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Regular Article

CLINICAL TRIALS AND OBSERVATIONS

Sorafenib plus intensive chemotherapy in newly diagnosed *FLT3*-ITD AML: a randomized, placebo-controlled study by the ALLG

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KEY POINTS

- **Sorafenib did not significantly improve EFS when added to intensive chemotherapy in patients with newly diagnosed *FLT3*-ITD AML.**
- ***FLT3*-ITD MRD clearance as assessed by next-generation sequencing has powerful prognostic utility in determining survival outcome.**

Sorafenib maintenance improves outcomes after hematopoietic cell transplant (HCT) for patients with FMS-like tyrosine kinase 3–internal tandem duplication (*FLT3*-ITD) acute myeloid leukemia (AML). Although promising outcomes have been reported for sorafenib plus intensive chemotherapy, randomized data are limited. This placebo-controlled, phase 2 study (ACTRN12611001112954) randomized 102 patients (aged 18-65 years) 2:1 to sorafenib vs placebo (days 4-10) combined with intensive induction: idarubicin 12 mg/m^2 on days 1 to 3 plus either cytarabine 1.5 g/m^2 twice daily on days 1, 3, 5, and 7 (18-55 years) or 100 mg/m^2 on days 1 to 7 (56-65 years), followed by consolidation and maintenance therapy for 12 months (post-HCT excluded) in newly diagnosed patients with *FLT3*-ITD AML. Four patients were excluded in a modified intention-to-treat final analysis (3 not commencing therapy and 1 was *FLT3*-ITD negative). Rates of complete remission (CR)/CR with incomplete hematologic recovery were high in both arms (sorafenib, 78%/9%; placebo, 70%/24%). With 49.1-months median follow-up, the primary end point of event-free survival (EFS) was not improved by sorafenib (2-year EFS 47.9% vs 45.4%; hazard ratio [HR], 0.87; 95% confidence interval [CI], 0.51-1.51; *P* = .61). Two-year overall survival (OS) was 67% in the sorafenib arm and 58% in the placebo arm (HR, 0.76; 95% CI, 0.42-1.39). For patients who received HCT in first remission, the 2-year OS rates were 84% and 67% in the sorafenib and placebo arms, respectively (HR, 0.45; 95% CI, 0.18-1.12; *P* = .08). In exploratory analyses, *FLT3*-ITD measurable residual disease (MRD) negative status (<0.001%) after induction was associated with improved 2-year OS (83% vs 60%; HR, 0.4; 95% CI, 0.17-0.93; *P* = .028). In conclusion, routine use of pretransplant sorafenib plus chemotherapy in unselected patients with *FLT3*-ITD AML is not supported by this study.

Introduction

FMS-like tyrosine kinase 3–internal tandem duplications (*FLT3*-ITDs) are present in up to 30% of patients with newly diagnosed adult acute myeloid leukemia (AML).[1,2] *FLT3*-ITD mutation is associated with increased relapse risk and inferior overall survival (OS).[3] Small molecule targeting of FLT3 kinase activity has proven successful, with improved OS demonstrated for midostaurin or quizartinib combined with intensive chemotherapy for newly diagnosed patients and for gilteritinib or quizartinib for patients with relapsed/refractory *FLT3*-mutated AML.[4-8]

Sorafenib is a small molecule multikinase FLT3, vascular endothelial growth factor receptor, platelet-derived growth factor receptor β, KIT, and RAF inhibitor that has been studied in several prospective trials in AML.[9-11] When combined with intensive chemotherapy for older patients from day 10 of induction until 3 days prior to commencement of the next cycle, sorafenib was associated with an increased rate of early death (17%).[12] In younger patients with newly diagnosed AML (including wild-type FLT3), an abbreviated course of sorafenib from days 10 to 19 combined with daunorubicin and cytarabine improved event-free survival (EFS) and was associated with an induction death rate of 3%, compared to 2% in the placebo-controlled arm.[13] As only 46 patients (17%) in the study were *FLT3*-ITD positive, the benefit of sorafenib in *FLT3*-ITD–positive AML remained unclear. Ravandi et al examined higher dose cytarabine combined with idarubicin and sorafenib from days 1 to 7 and showed a 93% response rate in patients with *FLT3*-mutant AML.[14] Two prospective studies found that maintenance therapy with sorafenib could reduce relapse risk after allogeneic hematopoietic cell transplant (allo-HCT).[15,16] Despite these generally encouraging findings, the role of sorafenib in frontline combination with intensive chemotherapy for patients with *FLT3*-ITD AML prior to HCT remains contentious.

A phase 2, multicenter, randomized, placebo-controlled, double-blind study was therefore undertaken to assess the safety and efficacy of sorafenib in combination with intensive induction and consolidation chemotherapy followed by HCT or sorafenib maintenance in patients with *FLT3*-ITD AML. To mitigate toxicity, a 7-day course of sorafenib/placebo was administered after idarubicin. High levels of circulating FLT3 ligand (FLT3L) in response to chemotherapy-induced cytopenia are known to inhibit FLT3 inhibitor activity.[17] We sought to minimize exposure of sorafenib to high levels of FLT3L by limiting sorafenib exposure to days 4 to 10 of induction. This study enabled the role of pre-HCT sorafenib to be specifically addressed, as maintenance therapy with sorafenib after HCT was not permitted, given that it was not standard of care when the study commenced.

Methods

Patient population

Patients aged 18 to 65 years with newly diagnosed AML were enrolled to the Australasian Leukaemia and Lymphoma Group (ALLG) National Blood Cancer Registry and rapidly screened via a network of accredited laboratories for *FLT3*-ITD mutation. To avoid delays in chemotherapy administration, sites were permitted to commence induction chemotherapy prior to knowledge of the *FLT3* result. Patients with AML (excluding acute promyelocytic leukemia) were eligible if the *FLT3*-ITD allelic ratio was ≥0.05 by capillary electrophoresis (CE) and trial-specific consent was obtained before day 4 of induction. Refer to supplemental Methods (available on the *Blood* website) for a list of study inclusion and exclusion criteria.

Randomization and treatment

The study schema is summarized in Figure 1. By day 4 of induction chemotherapy, enrolled patients were randomized 2:1 to induction chemotherapy combined with either sorafenib or placebo. Randomization was stratified according to age (18-55 and 56-65 years). Each patient was given a unique 5-digit study identification (ID) and randomization code. Drug allocation was determined by a center-specific randomization chart provided by the ALLG trial center to each site. Investigators and the ALLG trial center were blinded to the allocation. Patients aged 18 to 55 received IDAC-3 (cytarabine 1.5 g/m^2 IV for 2-4 hours twice daily on days 1, 3, 5, and 7 and idarubicin 12 mg/m^2 IV on days 1 to 3). Patients aged 56 to 65 years or with *FLT3*-ITD–positive core-binding factor (CBF) AML received 7 + 3 (cytarabine 100 mg/m^2 on days 1 to 7 and idarubicin 12 mg/m^2 IV days 1 to 3). Sorafenib or placebo 400 mg twice daily was administered orally days 4 to 10 for a total of 14 doses. A bone marrow biopsy was performed on day 28 ± 7 days to assess response according to International Working Group criteria.[18] Patients not achieving complete remission (CR) or CR with incomplete hematologic recovery (CRi) after induction were permitted to receive a second induction cycle identical to the first. If CR/CRi was not achieved after reinduction, protocol treatment was discontinued. Patients who achieved CR/CRi proceeded to receive consolidation chemotherapy. For consolidation, patients aged 18 to 55 years received 2 cycles of idarubicin 9 mg/m^2 IV on days 1 to 2, cytarabine 100 mg/m^2 continuous IV infusion on days 1 to 5, and etoposide 75 mg/m^2 IV on days 1 to 5. Patients aged 56 to 65 years received 2 cycles of IDAC-2 (cytarabine 1g/m^2 IV for 2-4 hours twice daily on days 1, 3, and 5 and idarubicin 12 mg/m^2 IV on days 1 to 2). Patients with CBF AML received 3 cycles of cytarabine 1.5 g/m^2 IV over 2 to 4 hours twice daily on days 1, 3, and 5. In all consolidation cycles, sorafenib or placebo 400 mg twice daily was administered orally from days 4 to 10 for a total of 14 doses. During maintenance, sorafenib or placebo 400 mg twice daily was administered no sooner than day 42 after commencing the last consolidation cycle, but no later than day 90, if awaiting recovery of neutrophils to 1.0 × 10^9/L and platelets to 75 × 10^9/L. Maintenance was delivered for up to 12 × 28–day cycles. At any time, patients could discontinue treatment and proceed to allo-HCT. Post-transplant sorafenib or placebo was not permitted. To avoid the known CYP3A4 interaction with sorafenib, strong CYP3A4 inhibitors, including posaconazole, were not permitted during induction. Antifungal prophylaxis consisted of ambisome 5 mg/kg IV twice weekly from day 4 throughout induction until neutrophil recovery to ≥0.5 × 10^9/L.[19] A future publication will detail the tolerability and efficacy of ambisome antifungal prophylaxis. During consolidation, concurrent use of strong azoles on sorafenib dosing days was prohibited.

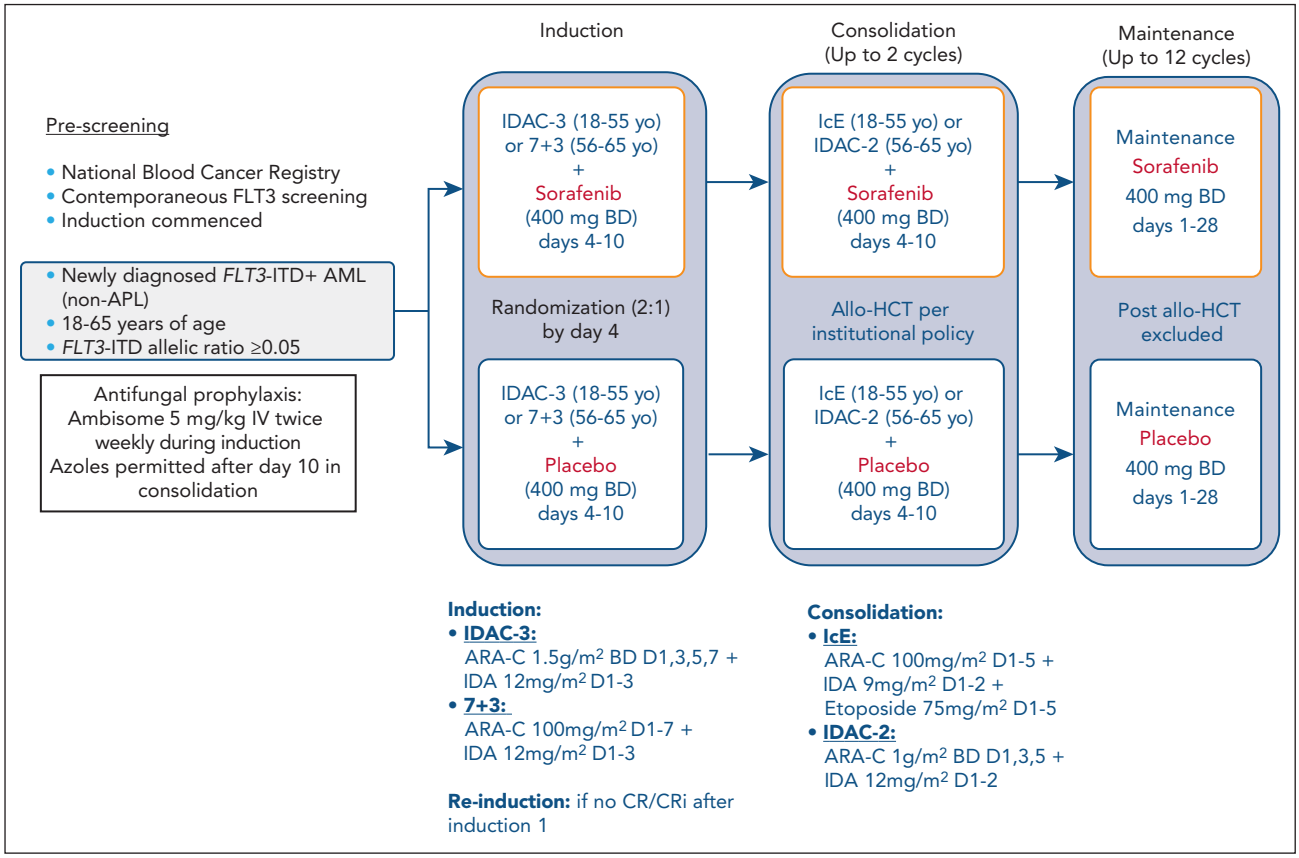

Figure 1. Study schema. APL, acute promyelocytic leukemia; BD, twice daily; IDA, idarubicin; IDAC, intermediate-dose cytarabine.

Trial oversight and support

The ALLG AMLM16 trial (ACTRN12611001112954) was a cooperative trial group study led by the ALLG. The ALLG safety and data monitoring committee and scientific advisory committee approved the study design. The Human Research and Ethics Committees of the participating centers reviewed and approved the study protocol. The trial was conducted in accordance with the Declaration of Helsinki. The trial was funded by the National Health and Medical Research Council of Australia and Leukaemia Foundation of Australia. Sorafenib and placebo were provided by Bayer, and liposomal ambisome was kindly supplied by Gilead.

Statistical design

The primary end point was EFS, measured from the date of randomization to the date of the earliest of 3 events: treatment failure (no CR/CRi achieved after up to 2 cycles of chemotherapy resulting in withdrawal from study), relapse, or death (or last follow-up for patients who were alive). The study design assumed a 2-year EFS for the standard treatment to be ~25%, with intention to randomize 99 patients in a 2:1 allocation ratio (66 patients to sorafenib and 33 patients to placebo). Using a 2-sided significance level (α) of 0.05, the study had 82% power to detect a hazard ratio (HR) from 0.5 to 0.661, corresponding to a 2-year EFS improvement in the sorafenib arm from 40% to 50%. The main analysis was planned after 81 EFS events had been accumulated. Although the overall power of the study to detect changes in the hazard rate corresponding to absolute increases in an EFS <25% at 2 years was not high, a sample size of 99 was considered large enough in this phase 2 study to provide an estimate of clinical efficacy.

Secondary study end points included safety, response rates, relapse-free survival (RFS; measured from the date of CR/CRi until relapse, death or last follow-up for patients who were alive), and OS, calculated from the date of randomization to the date of death or last follow-up date in patients who were alive. Kaplan-Meier survival curves were constructed to estimate the EFS, RFS, and OS for each treatment group using the log-rank test. The HR, 2-sided *P* value, and 95% confidence interval (CI) are presented. Cox regression was used for additional analyses involving the evaluation of prognostic factors. All eligible participants who were randomized, had *FLT3*-ITD mutation, and received at least 1 dose of study treatment were included in the analyses according to a modified intention-to-treat basis. Follow-up for disease and survival status was continued for at least 2 years after the accrual of the last patient.

Results

Patient characteristics

From January 2013 to May 2018, a total of 589 patients with AML aged 18 to 65 from 20 participating centers were registered and underwent *FLT3* testing (supplemental Figure 1). Of the 156 patients with *FLT3*-ITD allelic ratio ≥0.05, a total of 102 provided consent and were enrolled. The reasons why patients with *FLT3*-ITD AML were not enrolled in the trial were not prospectively collected.

Table 1. Baseline patient and leukemia characteristics

Variables	Sorafenib (n = 65)	Placebo (n = 33)	*P*
Age, median (range), y	49 (18-65)	50 (20-65)	.67
Sex, n (%)			.73
Female	37 (57)	20 (61)	
Male	28 (43)	13 (39)	
AML type, n* (%)			.46
De novo	62 (95)	33 (100)	
Secondary	2 (3)	0	
White cell count × 10^9/L, median (IQR)	35.7 (12.8-79.4)	33.1 (12.9-63.1)	.64
Platelet count × 10^9/L, median (IQR)	61 (43-79)	52 (33-89)	.78
MRC cytogenetic risk, n (%)			.79
Favorable	2 (3)	1 (3)	
Intermediate	61 (94)	32 (97)	
Adverse	1 (1)	0	
Not available	1 (1)	0	
FLT3-ITD allelic ratio, median (range)	0.46 (0.05-5.8)	0.52 (0.05-8.4)	.53
***FLT3*-ITD allelic ratio, n (%)**			.36
<0.5	36 (55)	15 (45)	
≥0.5 to <0.7	10 (15)	9 (27)	
≥0.7	19 (29)	9 (27)	
FLT3-ITD length (base pairs)†, median (range)	43.5 (15-282)	45 (18-195)	.8
***FLT3*-ITD insertion site†, n (%)**			
Juxtamembrane domain	124 (74)	44 (71)	
TKD1 β1-sheet	27 (16)	15 (24)	
TKD1 nucleotide binding loop	3 (2)	2 (3)	
TKD1 β2-sheet	13 (8)	1 (2)	
NPM1 detected, n/total available (%)	30/50 (60)	22/27 (81)	.07

IQR, interquartile range; MRC, Medical Research Council; TKD1, tyrosine kinase domain 1.

*One patient in the sorafenib group with insufficient information for the AML type to be assigned.

†Diagnostic DNA available for *FLT3*-ITD NGS for 52 patients in the sorafenib group and 28 patients in the placebo group.

Of the 102 patients randomized, 3 did not receive study treatment (2 sorafenib and 1 placebo). One additional patient was redesignated with *FLT3*-ITD–negative status after a testing error and excluded from analysis. Therefore, 98 patients formed the final analysis set. Baseline characteristics were balanced between both arms (Table 1). The median age was 49 years. The median white cell count at study entry was 35.7 × 10^9/L, and the proportion with high *FLT3*-ITD allelic ratio ≥0.7 was 29%. *FLT3*-ITD length and insertion site characteristics were similar between both arms. Concurrent *NPM1* mutation in the placebo arm was 81% compared with 60% in the sorafenib arm (P = .07). In the sorafenib and placebo arms, 78% and 79% of patients commenced consolidation and 32% and 27% commenced maintenance, respectively (supplemental Figure 1).

Clinical response

The overall response rate (CR/CRi) was 88% (57/65) in the sorafenib arm compared with 94% (31/33) in the placebo arm (P = .49); CR was achieved in 78% and 70%, respectively (P = .46; Table 2). After the first induction, 7 patients (11%) in the sorafenib arm had a partial response, with 3 subsequently achieving CR/CRi after a second cycle of chemotherapy and the remaining 4 patients exiting to receive off-study therapy and designated as treatment failures. Among younger patients receiving the more intensive IDAC-3 induction, CR rate in the sorafenib arm was 82%, compared with 65% in the placebo arm (P = .14; supplemental Table 1). Overall, 48 patients (74%) in the sorafenib arm and 21 (64%) in the placebo arm proceeded to HCT, with 40 (62%) in the sorafenib arm and 19 (58%) in the placebo arm transplanted in first remission. The median time to HCT from study randomization was 4.2 months (range, 1.6-35 months).

EFS, RFS, and OS

The primary end point was EFS. With a median follow-up time of 49.1 months, there was no difference in EFS (HR, 0.87; 95% CI, 0.51-1.51; Figure 2A; supplemental Table 2). Two-year EFS

Table 2. Clinical response

	Sorafenib (n = 65)	Placebo (n = 33)
Best response on study, n (%)		
Overall response rate (CR/CRi)	57 (88)	31 (94)
CR	51 (78)	23 (70)
CRi	6 (9)	8 (24)
PR	4 (6)	0
No response	3 (5)	1 (3)
Response not available	1 (2)	1 (3)
Response rates within 60 days, n (%)		
Overall response rate (CR/CRi)	55 (82)	31 (94)
CR	41 (63)	21 (64)
CRi	14 (22)	10 (30)
PR	6 (9)	0
No response	3 (5)	1 (3)
Response not available	1 (2)	1 (3)

CR, complete remission; CRi, CR with incomplete hematologic recovery; PR, partial remission.

in the sorafenib arm was 47.9% (95% CI, 35-60), compared with 45.4% (95% CI, 28-62) in the placebo arm (P = .61; Figure 2A). For RFS, the 2-year estimate for the sorafenib arm was 54% (95% CI, 40-66), compared with 40% (95% CI, 22-57) for the placebo arm (HR, 0.76; 95% CI, 0.4-1.4; Figure 2B). EFS limited to CR responders and landmarked at 60 days was also examined (EFS_{CR60}). The 2-year EFS_{CR60} rates were 38% in the sorafenib arm and 30% for the placebo arm (HR, 0.81; 95% CI, 0.49-1.34; supplemental Figure 2A). The 2-year RFS_{CR60} rates were 61% for sorafenib and 36% for the placebo (HR, 0.64; 95% CI, 0.31-1.31; supplemental Figure 2B).

The OS was not different between the arms, with a 2-year OS rate of 67% in the sorafenib arm compared with 58% for placebo (HR, 0.76; 95% CI, 0.42-1.39; Figure 2C; supplemental Table 2). To assess the impact of allo-HCT on outcome, landmark analyses on days 60, 90, and 120 were conducted to account for the time-dependent bias associated with HCT (supplemental Figure 3). Using the 120-day landmark as a representative example, the 2-year OS was significantly increased in the sorafenib arm for patients who received HCT in first remission compared with those not transplanted (84% vs 53%; HR, 0.35; 95% CI, 0.14-0.87; P = .02), indicating a benefit for allo-HCT as postremission therapy (Figure 2D). In contrast, there did not appear to be any benefit for HCT in the placebo arm. Next, we determined the impact of sorafenib vs placebo in patients achieving first remission and proceeding to allo-HCT. The 2-year OS rate was 84% in the sorafenib arm compared with 67% in the placebo arm (HR, 0.45; 95% CI, 0.18-1.12; P = .08). Similar outcomes were observed if survival from randomization was landmarked on days 60 or 90 (supplemental Figure 3A-C).

Safety

Considering adverse events across all treatment cycles occurring in at least 5% of patients, rates of grade ≥3 hematologic toxicities were similar in both treatment groups, with febrile neutropenia reported in 65% vs 55% in the sorafenib and placebo arms, respectively (Table 3). The commonest grade ≥3 nonhematologic toxicities in the sorafenib and placebo groups were infection/sepsis (55% vs 48%), rash (11% vs 24%), alanine aminotransferase increase (11% vs 18%), or enterocolitis (15% vs 15%), respectively. The frequency of palmar-plantar syndrome (any grade) was higher in the sorafenib arm (15% vs 6%; grade ≥3, 8% vs 0%), as was corrected QT interval prolongation (grade ≥3, 5% vs 0%). The 30-day mortality was 2% in the sorafenib arm (1 patient with sepsis/multiorgan failure) and 3% in the placebo arm (1 patient with cardiac arrest).

Correlative analysis of FLT3L and P-FLT3 inhibition and the impact of *FLT3*-ITD burden on outcome

To assess the variation in FLT3L levels during induction, peripheral blood plasma was collected on days 4, 10, 15, and 28, and FLT3L levels were assessed by enzyme-linked immunosorbent assay (supplemental Methods). FLT3L levels were low on day 4 of induction, rising to a peak by day 15, before returning to baseline in most cases by day 28. There was no difference in the pattern of FLT3L change in the sorafenib (Figure 3A) or placebo (Figure 3B) arms.

A phosphoprotein reporter assay was used to determine whether sorafenib levels achieved in vivo were sufficient to adequately suppress *FLT3*-ITD ex vivo. The plasma inhibitory assay (PIA) provides a pharmacodynamic assessment of circulating sorafenib, using patient-derived plasma to suppress phosphorylated FLT3 (P-FLT3) in a reporter cell line in vitro. FLT3 inhibitor suppression of P-FLT3 to <15% of baseline has previously been shown to correlate with improved clinical outcomes.[20] Plasma from days 4, 10, and 15 were analyzed using previously described methods.[21] Representative immunoblots indicative of either PIA response or nonresponse are shown in Figure 3C. Analysis of residual P-FLT3 on day 10 relative to day 4 was performed in 41 patients in the sorafenib arm with adequate PIA suppression to <15% demonstrated in 88% of cases (Figure 3C).

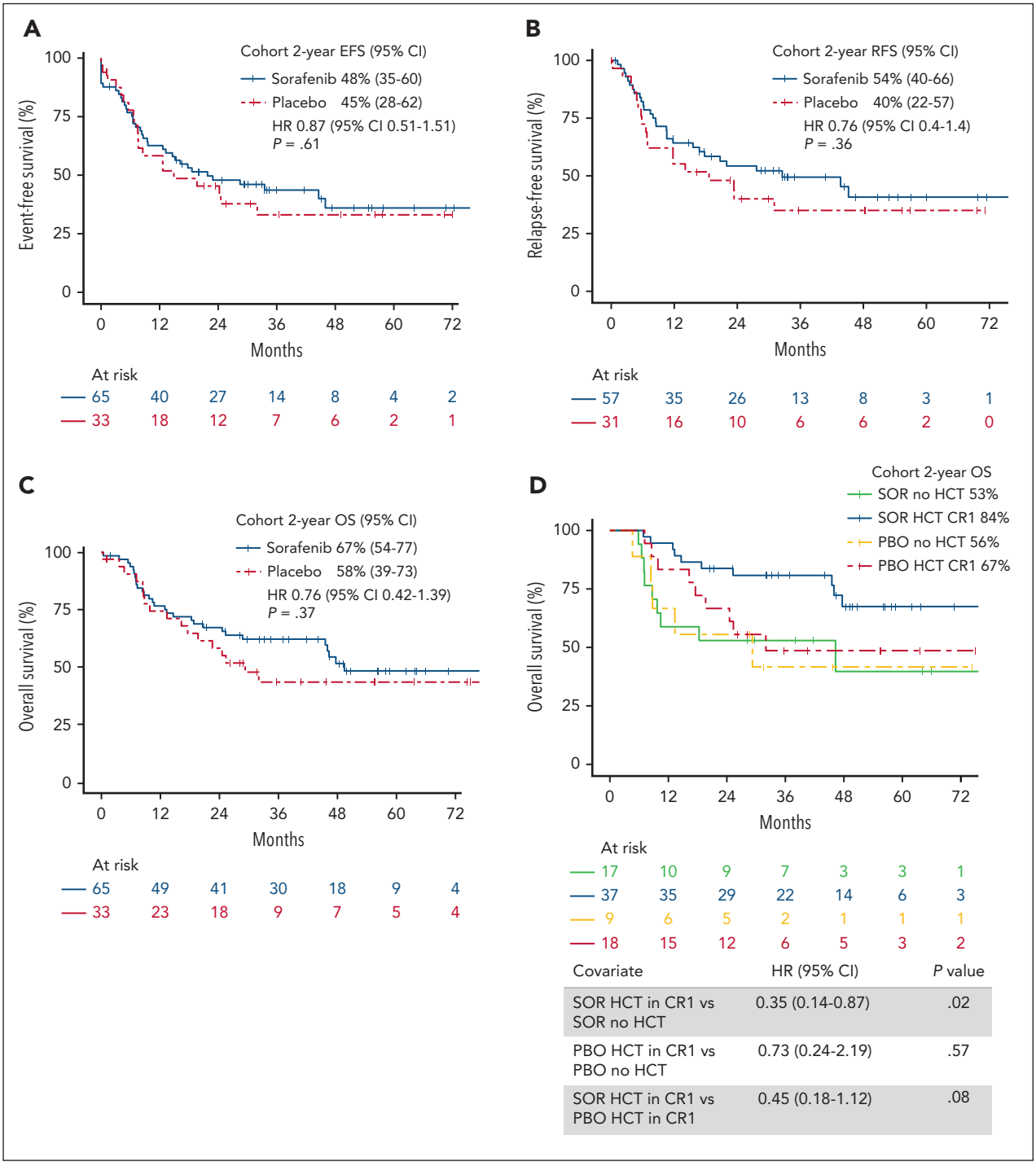

Figure 2. Kaplan-Meier estimates of survival outcomes according to the treatment arm. (A) Kaplan-Meier estimate of EFS in the sorafenib and placebo arms. (B) Kaplan-Meier estimate of RFS in the sorafenib and placebo arms. (C) Kaplan-Meier estimate of OS in the sorafenib and placebo arms. (D) Kaplan-Meier estimate of OS based on a landmark analysis at 120 days after randomization to SOR or PBO and HCT in hematologic remission. CR1, first remission; PBO, placebo; SOR, sorafenib.

To determine whether outcome was associated with *FLT3*-ITD burden, survival was examined relative to an *FLT3*-ITD allelic ratio threshold of 0.7. This boundary was prespecified in the statistical plan and concordant with analyses in the RATIFY study.[4] Patients with an *FLT3*-ITD allelic ratio ≥ 0.7 had a 2-year OS of 72% in the sorafenib arm vs 33% for the placebo (HR, 0.48; 95% CI, 0.17-1.34; Figure 3D). Among the patients with *FLT3*-ITD allelic ratio < 0.7, no separation of the OS curve was apparent (Figure 3E). Similar patterns were noted for patients categorized using an *FLT3*-ITD allelic ratio threshold of 0.5 (supplemental Figure 4A,B).

FLT3-ITD MRD

Among the 86 patients achieving CR/CRi response after induction, 74 (48, sorafenib and 26, placebo) had samples available for polymerase chain reaction–next-generation

Table 3. Summary of adverse events across all treatment cycles occurring in at least 5% of the patients

Events, n (%)	Sorafenib (n = 65)		Placebo (n = 33)	
CTCAE grade	All grades	Grade ≥3	All grades	Grade ≥3
Hematologic				
Thrombocytopenia	—	58 (89)	—	28 (85)
Neutropenia	—	58 (89)	—	27 (82)
Anemia	—	50 (77)	—	23 (70)
Febrile neutropenia	—	42 (65)	—	18 (55)
Nonhematologic				
Infection/sepsis	45 (69)	36 (55)*	18 (55)	16 (48)
Nausea/vomiting	28 (43)	4 (6)	11 (33)	0
Diarrhea	25 (39)	8 (12)	10 (30)	1 (3)
Constipation	4 (6)	0	1 (3)	0
Colitis/enterocolitis	11 (17)	10 (15)	7 (21)	5 (15)
Mucositis	17 (26)	6 (9)	4 (12)	2 (6)
Rash	35 (54)	7 (11)	18 (55)	8 (24)
Palmar-plantar syndrome	10 (15)	5 (8)	2 (6)	0
QTc prolongation	6 (9)	3 (5)	2 (6)	0
Cardiac arrest	—	—	1 (3)†	1 (3)†
Alanine aminotransferase increase	18 (28)	7 (11)	9 (27)	6 (18)
Bilirubin increase	13 (20)	6 (9)	7 (21)	3 (9)
Acute kidney injury	3 (5)	1 (2)	3 (9)	1 (3)
Hypokalemia	16 (25)	12 (18)	6 (18)	5 (15)
Hypophosphatemia	8 (12)	7 (11)	1 (3)	1 (3)
Fatigue	10 (15)	2 (3)	2 (6)	1 (3)
Hypertension	11 (17)	5 (8)	2 (6)	0
Blurred vision	4 (6)	0	0	0

CTCAE, Common Terminology Criteria for Adverse Events; QTc, corrected QT interval; SAE, serious adverse event.

*Includes 1 patient with *Klebsiella* sepsis who died in induction.

†Includes 1 patient with cardiac arrest who died in induction.

sequencing (PCR-NGS) measurable residual disease (MRD) assessment (supplemental Methods). *FLT3*-ITD MRD–negative status (<0.001%) after induction was associated with a significantly improved 2-year RFS (71% vs 37%; $P = .001$; Figure 4A) and 2-year OS (83% vs 60%; $P = .028$; Figure 4B). The proportion of patients who were *FLT3*-ITD negative after induction in the sorafenib and placebo arms were 44% and 31%, respectively (Figure 4D).[22] The *FLT3*-ITD clearance rate rose to 69% vs 54% in the sorafenib and placebo arms, respectively, after completing consolidation therapy (supplemental Figure 5A,B). The 2-year RFS was significantly higher in the sorafenib arm among patients rendered *FLT3*-ITD MRD–negative after induction (75% vs 46% for those still MRD-positive; $P = .015$; Figure 4C).

At first relapse, evaluable material was available for 31 of 38 patients. *FLT3*-ITD was negative by CE in 9 of 19 patients (47%) in the sorafenib arm, compared with 3 of 12 (25%) in the placebo arm (relative risk, 1.9; 95% CI, 0.72-5.75; Figure 4E). Using a more sensitive PCR-NGS assay at relapse, the proportion with *FLT3*-ITD negativity (<0.001%) in the sorafenib and placebo arms were 29% and 38%, respectively (Figure 4F). Although the overall proportion of those tested as *FLT3*-ITD positive by PCR-NGS in the 2 arms was similar (sorafenib 71% [10/14] vs placebo 63% [5/8]), 5 of 10 patients in the sorafenib arm had *FLT3*-ITD microclones at relapse, with a variant allele frequency ranging from 0.001% to 1%; below the CE detection threshold. Of these, 3 had the same *FLT3*-ITD variant at diagnosis, whereas a different *FLT3*-ITD clone emerged in the other 2 patients.

Neither *FLT3* D835 nor F691 gatekeeper variants were observed at relapse in the sorafenib arm, ruling out *FLT3* on-target resistance as a mechanism of failure after frontline sorafenib and chemotherapy.[23] These findings suggest that sorafenib in combination with chemotherapy is efficacious at suppressing but not eliminating *FLT3*-ITD–mutant clones and that alternative non-*FLT3* resistance mechanisms are possible.

Discussion

The FLT3 inhibitor midostaurin in combination with intensive chemotherapy for adults with *FLT3* mutated AML has been standard of care in the United States since 2017. The multikinase inhibitor sorafenib was approved by the US Food and Drugs Administration for advanced renal carcinoma in 2005. Sorafenib has potent activity against *FLT3*-ITD, with

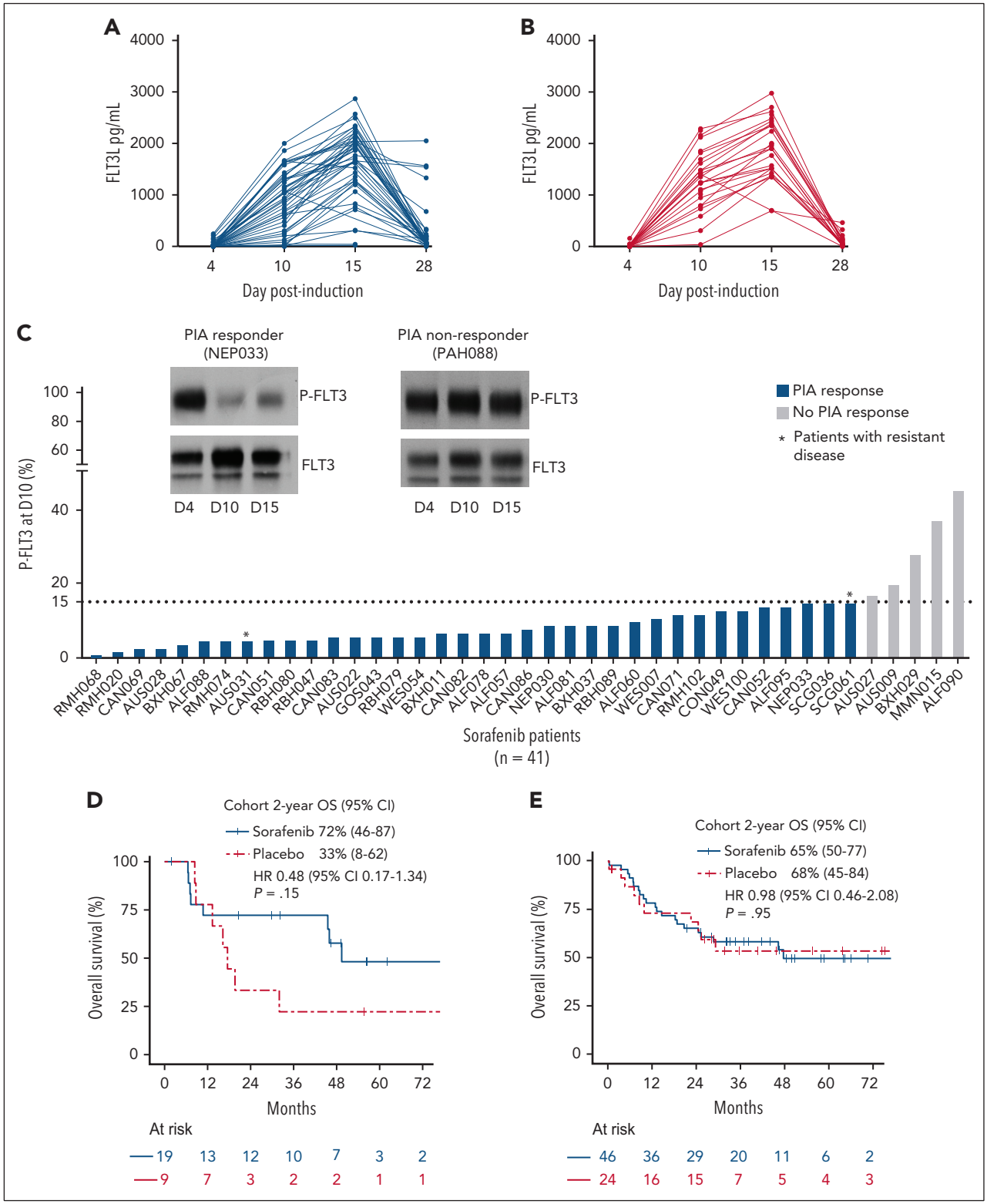

Figure 3. Correlative analysis of FLT3L levels, P-FLT3 levels, and survival outcome according to *FLT3*-ITD allelic burden. (A-B) Peripheral blood plasma FLT3L levels assessed on days 4, 10, 15, and 28 after induction in sorafenib (A) and placebo arms (B). (C) P-FLT3 levels at day 10 relative to day 4 with a representative example immunoblot of P-FLT3 changes in a PIA responder and nonresponder. Patients with a PIA response but without a clinical response are noted with an asterisk (*). (D-E) Kaplan-Meier estimate of OS in the sorafenib or placebo arms among patients with *FLT3*-ITD allelic ratio ≥0.7 (D) or <0.7. D, day (E).

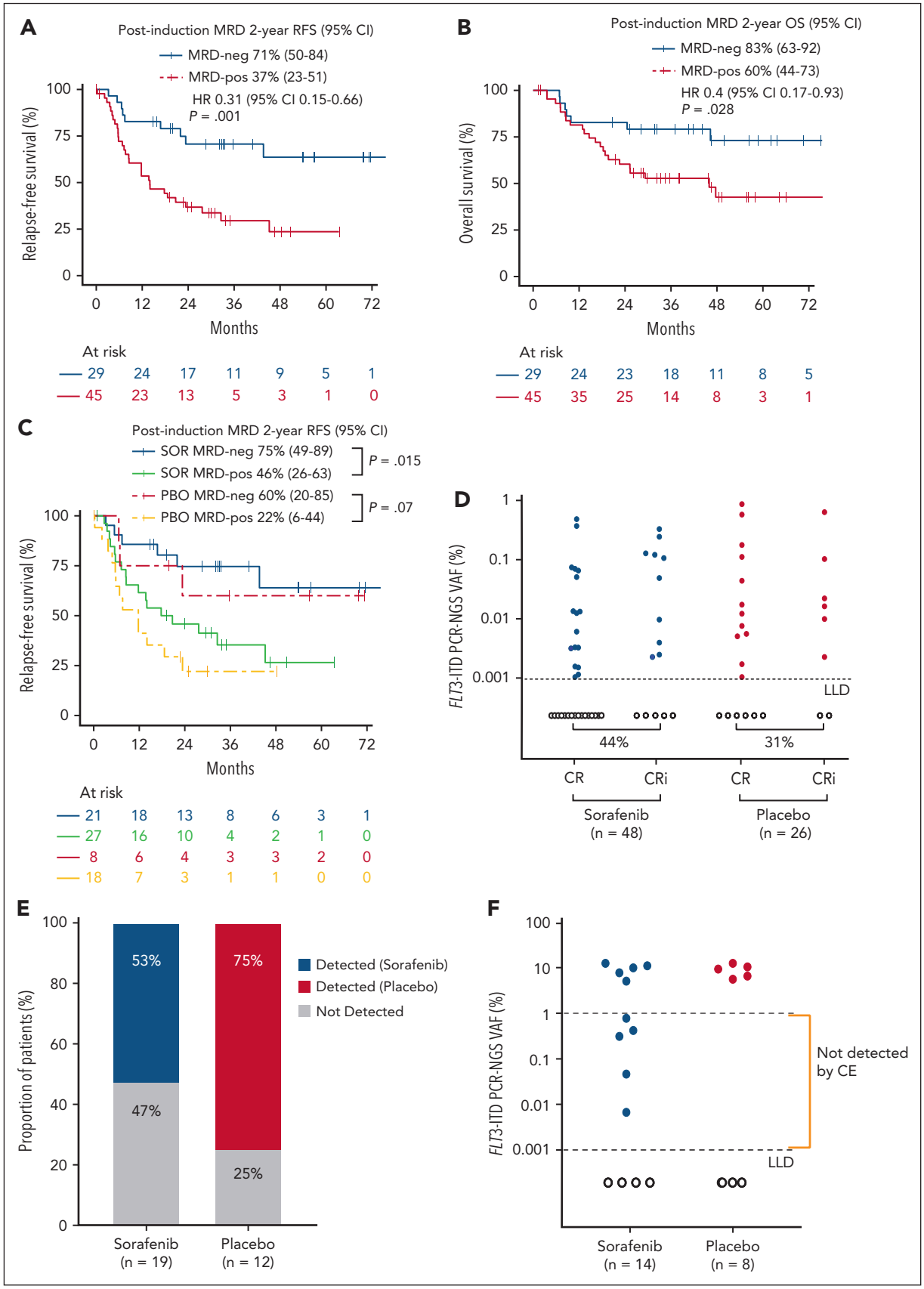

Figure 4. *FLT3*-ITD analysis using high-sensitivity PCR-NGS. (A-B) Kaplan-Meier estimate of RFS (A) and OS (B) according to postinduction *FLT3*-ITD MRD status assessed by PCR-NGS. (C) Kaplan-Meier estimate of RFS according to postinduction *FLT3*-ITD MRD status and treatment arm. (D) Scatter plot distribution of *FLT3*-ITD MRD after induction as indicated by variant allele frequency according to treatment arm and remission status. (E) *FLT3*-ITD status at relapse assessed by CE among patients in the

pharmacokinetic modeling indicating suppression of the kinase at an IC_{50} of 69.3 ng/mL, which is achieved by a sorafenib dose of 200 mg twice daily.[24] Although prolonged schedules of sorafenib have led to increased mortality during AML induction, our study confirmed that a shorter 7-day schedule was tolerable and associated with a 30-day mortality of 2%. Palmar-plantar erythrodysthesia, typically linked to dual inhibitors of platelet-derived growth factor receptor and vascular endothelial growth factor receptor, was observed in 15% of patients in the sorafenib arm, with grade ≥3 events recorded in 8%, potentially minimized by the short period of drug administration in the study. The rationale to administer sorafenib from days 4 to 10 was supported by FLT3L profiling showing a marked increase in ligand levels in both treatment arms by day 10, which peaked by day 15 and returned to baseline by day 28, consistent with prior observations.[17] Reassuringly, our correlative analyses confirmed that pharmacodynamically relevant levels of sorafenib were achieved by day 10 of induction, with a robust inhibition of P-FLT3 to <15% in 88%.

In this study, the primary end point of EFS was not improved by sorafenib. Although there was a nominally higher CR rate in the sorafenib arm (78% vs 70%), this was counterbalanced by a higher proportion of patients achieving CRi in the placebo arm (24% vs 9%). Using a more restricted definition of CR, as defined in the RATIFY study, CR by day 60 was achieved among 63% and 64% patients in the sorafenib and placebo arms, respectively, compared with 59% (midostaurin) and 54% (placebo) in the RATIFY trial. The high overall response rate in the placebo arm (CR/CRi, 94%) was surprising. Notably, the CR/CRi rate was particularly high (96%) among patients aged 18 to 55 years receiving the more intensive intermediate-dose cytarabine-based induction regimen. Prior studies have reported more favorable outcomes among patients with *FLT3*-ITD receiving idarubicin or higher doses of daunorubicin during induction. Another reason might have been the presence of *NPM1* mutation among 81% of patients in the placebo arm compared with 60% in the sorafenib arm. Concurrent use of azoles with sorafenib/placebo was prohibited to minimize the risk of higher sorafenib concentrations and potential toxicity associated with CYP3A4 inhibition. Although it is possible that omission of antifungal azoles could have blunted the overall clinical benefit of sorafenib in this study, the PIA studies suggest that the dose administered was pharmacodynamically sufficient. In any event, based on the high rate of response in the control arm of this study, any improvements in the response rate would likely have been marginal.

Before the introduction of midostaurin, the impact of HCT on OS in *FLT3*-ITD AML was unclear. Gale et al showed no significant improvement in survival with HCT in first remission using a donor-vs no donor comparison.[25] Schlenk et al identified a significant survival benefit for HCT only among patients with a *FLT3*-ITD allelic ratio ≥0.51.[26] In contrast, using a propensity-matched analysis for patients in first remission for at least 3 months, Oran et al demonstrated that HCT improved survival regardless of the *FLT3*-ITD allelic ratio.[27] In this study, the rate of allo-HCT at first remission was high in both arms (sorafenib, 62% and placebo, 58%). As expected, HCT in first remission improved survival compared with no transplant, across a series of landmark analyses for patients in the sorafenib arm. As a representative example, the 2-year OS in the sorafenib arm using a 120-day landmark was higher for those who received transplantation in first remission vs those who did not (84% vs 53%; HR, 0.35; 95% CI, 0.14-0.87; P = .02). Interestingly, a significant survival benefit for HCT was not evident in the placebo arm (Figure 2D; supplemental Figure 3).

In the RATIFY study, a trend for improved survival was suggested for patients who received HCT in first remission receiving prior midostaurin, compared with placebo (P = .07).[4] In this study, the 2-year OS for patients who received transplantation in first remission using a 120-day landmark was 84% vs 67% in the sorafenib and placebo arms, respectively (HR, 0.45; 95% CI, 0.18-1.12; P = .08). Although not significant, the limited study size was potentially a factor. Although it has been hypothesized that the enhanced benefit of midostaurin in relation to post-HCT outcomes might be attributable to lower levels of MRD achieved before transplant, MRD was not available in the RATIFY study. We recently demonstrated that pre-HCT *FLT3*-ITD MRD (≥0.001%) by PCR-NGS was strongly associated with increased relapse risk and reduced OS after transplantation.[22] In this study, *FLT3*-ITD MRD–negative status after induction was significantly associated with improved RFS and OS. Although this study was not adequately powered to show that sorafenib inhibition could enhance the rate of MRD response before transplant, future studies using other FLT3 inhibitors are awaited.

A notable finding in prior studies was the proportion of patients who were negative for *FLT3*-ITD at relapse after receiving midostaurin and intensive chemotherapy: 46% compared with 19% in patients who received placebo.[28,29] In this study, *FLT3*-ITD by CE was also frequently absent in the sorafenib arm (47% vs 25%), suggesting sorafenib had eradicated *FLT3*-ITD clones. Interestingly, using a more sensitive PCR-NGS assay, *FLT3*-ITD microclones (variant allele frequency, 0.001%-1%) were present in half the sorafenib-treated cases initially thought to be negative by CE (sensitivity 2%-5%). Pertinently, such *FLT3*-ITD microclones were not identified in the placebo cohort, although this observation requires validation in a larger cohort of patients.

Although we expected to see *FLT3*-TKD D835 variants at relapse in the sorafenib arm, this was not the case.[30] These findings suggest that treatment with sorafenib is capable of suppressing but not necessarily eliminating *FLT3*-ITD subclones and perhaps supporting the case for more prolonged maintenance therapy to sustain disease control. It remains unknown whether a *FLT3*-ITD microclone would be selected at subsequent relapse if a FLT3 inhibitor was not included at salvage.

In conclusion, this study confirms the safety of a 7-day schedule of sorafenib in combination with intensive induction and consolidation chemotherapy. The results of this phase 2 randomized study do not support the routine use of sorafenib in the pre-HCT setting for patients with *FLT3*-ITD AML. This report

Figure 4 (continued) sorafenib and placebo arms. Seven patients without available CE results at relapse were not included. (F) Presence of *FLT3*-ITD microclones with variant allele frequency (VAF) ≤ 1% in sorafenib-treated patients at relapse assessed by PCR-NGS. LLD, lower limit detection < 0.001%.

makes the new observation that *FLT3*-ITD monitoring by PCR-NGS represents a powerful prognostic marker for patients achieving clinical response, and our correlative data are generally supportive of the use of more potent FLT3 inhibitors in the management of patients with *FLT3*-mutant AML. Although sorafenib has been used in the post-HCT setting, this will likely change after positive validation of quizartinib in the QuANTUM-First study and recent preliminary data from the randomized BMT-CTN 1506 (MORPHO) study, which suggest that only patients with evidence of *FLT3*-ITD MRD detected before or after transplant benefit from gilteritinib maintenance in the post-HCT setting.[8,31]

Acknowledgments

The authors thank Shaun Fleming for information on statistical analysis tools.

This work was supported by grants from the Australian National Health and Medical Research Council (1048312 [A.H.W. and A.W.R.] and 2018071 [A.H.W.]) and Leukaemia Foundation Australia (A.H.W.).

Authorship

Contribution: A.H.W., J.F.S., and M.L. designed the research; G.K., K.M., S.H., C.Y.F., A.P.S., S.B.T., A.K.E., S.Y., J.D., M.L., P.M., I.B., J.T., G.C., C.T., E.V., U.H., D.H., H.J.I., N.M., S.R., A.B., A.W.R., and A.H.W. recruited patients to the study and analyzed study results; J.L., S.L., and J.R. performed the statistical analyses; S.L., I.S.T., D.M.O., and M.W. reviewed the diagnostic and molecular data; N.S.A., M.M., T.R., and M.L. performed the correlative studies; L.R. coordinated the trial data collection; S.L. and A.H.W. wrote the first version of the manuscript; and all authors read, reviewed, and approved the manuscript.

Conflict-of-interest disclosure: A.H.W. has served on advisory boards for Novartis, AstraZeneca, Astellas, Janssen, Amgen, Roche, Pfizer, AbbVie, Servier, Gilead, Bristol Myers Squibb, Shoreline, Macrogenics, and Agios; receives research funding to the Institution from Novartis, AbbVie, Servier, Janssen, BMS, Syndax, Astex, AstraZeneca, and Amgen; and serves on speaker's bureaus for AbbVie, Novartis, BMS, Servier, and Astellas. A.H.W., A.W.R., and N.S.A. are employees of the Walter and Eliza Hall Institute (WEHI); WEHI receives milestone and royalty payments related to the development of venetoclax. Current and past employees of WEHI may be eligible for financial benefits related to these payments, and A.H.W., A.W.R., and N.S.A. receive such financial benefits. A.W.R. is listed as an inventor on a patent related to venetoclax assigned to AbbVie and Genentech. The remaining authors declare no competing financial interests.

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Footnotes

Submitted 28 March 2023; accepted 1 August 2023; prepublished online on *Blood* First Edition 30 August 2023. https://doi.org/10.1182/blood.2023020301.

The deidentified data that support the findings of this study are available upon reasonable request from the corresponding author, Andrew H. Wei (andrew.wei@petermac.org).

The online version of this article contains a data supplement.

There is a *Blood* Commentary on this article in this issue.

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