)**. The most common newly emergent mutations detected at the time of relapse were RAS pathway mutations, which were identified in 4 patients (24%; *KRAS/NRAS,* n=2; *PTPN11*, n=1; *CBL*, n=1). The median VAF of these RAS pathway mutations was 14% (range 4%-37%). Other mutations newly detected at relapse included: *GATA2* in 3 patients (18%), spliceosome mutations in 2 patients (12%; *SF3B1*, n=1; *ZRSR2*, n=1), *IKZF1* in 2 patients (12%), and *FLT3* TKD mutation (VAF 5%) in 1 patient (6%).

Outcomes after relapse

Outcomes after relapse were poor. Among the 18 patients who received salvage therapy, the CR/CRi rate to first salvage was 22%. The median OS from relapse was only 6.1 months, with a 1-year OS of 28% **(Supplemental Figure 11)**. Outcomes were inferior in those with persistently detectable *FLT3* mutation by PCR as compared with those with *FLT3* wild type relapse (1-year OS: 0% versus 45%, respectively; P=0.03) **(Supplemental Figure 12).**

Discussion

Our data suggest that triplet regimens consisting of an HMA, venetoclax and a FLT3 inhibitor are an effective strategy for older patients with *FLT3*-mutated AML, resulting in a CR/CRi rate of 93% and median OS for *FLT3*-ITD and *FLT3*-TKD-mutated AML of 28.1 and 39.3 months, respectively. In contrast,

the reported median OS with azacitidine plus venetoclax from VIALE-A in these subgroups was 9.9 and 19.2 months, respectively.[2] The high response rates and durable remissions observed with these triplet regimens suggest a possible benefit compared with conventional "doublet" therapy and support the continued clinical development and dose optimization of these HMA, venetoclax and FLT3 inhibitor combinations.

Among patients treated with these triplet regimens, long-term outcomes were not impacted by age, *NPM1* co-mutation status, nor ELN 2022 risk. Importantly, even in patients ≥75 years age (a subgroup easiest to compare with VIALE-A[1]), a median OS of 28.1 months and an estimated 3-year OS rate of 49% were observed, suggesting that these triplet regimens can be delivered safely and were highly effective even in this older, less fit population. These triplet regimens may also be a reasonable frontline option for relatively fit patients 60-74 years of age with *FLT3*-mutated AML, including those planned for alloSCT in first remission. Of note, in a subgroup of patients >60 years of age who were enrolled in the QuANTUM-First study (all of whom were *FLT3*-ITD-mutated and were deemed suitable candidates for intensive chemotherapy), there was no clear benefit of the addition of quizartinib to intensive chemotherapy, possibly due to additional toxicity in the experimental arm.[18] Among older patients who were randomized to receive intensive chemotherapy plus quizartinib, the median OS was 17.5 months and the 3-year OS was ~35%. While challenging to compare across studies, it is notable that we observed a median OS of 31.3 months and a 3-year OS of 46% in patients <75 years of age with *FLT3*-ITD-mutated AML, suggesting comparable—or perhaps even superior—outcomes with the triplet regimen in a similar population. Randomized studies comparing these approaches (e.g. a FLT3 inhibitor in combination with intensive chemotherapy or with HMA plus venetoclax) in younger, alloSCT-eligible patients with *FLT3*-ITD mutated AML are planned and may shape our future approach to *FLT3*-mutated AML.

No difference in OS was observed based on alloSCT consolidation. AlloSCT in first remission improves OS in patients with *FLT3*-ITD-mutated AML and is generally recommended for younger, fit patients.[15] The lack of benefit of alloSCT in our study (including in the *FLT3*-ITD-mutated subgroup) may be related in part to the higher rate of transplant-related mortality (20%) in this older population. While alloSCT may still be appropriate for carefully selected older adults with *FLT3*-ITD-mutated AML, recent data also suggest that high-sensitivity NGS-based MRD testing may help identify patients in whom alloSCT may potentially be deferred with careful serial NGS MRD monitoring.[19,20] We observed that patients who achieved *FLT3*-ITD NGS MRD negativity within 4 cycles of the triplet regimen had relatively favorable long-term survival (3-year OS 61%), although there were not enough patients to evaluate the interaction of NGS MRD status and alloSCT. Thus, whether and how *FLT3*-ITD NGS MRD dynamics should impact decisions about alloSCT in patients receiving these triplet regimens remains unknown.

Baseline RAS pathway mutations were associated with worse long-term OS and were also the most common new mutations detected at relapse (newly detected in 24% of relapses). RAS pathway mutations have been previously described as mechanisms of resistance to both HMA plus venetoclax, to FLT3 inhibitors, and to venetoclax plus FLT3 inhibitors.[21-23] While inhibitors of key proteins in RAS signaling (e.g. MEK inhibitors such as trametinib) have been evaluated in AML, their efficacy has been largely disappointing.[24,25] Strategies using low-dose cytarabine-based regimens in combination with venetoclax may help to overcome RAS-mediated resistance mechanisms,[26] although the safety of adding FLT3 inhibitors to these regimens is not yet established.

Sixty-five percent of relapses in our study were driven by *FLT3* wild type clones, suggesting clonal escape as a major mechanism of secondary resistance to these regimens. The proportion of *FLT3* wild type relapses observed with these triplet regimens appears numerically higher than what has been reported with intensive chemotherapy plus a FLT3 inhibitor. For example, in younger patients with *FLT3*-mutated

AML receiving frontline intensive chemotherapy plus midostaurin, 46% relapses were *FLT3* wild type.[27] Whether this is reflective of meaningfully different patterns of relapse with these two approaches will need to be confirmed with larger datasets.

A notable limitation of our study is the heterogeneous pooled analysis from several clinical trials using different FLT3 inhibitors and dosing schedules. For example, 67% of patients in our analysis received frontline gilteritinib, and therefore the generalizability of our findings to triplet regimens with other FLT3 inhibitors is uncertain. Furthermore, as some of these studies are ongoing and have not yet been published, we were unable to provide outcomes data by specific FLT3 inhibitors (e.g. gilteritinib versus quizartinib). Despite these limitations, the pooled nature of our analysis provided a relatively large sample size (N=73), allowing for important subgroup analyses that are not feasible with the modest number of patients enrolled in these individual studies. Randomized studies are needed to more formally assess the potential superiority of a FLT3 inhibitor-containing triplet regimen versus the standard azacitidine and venetoclax doublet in *FLT3*-mutated AML (e.g. the ongoing MyeloMATCH trial: NCT06317649).

In summary, triplet regimens with an HMA, venetoclax, and a FLT3 inhibitor are effective in older adults with newly diagnosed *FLT3*-mutated AML, with response durations and survival outcomes that compare favorably to historical expectations of azacitidine plus venetoclax in a similar *FLT3*-mutated population. To further improve outcomes with these triplet regimens, novel strategies that address both *FLT3* wild type clonal escape and RAS-mediated resistance are needed.

References

1. DiNardo CD, Jonas BA, Pullarkat V, et al. Azacitidine and Venetoclax in Previously Untreated Acute Myeloid Leukemia. N Engl J Med. 2020;383(7):617-629.
2. Konopleva M, Thirman MJ, Pratz KW, et al. Impact of FLT3 Mutation on Outcomes after Venetoclax and Azacitidine for Patients with Treatment-Naïve Acute Myeloid Leukemia. Clin Cancer Res. 2022;28(13):2744-2752.
3. DiNardo CD, Tiong IS, Quaglieri A, et al. Molecular patterns of response and treatment failure after frontline venetoclax combinations in older patients with AML. Blood. 2020;135(11):791-803.
4. Short NJ, Nguyen D, Ravandi F. Treatment of older adults with FLT3-mutated AML: Emerging paradigms and the role of frontline FLT3 inhibitors. Blood Cancer J. 2023;13(1):142.
5. Ohanian M, Garcia-Manero G, Levis M, et al. Sorafenib Combined with 5-azacytidine in Older Patients with Untreated FLT3-ITD Mutated Acute Myeloid Leukemia. Am J Hematol. 2018;93(9):1136-1141.
6. Swaminathan M, Kantarjian HM, Levis M, et al. A phase I/II study of the combination of quizartinib with azacitidine or low-dose cytarabine for the treatment of patients with acute myeloid leukemia and myelodysplastic syndrome. Haematologica. 2021;106(8):2121-2130.
7. Wang ES, Montesinos P, Minden MD, et al. Phase 3 trial of gilteritinib plus azacitidine vs azacitidine for newly diagnosed FLT3mut+ AML ineligible for intensive chemotherapy. Blood. 2022;140(17):1845-1857.
8. Short NJ, Daver N, Dinardo CD, et al. Azacitidine, Venetoclax, and Gilteritinib in Newly Diagnosed and Relapsed or Refractory FLT3-Mutated AML. J Clin Oncol. 2024;42(13):1499-1508.
9. Musa Yilmaz MM, Nicholas Short, Sanam Loghavi, et al. PHASE I/II STUDY OF DECITABINE, VENETOCLAX, AND QUIZARTINIB TRIPLET COMBINATION IN FLT3-ITD MUTATED AML. presented at: EHA Library. 2025;4159219:S142
10. Maiti A, DiNardo CD, Daver NG, et al. Triplet therapy with venetoclax, FLT3 inhibitor and decitabine for FLT3-mutated acute myeloid leukemia. Blood Cancer J. 2021;11(2):25.
11. Bataller A, Short NJ, Daver N, et al. Phase 1/2 Study of Oral Decitabine/Cedazuridine With Venetoclax and Gilteritinib in Patients With Newly Diagnosed and Relapsed/Refractory Acute Myeloid Leukemia. EHA Library. 2024;(422243):S139.
12. Daver N, Perl AE, Wang E, et al. VICEROY: A Phase I/II Study of Gilteritinib, Venetoclax and Azacitidine Combination in Patients With Newly Diagnosed FLT3-Mutated Acute Myeloid Leukemia Ineligible for Intensive Induction Chemotherapy. EHA Library. 2024(421201):PB2454.
13. Short NJ, Kantarjian HM, Loghavi S, et al. Treatment with a 5-day versus a 10-day schedule of decitabine in older patients with newly diagnosed acute myeloid leukaemia: a randomised phase 2 trial. Lancet Haematol. 2019;6(1):e29-e37.
14. Luthra R, Patel KP, Reddy NG, et al. Next-generation sequencing-based multigene mutational screening for acute myeloid leukemia using MiSeq: applicability for diagnostics and disease monitoring. Haematologica. 2014;99(3):465-473.
15. Döhner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-1377.
16. Jen WY, Sasaki K, Ravandi F, et al. Impact of Measurable Residual Disease Clearance Kinetics in Patients with AML Undergoing Intensive Chemotherapy. Blood Adv. 2025;9(4):783-792.
17. Wang SA, Jorgensen JL, Hu S, et al. Validation of a 12-color flow cytometry assay for acute myeloid leukemia minimal/measurable residual disease detection. Cytometry B Clin Cytom. 2023;104(5):356-366.

18. Erba HP, Montesinos P, Kim H-J, et al. Quizartinib plus chemotherapy in newly diagnosed patients with FLT3-internal-tandem-duplication-positive acute myeloid leukaemia (QuANTUM-First): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2023;401(10388):1571-1583.
19. Ivey A, Hills RK, Simpson MA, et al. Assessment of Minimal Residual Disease in Standard-Risk AML. N Engl J Med. 2016;374(5):422-433.
20. Othman J, Potter N, Ivey A, et al. Postinduction molecular MRD identifies patients with NPM1 AML who benefit from allogeneic transplant in first remission. Blood. 2024;143(19):1931-1936.
21. Döhner H, DiNardo CD, Appelbaum FR, et al. Genetic risk classification for adults with AML receiving less-intensive therapies: the 2024 ELN recommendations. Blood. 2024;144(21):2169-2173.
22. McMahon CM, Ferng T, Canaani J, et al. Clonal Selection with RAS Pathway Activation Mediates Secondary Clinical Resistance to Selective FLT3 Inhibition in Acute Myeloid Leukemia. Cancer Discov. 2019;9(8):1050-1063.
23. Kennedy V, Peretz C, Lee P, et al. Multi-Omic Single-Cell Sequencing Reveals Genetic and Immunophenotypic Clonal Selection in Patients With FLT3-Mutated AML Treated With Gilteritinib/Venetoclax. Blood. 2022;140(Supplement 1):2244-2246.
24. Borthakur G, Popplewell L, Boyiadzis M, et al. Activity of the oral mitogen-activated protein kinase kinase inhibitor trametinib in RAS-mutant relapsed or refractory myeloid malignancies. Cancer. 2016;122(12):1871-1879.
25. Desikan SP, Ravandi F, Pemmaraju N, et al. A Phase II Study of Azacitidine, Venetoclax, and Trametinib in Relapsed or Refractory Acute Myeloid Leukemia Harboring RAS Pathway-Activating Mutations. Acta Haematol. 2022;145(5):529-536.
26. Bataller A, Kantarjian HM, Bazinet A, et al. Phase II Study of Cladribine with Low Dose Cytarabine and Venetoclax Alternating with Azacytidine and Venetoclax for Newly Diagnosed Acute Myeloid Leukemia. Blood. 2024;144(Supplement 1):56.
27. Schmalbrock LK, Dolnik A, Cocciardi S, et al. Clonal evolution of acute myeloid leukemia with FLT3-ITD mutation under treatment with midostaurin. Blood. 2021;137(22):3093-3104.

Table 1. Baseline characteristics of the study population

Characteristic (n=73)	N (%) / median [range]
Age, years	
Median [range]	70 [18-88]
≥75 years	26 (36)
Cytogenetics	
Diploid	36 (49)
Adverse	10 (14)
Others	22 (30)
Insufficient	5 (7)
ELN 2022 risk stratification	
Favorable	9 (12)
Intermediate	28 (38)
Adverse	36 (49)
FLT3 subtype	
ITD	58 (80)
TKD	14 (19)
ITD+TKD	1 (1)
FLT3 variant allelic frequency	
ITD	23 [1-80]
TKD	19 [2-57]
FLT3 inhibitor	
Gilteritinib	49 (67)
Quizartinib	18 (25)
Sorafenib	5 (7)
Midostaurin	1 (1)
Hypomethylating agent	
Azacitidine	35 (48)
Decitabine	38 (52)
Non-*FLT3* mutations[†]	
DNMT3A	34 (47)
NPM1	34 (47)
RUNX1	18 (25)
TET2	15 (21)
WT1	15 (21)
IDH2	12 (16)
BCOR	10 (14)
SRSF2	7 (10)
CEBPA	6 (8)
PTPN11	6 (8)

U2AF1	6 (8)
ASXL1	5 (7)
BCORL1	5 (7)
IDH1	5 (7)
NRAS	5 (7)
RAD21	5 (7)
SF3B1	5 (7)
STAG2	5 (7)
SMC1A	4 (5)
RAS* pathway mutation	19 (26)

† Mutations detected in ≥5% of patients

* Includes *KRAS, NRAS, PTPN11, CBL, NF1* and/or *BRAF* mutations

Abbreviations: ELN, European LeukemiaNet; ITD, internal tandem duplication; TKD, tyrosine kinase domain

Figure Legends

Figure 1. NGS measurable residual disease (MRD) for *FLT3*-ITD.

A. MRD after cycles 1-4.

B. cumulative rates of MRD negativity.

*MRD negativity was defined as *FLT3*-ITD $<5x10^{-5}$ (0.005%)

Figure 2. Outcomes by *FLT3* mutation subtype.

A. Relapse free survival.

B. Overall survival.

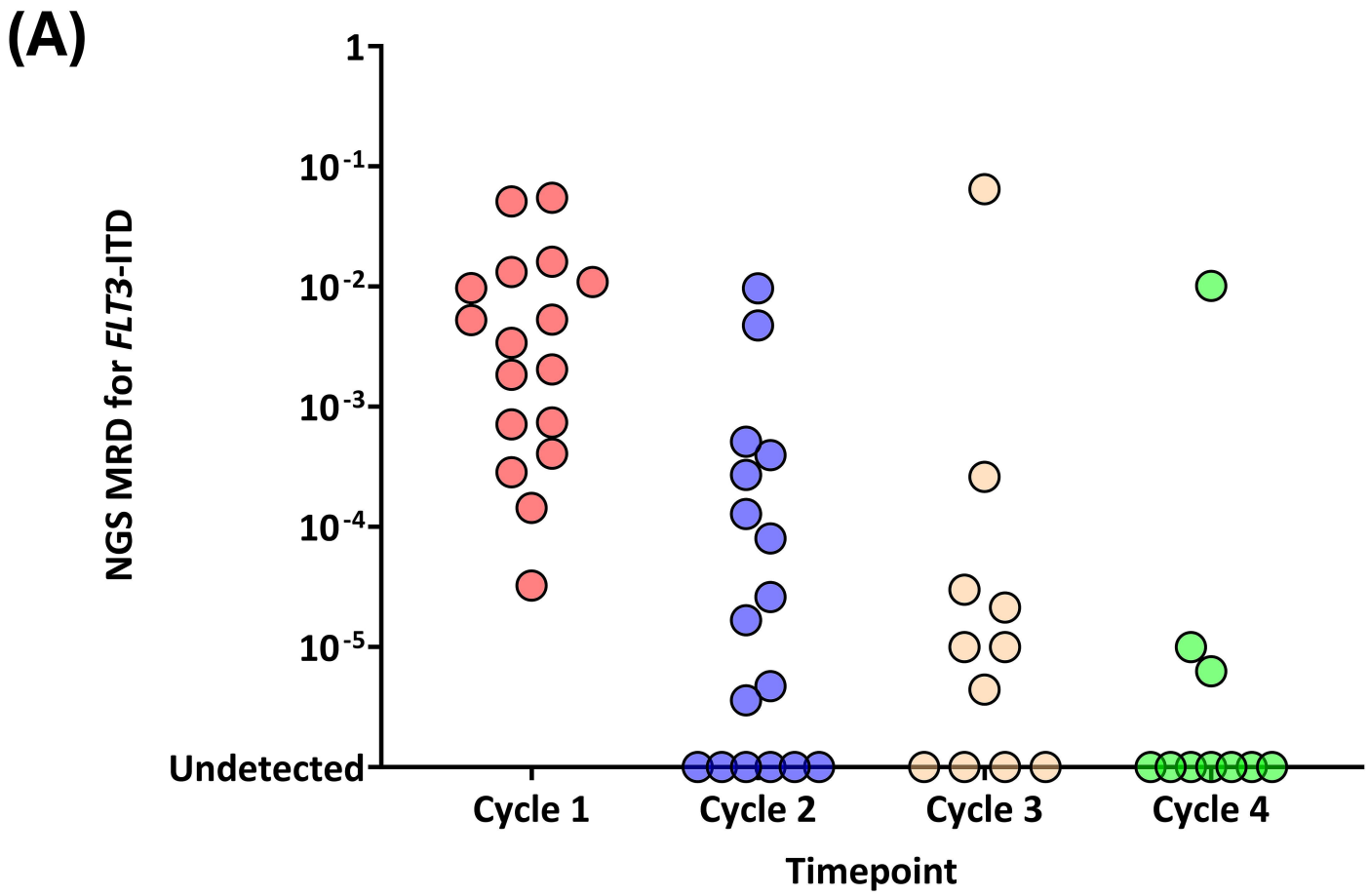
Figure 3. Overall survival by *RAS* pathway mutation status.

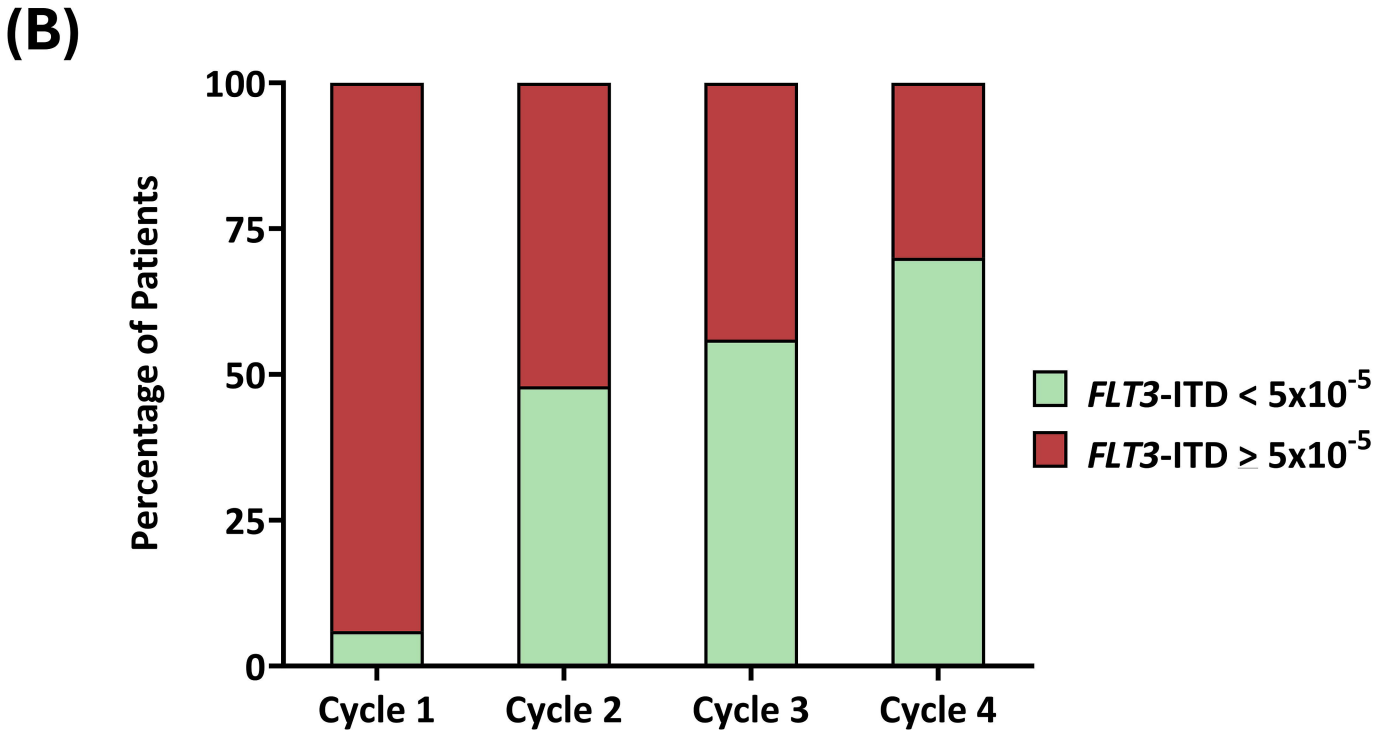
Figure 4. Outcomes of the study cohort, stratified by *FLT3*-ITD NGS MRD negativity within 4 cycles.

A. Relapse-free survival.

B. Overall survival.

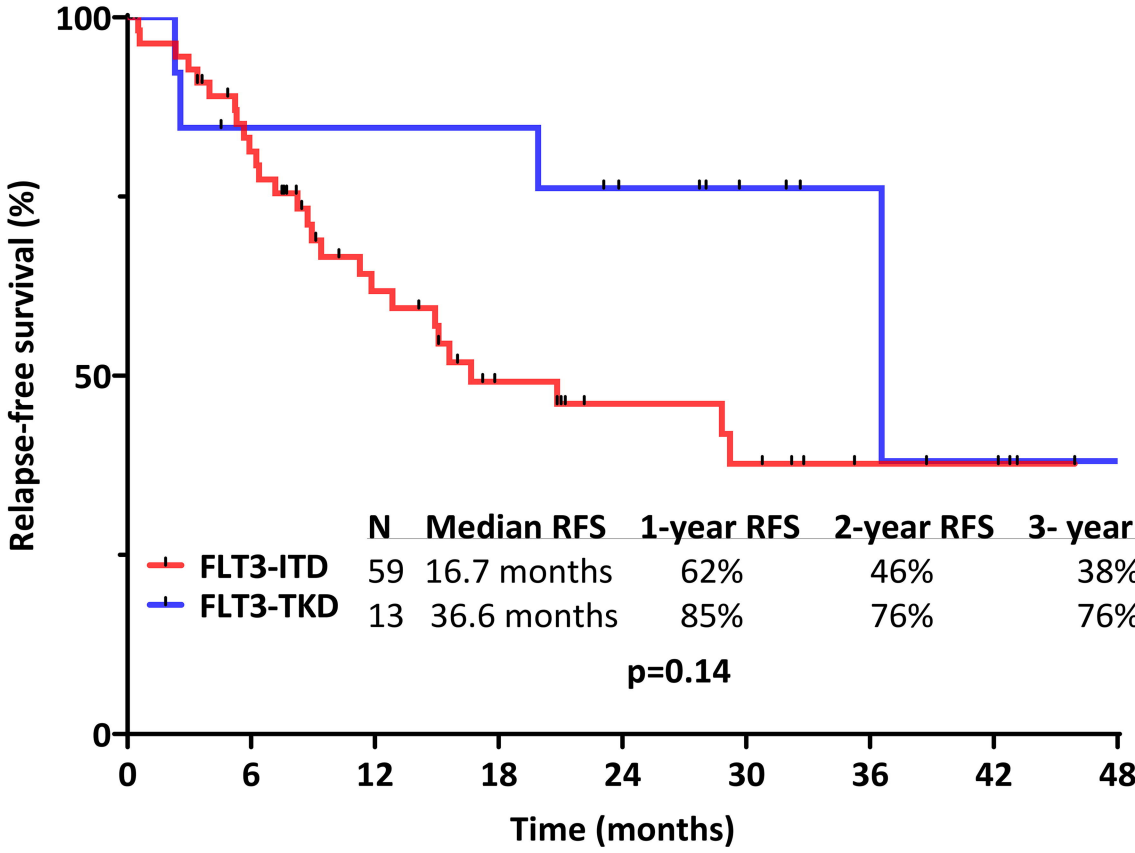
*MRD negativity was defined as *FLT3*-ITD $<5x10^{-5}$ (0.005%)



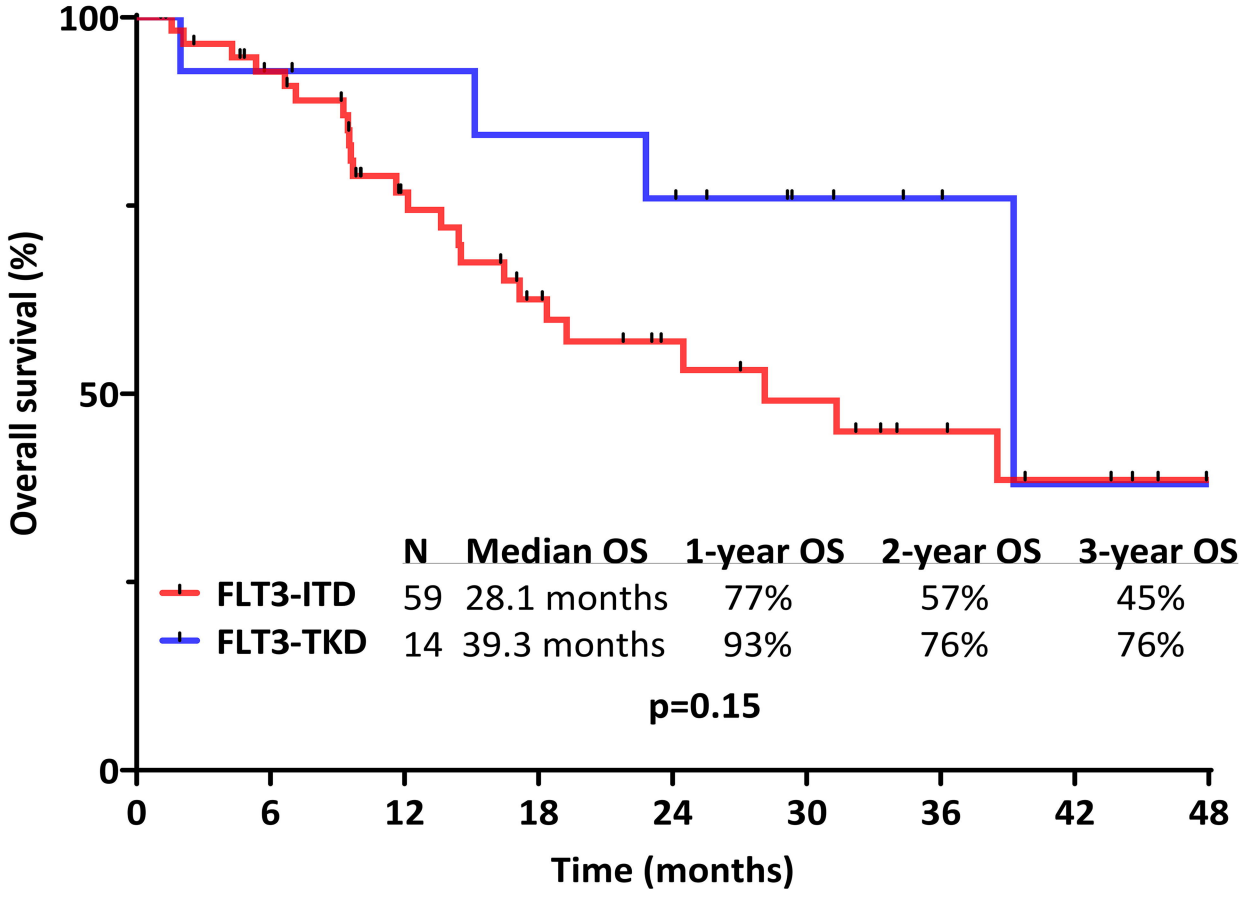


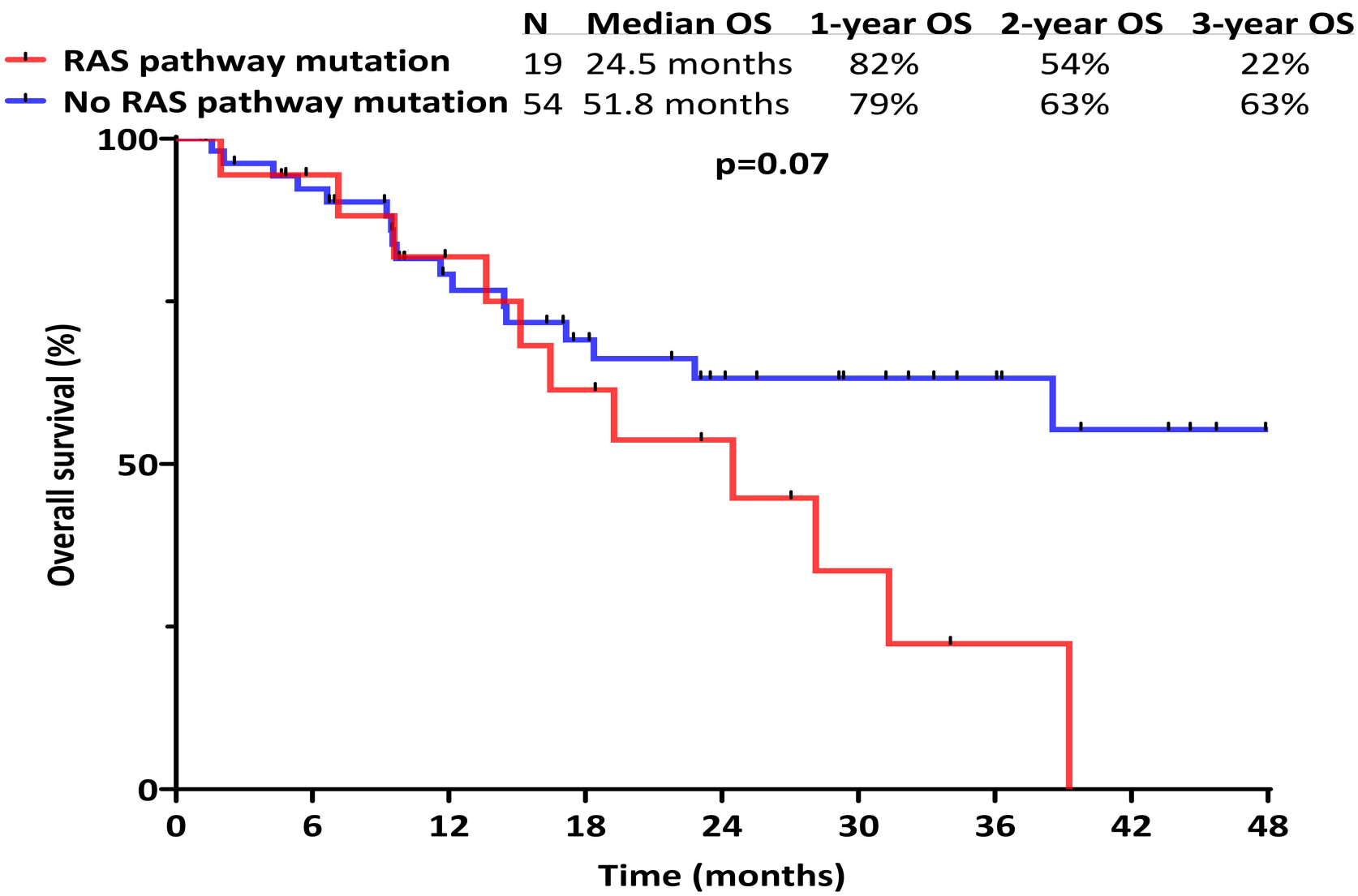
	Cycle 1	Cycle 2	Cycle 3	Cycle 4
Cumulative rate of NGS *FLT3*-ITD negativity	1/17 (6%)	10/21 (48%)	15/27 (56%)	23/33 (70%)

(A)


100
50
0
Relapse-free survival (%)
0
6
12
18
24
30
36
42
48
Time (months)
FLT3-ITD
FLT3-TKD
N Median RFS 1-year RFS 2-year RFS 3- year RFS
59 16.7 months 62% 46% 38%
13 36.6 months 85% 76% 76%
p=0.14

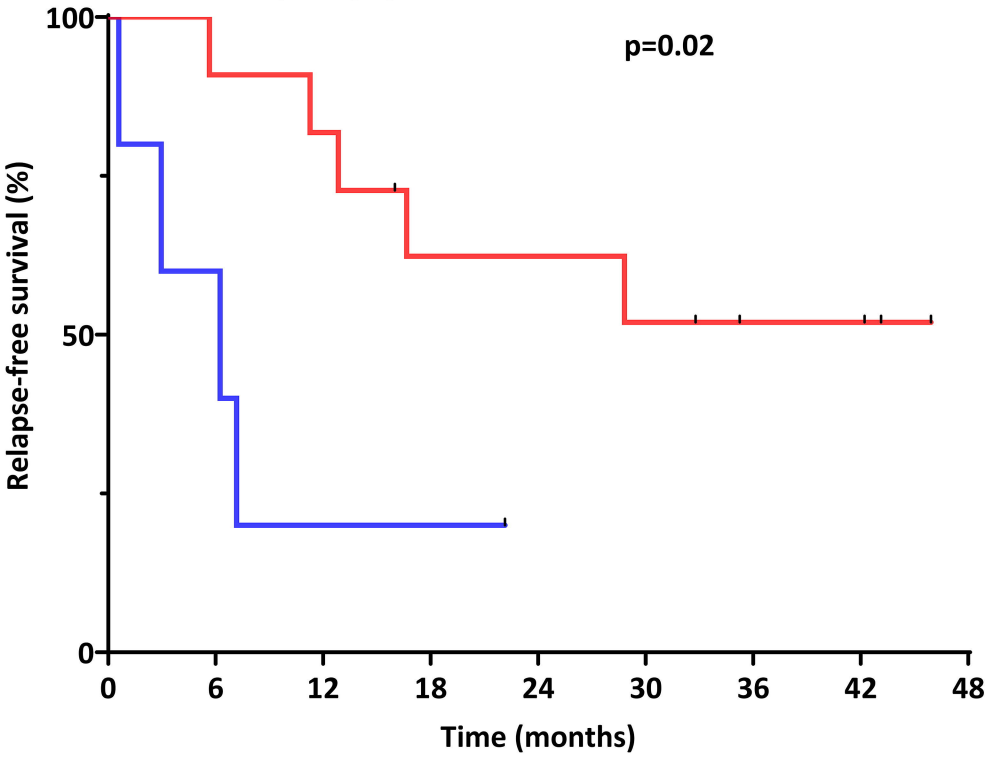
(B)


100
50
0
Overall survival (%)
0
6
12
18
24
30
36
42
48
Time (months)
FLT3-ITD
FLT3-TKD
N Median OS 1-year OS 2-year OS 3-year OS
59 28.1 months 77% 57% 45%
14 39.3 months 93% 76% 76%
p=0.15


N Median OS 1-year OS 2-year OS 3-year OS
RAS pathway mutation 19 24.5 months 82% 54% 22%
No RAS pathway mutation 54 51.8 months 79% 63% 63%
p=0.07
Overall survival (%)
100
50
0
0
6
12
18
24
30
36
42
48
Time (months)

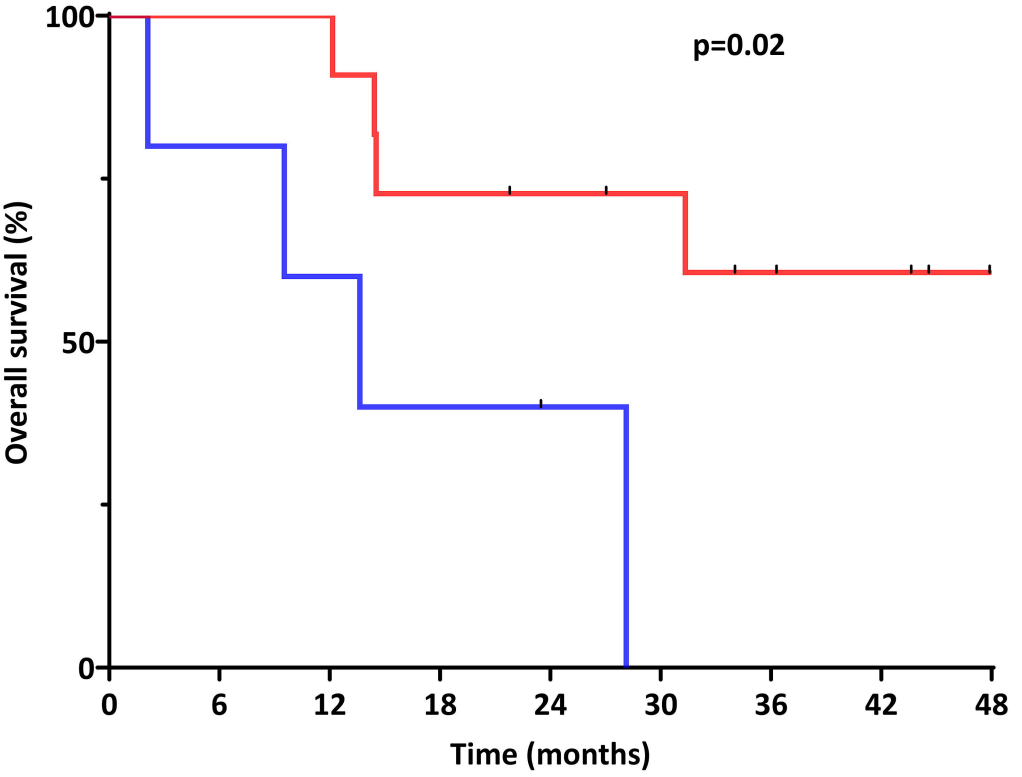
(A)

	N	Median RFS	1-year RFS	2-year RFS	3- year RFS
FLT3-ITD NGS MRD neg by cycle 4	11	Undefined	82%	62%	52%
FLT3-ITD NGS MRD pos by cycle 4	5	6.2 months	20%	20%	20%



(B)

	N	Median OS	1-year OS	2-year OS	3-year OS
FLT3-ITD NGS MRD neg by cycle 4	11	Undefined	100%	73%	61%
FLT3-ITD NGS MRD pos by cycle 4	5	13.6 months	60%	40%	0%



Title: Long-term outcomes in *FLT3*-mutated acute myeloid leukemia after frontline hypomethylating agent, venetoclax and a FLT3 inhibitor

Running title: Outcomes of triplet regimens in *FLT3*-mutated AML

Supplemental Table 1: Mutations included in the 81-gene targeted sequencing panel

Supplemental Table 2: Hematologic responses

Supplemental Table 3: Baseline characteristics of patients evaluated for the landmark analysis

Supplemental Table 4: Cytogenetic and molecular clonal evolution at time of relapse.

Supplemental Figure 1: Patient disposition

Supplemental Figure 2: Outcomes of the study cohort (A) Relapse-free survival, and (B) Overall survival

Supplemental Figure 3: Outcomes of the study cohort, stratified by age

Supplemental Figure 4: Impact of *FLT3* mutations on overall survival, stratified by age. (A) *FLT3-ITD* mutation, and (B) *FLT3-TKD* mutation

Supplemental Figure 5: Overall survival by subgroup. (A) *NPM1* co-mutation status, and (B) ELN 2022 risk stratification

Supplemental Figure 6: Overall survival stratified by *FLT3-ITD, NPM1, and DNMT3A* co-mutations status

Supplemental Figure 7: Overall survival stratified by receipt of hematopoietic stem cell transplantation (landmark analysis)

Supplemental Figure 8: Overall survival stratified by receipt of hematopoietic stem cell transplantation in patients <75 years of age (landmark analysis)

Supplemental Figure 9: Overall survival stratified by receipt of hematopoietic stem cell transplantation in patients with *FLT3-ITD* mutation (landmark analysis)

Supplemental Figure 10: Overall survival stratified by receipt of hematopoietic stem cell transplantation in patients with ELN adverse risk disease (landmark analysis)

Supplemental Figure 11: Overall survival after relapse

Supplemental Figure 12: Overall survival after relapse, stratified by *FLT3* mutation status by PCR at time of relapse

Supplemental Table 1. Mutations included in the 81-gene targeted sequencing panel

ANKRD26	*CBLB*	*EED*	*GFI1*	*JAK1*	*NF1*	*PTEN*	*SH2B3*	*SUZ12*
ASXL1	*CBLC*	*ELANE*	*GNAS*	*JAK2*	*NOTCH1*	*PTPN11*	*SMC1A*	*TERC*
ASXL2	*CEBPA*	*ETNK1*	*HNRNPK*	*JAK3*	*NPM1*	*RAD21*	*SMC3*	*TERT*
BCOR	*CREBBP*	*ETV6*	*HRAS*	*KDM6A*	*NRAS*	*RARA*	*SRSF2*	*TET2*
BCORL1	*CRLF2*	*EZH2*	*IDH1*	*KIT*	*PAX5*	*RUNX1*	*STAG1*	*TP53*
BRAF	*CSF3R*	*FBXW7*	*IDH2*	*KMT2A*	*PHF6*	*SETBP1*	*STAG2*	*U2AF1*
BRINP3	*CUX1*	*FLT3*	*IKZF1*	*KRAS*	*PIGA*	*SF1*	*STAT3*	*U2AF2*
CALR	*DDX41*	*GATA1*	*IL2RG*	*MAP2K1*	*PML*	*SF3A1*	*STAT5A*	*WT1*
CBL	*DNMT3A*	*GATA2*	*IL7R*	*MPL*	*PRPF40B*	*SF3B1*	*STAT5B*	*ZRSR2*

Supplemental Table 2. Hematologic responses

Response, N (%)	**N=73**
CRc (CR+CRi)	68 (93)
CR	60 (82)
CRi	8 (11)
MLFS	4 (6)
ORR (CR + CRi + MLFS)	72 (99)
Early death	1 (1)

Abbreviations: CRc, composite complete remission; CR, complete remission; CRi, complete remission with incomplete count recovery; MLFS, morphologic leukemia-free state; ORR, overall response rate

Supplemental Table 3. Baseline characteristics of patients evaluated for the landmark analysis

Characteristic[1]	Transplanted cohort (N=30)	Non-transplanted cohort (N=32)	Univariate p-value
Age, years			
Median [range]	67 [18-75]	72 [61-88]	**0.001**
≥75 years	1 (3)	14 (44)	**0.0002**
Cytogenetics			
Diploid	15 (60)	13 (41)	
Adverse	6 (20)	4 (13)	0.57
Others	7 (23)	12 (38)	
Insufficient	2 (7)	3 (22)	
ELN 2022 risk stratification			
Favorable	5 (17)	3 (9)	0.63
Intermediate	9 (30)	12 (38)	
Adverse	16 (53)	17 (53)	
FLT3 subtype			
ITD	26 (87)	24 (75)	0.41
TKD	4 (13)	7 (22)	
ITD+TKD	0	1 (3)	
FLT3 variant allelic frequency			
ITD	18 [1-53]	25 [3-75]	0.1
TKD	34 [26-57]	14 [13-51]	0.07
FLT3 inhibitor			
Gilteritinib	21 (70)	21 (66)	
Quizartinib	6 (20)	8 (25)	0.88
Sorafenib	3 (10)	2 (6)	
Midostaurin	0	1 (3)	
Hypomethylating agent			
Azacitidine	15 (50)	16 (50)	0.99
Decitabine	15 (50)	16 (50)	
Mutations[2]			
NPM1	13 (43)	15 (47)	0.78
DNMT3A	11 (37)	16 (50)	0.29
WT1	8 (27)	4 (13)	0.21
RUNX1	7 (23)	8 (25)	0.88
BCOR	5 (17)	5 (16)	0.91
IDH1	5 (17)	0	**0.01**

IDH2	4 (13)	5 (16)	0.99
TET2	3 (10)	10 (31)	0.06
ASXL1	3 (10)	2 (6)	0.79
NF1	3 (10)	0	0.06
RAD21	3 (10)	2 (6)	0.58
U2AF1	3 (10)	3 (9)	0.93
BCORL1	2 (7)	3 (9)	0.69
NRAS	2 (7)	1 (3)	0.51
PTPN11	2 (7)	3 (9)	0.69
SF3B1	2 (7)	2 (6)	0.94
SRSF2	2 (7)	5 (16)	0.26
STAG2	2 (7)	3 (9)	0.69
CEBPA	1 (3)	5 (16)	0.10
***RAS* pathway mutation[3]**	9 (30)	6 (19)	0.30

[1] Values are listed as median [range] or n (%)

[2] Mutations detected in ≥5% of the study cohort

[3] Includes *KRAS, NRAS, PTPN11, CBL, NF1* and/or *BRAF* mutations

Abbreviations: ELN, European LeukemiaNet; ITD, internal tandem duplication; TKD, tyrosine kinase domain

Supplemental Table 4. Cytogenetic and molecular clonal evolution at time of relapse.

Patient	Regimen	Cytogenetics at diagnosis	Cytogenetics at relapse	Cytogenetic evolution	Mutation(s) at diagnosis	Mutation(s) at relapse	Mutational evolution
#1	Azacitidine + venetoclax + gilteritinib	46,XX,t(7;11)(p15; p15)[20]	46,XX,t(7;11)(p15; p15)[17]/46XX[3]	No	**FLT3 ITD** RUNX1 F163fs KRAS Q22K	RUNX1 F163fs **NRAS G12A** **NRAS G13R** **GATA2 A372T**	Yes
#2	Azacitidine + venetoclax + gilteritinib	47,XY,+11[20]	47,XY,+11[8]/**49,idem,+14,+18[4]**/46,XY[8]	Yes	**FLT3 D835Y** **SF3B1 K700E** **SMC1A I9T** **NRAS G13C** DNMT3A R882H TET2 N1156Y **STAG2 K493fs**	DNMT3A R882H TET2 N1156Y **TET2 L1101fs** **ZRSR2 C326G** **GATA2 R307Q (11)**	Yes
#3	Azacitidine + venetoclax + gilteritinib	N/A	N/A	N/A	FLT3 ITD NPM1 W288fs IDH2 R140Q DNMT3A Splice	N/A	N/A
#4	Azacitidine + venetoclax + gilteritinib	N/A	N/A	N/A	**FLT3 ITD** U2AF1 S34F DNMT3A R882H TET2 Y1148fs BCOR R1480* **BCORL1 P334fs** PTPN11 N58Y	**FLT3 TKD** U2AF1 S34F DNMT3A R882H TET2 Y1148fs TET2 Q531* BCOR R1480* PTPN11 N58Y	Yes
#5	Azacitidine + venetoclax + gilteritinib	46, XY[20]	N/A	N/A	FLT3 ITD NPM1 p.W288fs IDH1 R132H DNMT3A R882	N/A	N/A
#6	Azacitidine + venetoclax + gilteritinib	46,XY,del(12)(p13 p12)[13]/46,XY[7]	46,XY,del(12)(p13 p12)[3]/47,**idem,+21**[1]/46,XY[6]	Yes	FLT3 D835E RUNX1 splice DNMT3 I705T	FLT3 D835E RUNX1 splice DNMT3 I705T **IKZF1 p.N159S**	Yes

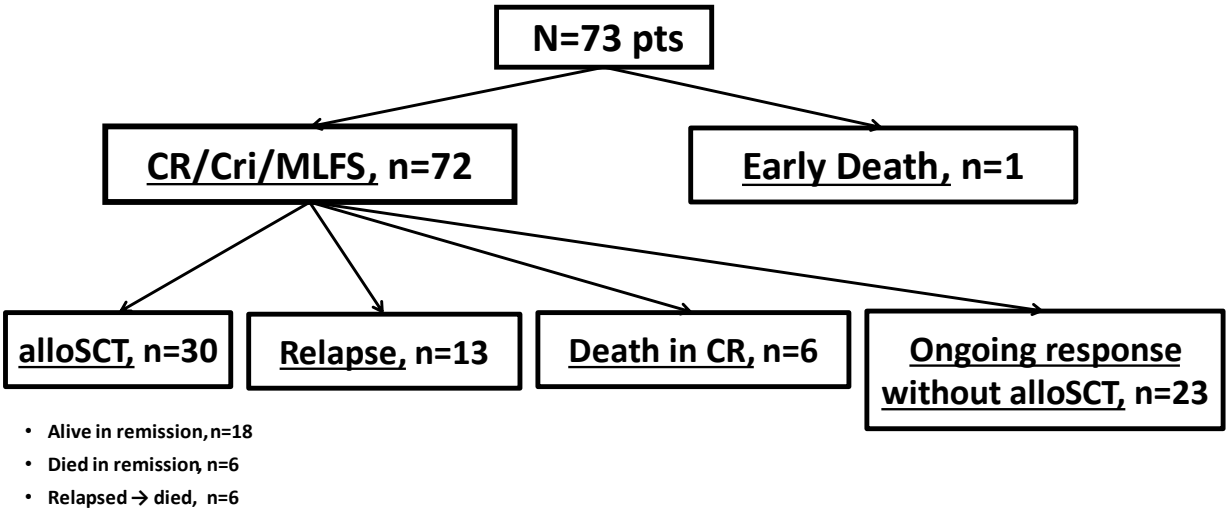
						PTPN11 p.G60R	
#7	Azacitidine + venetoclax + gilteritinib	N/A (FISH pos for KMT2A)	46,XY,t(11;19)(q23;p13.3)[20]	No	**FLT3 ITD** **STAG2 N58fs**	None	No
#8	Decitabine + venetoclax + quizartinib	46,XX,inv(3)(p23q26.2)[18]/45,idem,t(4;5)(q21;p15.1),-21[1]/47,XX,add(5)(p15.3),+13[1]	46,XX,inv(3)(p23q26.2)[3]/45**,idem,-7**[16]/46,XX[1]	Yes	**FLT3 ITD** RUNX1 H85fs RUNX1 G412S DNMT3A R882H SRSF2 P95L	DNMT3A R882H SRSF2 P95L **IKZF1 p.H163Q** **IKZF1 Y180_A181del** **IKZF1 N159K** RUNX1 H85fs RUNX1 G412S **RUNX1 I193N** **NRAS Q61H** **NRAS G13D**	Yes
#9	Decitabine + venetoclax + quizartinib	46,XX,add(12)(q24.3)[4]/46,XX[16]	46,XX[20]	No	**FLT3 ITD** SUZ12 F295S **RUNX1 R201** **TET2 R550*** TET2 T1554fs SRSF2 P95H PFH6 p.R225 CBL R420Q SH2B3 I446N	CBL R420Q SUZ12 F295S SH2B3 I446N SH2B3 S503fs ET2 R550* TET2 T1554fs SRSF2 P95H PFH6 p.R225 **GATA2 L305V**	Yes
#10	Decitabine + venetoclax + quizartinib	46,XX,t(4;7)(q21;q32),del(21)(q22)[20]	46,XX,t(4;7)(q21;q32)[5], 46XX[1]	No	FLT3 ITD WT1 T460_C461delinsS	FLT3 ITD WT1 T460_C461delinsS **CBL C384Y**	Yes
#11	Azacitidine + venetoclax + gilteritinib	46,XY,t(7;11)(p15;p15)[20]	46,XY,t(7;11)(p15;p15)[18]/46,XY[2]	No	**FLT3 D835** MPL L580fs WT1 R385fs RUNX1 A352fs	MPL L580fs WT1 R385fs RUNX1 A352fs	No

#12	Decitabine + venetoclax + gilteritinib	46, XX[20]	46,XX[20]	No	**FLT3 D835Y** WT1 M415fs **WT1 A387fs** **IDH2 R140Q** NRAS G13R BRINP3 S592N DNMT3A G413V **BCOR V1653A**	WT1 M415fs DNMT3A G413V NRAS G13R BRINP3 S592N	No
#13	Decitabine + venetoclax + sorafenib	46,XX,add(2)(q21), add(19)(p13.3)[1]/ 46,XX[19]	46,XX[19], 46,XY[1]	No	**FLT3 ITD** NPM1 W288fs **RAD21 I17N** TET2 Q1274fs TET2 V328fs	NPM1 W288fs WT1 G183V TET2 Q1274fs TET2 V328fs	Yes
#14	Decitabine + venetoclax + sorafenib	46,XX,t(3;6)(q26.2 ;q25)[20]	46,XX,t(3;6)(q26.2 ;q25)[9], 46XX[11]	No	**FLT3 ITD** BCORL1 Q1133 **PHF6 G10fs** ASXL1 S577*	**SF3B1 K666N** BCORL1 Q1133 ASXL1 S577*	Yes
#15	Decitabine + venetoclax + sorafenib	46, XX[20]	46,XX,**t(4;17)(q12; q25)[**15]/46,XX[5]	Yes	**FLT3 ITD** IDH1 R132C U2AF1 S34F **NRAS G12D** DNMT3A R882H BCOR Splice	IDH1 R132C U2AF1 S34F DNMT3A R882H BCOR Splice	No
#16	Decitabine + venetoclax + quizartinib	46,XY,t(10;12)(q24 ;p12)[1]/46,XY,del (16)(p11.2)[1]/46, XY[18]	47,XY**,+6,add(7)(q 32)[6]/47,idem[cp 2]**//46,XX[12]	Yes	FLT3 ITD **WT1 Q414fs** **BCOR Splice** BCORL1 R1145* **NF1 T1310fs**	FLT3 ITD BCORL1 R1145*	No
#17	Oral decitabine + venetoclax + gilteritinib	46, XX[20]	46,XX[20]	No	**FLT3 ITD** NPM1 W288fs IDH2 R140Q DNMT3 A R882H KIT D816V	NPM1 W288fs IDH2 R140Q DNMT3 A R882H KIT D816V	No

#18	Azacitidine + venetoclax + gilteritinib	46, XX[20]	47,XX, **+4**[20]	Yes	FLT3 ITD TET2 K1339* TET2 F1287V	FLT3-ITD TET2 K1339* TET2 F1287V	No
#19	Oral decitabine + venetoclax + gilteritinib	46, XY[20]	47,XY, +mar[1]/46,XY[9]	N/A	FLT3 ITD NPM1 W288fs IDH2 R140Q SRSF2 P95L	FLT3-ITD NPM1 W288fs IDH2 R140Q SRSF2 P95L	No

Bold font indicates changes detected between diagnostic and relapse samples.

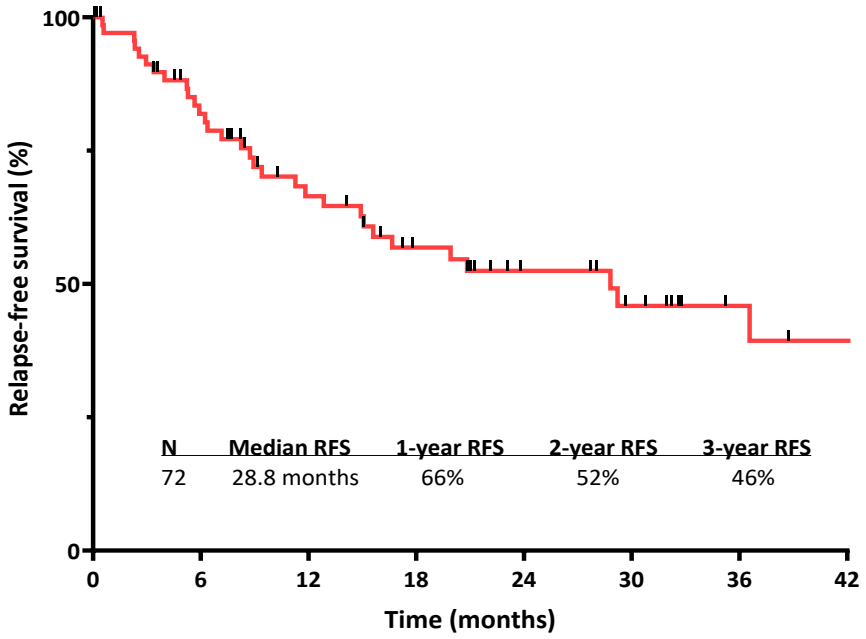
Supplemental Figure 1. Patient disposition



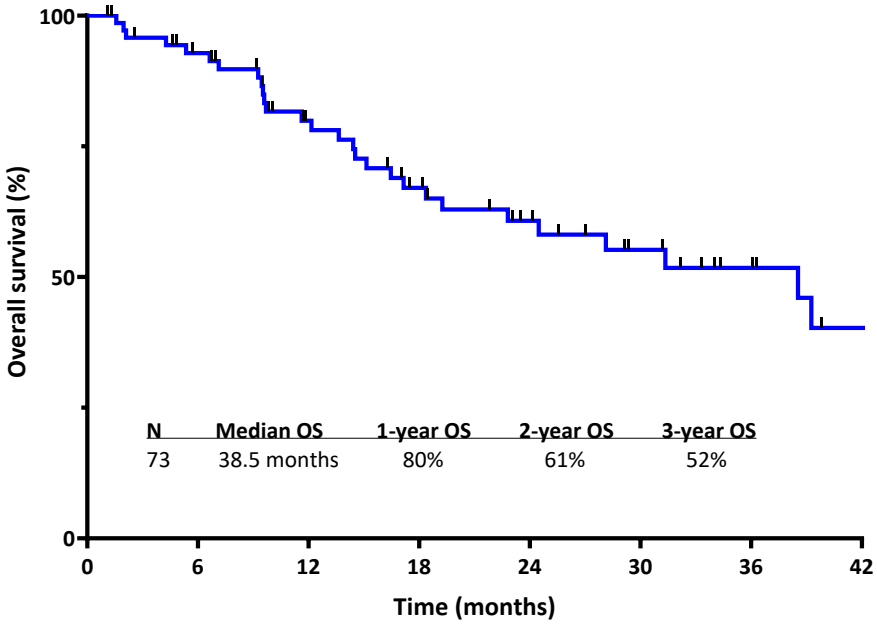
- **Alive in remission, n=18**
- **Died in remission, n=6**
- **Relapsed → died, n=6**

Supplemental Figure 2. Outcomes of the study cohort (A) Relapse-free survival, and (B) Overall survival

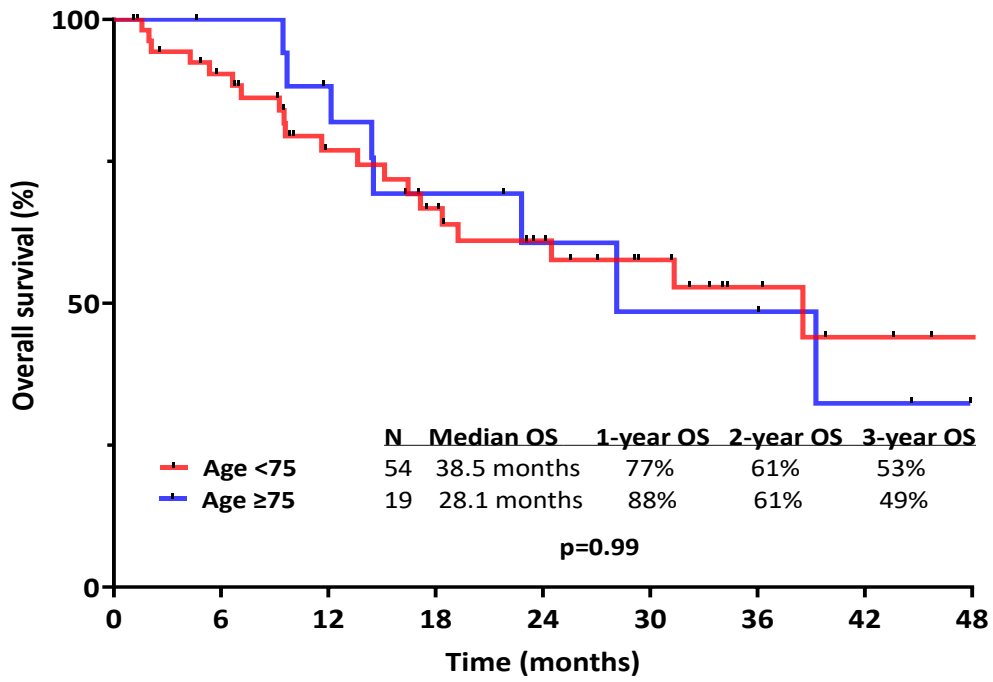
(A)



(B)

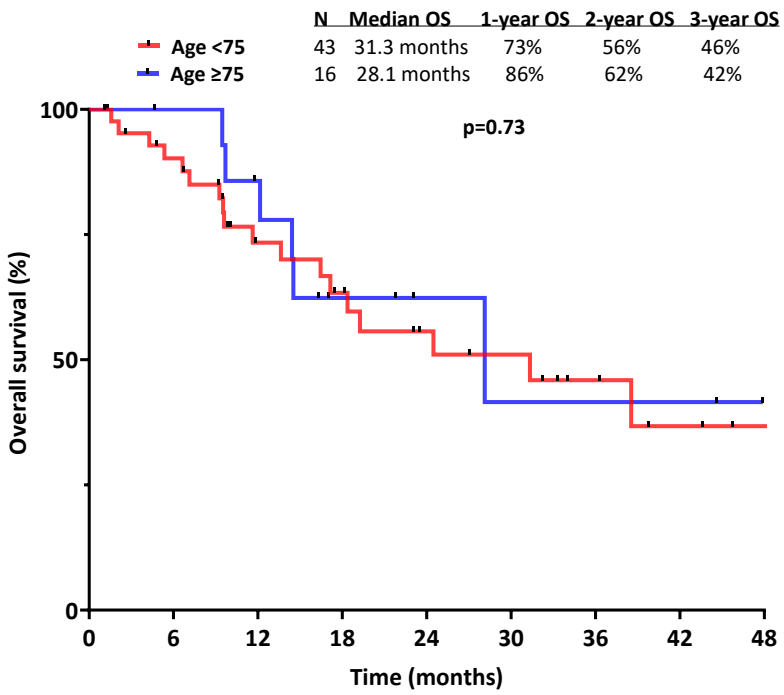


Supplemental Figure 3. Outcomes of the study cohort, stratified by age

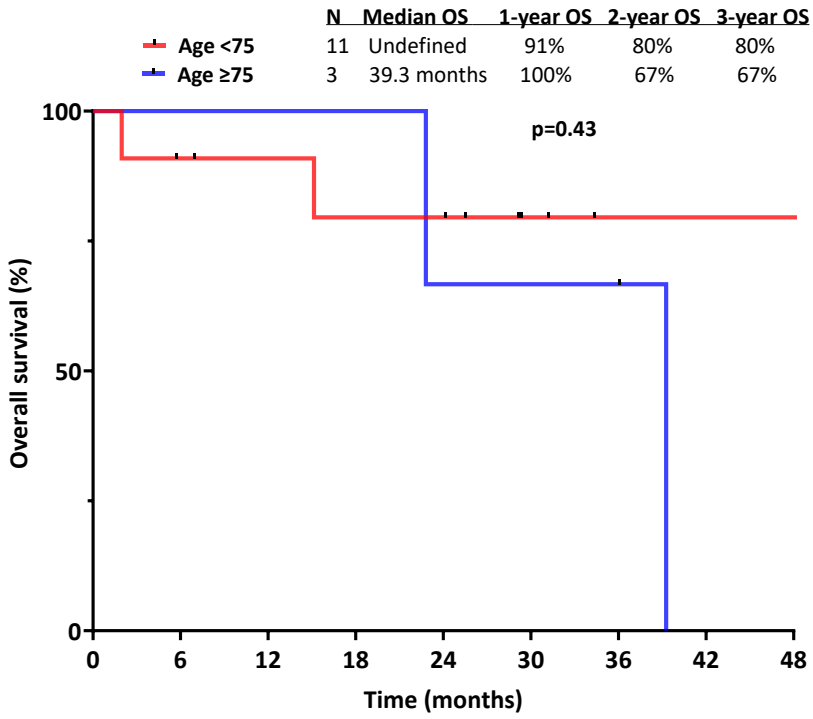


Supplemental Figure 4. Impact of *FLT3* mutations on overall survival, stratified by age. (A) *FLT3-ITD* mutation, and (B) *FLT3-TKD* mutation

(A)

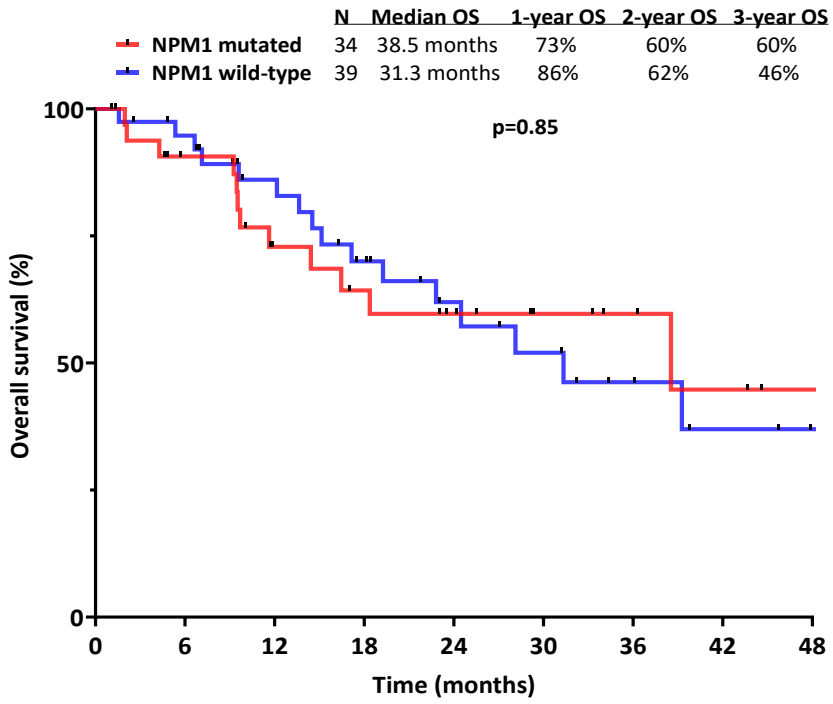


(B)

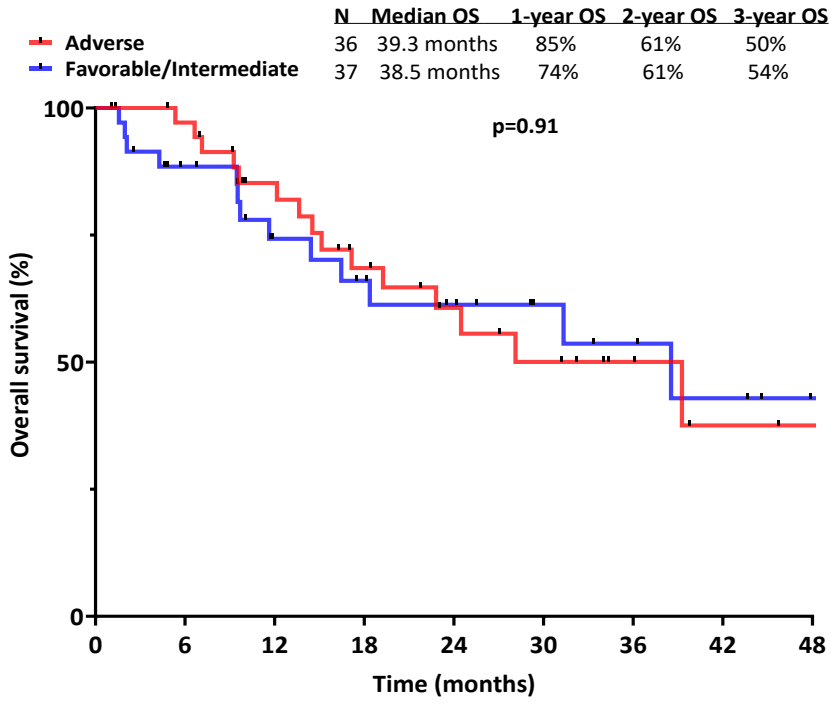


Supplemental Figure 5. Overall survival by subgroup. (A) *NPM1* co-mutation status, and (B) ELN 2022 risk stratification

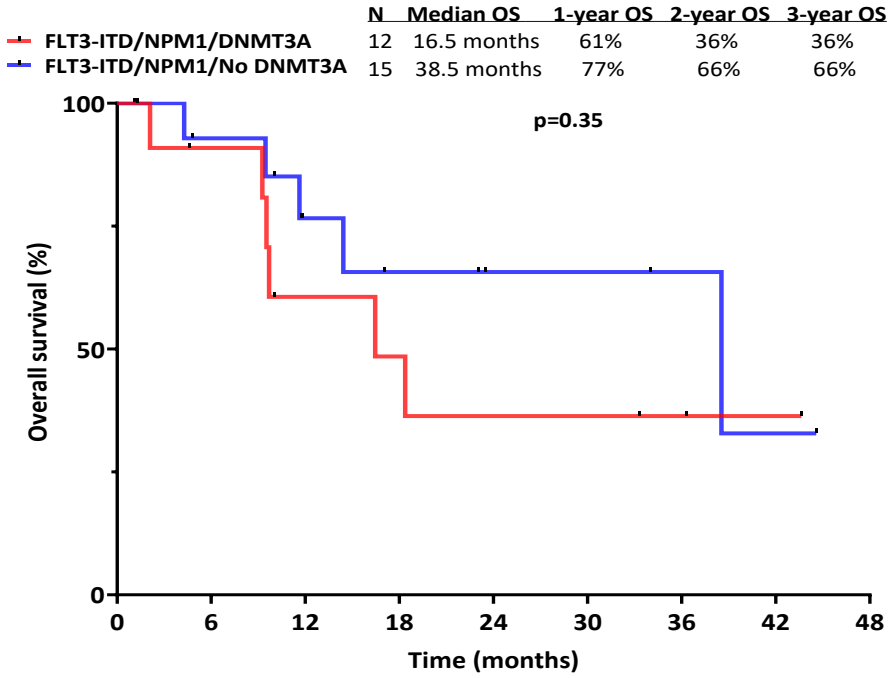
(A)



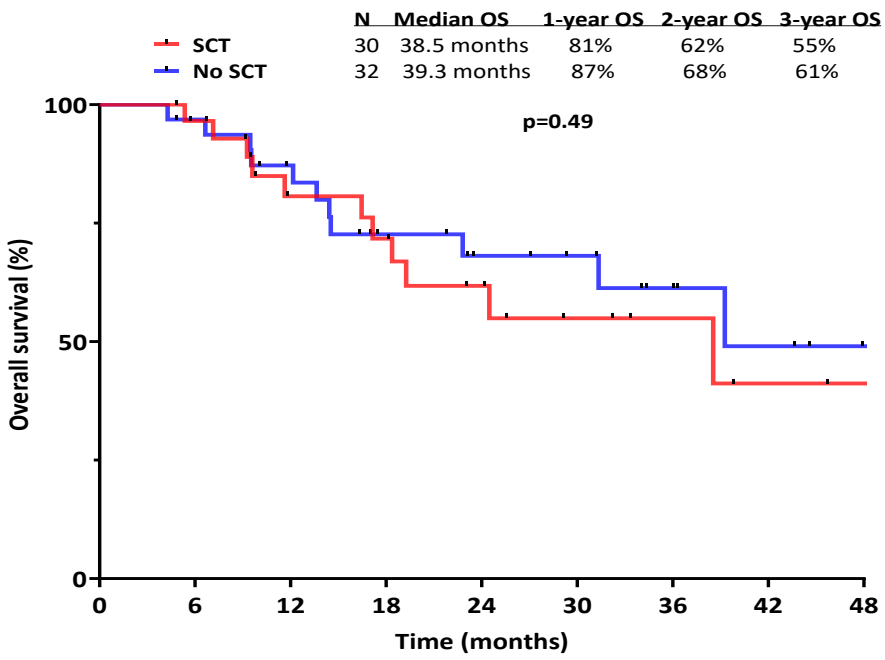
(B)



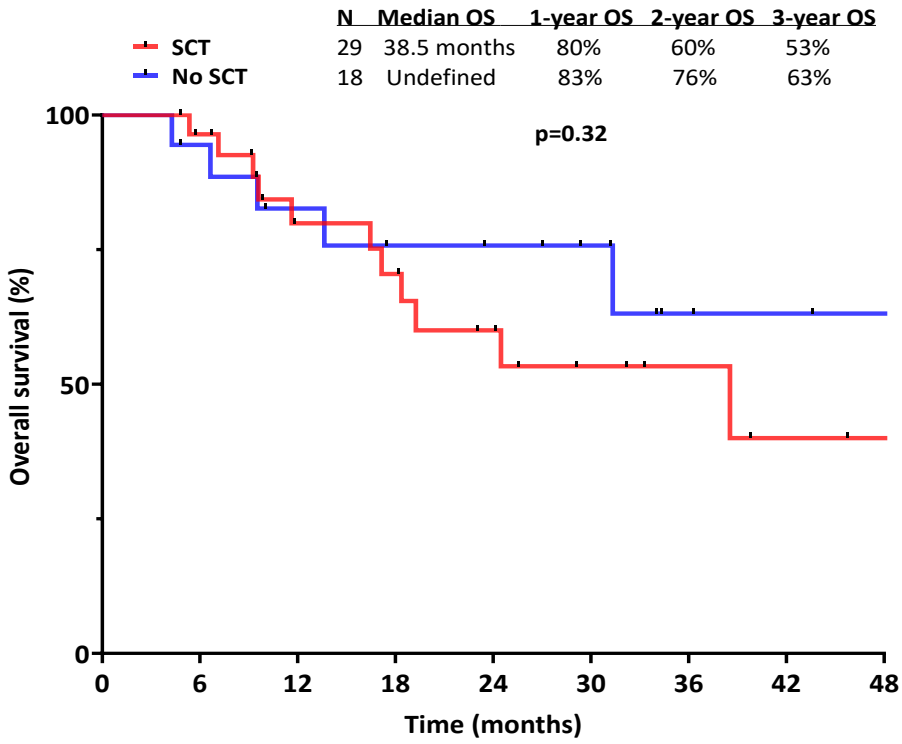
Supplemental Figure 6. Overall survival stratified by *FLT3-ITD, NPM1, and DNMT3A* co-mutations status



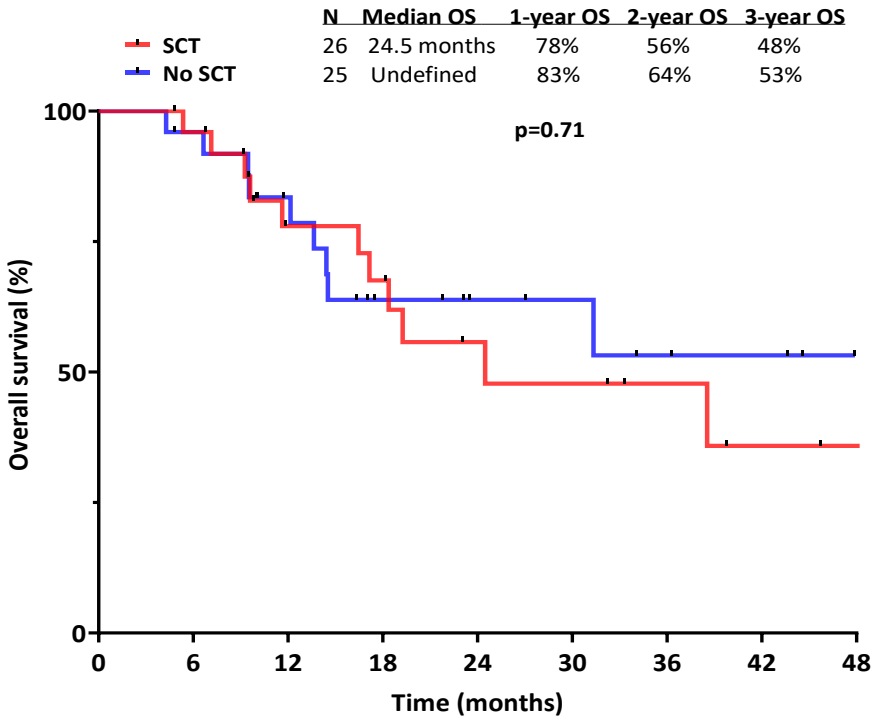
Supplemental Figure 7. Overall survival stratified by receipt of hematopoietic stem cell transplantation (landmark analysis)



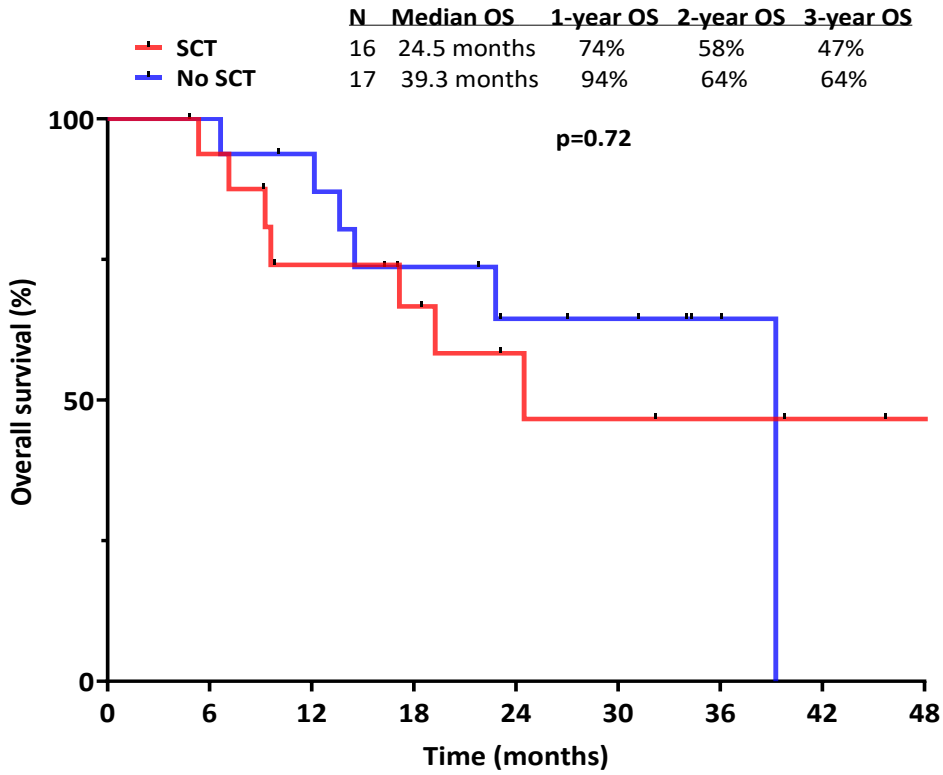
Supplemental Figure 8. Overall survival stratified by receipt of hematopoietic stem cell transplantation in patients <75 years of age (landmark analysis)



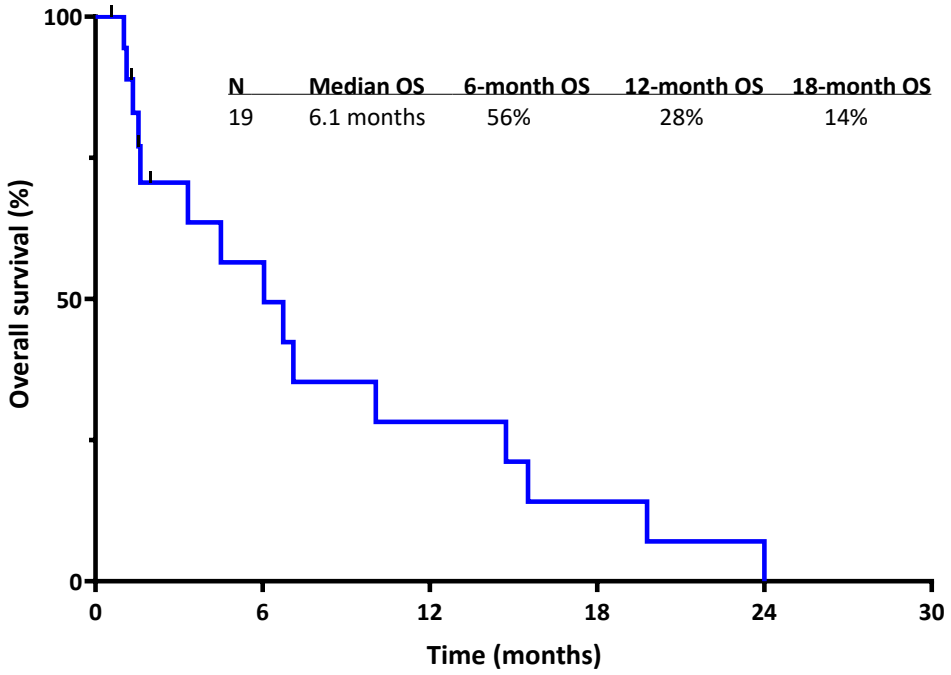
Supplemental Figure 9. Overall survival stratified by receipt of hematopoietic stem cell transplantation in patients with *FLT3-ITD* mutation (landmark analysis)



Supplemental Figure 10. Overall survival stratified by receipt of hematopoietic stem cell transplantation in patients with ELN adverse risk disease (landmark analysis)



Supplemental Figure 11. Overall survival after relapse



Supplemental Figure 12. Overall survival after relapse, stratified by *FLT3* mutation status by PCR at time of relapse