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ORIGINAL REPORT

Phase I/II Study of Combination Therapy With Sorafenib, Idarubicin, and Cytarabine in Younger Patients With Acute Myeloid Leukemia

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ABSTRACT

Purpose
To determine the efficacy and toxicity of the combination of sorafenib, cytarabine, and idarubicin in patients with acute myeloid leukemia (AML) younger than age 65 years.

Patients and Methods
In the phase I part of the study, 10 patients with relapsed AML were treated with escalating doses of sorafenib with chemotherapy to establish the feasibility of the combination. We then treated 51 patients (median age, 53 years; range, 18 to 65 years) who had previously untreated AML with cytarabine at 1.5 g/m^2 by continuous intravenous (IV) infusion daily for 4 days (3 days if > 60 years of age), idarubicin at 12 mg/m^2 IV daily for 3 days, and sorafenib at 400 mg orally twice daily for 7 days.

Results
Overall, 38 (75%) patients have achieved a complete remission (CR), including 14 (93%) of 15 patients with mutated FMS-like tyrosine kinase-3 (FLT3; the 15th patient had complete remission with incomplete platelet recovery [CRp]) and 24 (66%) of 36 patients with FLT3 wild-type (WT) disease (three additional FLT3-WT patients had CRp). FLT3-mutated patients were more likely to achieve a CR than FLT3-WT patients ($P = .033$). With a median follow-up of 54 weeks (range, 8 to 87 weeks), the probability of survival at 1 year is 74%. Among the FLT3-mutated patients, 10 have relapsed and five remain in CR with a median follow-up of 62 weeks (range, 10 to 76 weeks). Plasma inhibitory assay demonstrated an on-target effect on FLT3 kinase activity.

Conclusion
Sorafenib can be safely combined with chemotherapy, produces a high CR rate in FLT3-mutated patients, and inhibits FLT3 signaling.

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INTRODUCTION

The FMS-like tyrosine kinase-3 (FLT3) is a promising target in acute myeloid leukemia (AML).[1,2] Activating mutations of the kinase occur in about one third of patients with AML and are associated with leukocytosis, higher marrow blast percentage, higher likelihood of relapse, and shorter survival.[3-6] There are conflicting data on the association of these mutations with a lower complete remission (CR) rate and a higher induction death rate.[3,6]

An internal tandem duplication (ITD) in the juxtamembrane domain of the FLT3 gene occurs in approximately 25% of younger adult AML patients with the length of the duplicated DNA varying between 3 and > 400 base pairs.[7] Such in-frame mutations produce functional proteins with constitutive kinase activity leading to the activation of downstream signaling pathways including the STAT5 and MAP kinase pathways.[8] Variations in the ratio of wild-type (WT) to mutant allele levels, number of mutants of different sizes in the same patient, and the size of the inserted DNA have also been reported to be of prognostic significance.[3,5,6,9-12]

Constitutive phosphorylation of FLT3 in the absence of FLT3-ITD suggested the existence of other mechanisms of aberrant FLT3 signaling, including FLT3 tyrosine kinase domain (TKD) mutations and autocrine signaling by the FLT3 ligand and WT FLT3.[13-16] Mutations affecting codons 835 and 836 in the FLT3 second kinase domain occur in approximately 7% of patients and lead to its constitutive activation.[13,14] Other novel activating mutations within the activation loop of FLT3 kinase have been described.[16-18] More recently, point mutations

in the juxtamembrane domain of FLT3 have been reported as a new class of activating mutations.[19] Prognostic significance of these less common mutations remains unclear, with conflicting reports on FLT3-TKD mutations being associated with either a poorer or more favorable outcome.[20-23]

A number of small-molecule kinase inhibitors such as lestaurtinib (CEP-701), midostaurin (PKC412), and tandutinib (MLN518) block the autophosphorylation of FLT3 and lead to inhibition of cell proliferation and induction of apoptosis; they have demonstrated clinical activity in patients with AML, in particular those with mutations.[24-27] These drugs act synergistically with standard cytotoxic agents if used simultaneously with or after chemotherapy.[28]

Sorafenib is an oral small molecule, originally designed as an inhibitor of Raf-1 kinase targeting the RAF/MEK/ERK pathway; it has inhibitory properties against a number of other kinases including FLT3 and vascular endothelial growth factor receptor.[29-31] In preclinical studies, sorafenib induced dephosphorylation of MEK1/2 and ERK and induced apoptosis in AML cells.[32] Furthermore, sorafenib was 1,000- to 3,000-fold more potent in inducing apoptosis in Ba/F3 cells with FLT3-ITD or D835G mutations than those with WT FLT3.[33] In a mouse model of AML with mutant FLT3, sorafenib reduced the leukemic burden and prolonged survival.[33] It has been approved by the US Food and Drug Administration for the treatment of renal cell and hepatocellular carcinoma at a standard dose of 400 mg twice daily. In phase I studies and anecdotal use in patients with advanced AML, sorafenib was capable of producing significant clinical responses.[33,34] The objectives of this study were to determine the feasibility, safety, and efficacy of combining sorafenib with induction chemotherapy.

PATIENTS AND METHODS

Patient Eligibility

Patients with diagnosis of AML by WHO criteria who had relapsed after prior response (irrespective of number of prior salvage regimens) or were refractory to initial induction therapy with standard regimens were eligible to participate in the phase I part of the study. After the establishment of a safe dose of sorafenib in combination with chemotherapy, patients with previously untreated AML who were between the ages of 18 and 60 years were eligible. Patients older than age 60 years who had a low probability of 8-week mortality with intensive chemotherapy were also eligible, depending on the number of adverse risk factors (cytogenetics, performance status, antecedent hematologic disorder, organ function)[35]. For both phase I and II, patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2 and adequate cardiac, renal, and hepatic function with left ventricular ejection fraction $\geq$ 50%, creatinine $\leq$ 2.0 mg/dL, bilirubin $\leq$ 2.0 mg/dL, and liver transaminases less than three times the institutional upper limit of normal. All patients had to have reviewed and signed an appropriate informed consent approved by the institutional review board. Patients in the historical control group also signed an institutional review board–approved consent to participate in the study.

Treatment Regimen

The treatment included cytarabine at 1.5 g/m^2 given by continuous intravenous (IV) infusion daily for 4 days (3 days for patients older than age 60 years) as well as idarubicin at 12 mg/m^2 IV over 1 hour daily for 3 days. In phase I, sorafenib was administered during the first 7 days at escalating doses of 400 mg orally (PO) every other day, 400 mg PO daily, and 400 mg PO twice daily to cohorts of three patients. After the 400-mg twice daily dose was established as safe, all patients received this dose for the first 7 days of induction in phase II. Patients not achieving CR after one course could receive a second induction course if the treating physician determined this to be in the patients' best interest.

Patients achieving a CR could receive up to five cycles of consolidation (number chosen arbitrarily) with cytarabine at 0.75 g/m^2 IV over 24 hours daily for 3 days, idarubicin at 8 mg/m^2 IV over 1 hour daily for 2 days, and sorafenib at 400 mg PO twice daily for up to 28 days. Consolidation cycles were repeated every 4 to 6 weeks, depending on the recovery of neutrophil and platelet counts and toxicity. After the completion of consolidation courses, patients received maintenance sorafenib at 400 mg twice daily for a total of up to 1 year of sorafenib therapy (including the consolidation courses). Reductions to the doses of all three agents during consolidation and maintenance were allowed according to predetermined guidelines related to various adverse effects.

Response Criteria and Definitions

CR was defined by the presence of $<$ 5% blasts in the bone marrow (BM) with $> 1 \times 10^9$/L neutrophils and $> 100 \times 10^9$/L platelets in the peripheral blood (PB). Relapse was defined by recurrence of $>$ 5% blasts in a BM aspirate unrelated to recovery or by the presence of extramedullary disease. CR duration was calculated from the time of CR until relapse. Progression-free survival (PFS) was calculated from the beginning of treatment until an event including relapse, death during induction, or death in CR. Overall survival was calculated from the time of diagnosis until death.

Statistical Analysis

The overall trial objective was to provide an early assessment of efficacy of sorafenib when used in combination with idarubicin and cytarabine. Initially, escalating doses of sorafenib (up to the standard dose of 400 mg twice a day) were administered in cohorts of three patients. If grade 3 to 4 sorafenib-related toxicities were observed in more than two of six patients, the dose level would exceed the maximum tolerated dose. In the phase II study, we monitored the response rate and PFS as patients accrued and planned to stop the trial if we had evidence that the target CR of 70% or median PFS (7 months) could not be met. Formally, we planned to stop the study early (for futility) if there was less than a 2% chance that the median PFS rate was $\geq$ 7 months. On the basis of the above criterion, using simulations, we determined that if the true median PFS rate was $\geq$ 7 months, then there would be a $\leq$ 11% chance of declaring the treatment ineffective. Alternatively, if the true median PFS was $\leq$ 4 months, there would be a $>$ 80% chance of declaring the treatment ineffective.

Survival curves were plotted by the Kaplan-Meier method and compared using the log-rank test. Differences in subgroups by different covariates were evaluated using the χ^2 test for nominal values and the Mann-Whitney U test and Fisher's exact test for continuous variables.

RESULTS

Patient Characteristics

From October 2007 to February 2009, 61 patients with AML were enrolled, including 10 patients treated during phase I and 51 patients treated during phase II. Patient characteristics are summarized in Table 1. The median age of the patients treated in phase I was 34 years (range, 21 to 59 years). They had a median of two prior regimens (range, one to six prior regimens). Seven had FLT3 mutations, including one patient with FLT3-ITD and FLT3-TKD double mutants; in the phase I study, patients were targeted for the presence of FLT3 mutations.

In the phase II study, the median age was 53 years (range, 18 to 65 years). Eleven patients were older than 60 years of age, 11 patients had antecedent hematologic disorder, and five had unfavorable cytogenetics. Fifteen had FLT3 mutations, including 13 with FLT3-ITD (four with low mutation burdens) and two with FLT3-TKD (one with low mutation burden). The median presentation WBC was 5.2 $\times$ 10^9/L (range, 0.6 to 122.7 $\times$ 10^9/L). Eight patients were FLT3-ITD–positive/nucleophosmin-1 (NPM1) –negative. The median age of FLT3-mutated patients was 53 years (range, 20 to 65 years); nine had diploid

Table 1. Characteristics of Patients in Phase I and Phase II Trials

Characteristic	Phase I No.	Phase I %	Phase II No.	Phase II %
No. of patients	10		51	
Age, years				
Median	34		53	
Range	21-59		18-65	
> 60	0	0	11	22
WBC at presentation, 10^9/L				
Median	6.5		5.2	
Range	0.9-28.4		0.6-122.7	
Antecedent HD	0	0	11	22
Cytogenetics				
Diploid	2		22	
Intermediate risk	7		24	
Unfavorable risk	1		5	
No. of prior therapies				
Median	2			
Range	1-6		0	
FLT3-WT	3	30	36	71
FLT3-mutated				
ITD	6	60	13	25
TKD	0		2	4
Both	1	10	0	
FLT3 mutation burden				
High (> 25%)	5		10	
Low (≤ 25%)	2		5	
NPM1-mutated	3 of 7	43	12 of 50	24

Abbreviations: HD, hematologic disorder; FLT3-WT, FMS-like tyrosine kinase-3 wild type; ITD, internal tandem duplication; TKD, tyrosine kinase domain; NPM1, nucleophosmin-1.

Table 2. Response in Phase II Study

Response	FLT3 Mutational Status: Negative	Low	High	Positive (All)
CR	24	4	10	14
CRp	3	0	1	1
Early death	3	0	0	0
Resistant	6	0	0	0

Abbreviations: FLT3, FMS-like tyrosine kinase-3; CR, complete remission; CRp, CR with incomplete platelet recovery.

karyotype, two had +8, one was −5/−7, and three were miscellaneous. Their median presentation WBC was 18.6×10^9/L (range, 1.9 to 122.7×10^9/L).

Response and Outcome

Among the 10 patients in the phase I part of the study, four (40%) achieved a CR (three of seven patients with FLT3-ITD mutation compared with one of three with FLT3-WT). The other six patients either had refractory disease or died from complications of therapy. All four patients achieving a CR proceeded to an allogeneic stem-cell transplantation and all are still alive.

In the phase II part of the study, 51 patients were available for response assessment and 38 (75%) patients have achieved a CR, including 12 (92%) of 13 patients with FLT3-ITD (the thirteenth patient had CRp), two (100%) of two patients with FLT3-TKD, and 24 (66%) of 36 patients with FLT3-WT disease (three patients with FLT3-WT had CRp; Table 2). The difference between CR rate of FLT3-mutated and FLT3-WT patients was statistically significant ($P = .033$). Three patients died at induction and six were resistant to therapy (all FLT3-WT). CR was achieved after one induction cycle in 34 patients and after two induction cycles in four patients. Fourteen FLT3-mutated patients achieved CR/CRp after one cycle and one achieved CR/CRp after two cycles of induction. Altogether, seven patients have proceeded to an allogeneic stem-cell transplantation in the first CR, including four FLT3-mutated patients (three FLT3-ITD, one FLT3-TKD).

With a median follow-up of 54 weeks (range, 8 to 87 weeks) for all patients, the probability of survival at 6 months was 83%; at 12 months, it was 74% (Fig 1A). Figures 1B and 1C demonstrate the PFS for all patients and for patients with mutated FLT3; the CR duration for the latter is shown in Figure 1D. Among the patients with mutated FLT3, 10 have relapsed and five remain in CR with a median follow-up of 62 weeks (range, 10 to 76 weeks).

Toxicity

Assessment of toxicity and its attribution was based on baseline expectations and data available from sorafenib solid tumor studies.[36] The regimen was reasonably well tolerated with adverse events being, in general, similar to those expected in patients receiving induction chemotherapy with the idarubicin + cytarabine (IA) combination. Grade ≥ 3 adverse events thought to be possibly related to the addition of sorafenib during induction included hyperbilirubinemia in four patients, elevated transaminases (five), diarrhea (four), rash (two), pancreatitis (one), colitis (one), pericarditis (one), hand and foot syndrome (two), and elevated creatinine (one; Table 3).

Dynamics of Mutated FLT3 Levels During the Treatment

We examined the fate of the FLT3-mutated clone in BM aspirate samples taken 3 weeks after cycle 1 of treatment. Among 11 patients with available samples, six had complete regression of the FLT3-mutated clone, three had partial regression (as assessed by > two-fold change in the blast-normalized mutant allelic ratio), and two had no change in the ratio. Among the historical controls, 10 FLT3-mutated patients had available samples at 3 weeks, with six showing persistence of the FLT3-mutated clone, one with a decrease in the blast-normalized allelic ratio, and two with disappearance of the FLT3-mutated clone ($P = .04$, Fisher's exact test).

Plasma Inhibitory Assay and Comparison With Lestaurtinib and Midostaurin

To determine the efficacy of in vivo FLT3 inhibition from sorafenib with this dosing regimen, we performed plasma inhibitory activity assays using blood samples obtained from trial patients 12 hours after dosing with sorafenib on day 7. Previous studies have shown the utility of this method for other FLT3 inhibitors.[24,37,38] The results of plasma inhibitory activity assays for 10 trial patients are shown in Figure 2. All 10 patients displayed complete inhibition of phosphorylated FLT3 in this assay. This profound degree of in vivo

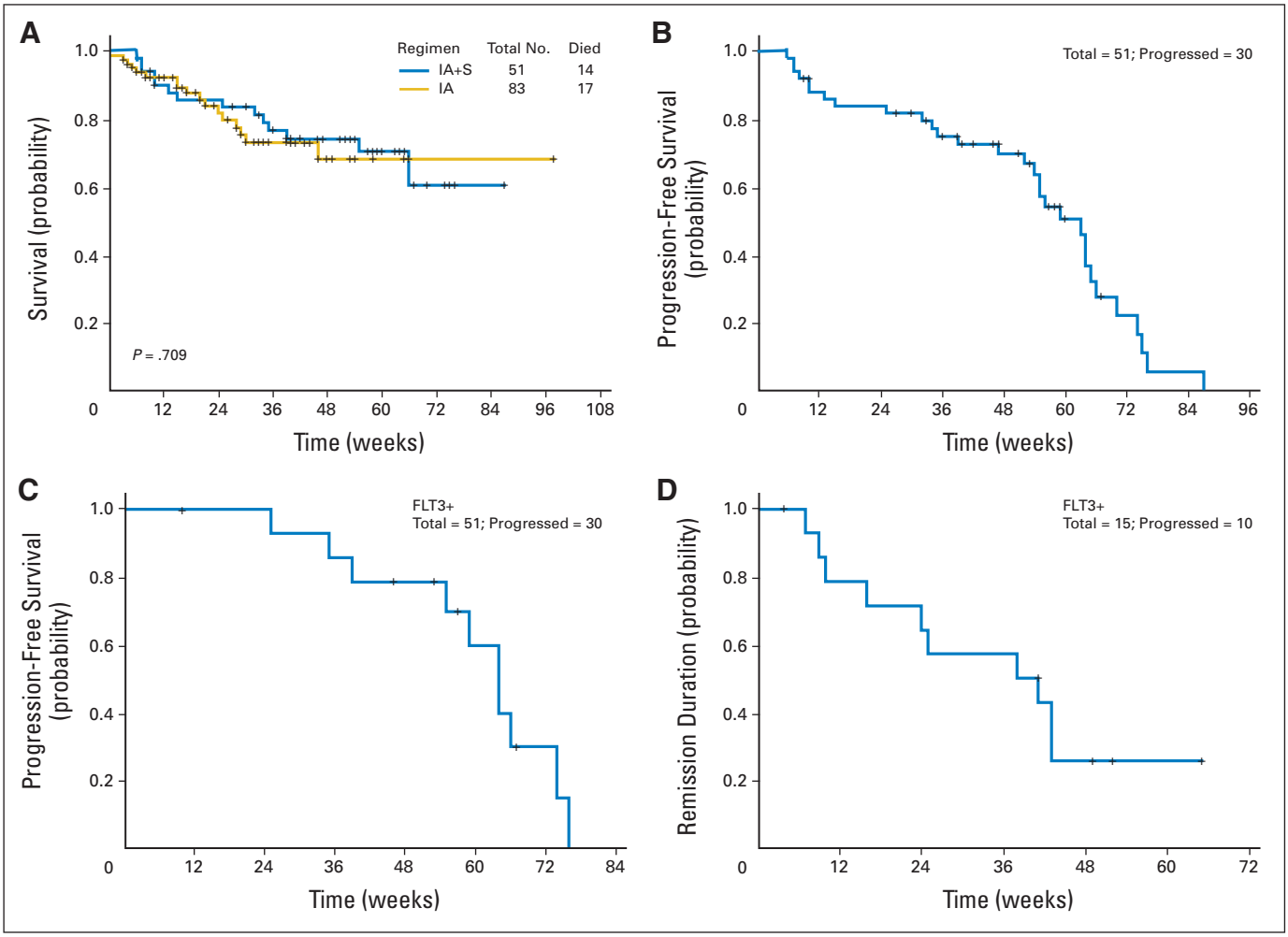

Fig 1. (A) Survival for patients treated with idarubicin and cytarabine plus sorafenib (IA + S) and a historical IA alone, (B) progression-free survival for the patients treated with IA + S, (C) progression-free survival for FMS-like tyrosine kinase-3–mutated (FLT3+) patients treated with IA + S, and (D) duration of complete remission for FLT3+ patients treated with IA + S.

FLT3 inhibition was not observed with other FLT3 inhibitors such as lestaurtinib, midostaurin, and KW-2449 (Fig 3).[24,38]

DISCUSSION

Dysregulated receptor tyrosine kinase function is implicated in the pathogenesis of a number of hematologic malignancies leading to a search for potential targets for kinase inhibition in AML.[39-41] The role of FLT3 kinase in leukemogenesis and the identification of its mutant forms and their adverse influence on the outcome of patients with AML suggest that its inhibitors are of potential therapeutic benefit.[1,42,43] This is further corroborated by the high level of expression of FLT3 receptor in most AML cells and demonstration of constitutive activation of FLT3 signaling not only through mutations but also by autocrine signaling in FLT3-WT AML.[16,44,45] Mutations of the FLT3 receptor gene are among the most common molecular abnormalities in AML; the presence of FLT3-ITD has been associated with shorter duration of remission and overall survival by a number of groups, whereas information on the influence of FLT3-TKD on outcome has been conflicting.[3-6,20,21,23]

Inhibition of FLT3 kinase in vitro prevents its autophosphorylation and activation of downstream signaling pathways (including STAT5 and MEK/ERK MAP kinase pathways), leading to apoptosis.[46-48] Small-molecule FLT3 kinase inhibitors have been developed with activity against FLT3-mutant cells. Furthermore, these agents act synergistically with chemotherapeutic agents such as idarubicin and cytarabine to induce cytotoxicity.[28,49] The relative efficacy and toxicity of these agents alone or in combination with chemotherapy remains to be determined and is likely dependent on a number of factors such as plasma protein binding, degree of residual FLT3 phosphorylation and downstream STAT5 and MAP kinase signaling, specificity against target FLT3 and the number of other kinases inhibited, and presence or absence of de novo or induced resistance.[37,46,48,50-52] For example, mutations in the kinase domain of FLT3 that generate FLT3-ITD-TKD double mutants have been shown to confer resistance to the small-molecule inhibitors.[50,52,53]

The variable potency of FLT3 inhibitors and demonstration of de novo and acquired resistance to FLT3 inhibitors justifies the identification of new agents active against mutant FLT3. The potential role of residual downstream signaling as a mechanism of resistance suggests

Table 3. Toxicity During Induction Course

Toxicity	No. of Patients With Grade 1 and 2	No. of Patients With Grade 3 and 4
GI effects (nausea and vomiting)	18	
Mucositis	7	2
Colitis		1
Anorexia	2	
Elevated liver enzymes	3	5
Elevated bilirubin	5	4
Rash	15	2
Bleeding	3	1
Diarrhea	23	4
Hand and foot syndrome	4	2
Cardiac/hypertension	3	4
Elevated creatinine	1	1
Weight gain/fluid overload	2	
Pulmonary effects		2
Pancreatitis		1

that agents with dual activity against FLT3 and its downstream targets, such as the MAP kinase pathway, may be beneficial. Sorafenib induces the dephosphorylation of MEK1/2 and ERK proteins and leads to apoptosis of AML cells via Bim-mediated activation of the intrinsic apoptotic pathway.[32] Furthermore, molecules that are less plasma protein bound may be more active in the clinical setting. Sorafenib is as potent as other available FLT3 inhibitors in culture media and is more potent in plasma possibly because of a lower degree of plasma protein binding (Fig 3 and Appendix Table A1, online only; data provided by M.L.).

The potential role of FLT3 tyrosine kinase inhibitors (TKIs) in the treatment of patients with AML and mutated FLT3 remains undefined. TKIs such as imatinib and dasatinib have been successfully combined with chemotherapy regimens to treat patients with Philadelphia chromosome–positive acute lymphoblastic leukemia.[54,55] In that disease, the target gene and its protein product with enhanced kinase activity has been clearly implicated as a pivotal pathogenic factor. In AML, the mutations of FLT3 gene lead to enhanced kinase activity of the protein product and constitutive activation of proliferative and prosurvival signals. However, such mutations are insufficient to lead to the AML phenotype without the presence of cooperative mutations in genes involved in cellular differentiation. This multiple hit theory suggests that the inhibition of FLT3 signaling on its own may not be sufficient to completely reverse the AML phenotype and perhaps combination with other agents with activity against other deregulated pathways in the leukemic cells may be necessary to produce long-lasting remissions. Agents such as sorafenib with activity against downstream signaling pathways may have an advantage over other FLT3 inhibitors without such activity.

We compared the results with those in a matched population of 83 patients with AML treated at our institution with a regimen identical to the one described above but without the addition of sorafenib. Eleven had FLT3-ITD mutation (five with low mutation burden), four had FLT3-TKD mutations, and one had both. The response rates (CR and CRp) for patients with mutated FLT3 (including both FLT3-ITD and FLT3-TKD patients) treated with the sorafenib-containing regimen were higher than those seen with IA alone (15 [100%] of 15 *v* 11 [79%] of 14; $P = .049$). There was no statistically significant difference in response rate when comparing all treated patients and, so far, with small numbers and short follow-up, no significant difference in survival, PFS, or CR duration when comparing all patients (Fig 1A) or only FLT3-mutated patients.

In conclusion, we were able to achieve a universal response in patients with mutated FLT3 and clearly demonstrated the on-target effect of sorafenib on FLT3 signaling. However, with a relatively short follow-up, several patients with mutated FLT3 have already relapsed. Clearly, so far, the addition of the potent TKI in induction, consolidation, and maintenance does not appear to prevent relapse. This may be related to the schedule of administration of

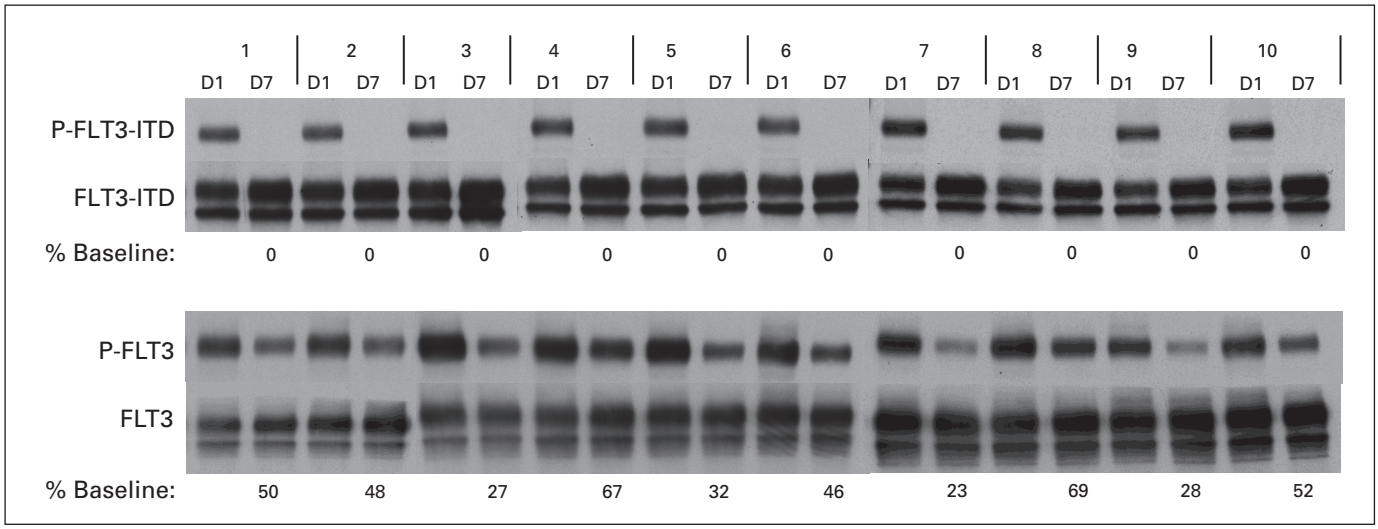

Fig 2. Plasma samples from patients were incubated with TF-ITD cells (upper blots) and SEMK2 FLT3 wild type (FLT3-WT) cells (lower blots). The cells were then lysed and FLT3 was immunoprecipitated and subject to sodium dodecyl sulfate polyacrylamide gel electrophoresis. After transfer, the membranes were probed with antiphosphotyrosine (upper rows of each blot). The membranes were stripped and reprobed with anti-FLT3 to confirm equal loading (lower rows). The percent baseline refers to the densitometric measurement of the day 7 (D7) sample compared with the D1 sample for each patient. P-FLT3, phospho-FLT3.

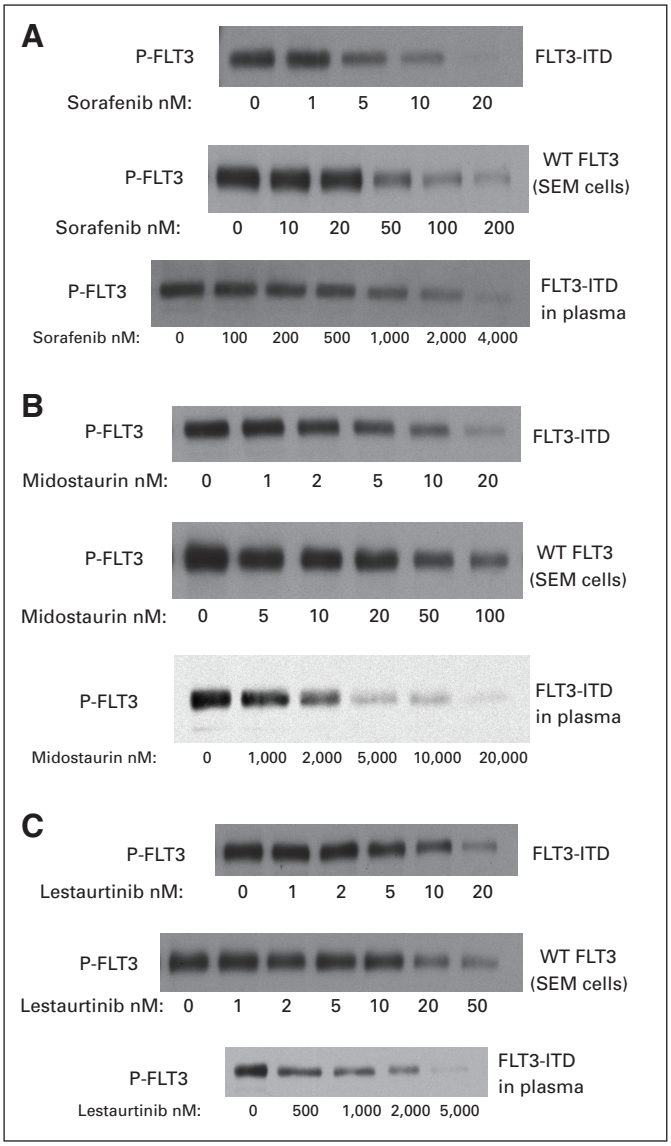

Fig 3. Effect of (A) sorafenib, (B) PKC412 (midostaurin), and (C) CEP-701 (lestaurtinib) on FMS-like tyrosine kinase-3 (FLT3) phosphorylation in TF-ITD cells (upper blots) and SEMK2 FLT3 wild type (FLT3-WT) cells (middle blots) in culture media (upper two blots) and plasma (lower blots).

sorafenib, development of resistance to sorafenib, and other potential factors limiting the beneficial effect of sorafenib. Randomized clinical trials are needed to demonstrate any prolongation of CR duration and survival in patients receiving FLT3 inhibitors. Similarly, mechanisms of resistance to FLT3 inhibitors such as development of mutations or the protective effects of a microenvironment will need to be better defined.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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