A phase Ib trial of mivavotinib (TAK-659), a dual SYK/FLT3 inhibitor, in patients with relapsed/refractory acute myeloid leukemia

Keith W. Pratz,[1] Jason Kaplan,[2] Moshe Levy,[3] Dale Bixby,[4] Patrick W. Burke,[4] Harry Erba,[5] Trisha M. Wise-Draper,[6] Gail J. Roboz,[7] Nikolaos Papadantonakis,[8] Trivikram Rajkhowa,[9] Daniela Hernandez,[9] Iwona Dobler,[10] Richard C. Gregory,[10] Cheryl Li,[10] Shining Wang,[10] Kate Stumpo,[10] Karuppiah Kannan,[10] Harry Miao[10] and Mark Levis[9]

[1]Abramson Cancer Center of the University of Pennsylvania, Philadelphia, PA; [2]Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL; [3]Baylor University Medical Center, Dallas, TX; [4]University of Michigan Rogel Cancer Center, Ann Arbor, MI; [5]Duke University School of Medicine, Durham, NC; [6]University of Cincinnati Cancer Center, Cincinnati, OH; [7]Weill Cornell Medical College, New York, NY; [8]Winship Cancer Institute of Emory University, Atlanta, GA; [9]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD and [10]Takeda Development Center Americas Inc. (TDCA), Cambridge, MA, USA

Correspondence: M. Levis
levisma@jhmi.edu

Received: April 27, 2022.
Accepted: October 5, 2022.
Early view: October 13, 2022.

https://doi.org/10.3324/haematol.2022.281216

Abstract

Mivavotinib (TAK-659) is an investigational type 1 tyrosine kinase inhibitor with dual activity against spleen tyrosine kinase (SYK) and FMS-like tyrosine kinase 3 (FLT3). We conducted a phase Ib study to investigate the safety, tolerability, and efficacy of mivavotinib in patients with refractory and/or relapsed (R/R) acute myeloid leukemia (AML). Both daily (QD) and twice daily (BID) dosing regimens were evaluated. A total of 43 patients were enrolled, and there were 5 complete responses (4 with incomplete count recovery). In the QD dosing regimen, the maximum tolerated dose (MTD) was not reached up to 160 mg QD per protocol; 140 mg QD was identified as the recommended phase II dose. In the BID dosing regimen, the MTD was 60 mg BID. Thirty patients (70%) experienced a bleeding event on study; the majority were grades 1 or 2, were resolved without mivavotinib modification, and were not considered related to study treatment. Eleven patients (26%) experienced grade ≥3 bleeding events, which were observed most frequently with the 80 mg BID dose. We conducted platelet aggregation studies to investigate the potential role of mivavotinib-mediated SYK inhibition on platelet function. The bleeding events observed may have been the result of several confounding factors, including AML disease status, associated thrombocytopenia, and high doses of mivavotinib. Overall, these findings indicate that the activity of mivavotinib in R/R AML is modest. Furthermore, any future clinical investigation of this agent should be undertaken with caution, particularly in thrombocytopenic patients, due to the potential bleeding risk of SYK inhibition. ClinicalTrials.gov: NCT02323113.

Introduction

Acute myeloid leukemia (AML) is a genetically heterogenous hematologic malignancy that arises from the sequential development of key mutations within hematopoietic stem/progenitor cells.[1] Most patients with AML are >60 years of age, and overall prognosis is poor. Although standard-of-care treatment remained almost unchanged for decades, several new agents have recently been approved. Most new therapies are molecularly targeted and are either approved for, or most effective in, genetically-defined AML subtypes.

FMS-like tyrosine kinase 3 (FLT3) is a receptor tyrosine kinase which is important for hematopoiesis; it is expressed on blasts in most cases of AML.[2] Internal tandem duplication (ITD) mutations of FLT3 are among the most common mutations found in AML and are associated with a poor prognosis. Additional activating mutations are found in the tyrosine kinase domain (TKD) and at non-canonical sites.[3] Sustained *in vivo* inhibition of FLT3 is necessary to achieve clinical benefit with this targeted approach.[4,5] Two type I tyrosine kinase inhibitors, midostaurin and gilteritinib, have been approved for the treatment of newly diagnosed and relapsed/refractory (R/R) FLT3-mutated AML, respectively.[6,7] Gilteritinib has single-agent activity in R/R AML, although most recipients ultimately succumb to the disease.[7] Resistance to FLT3 inhibitors occurs through diverse mechanisms, e.g., emergence of FLT3 gatekeeper

mutations, or RAS-pathway mutations.[8]

Spleen tyrosine kinase (SYK) is a non-receptor tyrosine kinase crucial to the adaptive immune response.[9] Pre-clinical evidence suggests SYK overexpression contributes to FLT3-ITD-mediated transformation and resistance to FLT3 inhibitors,[10] suggesting that inhibition of SYK could potentiate (and possibly broaden) the clinical activity of FLT3 inhibition. In addition, data suggest SYK plays a significant role in regulating Hoxa9/Meis1-driven AML,[11] encouraging its positioning as another target in the treatment of a biomarker-specific subset of AML patients.

Mivavotinib (TAK-659) is an investigational type 1 tyrosine kinase inhibitor, which binds competitively to the adenosine triphosphate-binding site of an active tyrosine kinase,[12] with dual activity against SYK and FLT3.[13] In a first-in-human study, mivavotinib induced clinical responses with generally manageable toxicity in patients with R/R B-cell lymphoma.[14] We hypothesized that we could identify a mivavotinib dose that would result in sustained *in vivo* FLT3 inhibition, thereby achieving dual SYK/FLT3 inhibition in AML. This phase Ib study aimed to determine the safety, tolerability, maximum tolerated dose (MTD), and recommended phase II dose (RP2D) of mivavotinib in patients with R/R AML.

Methods

Study design

This was a phase Ib, multicenter, open-label, dose-escalation study of single agent, oral mivavotinib in patients with R/R AML. The primary objective was to determine the MTD/RP2D of mivavotinib. Secondary objectives included characterizing the pharmacokinetic (PK) profile of mivavotinib. An exploratory objective was to determine the optimal FLT3 inhibitory dosing regimen of mivavotinib. The planned phase II expansion of this study was not conducted.

Patients received oral mivavotinib, once (QD) or twice (BID) daily, in 28-day cycles until disease progression or unacceptable toxicity. The starting dose of mivavotinib was 60 mg QD, which had previously been determined to be safe and tolerable.[14] QD dose escalation followed 20 mg increments; evaluation of >20 mg increments (not exceeding 100%), alternative dosing regimens, and expansion of existing dose levels for up to 12 evaluable patients, was allowed. Based on *ex vivo* plasma inhibitory activity (PIA) assay data suggesting non-sustained 90% FLT3 inhibition *in vivo* with QD dosing, patients were also enrolled to receive mivavotinib BID at a starting dose of 80 mg, followed by a group of patients who received 60 mg BID. Dose escalation was to continue in a 3+3 design until the MTD was reached, or the RP2D if different from the MTD. Patients were followed for 28 days after the last mivavotinib dose, or until initiation of subsequent anticancer therapy, whichever occurred first.

The MTD was determined based on dose-limiting toxicities (DLT) defined as any of the adverse events (AE) listed in the *Online Supplementary Appendix* occurring in Cycle 1 and considered by the investigator to be at least possibly related to mivavotinib.

This study was conducted in compliance with the principles of the Declaration of Helsinki, Good Clinical Practice standards, applicable regulatory requirements, and the International Conference on Harmonization guidelines. It was approved by the institutional review boards (IRB) and/or independent ethics committees (IEC). All patients provided informed consent.

Study population

Patients ≥18 years with histologically confirmed primary/secondary AML who were unlikely to benefit from standard therapies or who refused standard treatment were enrolled. Eligibility criteria are detailed in the *Online Supplementary Appendix*.

Assessments

Response was evaluated using the Revised Recommendations of the International Working Group for AML.[15] Modifications to these response criteria are defined in the *Online Supplementary Appendix*. Bone marrow biopsies and/or aspirates for disease response monitoring were performed at screening, at the end of Cycles 1, 2, and 4, and as clinically indicated beyond Cycle 4. AE were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03. Pharmacokinetic and pharmacodynamic methods and statistical analyses are described in the *Online Supplementary Appendix*.

Results

Patients' characteristics and treatment

Between April 2015 and March 2018, 43 patients were enrolled and received ≥1 dose of mivavotinib (60 mg QD, n=4; 100 mg QD, n=7; 120 mg QD, n=4; 140 mg QD, n=5; 160 mg QD, n= 9; 80 mg BID, n= 6; 60 mg BID n=8). In general, patients were heavily pre-treated, and 70% had a poor risk status according to the European LeukemiaNet classification (assessed locally) (Table 1).[1] During trial design, next-generation sequencing was generally unavailable, and data were not available for most patients. Median age was 65 years; 74% of patients were White, most (84%) had received intensive therapy, and 7 (16%) had received treatment with an FLT3 inhibitor. Fifty-one percent of patients had centrally assessed FLT3 mutation at enrollment (56% as assessed locally); 6 (14%) patients had *NPM-1* co-mu-

tation (Table 1). At data cutoff, all patients had discontinued treatment due to AE or disease progression.

Dose-limiting toxicities and maximum tolerated dose determination

Thirty-four patients were evaluable for MTD determination (completed ≥75% of planned dosing in Cycle 1 and/or experienced a DLT). In the QD cohort, the DLT at 160 mg QD was associated with asymptomatic increase in amylase (grade 3) and lipase (grade 4) in one patient. Among 9 patients receiving 160 mg QD, 4 could not complete the DLT period: one died of infection/progressive disease (PD), 2 died of hemorrhagic events assessed as unrelated to mivavotinib, and another experienced a DLT. Since only one DLT was reported, the MTD was not reached per protocol. However, based on consensus from the study investigators that 160 mg QD was not well tolerated (due to grade 2 AE, such as increased transaminase and amylase/lipase), it was decided that doses above this should not be investigated. The RP2D was, therefore, determined to be 140 mg QD, supported by clinical efficacy (the first QD dose at which an objective response was observed), initial evidence of at least 90% FLT3 inhibition by Cycle 1 day 15, and no DLT or grade 2 AE.

In the 80 mg BID cohort, one of 3 patients experienced gastrointestinal (GI) bleeding (grade 3) as a DLT, and the cohort was expanded to 3 additional patients. Of these, one experienced asymptomatic increase in amylase (grade 3) and lipase (grade 4) as DLT, and another experienced both a GI bleed (grade 3) and a grade 5 intracranial hemorrhage (assessed as unrelated to treatment by the investigator). Therefore, this dose was considered to be above the MTD.

For the final cohort, 8 patients were treated at 60 mg BID. While no DLT were identified in the first 3 patients, the sponsor and investigators agreed to further expand the dose level to gather additional safety and efficacy data. No episodes of DLT were observed, and this dose level was determined to be the BID MTD.

Treatment exposure and safety

All 43 patients were evaluable for safety (having received ≥1 mivavotinib dose). The median number of mivavotinib cycles was 2.0 for both the QD and BID regimens (QD range, 1-11; BID range, 1-6); 23% of patients received ≥4 treatment cycles and the median duration of treatment was 7.9 weeks (range, 1-44) QD *versus* 4.6 weeks (range, 2-22) BID.

All patients (100%) experienced ≥1 treatment-emergent adverse event (TEAE); 36 patients (84%) experienced ≥1 TEAE that was assessed by the investigator as related to mivavotinib. The most frequently reported TEAE were febrile neutropenia (60%), increased aspartate aminotransferase (AST) (51%), diarrhea (40% [generally grade 1 or 2]), increased amylase (37%), fatigue (35%), increased alanine aminotransferase (ALT) (33%), and increased lipase (33%) (Table 2). Increased enzyme levels were generally asymptomatic and consistent with previous observations.[14] Dose interruptions for these events were infrequent.

The overall frequencies of grade ≥3 TEAE and drug-related grade ≥3 TEAE were 98% and 49%, respectively. The most frequent grade ≥3 AE were febrile neutropenia (56%), anemia (28%), increased amylase (21%), increased lipase (21%), and a drop in platelet count (21%). Overall, 41 pa-

Table 1. Baseline demographics and disease characteristics.

	Patients (N=43)
Median age, years (range)	65 (25-86)
Male, N (%)	23 (53)
ECOG PS, N (%)	
0	7 (16)
1	35 (81)
Not done	1 (2)
Risk status,* N (%)	
Favorable	1 (2)
Intermediate	7 (16)
Poor	30 (70)
Unknown	5 (12)
Mutation status, N (%)	
FLT3-WT	13 (30)
FLT3-ITD	12 (28)
FLT3-TKD	6 (14)
FLT3-ITD/TKD	4 (9)
Previous therapy, N (%)	
Intensive†	36 (84)
Non-intensive‡	6 (14)
Unknown	1 (2)
N lines of previous therapy, median (range)	3 (1-8)
Prior allogeneic transplant, N (%)	7 (16)
Prior FLT3 inhibitor, N (%)	7 (16)§
Co-mutations, N (%)	
NPM1	6 (14)
IDH1	2 (5)
IDH2	2 (5)
DNMT3A	2 (5)
Other gene	12 (28)

*Risk status conforms to the European LeukemiaNet classification system but confined to karyotype only because next-generation sequencing results were not available. †Intensive previous therapy defined as any patient who received 7+3, high-dose chemotherapy and/or allogeneic transplant ± azacitidine or dacogen. ‡Non-intensive previous therapy defined as any patient who received azacitadine only, dacogen only, or both. §Seven patients had prior exposure to other FLT3 inhibitors including sorafenib (N=5), midostaurin (N=1), and both sorafenib and midostaurin (N=1). N: number; ECOG PS: Eastern Cooperative Oncology Group performance status; FLT3: FMS-like tyrosine kinase 3; ITD: internal tandem duplication; TKD: tyrosine kinase domain; WT: wild-type.

Table 2. Treatment-emergent adverse events occurring in >20% of all patients.

Preferred term	Grade 1 or 2 N (%)	Grade ≥3 N (%)	Overall total N (%)
Patients with any TEAE*			43 (100)
Febrile neutropenia	2 (4)	24 (56)	26 (60)
Increased AST	14 (32)	8 (19)	22 (51)
Diarrhea	17 (40)	-	17 (40)
Increased amylase	7 (16)	9 (21)	16 (37)
Fatigue	11 (26)	4 (9)	15 (35)
Increased ALT	11 (26)	3 (7)	14 (33)
Increased lipase	5 (12)	9 (21)	14 (33)
Headache	13 (30)	-	13 (30)
Anemia	-	12 (28)	12 (28)
Petechiae	12 (28)	-	12 (28)
Nausea	10 (23)	1 (2)	11 (26)
Pyrexia	9 (21)	2 (5)	11 (26)
Hypophosphatemia	4 (10)	7 (16)	11 (26)
Epistaxis	11 (26)	-	11 (26)
Dizziness	11 (26)	-	11 (26)
Cough	10 (23)	-	10 (23)
Stomatitis	6 (14)	3 (7)	9 (21)
Chills	9 (21)	-	9 (21)
Drop in platelet count	-	9 (21)	9 (21)
Hypocalcemia	7 (16)	2 (5)	9 (21)

*A treatment-emergent adverse event (TEAE) was defined as any adverse event occurring on or after day 1 of Cycle 1 of treatment with mivavotinib. N: number; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

tients (95%) experienced a serious AE (SAE); SAE were drug-related in 12 patients (28%). The most frequent drug-related SAE was gastric hemorrhage, which occurred in 3 patients (7%) who received 80 mg BID.

Thirty of 43 patients (70%) experienced a bleeding event while on study (Figure 1; *Online Supplementary Table S1*); only epistaxis occurred with a frequency to be included within the TEAE threshold of >20% of patients (low-grade events, common in patients with AML) (Table 2). The majority were grades 1 or 2, resolved without mivavotinib dose modification and were not considered at the time as being related to mivavotinib. Eleven patients (26%) experienced grade ≥3 bleeding events that were most frequently observed at 80 mg BID (Table 3), which is consistent with this dose surpassing the MTD. Thirteen patients experienced bleeding involving the GI tract: 7 patients had events that were considered grade ≥3 (100 mg QD, n=1; 120 mg QD, n=1; 140 mg QD, n=1; 160 mg QD, n=1; 80 mg BID, n=3), and some patients experienced recurrent events. Seven patients experienced intracranial bleeding, 4 of which were considered grade ≥3. Median platelet count of patients with any bleeding event on the study day preceding the event was $25x10^9$/L (range, 5-$375.3x10^9$/L) compared with a median platelet count $32x10^9$/L (range, 3-$375x10^9$/L) of all patients at any given time. The median day of occurrence of all bleeding events was day 22 (range, -1 to 262).

Twenty-nine patients (67%) discontinued mivavotinib due to a TEAE. Hemorrhagic events were the most common, with 9 patients having a GI-related or central nervous system-related bleeding event leading to study discontinuation (60 mg QD, n=1; 140 mg QD, n=2; 160 mg QD, n=2; 80 mg BID, n=4). Of these, 5 were considered related to treatment with mivavotinib: grade 4 GI hemorrhage (140 mg QD, n=1); grade 3 GI hemorrhage (160 mg QD, n=1); grade 3 gastric hemorrhage, grade 3 subdural hematoma, and grade 3 gastric hemorrhage (80 mg BID, n=3).

There were 26 on-study deaths (any death occurring after informed consent and ≤28 days after the last dose of mivavotinib), one of which was considered related to mivavotinib (multi-organ failure). Other causes of death included progression of AML (n=11), cardiac arrest/failure (n=5), infection (n=3), neutropenia (n=1), pulmonary edema (n=1), respiratory failure (n=1), gastric hemorrhage (n=1), intracranial hemorrhage (n=1), and not specified (n=1).

Pharmacokinetics and pharmacodynamics

Following 60-160 mg QD or 60-80 mg BID, mivavotinib was

rapidly absorbed with a median t_{max} of 1-3 hours and a geometric mean C_{max} of 74-322 ng/mL. Plasma exposure of mivavotinib was generally dose proportional in the dose ranges studied (Figure 2A, B). Following BID dosing, the pre-dose concentrations of mivavotinib on Cycle 1 day 15 were slightly higher than the pre-dose concentration following QD dosing across the dose groups, suggesting a slightly higher accumulation in C_{trough} with BID dosing.

Mivavotinib was specifically developed as an inhibitor of SYK, with a half maximal inhibitory concentration (IC_{50}) of 2-3.2 nM (0.7-1.1 ng/mL) in kinase assays.[13] The evaluation of additional kinases revealed that mivavotinib had activity against FLT3 isoforms ranging from 4.6-22 nM (1.6-7.6 ng/mL). In the previously published study of mivavotinib in lymphoma patients (where the target was SYK), the dose identified for expansion was 100 mg QD.[14] A large body of data on FLT3 inhibition has established that responses in AML patients with FLT3 activating mutations correlate with sustained inhibition of pFLT3 to 15% of baseline (and preferably even lower).[15,16-19] Using Molm14 cells (a human AML cell line which expresses an FLT3-ITD mutation[20]) incubated in human plasma spiked with increasing concentrations of mivavotinib, we determined the IC_{50} of mivavotinib in plasma against FLT3-ITD to be approximately 80 nM (27.6 ng/mL) (Figure 2C). From this dose-response curve, we estimated that the target mean trough concentration in patient plasma for optimal efficacy against FLT3-mutant AML with >85% suppression of FLT3-ITD signaling would be approximately 400-500 nM (137.8-172.2 ng/mL). Steady state trough samples taken from trial participants on day 15 of Cycle 1 prior to mivavotinib administration were analyzed for mivavotinib concentrations (Figure 2D). A dose of 80 mg BID came closest to achieving this mean target concentration.

We used a plasma inhibitory activity (PIA) assay for FLT3 to estimate the degree of *in vivo* FLT3 inhibition in patients dosed with mivavotinib. This is a well-established assay that has been used in the development of other FLT3 in-

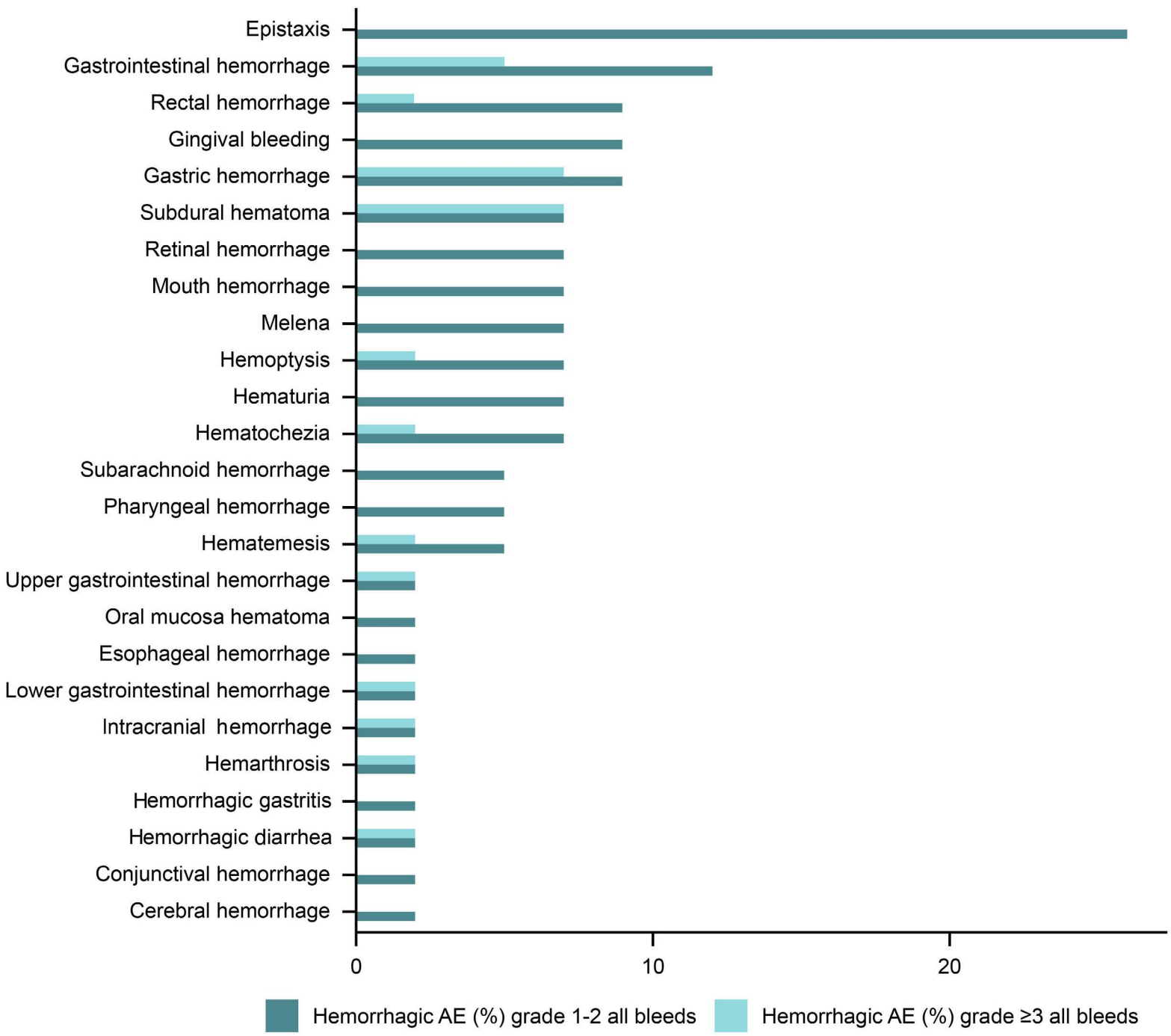

Figure 1. Number of hemorrhagic events on study (grade 1 or 2 and grade ≥3). AE: adverse event.

Table 3. Grade ≥3 hemorrhagic events on study according to mivavotinib dose.

Hemorrhagic event, N (%)	60 mg QD N=4	100 mg QD N=7	120 mg QD N=4	140 mg QD N=5	160 mg QD N=9	60 mg BID N=8	80 mg BID N=6	Total N=43
Patients with grade ≥3 bleeding	1 (25)	2 (29)	1 (25)	1 (20)	2 (22)	0	4 (67)	11 (26)
Gastric hemorrhage	-	-	-	-	-	-	3 (50)	3 (7)
Subdural hematoma	1 (25)	-	-	-	1 (11)	-	1 (17)	3 (7)
Gastrointestinal hemorrhage	-	-	-	1 (20)	1 (11)	-	-	2 (5)
Hematochezia	-	-	1 (25)	-	-	-	-	1 (2)
Hemoptysis	-	-	-	-	-	-	1 (17)	1 (2)
Rectal hemorrhage	-	-	-	-	-	-	1 (17)	1 (2)
Hemorrhagic diarrhea	-	1 (14)	-	-	-	-	-	1 (2)
Hemarthrosis	-	1 (14)	-	-	-	-	-	1 (2)
Hematemesis	-	-	-	-	-	-	1 (17)	1 (2)
Intracranial hemorrhage	-	-	-	-	-	-	1 (17)	1 (2)
Lower gastrointestinal hemorrhage	-	1 (14)	-	-	-	-	-	1 (2)
Upper gastrointestinal hemorrhage	-	-	-	-	1 (11)	-	-	1 (2)

N: number; BID: twice daily; QD: once daily.

hibitors.[4] Plasma samples are collected at trough time points from patients taking the inhibitor long enough to achieve steady state exposure. An FLT3-ITD-expressing cell line is incubated in the plasma, and the degree of FLT3 inhibition observed relative to control or baseline plasma is quantified.[21] For other FLT3 inhibitors, the assay correlates well with the degree of inhibition achieved in leukemic blasts circulating in the patient, and this appears to be the case for mivavotinib. The degree of FLT3 inhibition observed in the PIA assay for trial participants generally matched PK data (Figure 3A).

During the study, it was evident from selected PIA analysis at trough time points that sustained *in vivo* FLT3 inhibition was achieved after BID dosing when compared to QD dosing with the same amount of total daily dose. The results are consistent with an expected higher steady state trough concentration after BID dosing, while the total daily drug exposure remained unchanged compared with QD dosing (Figure 3B). In accordance with the PK data, 80 mg BID resulted in the most effective FLT3 inhibition.

Efficacy

Thirty-three patients (76.7%) were evaluable for response. Five patients had complete responses (4 with incomplete count recovery, one complete response; overall rate, 15%), 19 had stable disease, 5 had PD, and 4 had clinical benefit despite PD. Response was first observed at the 140 mg QD dose, with 2 subsequent responses at the 160 mg QD dose, and one in each of the 60 mg and 80 mg BID cohorts. One responding patient had prior exposure to an FLT3 inhibitor (sorafenib/midostaurin). Responses were primarily observed in patients with an FLT3-ITD mutation based on central testing. Results for two responding patients had unquantifiable FLT3-ITD, so the true mutation status of these patients is unknown. (Local results reported the FLT3 status as wild-type [WT] in one patient and unknown in the other). No responding patients had FLT3-WT or FLT3-TKD mutations according to central testing. One responding patient had an NPM1 mutation (NPM1 mutation status was unavailable for most patients, including the remaining 4 responders). Of 19 patients with FLT3 mutations who were treated at 120, 140, or 160 mg QD or BID, 3 had objective responses with incomplete count recovery (CRi) and 13 experienced a reduction in marrow blast percentage (range, 22.5-98.6%).

Additional antileukemic activity was observed in 21 of 33 patients with a ≥50% reduction in peripheral blast count from baseline across all dose levels, including 12 of 21 with QD dosing and 9 of 12 with BID dosing. These peripheral blast reductions included both FLT3-WT and FLT3-mutated patients. These findings are similar to those in midostaurin monotherapy, which has been shown to frequently reduce peripheral blood blast counts in FLT3-WT and FLT3-mutated patients, but which has a more limited influence on bone marrow blast reductions and clinical response.[22]

The duration of therapy, with responses and reasons for discontinuing mivavotinib are shown in Figure 4A. Further details of patients who responded to treatment are provided in the *Online Supplementary Appendix*.

Of the 33 patients evaluable for response, 19 harbored an FLT3 mutation (either ITD, TKD or both), 8 were FLT3-WT, and the status of FLT3-ITD was not determined/unknown for the remaining patients. The best change in the marrow blast percentage for these 33 patients is shown in Figure 4B. The results are consistent with what might be expected of an FLT3 inhibitor, but there were also modest reductions in select patients who were FLT3-WT, or whose FLT3-ITD status was not determined/unknown.

Effect of mivavotinib on platelet aggregation

In response to the frequency of bleeding events reported, and emerging data regarding the role of SYK in platelet function, we investigated the effect of mivavotinib on human platelet aggregation. An *in vitro* assay was used based on the principle that human platelets in samples of platelet rich plasma will aggregate in the presence of adenosine diphosphate (10 μM) or collagen (2 μg/mL). The mean C_{max} of 574 ng/mL mivavotinib, observed in the 140 mg QD cohort and corresponding to 1.07 μM, was used to

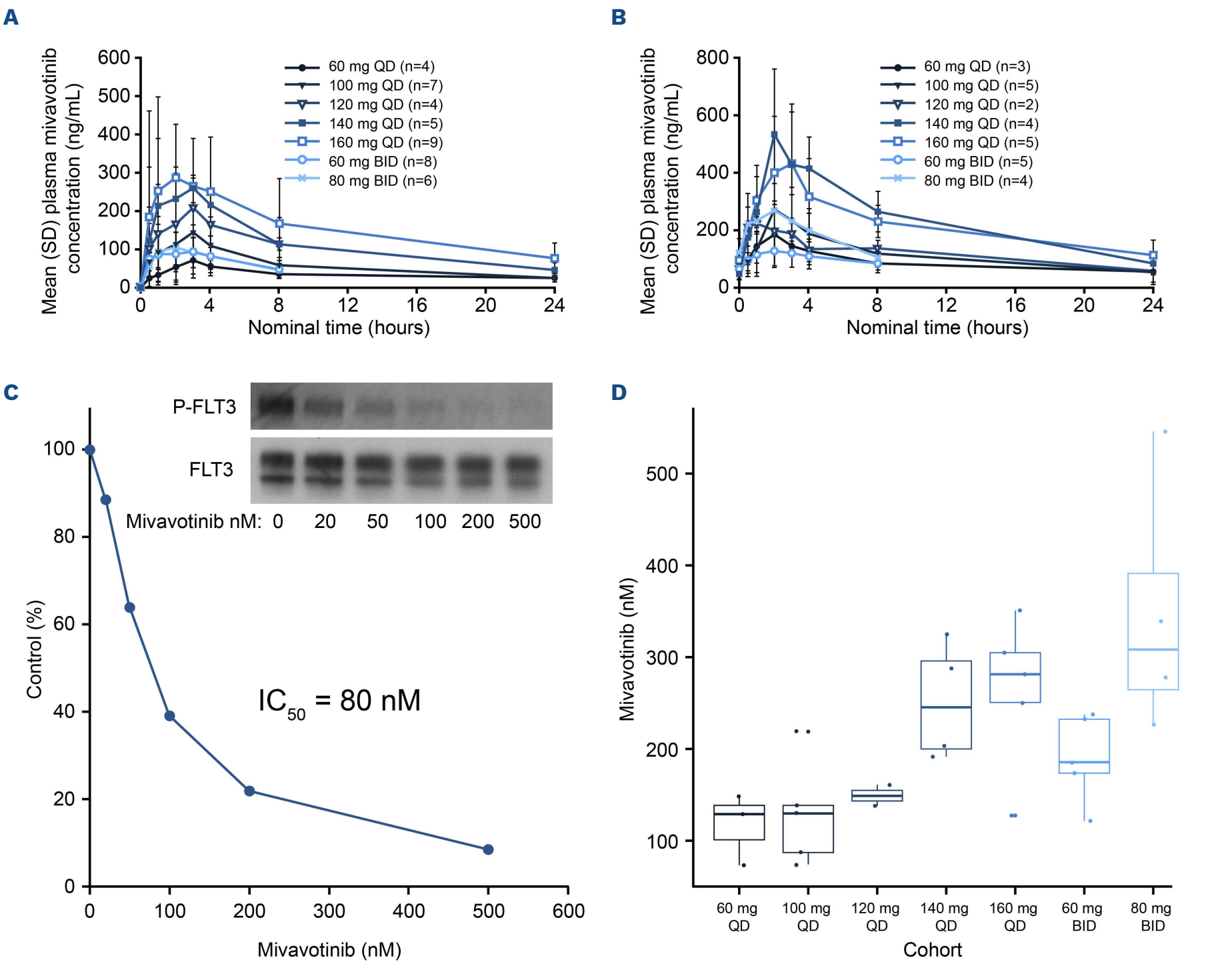

Figure 2. Pharmacokinetics of mivavotinib. (A) Plasma concentration-time profiles of mivavotinib in patients with acute myeloid leukemia (AML) following a single dose on Cycle 1 day 1. (B) Plasma concentration-time profiles of mivavotinib in patients with AML following a single dose on Cycle 1 day 15. (C) Dose-response experiment for inhibition of tyrosine kinase 3 (FLT3) phosphorylation by mivavotinib. Mivavotinib was spiked at the indicated concentrations into normal donor plasma. Molm-14 cells were incubated for one hour and analyzed for FLT3 phosphorylation by immunoblotting (inset) as described in the Methods. Densitometric analysis of the immunoblot is plotted on the graph, and regression analysis after linear conversion yielded an estimate of the half maximal inhibitory concentration (IC_{50}) at 80 nM (27.6 ng/mL). The experiment was performed 3 times, and a representative blot is shown. (D) Plasma samples from trial participants were collected prior to study drug administration on day 15 of Cycle 1. Mivavotinib concentration was determined by mass spectrometry (see *Online Supplementary Methods*) and the concentrations for each patient are plotted according to dose level. The solid black line represents the mean concentration for that group. SD: standard deviation; QD: once daily; BID: twice daily.

select the mivavotinib concentration. Concentrations tested were 10-fold higher and lower, resulting in concentrations of 0.107, 1.07, and 10.7 μM mivavotinib. The 1.07 μM mivavotinib plasma concentration prevented aggregation caused by collagen; 10.7 μM mivavotinib prevented aggregation caused by either adenosine diphosphate (Figure 5A) or collagen (Figure 5B). Mivavotinib plasma concentrations of 1.07 μM or above may have the potential to inhibit platelet aggregation, thus making severely thrombocytopenic patients more susceptible to a hemorrhagic event. Though inhibition of platelet aggregation was not observed at the lower concentration (0.107 μM) in the *in vitro* assay, it does not rule out the possibility of lower doses of mivavotinib having a similar effect in this patient population.

Discussion

The primary objective of this study was to determine the MTD/RP2D of mivavotinib in patients with R/R AML who either were unlikely to achieve a durable remission from standard therapies or who declined to undergo standard treatment. The patients enrolled in the study were mainly elderly, heavily pre-treated, and with a poor risk status. Dosing was switched from QD to BID based on preliminary population PK data that suggested that trough concentration was higher with BID dosing. A higher trough concentration was anticipated to offer more consistent >90% pFLT3 inhibition, thought to be necessary for FLT3-driven efficacy. However, neither selected RP2D, 140 mg QD or 60 mg BID, achieved a consistent or sustained 90% FLT3 inhibition.

We conclude that mivavotinib has clinical activity as a dual SYK/FLT3 inhibitor. When daily doses of mivavotinib were increased to a point of FLT3 inhibition, marrow blast reduction was observed in patients with FLT3-ITD mutations, establishing its efficacy as an FLT3 inhibitor. In addition, signs of antileukemic activity were observed in patients without FLT3 mutations and without sustained 90% FLT3 inhibition, suggesting SYK inhibition. While better initial disease control was achieved in select patients at the higher mivavotinib QD and BID doses, response durations were short, and high-grade AE occurred, resulting in either dose modification or discontinuation of mivavotinib. Overall, patients received mivavotinib for a median of 6.7 weeks, with 67% discontinuing due to an AE or death. Previously developed FLT3 inhibitors have been observed to induce a reduction in marrow blasts, typically within 1-2 months of therapy. R/R AML patients treated with gilteritinib required a median of 48 days to achieve their best response.[18] Therefore, mivavotinib may have induced a higher response rate if it were not for the toxicity induced at the highest dose levels, particularly the 80 mg BID regimen, which was considered above the MTD.

The overall safety profile observed in AML patients was generally consistent with the known safety profile of mivavotinib; events were typically associated with underlying AML. Major hemorrhagic events occurred at a rate beyond what might be reasonably expected in this population, particularly at the highest doses of mivavotinib. This was evident based on DLT identification and following the find-

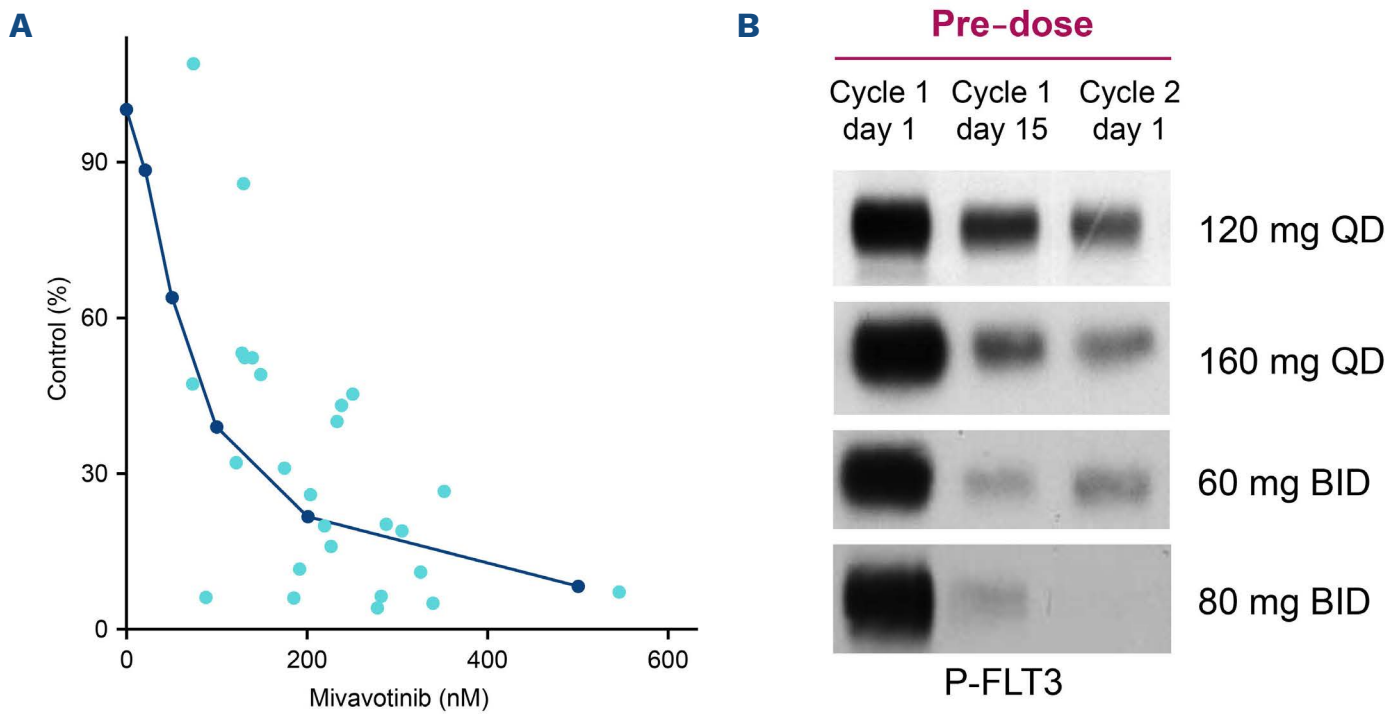

Figure 3. Pharmacodynamics of mivavotinib. (A) Plasma inhibitory activity (PIA) results plotted against mivavotinib concentrations. Plasma samples from Cycle 1 day 15 were available from 35 patients. The plasma was used for PIA analysis and pharmacokinetic (PK) analysis. PIA results, expressed as percentage of FLT3 phosphorylation relative to baseline, are plotted for each patient on the y-axis, and the concentration of mivavotinib for that same sample is plotted on the x-axis. The dose-response curve from Figure 1A is overlaid for reference. (B) Representative immunoblots of phosphorylated FLT3 (pFLT3) from patients receiving once daily (QD) or twice daily (BID) mivavotinib, demonstrating more sustained FLT3 inhibition with twice daily dosing. Plasma samples were collected prior to study drug administration on the indicated day.

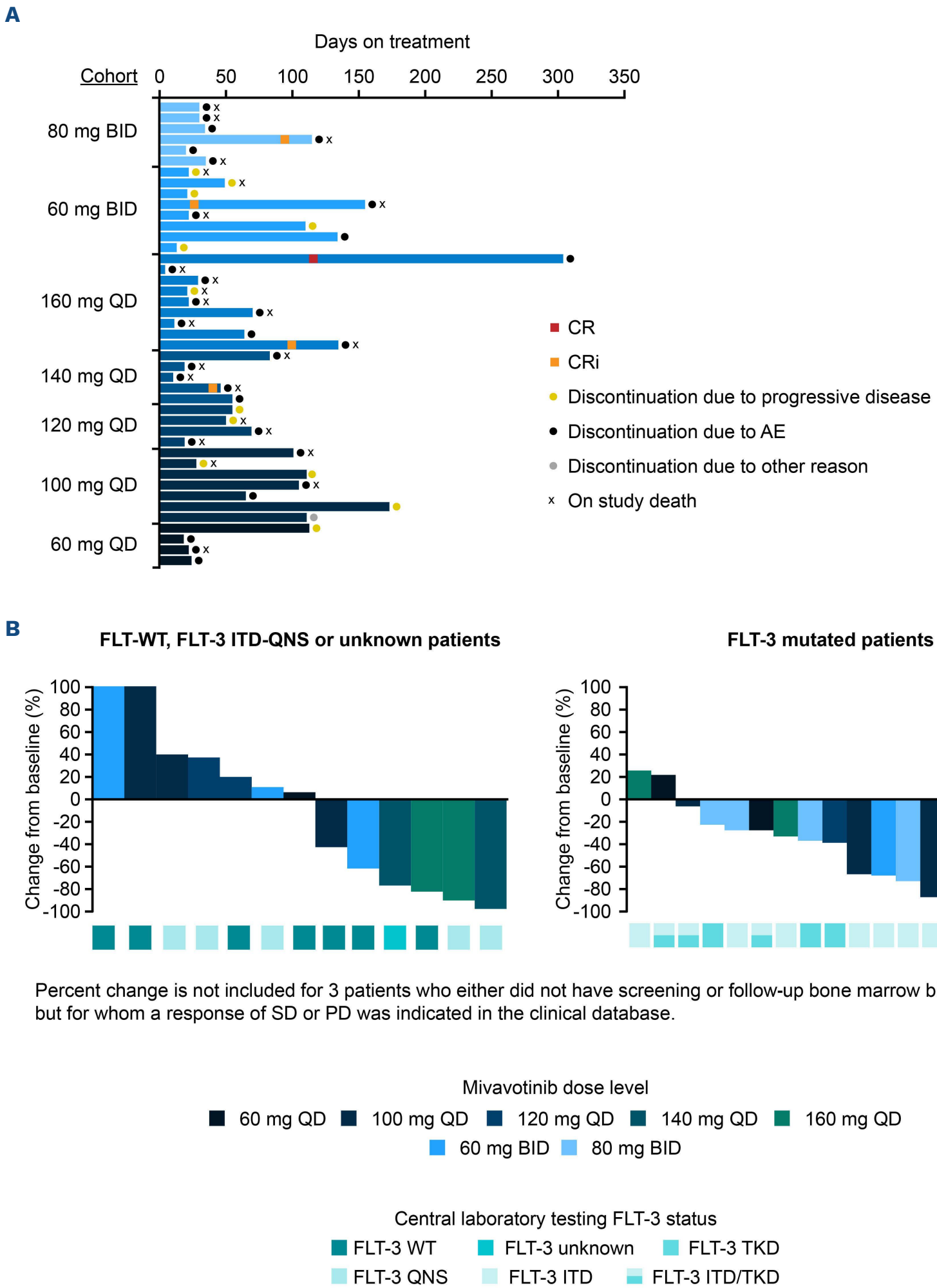

Figure 4. Clinical activity of mivavotinib. (A) Swimmer plot for all study participants. The number of days of each patient's treatment is shown in individual columns. An "X" at the end of a column indicates that the study treatment ended with the death of the patient. ● Reason for discontinuation of treatment: either disease progression; a treatment-emergent adverse event (TEAE; hemorrhagic or other), in some cases followed by the death of the patient if it occurred within 30 days of stopping treatment; or 'other' (one patient discontinued treatment because the study drug had been withheld for a prolonged period of time due to serious adverse event [SAE]). (B) Waterfall plot for best change in marrow blast percentage for all response evaluable patients. CR: complete response; Cri: incomplete count recovery; AE: adverse event; QD: once daily; BID: twice daily; SD: stable disease; PD: progressive disease.

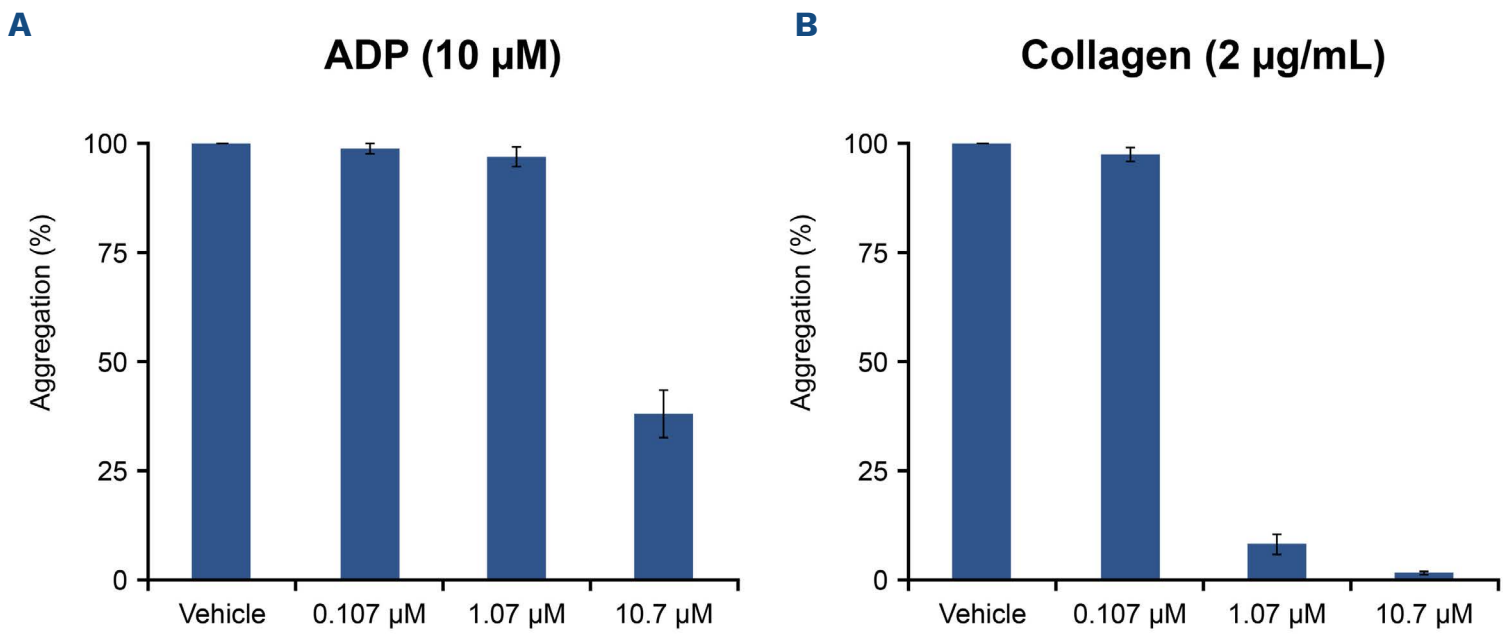

Figure 5. The effect of mivavotinib on human platelet aggregation driven by adenosine diphosphate or collagen. The effect of mivavotinib on human platelet aggregation driven by (A) adenosine diphosphate (ADP) (10 µM) or (B) collagen (2 µg/mL). Mivavotinib concentrations tested were 10-fold higher and lower than the Cmax of 574 ng/mL, observed in the 140 mg daily (QD) cohort (corresponding to 1.07 µM), resulting in concentrations of 0.107, 1.07 and 10.7 µM mivavotinib. Error bars are representative of the Standard Error of the Mean.

ing that the 80 mg BID dose was, in large part, not tolerated due to these events. Eleven patients had grade ≥3 bleeding events, 2 of which were fatal. Given the nature of R/R AML, it is not uncommon to encounter major and/or fatal bleeding events.[23] Due to time constraints and the number of study sites, bleeding events were not evident until the data were later reviewed. After bleeding events had been reviewed, *in vitro* studies were undertaken to mechanistically characterize their potential relationship with mivavotinib.

This study of mivavotinib had been initiated before the effects of SYK and Bruton's tyrosine kinase (BTK) inhibition on platelet function were known. Activated B-cell receptor signaling is carried forward by associating with transmembrane proteins containing immunoreceptor tyrosine-based activation motifs, which recruit and activate SYK. SYK then activates phospholipase Cγ2.[9] This central role in signaling makes SYK an attractive target for B-cell malignancies, an approach that has been validated in clinical studies of SYK inhibitors in lymphoma and chronic lymphocytic leukemia.[24-26] However, platelet collagen receptor glycoprotein VI signaling is also carried out via an immunoreceptor tyrosine-based activation motif-containing protein and SYK.[27] There is now a substantial body of literature supporting the role of SYK in mediating platelet aggregation and activation, and SYK inhibitors are postulated to have the potential to induce platelet dysfunction (and, therefore, bleeding).[28] BTK also regulates phospholipase Cγ2 in both B-cell and platelet signaling, and BTK inhibitors have been associated with increased bleeding risk.[29,30] Our data demonstrating the effect of mivavotinib on platelet aggregation may offer an explanation for the hemorrhagic DLT and increase in bleeding events at the 80 mg BID dose level.

Mivavotinib had been previously characterized as having single-agent efficacy with an acceptable toxicity profile in B-cell lymphoma.[14] An important difference between lymphoma and AML patients, however, is that AML patients often have thrombocytopenia. Patients who suffered hemorrhagic events in the present study had a median platelet count of $25x10^9$/L, typical for a population of relapsed AML patients. Entospletinib is another SYK inhibitor with activity against FLT3 that is under investigation for AML,[31] although, as far as we are aware, there have been no reports of excessive bleeding.[32] We hypothesize that increasing the dose of mivavotinib in an attempt to augment FLT3 inhibition might have gone over the threshold of affecting platelet function.

While it may be possible that mivavotinib inhibits platelet aggregation at high-dose levels, resulting in hemorrhagic events, several confounding factors remain. First, in addition to being severely myelosuppressed, several patients experiencing both major and non-major GI or central nervous system hemorrhagic events had either a prior history of such events, or predisposing conditions, which supports the fact that several events were not considered to be related to treatment. In addition, concomitant administration of hydroxyurea was allowed through Cycle 1, if needed, to control circulating blasts; hydroxyurea is known to affect the GI mucosa, adding another layer of complexity to the interpretation of GI bleeding events.

In pre-clinical toxicology studies of mivavotinib, healthy animals were administered mivavotinib daily by oral gavage for 14 days to 3 months. One of the consistent toxicities in rats and dogs was GI mucosal hemorrhage, which, while present at doses of ≥30 mg/kg in rats and ≥3 mg/kg in dogs, was dose limiting in dogs only at ≥10 mg/kg (3-fold clinical C_{max} exposure associated with bleeding AE). Although the impact of mivavotinib on platelet function in

rats and dogs is not known, the presence of dose-limiting toxicity related to hemorrhage at exposures several fold higher than those at which bleeding AE were noted clinically supports a role for high doses of mivavotinib in hemorrhagic events.

Our central hypothesis for this trial, based on pre-clinical AML studies, was that SYK inhibition would potentiate the responses induced by FLT3 inhibition and possibly decrease the development of resistance. While early signs of clinical activity suggested this was possible, most patients were not on study long enough to confirm this hypothesis. Despite several confounding factors, our findings nonetheless raise important potential concerns about the general feasibility of high-dose SYK inhibition in any patient population with severe thrombocytopenia. This caution may also apply to the use of BTK inhibitors in such patients. For these reasons, any future studies of mivavotinib in AML, if they take place at all, should re-evaluate dose selection and schedule to optimize the benefit *versus* risk profile.

Disclosures

KWP has received consultancy fees from AbbVie, Astellas, Boston BioMedical, Bristol Myers Squibb, Novartis, Jazz Pharmaceuticals, and Celgene, and institutional research funding from AbbVie, Astellas, Agios, Daiichi Sankyo, and Millennium; JK is now employed by Ayala Pharma; ML has been a speaker/consultant for AbbVie, Amgen, AZ, Beigene, BMS, Dova, Epizyme, Gilead, GSK, Janssen, Jazz, Karyopharm, Sanofi, Seagen, Takeda, and TG Therapeutics; PB has received funding from Millennium-Takeda for a separate clinical trial; HE has received support for the work from Takeda, and support outside this work from AbbVie, Agios, Astellas, Bristol Myers Squibb, Celgene, Daiichi Sankyo, Glycomimetics, Immunogen, Incyte, Jazz, Kura Oncology, Macrogenics, Novartis, Pfizer, Servier, Syros, Takeda, and Trillium; TMW-D has received research funding from Merck & Co, BMS, Tesaro/GSK, Janssen, Isoray, AstraZeneca/MedImmune, and Caris Life Sciences, has held a consultancy role for Caris Life Sciences, Rakuten, Exicure, Shattuck Labs, and Merck & Co, holds stock/ownership for High Enroll, has received honoraria from Physician Education Resource, and travel/accommodation/expenses from Merck & Co, BMS, Bexion, AstraZeneca/MedImmune, Caris Life Sciences, Lilly, and Tesaro; GJR has held a consultancy role or has sat on the advisory board or on the Data and Safety Monitoring Committee for the following companies: Actinium, AbbVie, Agios, Amgen, Astellas, AstraZeneca, Bristol Myers Squibb, Blueprint Medicines, Bluebird Bio, Celgene, Glaxo SmithKline, Janssen, Jasper Therapeutics, Jazz, MEI Pharma (IDMC Chair), Mesoblast, Novartis, Pfizer, Syndax, and Takeda (IRC Chair), and has received research support from Janssen; NP has sat on the advisory board and received honoraria from CTI BioPharma, and indirect research support from AbbVie, Takeda, Gilead, and Immunogen; ID, RCG, CL, SW, KS, KK, and HM are employees of Takeda; ML has received support outside this work from AbbVie, Amgen, Astellas, Bristol-Myers-Squibb, Daiichi-Sankyo, FujiFilm, Glaxo-Smith-Kline, and Jazz; DB, TR and DH have no conflicts of interest to disclose.

Contributions

KWP, KS, KK and ML contributed to study conception and design; KWP, JK, ML, DB, HE, GJR, NP, TR, DH, KS and ML contributed to collection and assembly of data; KWP, ML, PB, TMW-D, GJR, ID, RCG, CL, SW, KS, KK, HM and ML contributed to data analysis and interpretation; KWP, DB, PB, NP, TR, ID, SW, KS, HM, ML, GJR and DH contributed to the drafting of the manuscript; KWP, JK, ML, HE, TMW-D, RCG, CL, KK and ML contributed to writing the manuscript. All authors approved the manuscript for submission and agree to be accountable for all aspects of the work, which includes ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Acknowledgments

We thank all patients and their families, and investigators at all clinical sites for their participation in the study. We thank Ravi Peri and Francis Wolenksi for their contribution to the study.

Funding

This study is funded by Takeda Development Center Americas Inc. (TDCA). Medical writing support for the development of this manuscript, under the direction of the authors, was provided by Clair Clowes, MPhil., of Ashfield MedComms, an Ashfield Health company, funded by Takeda Pharmaceuticals U.S.A. Inc.

Data-sharing statement

Requests for de-identified datasets for the results reported in this publication will be made available to qualified researchers following submission of a methodologically sound proposal. Data will be made available for such requests following online publication of this article and for one year thereafter in compliance with applicable privacy laws, data protection, and requirements for consent and anonymization. Calithera does not share identified participant data or a data dictionary.

References

1. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424-447.
2. Levis M, Small D. FLT3: ITDoes matter in leukemia. Leukemia. 2003;17(9):1738-1752.
3. Ambinder AJ, Levis M. Potential targeting of FLT3 acute myeloid leukemia. Haematologica. 2021;106(3):671-681.
4. Levis M, Brown P, Smith BD, et al. Plasma inhibitory activity (PIA): a pharmacodynamic assay reveals insights into the basis for cytotoxic response to FLT3 inhibitors. Blood. 2006;108(10):3477-3483.
5. Pratz KW, Cortes J, Roboz GJ, et al. A pharmacodynamic study of the FLT3 inhibitor KW-2449 yields insight into the basis for clinical response. Blood. 2009;113(17):3938-3946.
6. Stone RM, Mandrekar SJ, Sanford BL, et al. Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation. N Engl J Med. 2017;377(5):454-464.
7. Perl AE, Martinelli G, Cortes JE, et al. Gilteritinib or chemotherapy for relapsed or refractory FLT3-mutated AML. N Engl J Med. 2019;381(18):1728-1740.
8. McMahon CM, Ferng T, Canaani J, et al. Clonal selection with RAS pathway activation mediates secondary clinical resistance to selective FLT3 inhibition in acute myeloid leukemia. Cancer Discov. 2019;9(8):1050-1063.
9. Mocsai A, Ruland J, Tybulewicz VL. The SYK tyrosine kinase: a crucial player in diverse biological functions. Nat Rev Immunol. 2010;10(6):387-402.
10. Puissant A, Fenouille N, Alexe G, et al. SYK is a critical regulator of FLT3 in acute myeloid leukemia. Cancer Cell. 2014;25(2):226-242.
11. Mohr S, Doebele C, Comoglio F, et al. Hoxa9 and Meis1 cooperatively induce addiction to Syk signaling by suppressing miR-146a in acute myeloid leukemia. Cancer Cell. 2017;31(4):549-562.
12. Dar AC, Shokat KM. The evolution of protein kinase inhibitors from antagonists to agonists of cellular signaling. Ann Rev Biochem. 2011;80:769-795.
13. Lam B, Arikawa Y, Cramlett J, et al. Discovery of TAK-659 an orally available investigational inhibitor of spleen tyrosine kinase (SYK). Bioorg Med Chem Lett. 2016;26(24):5947-5950.
14. Gordon LI, Kaplan JB, Popat R, et al. Phase I study of TAK-659, an investigational, dual SYK/FLT3 inhibitor, in patients with B-cell lymphoma. Clin Cancer Res. 2020;26(14):3546-3556.
15. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol. 2003;21(24):4642-4649.
16. Smith BD, Levis M, Beran M, et al. Single-agent CEP-701, a novel FLT3 inhibitor, shows biologic and clinical activity in patients with relapsed or refractory acute myeloid leukemia. Blood. 2004;103(10):3669-3676.
17. Cortes JE, Kantarjian H, Foran JM, et al. Phase I study of quizartinib administered daily to patients with relapsed or refractory acute myeloid leukemia irrespective of FMS-like tyrosine kinase 3-internal tandem duplication status. J Clin Oncol. 2013;31(29):3681-3687.
18. Perl AE, Altman JK, Cortes J, et al. Selective inhibition of FLT3 by gilteritinib in relapsed or refractory acute myeloid leukaemia: a multicentre, first-in-human, open-label, phase 1-2 study. Lancet Oncol. 2017;18(8):1061-1075.
19. Smith CC, Levis MJ, Frankfurt O, et al. A phase 1/2 study of the oral FLT3 inhibitor pexidartinib in relapsed/refractory FLT3-ITD-mutant acute myeloid leukemia. Blood Adv. 2020;4(8):1711-1721.
20. Kelly LM, Yu JC, Boulton CL, et al. CT53518, a novel selective FLT3 antagonist for the treatment of acute myelogenous leukemia (AML). Cancer Cell. 2002;1(5):421-432.
21. Levis M, Perl AE. Gilteritinib: potent targeting of FLT3 mutations in AML. Blood Adv. 2020;4(6):1178-1191.
22. Fischer T, Stone RM, Deangelo DJ, et al. Phase IIB trial of oral midostaurin (PKC412), the FMS-like tyrosine kinase 3 receptor (FLT3) and multi-targeted kinase inhibitor, in patients with acute myeloid leukemia and high-risk myelodysplastic syndrome with either wild-type or mutated FLT3. J Clin Oncol. 2010;28(28):4339-4345.
23. Ho G, Jonas BA, Li Q, et al. Early mortality and complications in hospitalized adult Californians with acute myeloid leukaemia. Br J Haematol. 2017;177(5):791-799.
24. Friedberg JW, Sharman J, Sweetenham J, et al. Inhibition of Syk with fostamatinib disodium has significant clinical activity in non-Hodgkin lymphoma and chronic lymphocytic leukemia. Blood. 2010;115(13):2578-2585.
25. Sharman J, Hawkins M, Kolibaba K, et al. An open-label phase 2 trial of entospletinib (GS-9973), a selective spleen tyrosine kinase inhibitor, in chronic lymphocytic leukemia. Blood. 2015;125(15):2336-2343.
26. Andorsky DJ, Kolibaba KS, Assouline S, et al. An open-label phase 2 trial of entospletinib in indolent non-Hodgkin lymphoma and mantle cell lymphoma. Br J Haematol. 2019;184(2):215-222.
27. Rayes J, Watson SP, Nieswandt B. Functional significance of the platelet immune receptors GPVI and CLEC-2. J Clin Invest. 2019;129(1):12-23.
28. Series J, Ribes A, Garcia C, et al. Effects of novel Btk and Syk inhibitors on platelet functions alone and in combination in vitro and in vivo. J Thromb Haemost. 2020;18(12):3336-3351.
29. Shatzel JJ, Olson SR, Tao DL, et al. Ibrutinib-associated bleeding: pathogenesis, management and risk reduction strategies. J Thromb Haemost. 2017;15(5):835-847.
30. Caron F, Leong DP, Hillis C, Fraser G, Siegal D. Current understanding of bleeding with ibrutinib use: a systematic review and meta-analysis. Blood Adv. 2017;1(12):772-778.
31. Currie KS, Kropf JE, Lee T, et al. Discovery of GS-9973, a selective and orally efficacious inhibitor of spleen tyrosine kinase. J Med Chem. 2014;57(9):3856-3873.
32. Walker AR, Byrd JC, Blachly JS, et al. Entospletinib in combination with induction chemotherapy in previously untreated acute myeloid leukemia: response and predictive significance of HOXA9 and MEIS1 expression. Clin Cancer Res. 2020;26(22):5852-5859.