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Review Article

FLT3-ITD Allelic Ratio and Response to Midostaurin-Based Induction in Newly Diagnosed Acute Myeloid Leukemia

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Summary

The FLT3-ITD allelic ratio was central to risk classification for five years and has now been removed from it. The trial that established midostaurin as standard of care had already shown that benefit did not track the ratio, and the measurement proved too poorly reproducible between laboratories to survive.

Objectives: To review the published evidence on the FLT3-ITD allelic ratio in newly diagnosed acute myeloid leukaemia: what it predicted before FLT3 inhibition, how midostaurin benefit distributed across ratio subgroups, why it was withdrawn from risk classification, and what has replaced it.

Material and Methods: Narrative review of randomised trials, correlative analyses, consensus classifications and validation studies indexed in PubMed and the major haematology journals. Studies were eligible if they reported outcomes stratified by FLT3-ITD allelic ratio, the effect of FLT3-directed therapy in defined mutation subgroups, or the performance of risk classifications incorporating the ratio. No new patient data were generated and no patients were recruited; every estimate quoted is one published by the original investigators.

Results: In the randomised trial of 717 patients that established midostaurin, median overall survival was 74.7 months against 25.6 months with placebo (HR 0.78, 95% CI 0.66-0.93). Benefit was similar across mutation subgroups, with hazard ratios of 0.65 (95% CI 0.39-1.08) for FLT3-TKD, 0.81 (0.60-1.11) for low allelic ratio ITD and 0.80 (0.57-1.12) for high allelic ratio ITD, so the ratio did not identify who benefited. The 2017 classification had defined four NPM1/FLT3-ITD genotypes using a ratio cutoff of 0.5, and retrospective analysis confirmed both that these genotypes separated survival and that midostaurin benefited all three resulting risk groups. Five-year survival on midostaurin was 73, 52 and 43 per cent in favourable, intermediate and adverse groups. The 2022 revision removed the allelic ratio entirely, citing limited reproducibility, and now assigns all FLT3-ITD disease to intermediate risk irrespective of ratio or NPM1 status.

Conclusion: The allelic ratio was prognostic in the era before FLT3 inhibition and is no longer used, for two independent reasons: midostaurin benefit does not depend on it, and the measurement itself cannot be reproduced reliably enough between laboratories to bear clinical weight.

Abbreviations: AML – Acute Myeloid Leukaemia, FLT3 – FMS-Like Tyrosine Kinase 3, ITD – Internal Tandem Duplication, TKD – Tyrosine Kinase Domain, AR – Allelic Ratio, NPM1 – Nucleophosmin 1, ELN – European LeukemiaNet, MRD – Measurable Residual Disease, alloHCT – Allogeneic Haematopoietic Cell Transplantation, CR1 – First Complete Remission, OS – Overall Survival, EFS – Event-Free Survival, TKI – Tyrosine Kinase Inhibitor.

Keywords: Acute Myeloid Leukaemia; FLT3-ITD; Allelic Ratio; Midostaurin; Risk Stratification

Introduction

Internal tandem duplications of the FLT3 gene occur in roughly a quarter to a third of newly diagnosed acute myeloid leukaemia and have long been associated with poor outcome after conventional chemotherapy [1,2]. The duplication produces constitutive activation of a receptor tyrosine kinase, and the proportion of mutant to wild-type alleles, the allelic ratio, was proposed as a measure of how far the leukaemic clone depended on that activation [3].

The 2017 European LeukemiaNet recommendations formalised the idea, defining four genotypes from the combination of allelic ratio and NPM1 mutation status and distributing them across favourable, intermediate and adverse risk categories [4]. A high ratio, conventionally 0.5 or above, moved a patient toward adverse risk and toward allogeneic transplantation in first remission [4,5].

Almost simultaneously, midostaurin became the first targeted agent approved for acute myeloid leukaemia on the strength of a randomised trial adding it to standard induction [1,6,7]. The two developments interacted in a way that was not anticipated: an agent that improves outcome across the whole mutated population weakens the case for using a marker to subdivide it.

This review sets out what the allelic ratio predicted, how the midostaurin benefit distributed across ratio subgroups, and why the 2022 revision removed the measurement from risk classification altogether [8].

Material and Methods

Search strategy: PubMed and the archives of the major haematology journals were searched for studies of the FLT3-ITD allelic ratio and FLT3-directed therapy. Search terms combined acute myeloid leukaemia with FLT3-ITD, allelic ratio, mutant to wild-type ratio, midostaurin, RATIFY, European LeukemiaNet, risk stratification and FLT3 inhibitor. Reference lists of retrieved trials and classification documents were screened by hand.

Eligibility: Studies were included if they reported outcomes stratified by FLT3-ITD allelic ratio, the effect of FLT3-directed therapy within defined mutation subgroups, or the validation and revision of risk classifications incorporating the ratio. Trials in relapsed or refractory disease are cited where they inform the interpretation of frontline findings and are identified as such.

Data extraction and handling: Design, cohort size, the allelic ratio cutoff applied, NPM1 status and reported effect estimates with confidence intervals were extracted as published. The cutoff is stated alongside every figure, because trials and classifications have used both 0.5 and 0.7 and the resulting subgroups are not the same. No pooling or re-analysis was performed and no patient-level data were obtained. Figures 1 to 4 and Tables 1 to 3 reproduce published values only. This is a narrative synthesis and was not registered as a systematic review.

Results

What midostaurin achieved: The randomised, placebo-controlled trial enrolled 717 analysable patients aged 18 to 60 with FLT3-mutated disease and added midostaurin to daunorubicin and cytarabine induction, consolidation and twelve months of maintenance. Median overall survival was 74.7 months against 25.6 months with placebo, with a hazard ratio of 0.78 (95% CI 0.66-0.93)

(Figure 2) [1,6]. Event-free survival was 8.2 against 3.0 months and disease-free survival 26.7 against 15.5 months (Figure 4) [6].

The result made midostaurin the first targeted agent approved in this disease and the first combination to improve survival in several decades [7,9]. Earlier phase II work had established activity as a multikinase inhibitor with effects beyond FLT3, including on KIT [10,11].

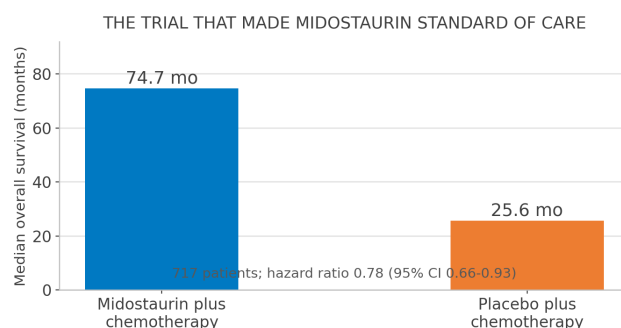


Figure 2: Median overall survival in the randomised trial of midostaurin added to standard induction [1,6]

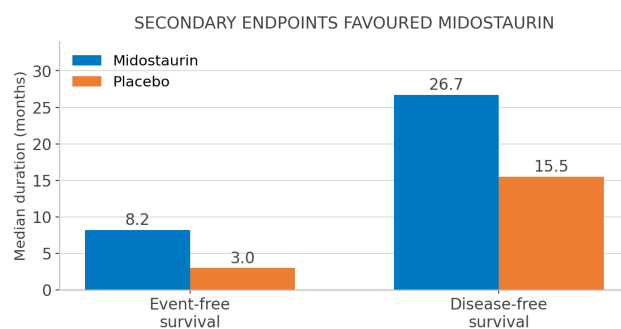


Figure 4: Secondary survival endpoints in the same trial [6]

Benefit did not track the allelic ratio: Randomisation was stratified by allelic ratio using a cutoff of 0.7, and the trial reported outcomes separately for tyrosine kinase domain mutations, low-ratio duplications and high-ratio duplications. Hazard ratios for overall survival were 0.65 (95% CI 0.39-1.08) for FLT3-TKD, 0.81 (0.60-1.11) for low-ratio ITD and 0.80 (0.57-1.12) for high-ratio ITD (Figure 1) [6].

The three intervals overlap almost completely. Whatever else the allelic ratio measures, it did not identify which patients benefited from adding a FLT3 inhibitor. When the same endpoints were examined for event-free survival, the benefit appeared concentrated in the tyrosine kinase domain subgroup rather than in either duplication group [6], which is the opposite of what a dose-dependent effect on FLT3 signalling would predict.

The trial investigators offered a mechanistic explanation. Midostaurin is a multikinase inhibitor rather than a selective FLT3 antagonist, so benefit in low-ratio disease, where mutations other

than FLT3 may be driving the leukaemia, could reflect inhibition of alternative pathways [6,10]. Conversely, samples from relapsed patients with high mutant burden proved more sensitive to selective FLT3 inhibition than diagnostic samples with lower ratios [6].

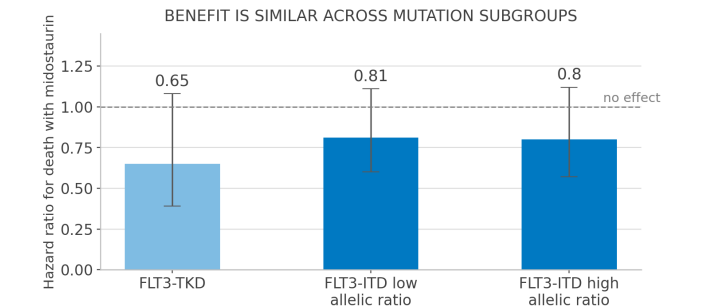


Figure 1: Hazard ratio for death with midostaurin by FLT3 mutation subgroup [6]

Study	n	Focus	Principal reported finding
Stone et al. 2017 [1]	717	RATIFY	Median survival 74.7 against 25.6 months
Döhner et al. 2017 [4]	Consensus	ELN 2017	Four NPM1/FLT3-ITD genotypes defined by ratio
Döhner K et al. 2020 [12]	RATIFY	Genotypes	0.5 best discriminant; benefit across all risk groups
Döhner et al. 2022 [8]	Consensus	ELN 2022	Allelic ratio removed from classification
Perl et al. 2019 [15]	371	ADMIRAL	Gilteritinib superior in relapsed disease
Erba et al. 2023 [17]	Randomised	QuANTUM-First	Quizartinib added to frontline chemotherapy

Table 1: Principal studies reviewed, with focus and the finding as reported by the original investigators

The 2017 genotypes and their validation: The four genotypes combined NPM1 mutation status with allelic ratio: NPM1-mutated with low ratio was favourable, NPM1-mutated with high ratio and NPM1-wild-type with low ratio were intermediate, and NPM1-wild-type with high ratio was adverse [4]. Retrospective analysis of the randomised trial population tested this directly and found that overall survival differed significantly across the resulting risk groups, and that 0.5 rather than the trial's stratification cutoff of 0.7 was the better discriminant [12].

The same analysis found a consistent beneficial effect of midostaurin across all three risk groups in a multivariable model [12]. Five-year survival on midostaurin was 73 per cent in the favourable group, 52 per cent in the intermediate group and 43 per cent in the adverse group (Figure 3) [12,13]. The classification

therefore worked as prognosis and failed as prediction: it sorted patients by outcome but not by who should receive the drug.

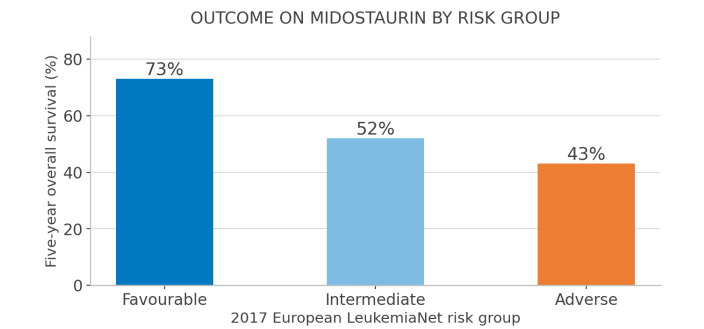


Figure 3: Five-year overall survival on midostaurin by 2017 risk group [12,13]

Why the ratio was withdrawn: The 2022 revision removed the allelic ratio from risk classification and now assigns all FLT3-ITD disease to intermediate risk irrespective of ratio or NPM1 co-mutation, with allogeneic transplantation recommended in first remission [8]. Two reasons were given, and they are independent of one another.

The first is technical. Measuring the allelic ratio requires quantifying mutant against wild-type product, and the reproducibility of that measurement between laboratories proved limited [8,14]. A variable that cannot be measured consistently cannot carry clinical weight however strong its biological rationale, and this is the reason the classification documents themselves emphasise [8].

The second is that FLT3-directed therapy changed the prognostic landscape the ratio was describing. If midostaurin benefits high and low ratio patients similarly [6], then a classification built to separate them is sorting patients into categories that no longer imply different treatment. Validation work on the revised classification confirms that removing the ratio does not degrade prognostic performance [14,16].

Genotype	2017 risk group	2022 risk group
NPM1-mutated, low allelic ratio	Favourable	Intermediate [4,8]
NPM1-mutated, high allelic ratio	Intermediate	Intermediate [4,8]
NPM1-wild-type, low allelic ratio	Intermediate	Intermediate [4,8]
NPM1-wild-type, high allelic ratio	Adverse	Intermediate [4,8]

Table 2: How the four genotypes were reclassified between the 2017 and 2022 recommendations

What has taken its place: Measurable residual disease has assumed much of the stratifying role the ratio once held. NPM1 mutation provides a reliable target for molecular monitoring in the majority of FLT3-ITD patients, and residual disease status after treatment refines

prognosis further and informs post-remission strategy [12,18,19]. Quantitative NPM1 monitoring in standard-risk disease carries strong prognostic weight [20], and persistent mutations detected by sequencing predict relapse independently [21].

The shift is from a variable measured once at diagnosis to one measured repeatedly during treatment, which parallels what happened in acute lymphoblastic leukaemia a decade earlier [22,23]. It has the practical advantage that the measurement responds to therapy, so it can inform decisions rather than only describe risk.

More selective FLT3 inhibitors have also arrived. Gilteritinib improved survival against salvage chemotherapy in relapsed and refractory disease [15], with post-transplant maintenance associated with low relapse rates [24], and quizartinib has been tested both in the relapsed setting [25] and added to frontline chemotherapy [17]. Whether the allelic ratio predicts benefit from these more selective agents, as it did not for midostaurin, is a separate question that the frontline data do not yet answer.

Discussion

The trajectory here is unusual and worth stating plainly. A biomarker was incorporated into an international risk classification in 2017, validated against trial data in 2020, and removed from the classification in 2022 [4,8,12]. That is a short life for a variable that determined transplant decisions, and the reasons for its removal are instructive.

The predictive failure came first and was visible in the pivotal trial itself. Hazard ratios of 0.81 and 0.80 for low and high ratio disease, with almost entirely overlapping confidence intervals, say that the ratio does not identify who benefits from midostaurin [6]. A marker that sorts prognosis but not treatment response has limited use once an effective treatment exists for the whole group.

Reproducibility as a disqualifying problem

The technical reason is the more general one. An allelic ratio is a quantitative comparison of two polymerase chain reaction products, and its value depends on primer design, fragment length, and how the assay handles multiple or long duplications [8,14]. Laboratories using different methods produced different ratios for the same disease, which is why the classification committee withdrew it [8].

This is the same problem that afflicts flow cytometric residual disease thresholds and, in a different form, histological grading: a measurement with a sound biological basis can still be unusable if it cannot be reproduced across the laboratories that will apply it. The lesson is that analytical validation deserves the weight given to clinical validation, and it usually receives less.

Study limitations

The limitations belong to the underlying literature. The pivotal trial enrolled only patients aged 18 to 60, so its findings cannot be extrapolated directly to older adults, in whom separate evaluation was required [1,6,11]. Subgroup analyses by mutation type were exploratory and the trial was not powered to detect differences between them, so overlapping confidence intervals indicate absence of evidence rather than evidence of absence [6]. The genotype validation was retrospective and conducted within a trial population selected for fitness [12]. Different allelic ratio cutoffs, 0.5 and 0.7, were used for classification and for trial stratification respectively, so subgroups are not directly comparable across studies [6,12]. And the revised classification is recent enough that long-term validation is still accumulating [14,16].

Two gaps deserve naming. No trial has tested whether allelic ratio predicts benefit from the more selective second-generation inhibitors, where a dose-dependent relationship might exist that midostaurin's multikinase activity obscured [15,17]. And no study reviewed here reports whether removing the ratio from classification changed transplant allocation in practice, which is the decision it was principally used to inform [8].

Conclusion

The FLT3-ITD allelic ratio predicted outcome in the era before FLT3 inhibition and was built into the 2017 risk classification on that basis. The trial that made midostaurin standard of care showed benefit that did not depend on it, with hazard ratios of 0.81 and 0.80 for low and high ratio disease against 0.65 for tyrosine kinase domain mutations, and confidence intervals that overlap almost entirely. Retrospective analysis confirmed that the genotypes derived from the ratio separated survival, and that midostaurin benefited every group. The 2022 revision removed the ratio, primarily because it could not be measured reproducibly between laboratories, and now assigns all FLT3-ITD disease to intermediate risk. A marker can be biologically real, prognostically valid and still unusable if the assay behind it does not travel, and that is the more transferable lesson from this episode.

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Conflict of Interest

The authors declare no competing interests.

Ethics Statement

This article reviews previously published studies only. No new patient data were collected and no participants were recruited, so ethical approval was not required.

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