# FLT3 inhibitors potentially improve response rates in acute myeloid leukemia harboring t(6;9)(*DEK::NUP214*): the Mayo Clinic experience

Translocation (6;9)(p23;q34.1) is detected in 1% of acute myeloid leukemia (AML) cases and is considered a high-risk subtype by European Leukemia Network (ELN) 2022 criteria.[1] Compared to AML overall, t(6;9) AML affects younger adults with a median age of 35-38 years at diagnosis, and is historically associated with poor overall survival (OS) of roughly 14 months.[2,3] Most cases (62-88%) harbor internal tandem duplications of fms-like tyrosine kinase 3 (*FLT3*-ITD).[2-6] While preliminary data suggest that FLT3 inhibitors (FLT3i) might provide benefit,[7,8] this has not yet been confirmed in larger studies. Although outcomes have improved with allogeneic hematopoietic cell transplantation (alloHCT),[6,9,10] survival remains dismal for patients ineligible for transplant.[3] This report describes a three-site experience with t(6;9) AML at an academic tertiary care center with a large HCT referral program.

After institutional review board approval, all patients with t(6;9)(p23;q34.1) AML seen across the three Mayo Clinic sites in the US between 2010-2023 were identified via retrospective chart review; this included eight previously-published patients.[11] A separate AML cohort excluding t(6;9) was curated pragmatically over a similar time frame. Data from the time of diagnosis or presentation to Mayo Clinic were abstracted. Mutation assessment was performed as described.[12] When necessary, *FLT3* allelic ratio was estimated from the variant allele fraction (VAF).[12,13] Classification and response were assessed by ELN 2022 criteria.[1] Measurable residual disease (MRD) was assessed by t(6;9)(p23;q34.1) specific fluorescence *in situ* hybridization (FISH) of ≥500 nuclei (sensitivity 0.6%) or multiparametric flow cytometry (MFC; sensitivity 0.01%). Categorical variables were compared by Fisher exact or Pearson $\chi^2$ tests and continuous variables by Mann-Whitney U tests or two-way ANOVA with Tukey correction. Univariate and multivariate analyses utilized Cox proportional hazards models. Survival was assessed via the Kaplan-Meier method with log-rank comparisons. Calculations were performed with BlueSky Statistics (v10.3.1) or GraphPad Prism (v.10.1.2). $P<0.05$ was considered significant.

Twenty-one patients (12 females, 57%) with t(6;9) AML were identified with median age 39 years (Table 1). Anemia was common (N=19, 90%) and thrombocytopenia was universal. Peripheral blasts were identified in 19 (90%) patients. Cytogenetic analyses identified isolated t(6;9)(p23;q34.1) in eight (40%) cases, whereas three (15%) exhibited a complex karyotype. Most cases (N=14, 70%) harbored *FLT3*-ITD mutations (median VAF 39%), which were the sole mutations identified in all 14 cases that harbored them (*Online Supplementary Figure S1A, B*). No FLT3 tyrosine kinase domain mutations were detected.

Most (N=19, 90%) received frontline induction therapy with cytarabine plus an anthracycline (*Online Supplementary Table S1*). Six of 14 (43%) patients harboring *FLT3*-ITD received a FLT3i during induction. Four (29%) additional patients received a FLT3i with consolidation or salvage therapy. Following induction, 12 of 19 (63%) evaluable patients achieved a compete response (CR) or CR with either partial (CRh) or incomplete (CRi) hematologic recovery. An additional five patients achieved CR/CRh/CRi after salvage or consolidative chemotherapy (Table 1). All *FLT3* wild-type (WT) cases eventually achieved a CR/CRh/CRi for an overall response rate (ORR) of 100%. Amongst *FLT3*-ITD cases, the ORR was 77%. Sixteen patients (76%) proceeded to alloHCT.

The role of MRD assessment in adverse-risk AML is not well defined.[14] Because methodology has evolved over time, the present analysis considered MRD based on mean fluorescense intensity (MFC) (N=4) or FISH (N=11) testing within 60 days of induction, prior to transplant, or at post-transplant day +100 (*Online Supplementary Figure S1C*). After induction, only two of 11 (18%) patients achieving morphologic CR had undetectable MRD. Ten of 11 (91%) evaluable patients were MRD-negative prior to alloHCT. All evaluable patients were MRD-negative at post-transplant day +100. Of the four patients with MRD testing strictly by MFC, all were MRD-negative prior to HCT, and the three evaluable cases remained MRD-negative on day +100 (*Online Supplementary Figure S1D*). When both MFC and FISH were performed, all results were concordant.

Eight patients (38%) were deceased at last follow-up. With median follow-up of 72 months, the 2-year OS of the cohort was 71% while the median OS (mOS) was not reached (NR; Figure 1A).

The mOS was NR irrespective of *FLT3* status ($P=0.45$; Figure 1B). Amongst *FLT3*-ITD cases, OS was numerically longer (NR *vs*. 24 months, $P=0.17$; Figure 1C) and there were fewer deaths (1 *vs*. 5) amongst those who received a FLT3i during induction (Table 2). Accordingly, the 2-year OS was numerically higher for those receiving a FLT3i with induction (83% *vs*. 50%). Although statistically insignificant, these trends again suggest that FLT3i benefit a subgroup of patients.

AlloHCT significantly prolonged survival compared to those who were not transplanted (mOS NR *vs*. 19 months; $P=0.0001$; Figure 1D). Two-year survival rates were also superior with alloHCT (88% *vs*. 20%; Table 2). Moreover, alloHCT was beneficial irrespective of *FLT3* status ($P<0.03$ for both compari-

sons; Figure 1E, F). There was no difference in survival when patients were stratified by myeloablative *versus* non-myeloablative conditioning intensity (*P*=0.55) or conditioning regimen (*P*=0.40). Transplant-related mortality (TRM) was 6%, as one patient expired on post-HCT day +32 from sinusoidal obstruction syndrome.

Analogous results were obtained when patients with complex cytogenetics (N=3) were excluded, with mOS of 81 months and 2-year OS of 65% for the remaining patients. There was again no difference in mOS when stratified by *FLT3* status (*P*=0.21). AlloHCT similarly improved mOS (NR *vs*. 19 months; *P*=0.0014), with 2-year OS of 83% *versus* 20% for those who did and did not undergo HCT, respectively.

There was no difference in OS based on MRD status after induction (*P*=0.64) or prior to alloHCT (*P*=0.75; *Online Supplementary Figure S1E, F*). When stratified by MRD-negativity at any time prior to HCT (if transplanted), there was a trend toward improved survival compared to MRD-positive patients (mOS NR *vs*. 29 months; *P*=0.069; *Online Supplementary Figure S1G*). Accordingly, the 2-year survival was higher (90% *vs*. 60%) with fewer deaths (1 *vs*. 3) amongst MRD-negative patients compared to positive patients (Table 2). However, these analyses are confounded, as all patients who achieved MRD negativity proceeded to alloHCT. Amongst the four patients with MRD testing by MFC, the mOS was NR with 2-year OS of 75% (*Online Supplementary Figure S1H*).

Survival of the t(6;9) AML cohort was better than anticipated; therefore, these outcomes were compared to those from a

**Table 1.** Patient demographics.

| Metric | Evaluable cases N | Result |
|---|---|---|
| **Demographics** | | |
| Age in years at diagnosis, median (range) | 21 | 39 (15-67) |
| Male, N (%) | 21 | 9 (43) |
| Female, N (%) | 21 | 12 (57) |
| **Laboratory parameters** | | |
| Hemoglobin g/dL, median (range) | 21 | 8.6 (5.8-13.0) |
| Mean corpuscular volume fL, median (range) | 20 | 98.6 (82.7-110.1) |
| Platelet count x$10^9$/L, median (range) | 21 | 42 (18-89) |
| White blood cell count x$10^9$/L, median(range) | 21 | 9.7 (0.5-99.5) |
| Absolute neutrophil count x$10^9$/L, median (range) | 20 | 1.73 (0.12-16.73) |
| Peripheral blood blasts, median % (range) | 21 | 20 (0-89) |
| Bone marrow blasts, median % (range) | 19 | 63 (7-90) |
| **Cytogenetic parameters** | | |
| *DEK::NUP214* FISH nuclei, median % (range) | 14 | 78.1 (24.8-98.2) |
| Isolated t(6;9)(p23;q34.1), N (%) | 20 | 8 (40) |
| Complex karyotype, N (%) | 20 | 3 (15) |
| **Mutation status** | | |
| Number of mutations, median (range) | 21 | 1 (0-2) |
| *FLT3*-ITD, N (%) | 20 | 14 (70) |
| *FLT3*-ITD allelic ratio, median (range) | 14 | 0.5 (0.05-7.3) |
| *FLT3*-ITD variant allele fraction, median (range) | 14 | 39 (10-88) |

| Metric | Evaluable cases N | Result |
|---|---|---|
| **Treatment** | | |
| Anthracycline-based induction in first line, N (%) | 21 | 19 (90) |
| Azacitidine plus venetoclax in first line, N (%) | 21 | 1 (5) |
| Received a FLT3i with induction,[1] N (%) | 14 | 6 (43) |
| Received a FLT3i in later lines only,[2] N (%) | 14 | 4 (29) |
| **Allogeneic HCT parameters** | | |
| Underwent allogeneic HCT, N (%) | 21 | 16 (76) |
| Myeloablative conditioning, N (%) | 16 | 13 (81) |
| Busulfan and cyclophosphamide conditioning, N (%) | 16 | 5 (31) |
| Busulfan and fludarabine conditioning, N (%) | 16 | 5 (31) |
| Matched related donor, N (%) | 16 | 5 (31) |
| Matched unrelated donor, N (%) | 16 | 6 (38) |
| Mobilized peripheral blood stem cell source, N (%) | 16 | 14 (88) |
| **Response** | | |
| Achieved CR, CRh, or CRi after induction, N (%) | 19 | 12 (63) |
| Achieved CR, CRh, or CRi at any time, N (%) | 20 | 17 (85) |
| Lines of therapy to first CR, CRh, or CRi, median (range) | 17 | 1 (1-3) |
| Total lines of therapy received, median (range) | 20 | 3 (1-8) |
| **Outcomes** | | |
| Deceased, N (%) | 21 | 8 (38) |
| Transplant related mortality, N (%) | 16 | 1 (6) |

[1]Patients who received a FLT3 inhibitor (FLT3i) with induction therapy may have also received a FLT3i during consolidation or in later lines of therapy (*Online Supplementary Table S1*); [2]indictates patients with a *FLT3* mutation who did not receive a FLT3i with induction therapy, but subsequently received one during either consolidation or salvage. *FISH*; fluorescence *in situ* hybridization; *FLT3*-ITD: fms-like tyrosine kinase 3 internal tandem duplications; HCT: hematopoietic cell transplantation; CR: complete response; CRh: complete response with partial hematologic recovery; CRi: complete response with incomplete hematologic recovery.

separate non-t(6;9) AML cohort (N=160; *Online Supplementary Table S2*). Patients were classified as ELN favorable (N=17, 11%), intermediate (N=61, 38%), or adverse (N=82, 51%) risk.[1] At last follow-up, 47 (29%) patients had undergone alloHCT and 114 (71%) had died. With median follow-up of 87 months, the mOS was 19 months (95% confidence interval [CI]: 15-28) with

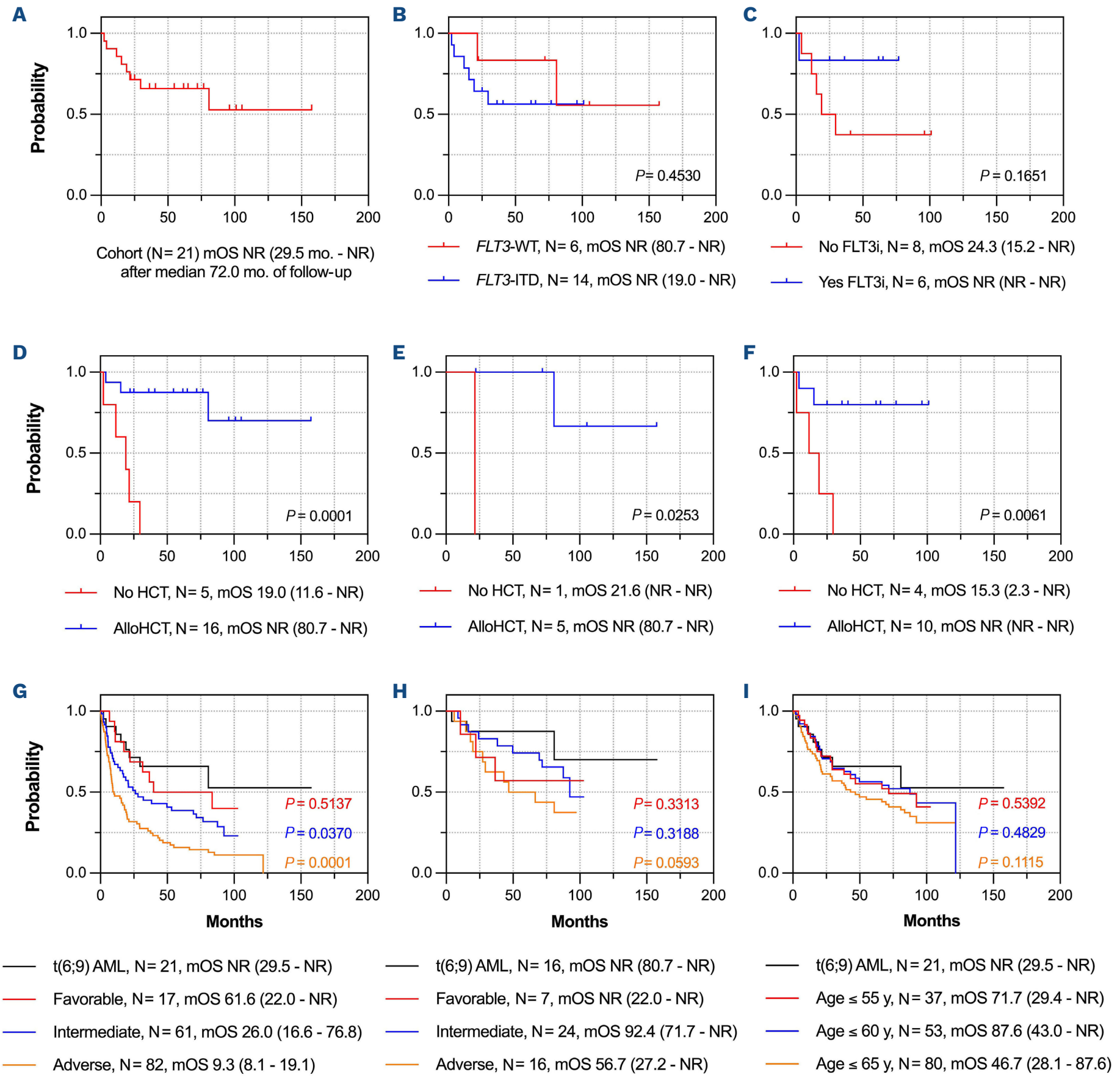


**Figure 1. Overall survival in t(6;9)(*DEK::NUP214*) acute myeloid leukemia and compared to European Leukemia Network risk groups.** (A) The median overall survival (mOS) of the entire cohort. (B-F) mOS of fms-like tyrosine kinase 3 (*FLT3*) wild-type *versus FLT3* internal tandem duplications (*FLT3*-ITD) cases (B); *FLT3*-ITD-positive cases receiving a FLT3 inhibitor (FLT3i) *versus* those that did not (C); t(6;9) acute myeloid leukemia (AML) patients undergoing allogeneic hematopoietic cell transplantation (alloHCT) *versus* those who did not (D); cases with wild-type *FLT3* who underwent allogeneic hematopoietic cell transplantation (alloHCT) *versus* those who did not (E); and those with a *FLT3*-ITD mutation who underwent alloHCT compared to those who did not (F). (G) Comparison of OS in the t(6;9) AML cohort *versus* the ELN favorable, intermediate, and adverse-risk groups. (H) Comparison of OS in the t(6;9) AML subset who underwent alloHCT *versus* the European Leukemia Network (ELN) favorable, intermediate, and adverse-risk groups who also underwent alloHCT. (I) Comparison of OS in the t(6;9) AML cohort *versus* age-restricted subgroups of the comparison cohort; the median (range) ages of the 3 subgroups are 38 (18-55), 50 (18-60), and 56 (18-65) years (y), respectively. In (G-I), *P* values depict the comparison between the t(6;9) AML cohort and the color-matched subgroup of the comparison cohort. All survival times are denoted as median (95% confidence interval) in months (mo).

**Table 2.** Treatment responses and survival outcomes.

| | ORR %[a] | MRD$^-$ %[b] | Deceased % | 2-year OS % | mOS in months | *P*[c] |
|---|---|---|---|---|---|---|
| Cohort | 85 | 56 | 38 | 71 | NR | - |
| ***FLT3-ITD* status** | | | | | | |
| Negative | 100 | 50 | 33 | 83 | NR | 0.4530 |
| Positive | 77 | 58 | 43 | 64 | NR | |
| Induction FLT3i | 83 | 83 | 17 | 83 | NR | 0.1651 |
| No induction FLT3i | 71 | 33 | 63 | 50 | 24 | |
| **Allogeneic HCT** | | | | | | |
| Received HCT | 100 | 77 | 19 | 88 | NR | 0.0001 |
| No HCT | 40 | 0 | 100 | 20 | 19 | |
| **MRD status[d]** | | | | | | |
| Negative | N/A | N/A | 10 | 90 | NR | 0.0688 |
| Positive | N/A | N/A | 60 | 60 | 29 | |

Percentage denominators are based on the number of evaluable patients for the specified metric. [a]The overall response rate (ORR) includes patients with complete response (CR), CR with partial hematologic recovery (CRh), and CR with incomplete hematologic recovery (CRi). ORR and measurable residual disease (MRD) status are presented as the best response attained. For patients who underwent allogeneic hematopoietic cell transplantation (HCT), these data represent the best response achieved prior to HCT, as all patients who underwent HCT and were subsequently evaluable achieved a $CR_{MRD-}$ status thereafter. MRD was assessed by multiparametric flow cytometry (MFC) or fluorescence *in situ* hybridization (FISH) as described in the text. [b]The denominator for MRD-negative percentages include both patients with MRD and persistent disease. [c]*P* values are for median OS comparisons. [d]These rows consider only patients who achieved morphologic remission and were evaluable for MRD status. *FLT3*-ITD: fms-like tyrosine kinase 3 internal tandem duplications; FLT3i: fms-like tyrosine kinase inhibitor; N/A: not applicable; NR: not reached; OS: overall survival; mOS: median OS.

2-year OS of 44%. Across the three ELN risk categories, the mOS was 62, 26, and 9 months with 2-year OS of 69%, 53%, and 31%, respectively (Figure 1G). AlloHCT provided a significant survival benefit in the intermediate- and adverse-risk groups (each *P*<0.0001) but not in the favorable-risk group (*P*=0.39), as expected for favorable-risk disease.[15]

Surprisingly, patients with t(6;9) AML fared better than the ELN adverse-risk comparison group (Figure 1G). Rather, the mOS of t(6;9) patients approximated that of the favorable-risk group (NR *vs*. 62 months; *P*=0.51) with 2-year OS of 71% *versus* 69%, respectively. However, patients with t(6;9) AML benefitted from alloHCT whereas those with favorable-risk disease in the comparison cohort did not. Indeed, t(6;9) AML patients who received alloHCT fared similarly to transplanted intermediate-risk patients, with 2-year OS of 88% and 87%, respectively (*P*=0.32; Figure 1H). Survival was also similar between t(6;9) and non-t(6;9) patients of comparable age (Figure 1I). These data raise the question as to whether t(6;9) AML should be reclassified as intermediate-risk, particularly for those treated with FLT3i and alloHCT. Notably, the 2022 ELN classification schema now categorizes AML with *FLT3*-ITD (without adverse-risk genetic lesions) in the intermediate-risk group.[1]

Attempts were made to identify parameters associated with OS. Univariate Cox regression identified MCV (*P*=0.045) and the peripheral blood blast percentage (*P*=0.011) as adverse prognostic factors while receipt of alloHCT was beneficial (*P*=0.0024). Neither *FLT3* status, induction FLT3i use, nor best MRD status correlated with OS. In the multivariate model of significant factors, only alloHCT retained significance (hazard ratio=0.11, 95% CI: 0.02-0.68; *P*=0.017).

Due to the rarity of t(6;9) AML, this study was underpowered to determine whether FLT3i or MRD negativity truly improve survival. Moreover, because this dataset spans an era of evolving MRD assessment standards and few patients had MRD testing by contemporary methods, larger combined analyses are needed to establish the role of frontline FLT3i and MRD monitoring in this setting. Furthermore, the mOS of this cohort (NR) is longer than previously reported (14-27 months),[2,3,6] likely signifying transplant referral bias, as alloHCT improves outcomes in t(6;9) AML.[6,9-11] Within these limitations, alloHCT significantly improved mOS in this cohort irrespective of *FLT3* or MRD status, and multivariate analysis identified alloHCT as the only prognostic factor.

In conclusion, t(6;9) AML has poor OS in the absence of alloHCT, and all eligible patients should be considered for transplant in first remission irrespective of MRD status. Although a definitive benefit has yet to be proven, FLT3i are an enticing avenue to improve response rates in *FLT3*-ITD-positive cases. Collectively, these interventions may provide sufficient benefit to reclassify t(6;9) AML as an intermediate-risk disease and need to be validated in larger studies.

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No conflicts of interest to disclose.

**Contributions**

CMC collected data, performed the analyses, drafted the manuscript, and collaboratively designed the study. ANS, PTG, and AA assisted with data collection. SHK and AA collaboratively designed and oversaw the study. All co-authors provided patient care, assisted with data collection, and critically reviewed the manuscript. All authors approved the final draft.

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Original data will be provided to collaborating investigators upon reasonable request to the corresponding authors after requisite institution review board approval.

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